icpm 10 & IWPCT 9

BOOK OF ABSTRACTS

10TH INTERNATIONAL CONFERENCE ON PLASMA MEDICINE
&
9TH INTERNATIONAL WORKSHOP ON PLASMA FOR CANCER TREATMENT

8-13 September 2024
Portorose, Slovenia

BOOK OF ABSTRACTS

10th INTERNATIONAL CONFERENCE ON PLASMA MEDICINE

&

9th INTERNATIONAL WORKSHOP ON PLASMA FOR CANCER TREATMENT

Organized by**: Jozef Stefan Institute**

Date**: 8 – 13 September, 2024**

Venue**: Conference centre LifeClass Hotels & Spa Portorož, Portorose, Slovenia**

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Editors: Urška Kisovec, Nataša Hojnik, Martina Modic, Uroš Cvelbar

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WELCOME NOTE

Dear Participants,

On behalf of the organizing committee, it is with great excitement and anticipation that I welcome you to the 10th International Conference on Plasma Medicine (ICPM10), held in conjunction with the traditional summer school and the 9th International Workshop on Plasma for Cancer Treatment (IWPCT9).

ICPM10 and IWPCT9 continue the legacy of providing a vital platform for the exchange of knowledge and ideas in the multidisciplinary field of plasma biomedicine. As we gather in this beautiful setting, we aim to foster collaboration among experts from diverse fields such as plasma physics, medicine, biology, biochemistry, pharmacy, agriculture, and food science. Together, we will explore the cutting-edge developments and technological challenges in plasma medicine, a rapidly advancing field that promises significant therapeutic breakthroughs.

This conference offers a unique opportunity for professionals and researchers to engage in meaningful discussions, share insights, and establish international collaborations that will drive the future of plasma technology in medicine and beyond. We are confident that the knowledge and experiences shared during this event will contribute to the advancement of our field and inspire innovative solutions to the complex challenges we face. We hope you find this event both inspiring and rewarding.

Sincerely,

Prof. Dr. Uroš Cvelbar ,

head of the Organising Committee

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PROGRAMME

Monday, 9 September 2024

Section: PLASMA FOR CANCER THERAPY
Hall: **IWPCT**

08:55 **Introduction**
Vandana Miller

09:00 **Non-thermal gas plasma for cancer treatment and immunomodulations**
Nagendra Kaushik

09:45 **Welcome**
Vandana Miller

PLENARY SESSION
Hall: **IWPCT**
Chair: **Lars Boeckmann**

10:00 PL-1 **Cancer therapy using plasma-activated solutions** 2
Hiromasa Tanaka, Masaaki Mizuno, Kenji Ishikawa, Hiroaki Kajiyama, Shinya Toyokuni, and Masaru Hori

Section: **PLASMA FOR CANCER THERAPY**
Hall: **IWPCT**
Chair: **Thomas von Woedtke**

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11:00 IT-1 **Pleiotropic therapeutic effects of cold atmospheric plasma on cholangiocarcinoma** 8
Laura Fouassier

11:30 Mon-O-1-2 **Investigating the Effects of Plasma-Treated Hydrogel for Dual Oxidative Stress and Doxorubicin Delivery with an *in ovo* Sarcoma Model** 21
Milica Živanić, Albert Espona-Noguera, Angela Privat-Maldonado, Patrik Matušů, Francesco Tampieri, Evelien Smits, Abraham Lin, Annemie Bogaerts, Cristina Canal

12:00 13:00 **Woman in plasma medicine group meeting**

Section: **PLASMA-BASED DECONTAMINATION AND STERILIZATION**
Hall: **ICPM Session 2**
Chair: **Paula Bourke**

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Eloïse Mestre, Inna Orel, Daniel Henze, Laura Chauvet, Sebastian Burhenn, Sébastien Dozias, Fabienne Brulé-Morabito, Judith Golda, Claire Douat

14:45	Mon-O-2-8	**Liquid laboratory biowaste sterilization by cold plasma: prospects and obstacles** Aleksandra Lavrikova, Fabio Avino, Rita Agus, Vivianne Padrun, Laurence Winkel, Andrew C. Oates, Ivo Furno	41
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15:45	Mon-O-2-12	***Candida albicans* biofilm eradication through cold atmospheric plasma: The impact of voltage and exposure time in the antifungal mode of action** Manca Lunder, Rok Fink, Sebastian Dahle	45
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Section: **REGULATORY ISSUES AND STANDARDS IN PLASMA MEDICINE**
Hall: **ICPM Session 2**
Chair: **Eun-ha Choi**

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Section: **PLASMA FOR CANCER THERAPY**
Hall: **IWPCT**
Chair: **Audrey Glory**

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17:45	Mon-O-1-10	**Identification of gas plasma resistance mechanisms in human squamous cell carcinoma cells** Julia Berner, Lea Miebach, Marcel Kordt, Kristian Wende, Brigitte Vollmar, Sander Bekeschus	29
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Section: **REGULATORY ISSUES AND STANDARDS IN PLASMA MEDICINE**
Hall: **ICPM Session 2**
Chair: **Eun-ha Choi**

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PLENARY SESSION
Hall: **IWPCT**
Chair: Uroš Cvelbar

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Tuesday, 10 September 2024

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Chair: **Gerrit Kroesen**

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Section: **PLASMA LIQUID INTERATIONS, PLASMA-ACTIVATED LIQUIDS**
Hall: **ICPM Session 1**
Chair: **Annemie Bogaerts**

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12:00	Tue-O-4-4	**Chemical and Bactericidal Properties of Cell Culture Media DMEM and RPMI Modified by He/O_2 Plasma Treatment** Petr Lukeš, Barbora Tarabová, Zuzana Kovalová, Vít Jirásek	57

Section: **PLASMA SURFACE INTERACTIONS/MODIFICATIONS FOR BIOMEDICAL APPLICATIONS**
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Chair: **Lenka Zajíčková**

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11:45	IT-6	**Hydrogels in plasma medicine: focus on the chemical interactionbetween reactive species and biopolymers** Francesco Tampieri, Alfio G. Arcoria, Albert Espona-Noguera, Cristina Canal	13

12:30 – 13:30 **Lunch Break**

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Section: **PLASMA LIQUID INTERATIONS, PLASMA-ACTIVATED LIQUIDS**
Hall: **ICPM Session 1**
Chair: **Petr Lukeš**

Section: **PLASMA MEDICAL APPLICATIONS – CLINICAL AND ANIMAL STUDIES**
Hall: **ICPM Session 2**
Chair: **Thomas von Woedtke**

Wednesday, 11 September 2024

PLENARY SESSION
Hall: **ICPM Session 1**
Chair: **Masaru Hori**

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Section: **FUNDAMENTALS OF ATMOSPHERIC PLASMA**
Hall: **ICPM Session 1**
Chair: **Michael Keidar**

10:45	IT-7	**Innovative Solutions in Agriculture and Biomedicine: Leveraging Electrical Discharges in Gases for Plasma Applications** Sabnaj Khanam, Madeeha Iqbal, Khadija Akter, Juie Rana, Anchal Bhatnagar, Young Jun Hong, Eun Ha Choi, Ihn Han	14
11:15	Wed-O-7-1	**Online diagnostics of the production of reactive species in a micropulsed plasma jet** Leonardo Zampieri, Emilio Martines	129
11:30	Wed-O-7-2	**Establishing a set of measurements to monitor plasma source duplicates in collaborative groups through systematic investigation and correlation of parameters** Helena Jablonowski, Ulfilas Hoffmann, Robert Bansemer, Katayoon Hadian Rasnani, Michael Schmidt, Raphael Rataj1, Sander Bekeschus, Torsten Gerling , Klaus-Dieter Weltmann, Thomas von Woedtke	130
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Section: **PLASMA-CELL AND PLASMA-TISSUE INTERACTIONS – BIOLOGICAL AND BIOCHEMICAL REACTIONS**
Hall: **ICPM Session 2**
Chair: **Kristian Wende**

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12:30 – 13:30 **Lunch Break**
13:45 – 21:30 **Excursion + Dinner**

Thursday, 12 September 2024

PLENARY SESSION
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Chair: **Hiromasa Tanaka**

10:00	PL-5	**From transient and localized plasma surface interaction to deep tissue biological effects in Plasma Medicine** E. Robert, J.G. Bauzin, G. Collet, S. Dozias, P. Escot Bocanegra, A. Hocine, F. Prieur, J.M. Pouvesle, A. Rouillar1, A. Stancampiano, V. Vijayarangan	6

Section: **PLASMA-CELL AND PLASMA-TISSUE INTERACTIONS – BIOLOGICAL AND BIOCHEMICAL REACTIONS**
Hall: **ICPM Session 1**
Chair: **Steffen Emmert**

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Section: **FUNDAMENTALS OF ATMOSPHERIC PLASMA**
Hall: **ICPM Session 2**
Chair: **Uroš Cvelbar**

12:30 – 13:30 **Lunch Break**

14:00 – 16:45 **POSTER SESSION**

16:45 – 17:00 **Coffee Break**

Section: **PLASMA-CELL AND PLASMA-TISSUE INTERACTIONS – BIOLOGICAL AND BIOCHEMICAL REACTIONS**
Hall: **ICPM Session 1**
Chair: **Theresa Freeman**

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Section: **PLASMA FOR PHARMACEUTICAL APPLICATIONS, BIOCHEMICAL AND BIOMOLECULAR ENGINEERING**
Hall: **ICPM Session 2**
Chair: **Matteo Gherardi**

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Chair: **Nevena Puać**

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Section: **PLASMA FOR PHARMACEUTICAL APPLICATIONS, BIOCHEMICAL AND BIOMOLECULAR ENGINEERING**
Hall: **ICPM Session 2**
Chair: **Eric Robert**

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PLENARY PRESENTATIONS

(PL)

Cancer therapy using plasma-activated solutions

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Plasma-activated solutions (PAS) refer to solutions irradiated with low-temperature plasma [1, 2]. Research on PAS is explosively advancing worldwide, focusing on component analysis and elucidating mechanisms of action for applications in fields such as medicine and agriculture. We have previously invented Plasma-activated medium (PAM) [3] and Plasma-activated Ringer's lactate solution (PAL) [4] with cancer therapy as the main purpose. We have been conducting research on the components of PAL and the intracellular molecular mechanisms of cell death induced by PAM/PAL. We further demonstrated the safety and efficacy of PAL in wound healing at the animal experimental level. Last year, we initiated a specific clinical study (Phase I equivalent) as a first-in- human at Nagoya University Hospital.

It has been previously demonstrated that interactions between water, air, and low-temperature plasma lead to the generation of substances such as hydrogen peroxide and nitrite ions in solution. Furthermore, we have discovered that when solutions containing organic compounds such as PAM and PAL interact with low-temperature plasma, new organic compounds are synthesized, which we believe contribute to the selective cytotoxic effects on cancer cells. Additionally, through comprehensive analyses of gene expression profiles and metabolic profiles of cells treated with PAM/PAL, we are beginning to elucidate the impact of PAM/PAL on signaling pathways within the cells.

In the future, elucidating the conditions under which specific plasma-activated solutions (PAS) can be generated and further understanding how PAS produced under different conditions can modify signaling pathways presents a big challenge. However, if achieved, it opens up the possibility of designing PAS capable of modifying signaling pathways crucial for various cellular processes. This not only holds promise for cancer treatment but also for regenerative medicine and controlling cell fate determination. We believe that by designing PAS to modify signaling pathways effectively, a new frontier in plasma pharmacy can be pioneered.

This work was partly supported by a Grant-in-Aid for Specially Promoted Research (No. 19H05462), a Grant-in-Aid for Scientific Research (B) (No. 21H01072), and a Grant-in-Aid for Scientific Research (A) (No. 24H00202) from the Ministry of Education, Culture, Sports, Science and Technology of Japan as well as the Joint Usage/Research Program of the Center for Low- temperature Plasma Science, Nagoya University, and the Plasma Bio Consortium.

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PL-2

Star Wars in Dermatology – Plasma Medicine?

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Cold atmospheric pressure plasma (CAP) comprises a multitude of active components such as charged particles, electric current, UV radiation, and reactive gas species, which can act synergistically. Anti-itch, antimicrobial, anti-inflammatory, tissue stimulating, blood flow enhancing as well as proapoptotic effects were demonstrated in *in vivo* and *in vitro* experiments [1]. The combination of the different active agents and their broad range of positive effects on various diseases, especially easily accessible skin diseases, render plasma attractive for applications in medicine. One upcoming field of indications for plasma therapy is inflammatory dermatoses and percutaneous drug delivery. Preliminary studies indicate effects of plasma therapy towards amelioration of acne, especially improvement of acne scarring. We conducted an investigator-initiated trial and showed marked improvement of rosacea inflammation on the face after plasma application [2]. Several groups showed that plasma increases skin barrier permeability by widening of intercellular spaces and may allow skin pretreatment to enhance drug penetration in a sense of plasma oration. Thus, plasma pre-treatment may enhance treatment efficacies of topical applications.
Secondly, the potential use of CAP in cancer treatment has gained increasing interest. Especially the enhanced selective killing of tumor cells compared to normal cells has prompted researchers to elucidate the molecular mechanisms for the efficacy of CAP in cancer treatment. Plasma has been shown to induce proapoptotic effects more efficiently in tumor cells compared to the benign counterparts, leads to cellular senescence, and – as shown *in vivo* – reduces skin tumors [3]. Reactive oxygen and nitrogen species are considered the most important components mediating these anticancerous effects [4].
With respect to wound treatment, plasma is already in routine use, incorporated in complex and multimodal treatment algorithms. Several multicenter studies in the last years proofed significant accelerated wound healing with the addition of plasma. This is already incorporated in the new *Textbook of Good Clinical Practice in Cold Plasma Therapy* [5] as well as in the first clinical guideline for plasma medicine [6]. Now, automated plasma treatment as well as KI-based wound documentation are further developed.

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PL-3

Plasma for Biology and Medicine – Now and Next

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Plasma medicine is on the move! Ever since the approval of medical gas plasma technology for wound healing in Europe in 2013, a number of new subfields have evolved and are substantiated by intriguing publications. In medicine, new applications in dermatology, orthopedics, surgery, oncology, and dentistry of plasmas alone or in combination therapies are on the rise, explored by preclinical evaluations. Plasmas are also increasingly recognized as versatile tools for biology and biochemistry. This non-exhaustive plenary lecture will provide an overview of existing and upcoming trends.

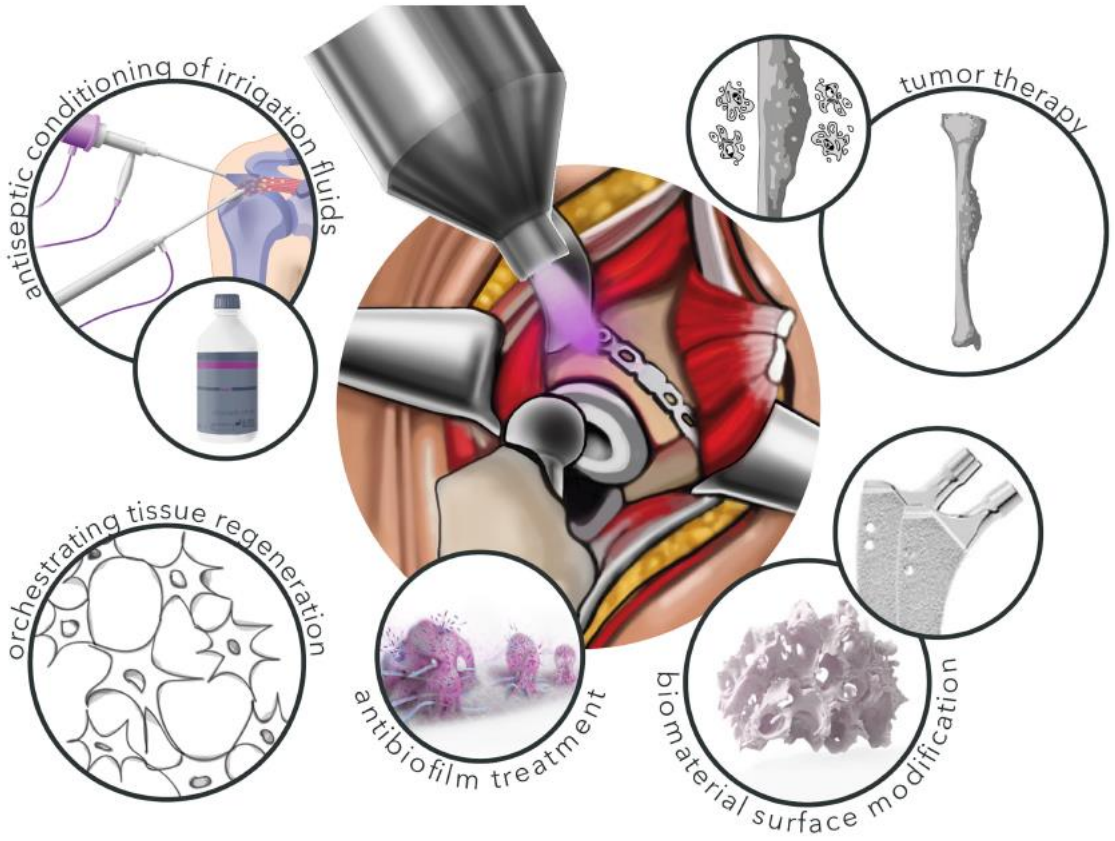

Fig. 1. The field of orthopedics exemplifies well the multifaceted applications of gas plasma technology in medicine [1].

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PL-4

Cold plasma as a combinatorial therapy to combat orthopaedic infection

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Non-antibiotic options are needed to combat biofilm formation in clinical settings, as both a means of preventing and treating areas prone to and with active infections, respectively. Cold plasma, an energized gas containing a cocktail of reactive oxygen and nitrogen species, may provide such a strategy. *In vitro*, cold plasmas have been shown to have antimicrobial properties through direct bacterial killing and modulation of virulence factors. Additional studies have implicated the potential immunostimulatory effects of cold plasma. Clinical studies using cold plasma to heal diabetic ulcers and non-healing wounds have also shown successful outcomes. Unfortunately, to date, there are few reports of the *in vivo* effects of cold plasma treatment to combat or treat surgical infections. The surgical strategy for cold plasma would generally permit only a single treatment, as opposed to the multiple treatments employed in skin wounds. The surgical site environment and tissue types involved are also more complex. Thus, many questions remain unanswered as to the *in vivo* efficacy and mechanism of action of cold plasma treatment in this area.

As part of a multi-center investigation, the efficacy of cold plasma for treating infection in an *in vivo* rat model, was undertaken. Infection mitigation following cold plasma treatment alone and in combination with plasma activated liquids and with antibiotics is being assessed by measurement of colony forming units (CFU), qPCR, Westerns, cytokine arrays, IHC, RNA sequencing, and histology. Interestingly, early experiments employing cold plasma alone showed little or no reduction in CFUs at 4- or 14-days post treatment. However, RNAseq data revealed cold plasma treatment significantly enriched genes associated with innate immune cell response. Cytokines were also increased at the site of infection, coordinating with increased myeloperoxidase protein and positive cells. Surprisingly, draining lymph nodes in plasma-treated animals were markedly smaller than untreated animals, which had significant lymphadenomegaly. These results and how cold plasma treatment in combination with standard clinical antibiotic therapies will be further discussed.

PL-5

From transient and localized plasma surface interaction to deep tissue biological effects in Plasma Medicine

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It is obvious that during so-called direct plasma treatments for biomedical applications, the plasma delivery consists in a transient interaction with the upper surface of tissues. Nevertheless, from the pioneer *in vivo* demonstrations of plasma therapeutic benefits, it is well known but still highly questioning on how such surface plasma interaction may lead to deep tissue response, e.g., regression/ablation [1,2]- apoptosis induction [1]- signaling [3] in subcutaneous tumors, tissue regeneration for thick wounds [4], deep tissue oxygenation [5, 6].
The presentation first summarizes reactive and charged species generation, electric field features, micro-current characteristics and thermal input when targets of various nature and relevant for biomedical applications are exposed to plasma jets. A focus is proposed on the broad timescale range to be considered according to these different plasma components. Then *in vitro* and *ex vivo* transient permeabilization and localized or distant tissue oxygenation are documented. The presented results illustrate the potentialities of non-thermal plasma exposure for deep, instantaneous, delayed, transient or persisting tissue response and its possible combination with other non-plasma-based therapeutic protocols in oncology or wound care.

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INVITED TALKS

(IT)

Pleiotropic therapeutic effects of cold atmospheric plasma on cholangiocarcinoma

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Introduction. Cholangiocarcinoma (CCA) is a rare tumor of the bile ducts characterized by a poor prognosis and a rich desmoplastic stroma. As the efficacy of systemic palliative chemotherapies remain quite limited, it is mandatory to develop new therapeutic options against CCA. In this outlook, cold atmospheric plasma (CAP) shows promises in oncology. Generated from the partial ionization of a gas, CAP generates reactive oxygen and nitrogen species that exert deleterious cellular effects leading to cell death or dysfunction. **Methods.** Human cell lines of CCA and of its microenvironment, namely cancer-associated fibroblasts (CAFs) and tumor-endothelial cell (TECs), cultured in 2D or in 3D (spheroids), were treated directly with CAP. In tumor cells, immunogenic cell death (ICD) induction was evaluated *in vivo* through vaccination assays and *in vitro* by measuring the release of DAMPs in the extracellular environment. Concerning the stromal compartment, alterations in phenotype and cell functions were analyzed by live-imaging microscopy *in vitro*. **Results.** We showed that CAP induces antitumor effects that can be direct (i.e tumor cell death) and indirect (i.e stromal cell dysfunctions, activation of immunosurveillance). As proof of the direct antitumor effects, we demonstrated that CAP-triggered oxidative stress decreases tumor cell viability and led to the release of ICD key messengers which stimulate the tumor-surrounding immune cells and promote antitumor immunity, as we demonstrated *in vivo*. Interestingly, CAP also exhibited indirect antitumor effects by reducing CAF activation, impeding their migration, and inhibiting TEC angiogenic profiles. **Conclusion.** CAP opens perspectives for local treatment of CCA. In order to enhance the translational relevance of the technology for the patients, the plasma source has been miniaturized to deliver the plasma *in situ via* an endoscope (patented). In close collaboration with clinicians, feasibility and safety studies of the endoscopic plasma probe in a large live animal model are underway, prior to the launch of a clinical trial in humans with CCA.

IT-2

Application of non-thermal plasma during tumorectomies: from the lab to the clinic

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Local recurrence (LR), i.e., the resurgence of a tumor in the same place as the primary tumor, can significantly decrease both long-term survival and quality of life. Despite achieving a macroscopically complete resection, patients with locally recurrent disease still experience inferior oncologic outcomes [1]. The use of adjuvant radiotherapy (RT) has been proven to decrease the risk of LR and to increase overall survival rates [2]. As the cancer cell load is highest in the peritumoral area, it dictates the dose of RT needed to eradicate the last cells to obtain local control. However, increasing RT dose to normal tissues is associated with higher risks and severities of side effects. It is therefore important to find new strategies to reduce the cancer cell burden in the surgical cavity while safely reducing RT dose and toxicity. One of these strategies could be to use intra-operative non-thermal plasma (NTP) treatment to improve the local control of solid cancers.

To test this hypothesis, the NTP applicator Convertible Plasma Jet (CPJ) from NexPlasmaGen Inc was used in vivo on a recurrence model of triple negative breast cancer (TNBC) and murine fibrosarcoma. To do so, immunocompromised NodScid mice were orthotopically injected with MDA-MB-231 TNBC cells, and immunocompetent C57Bl/6 mice were injected sub-dermally with MCA-205 fibrosarcoma. After 2 to 3 weeks, most of the tumor is removed, leaving only a 2x2x2mm piece to simulate remaining tumor cells on a surgical bed. These remaining cells will then be treated with NTP to measure its impact on local recurrence compared to non-treated controls. At necropsy, tumors treated with NTP were at least 40% smaller than the untreated controls, for both TNBC and fibrosarcoma. These results show that NTP can kill solid cancer cells in vivo in a single treatment and could therefore help secure the tumor bed post tumorectomy.

The next step is a first-in-human study to evaluate the safety and non-toxicity of NTP used intra-operatively. A maximum of 30 breast cancer patients are being recruited at the CHUM and their tumor beds treated with NTP immediately after the removal of the tumor. They are then monitored for adverse events, pain indices and cosmetic appearance during a 3-month follow-up period. Correlative samples are collected to evaluate the efficacy of NTP ex vivo. If the safety and nontoxicity of NTP is demonstrated during this first-in-human study, a phase II clinical study will be launched to evaluate the impact of NTP as an adjuvant for breast cancer treatment to reduce LR.

This work was supported by Quebec Ministry of Economy and Innovation (PSO- 55896 /INVA-043) and Quebec Breast Cancer Foundation.

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Physical Plasma in Clinics - Requirements, Tech-transfer, Acceptance

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Non-invasive physical plasma (NIPP) represents an interesting therapeutic option for acute vs. chronic benign diseases as well as for curative vs. palliative malignant diseases both as a direct and indirect application. At the Division for Plasma Medicine and Medical Technology at the Department of Women's Health in Tübingen, NIPP has been developed for several years, physicochemically and biologically characterized, and evaluated by clinical studies. Successful examples of clinical application of NIPP technologies include preventive aspects in oncology, chronic inflammation and interventional medicine.
Preclinical and clinical studies by our working group showed that NIPP enables effective, painless therapy of low-grade (LSIL) and high-grade (HSIL) intraepithelial neoplasia of the cervix and vulva without interfering tissue integrity [1,2]. Furthermore, Plasma activated liquids (PAL) may be effective in context of preventing postoperative intraabdominal adhesions via the selective inhibition of activated fibroblasts and the expression of pro-adhesive factors, cytokines and extracellular matrix.
The clinical translation process in plasma medicine includes the identification of medical burden, the indication-based development of plasma sources, the biomedical characterization of cellular mechanisms, the early knowledge of regulatory aspects and corresponding experimental planning and documentation as well as well as the appropriate choice of clinical study concepts and the early collaboration with clinical partners.
The combination of plasma medicine with methods for molecular image recognition and image integration using AI approaches enables personalized and disease-specific in vivo application.

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Atmospheric pressure plasmas strategies for medical important biofilm inactivation

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Medical biofilms are of great importance due to their impact on health and disease management. These complex, multi-species microbial communities adhere to surfaces and are enveloped in a protective matrix, making them highly resistant to conventional antimicrobial treatments. The persistence and resilience of biofilms are major contributors to chronic infections, particularly in medical settings where they can colonize medical devices such as catheters, implants, and prosthetics. Biofilms offer bacteria a protection from the immune system and increase their resistance to antibiotics by up to 1000 times compared to planktonic bacteria [1]. As antibiotic resistance continues to rise globally, understanding and targeting biofilms becomes critical. Innovative approaches and research are urgently needed to develop new strategies for preventing biofilm formation and effectively treating biofilm-associated infections, ultimately safeguarding public health and improving clinical outcomes.
This contribution is based on the recent progress and investigation of cold atmospheric pressure plasma technology (CAP) for microbial deactivation. CAP generates UV, electric fields, charged particles and reactive oxygen and nitrogen species, such as ozone (O_3), hydroxyl radical (OH·), singlet oxygen $^1O_2$, superoxide O_2^-·, atomic oxygen O, organic radicals RO·, RO_2·, atomic nitrogen, N, nitric oxide NO_2·, nitrogen dioxide NO_2·. All of these molecules have been reported to contribute to the efficiency of plasma-assisted inactivation of biological materials. Typically, biofilms are mainly surrounded by water environment, which presents a certain barrier for plasma species in gaseous phase to directly attack microbial cells. ROS and RNS generated in the gaseous phase are transported through the plasma-liquid interface where the secondary RONS in liquid medium are formed. In so called plasma activated water (PAW), species such as OH·, hydrogen peroxide, nitrites and nitrates, peroxynitrites/peroxynitrous acid ($ONOO^-$/ONOOH), peroxynitrates/peroxynitric acid (O_2NOO^-/O_2NOOH), superoxide/perhydroxyl radical $O_2^{\cdot-}$/HO_2· and ozone are present, depending on the plasma operating conditions.
In this research we used atmospheric pressure air plasma with surface barrier discharge electrode configuration to create plasma activated water (PAW), which was used to treat *E. coli* biofilms. Biofilms were exposed to RONS for a different period of time in order to evaluate the time needed for complete kill. In the second part of the study, we investigated the influence of reactive species on individual biofilm components, such as lipids, polysaccharides, proteins and DNA.
This work was supported by ARRS National Grants no. J2-50047 and J2-4451 and BI-US/22-24-080.

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Plasma-Liquid Interactions in The Presence of Organic Matter – Including Chemical, Biological, and Electrical Interactions

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In plasma medicine, we are facing truly interactive behavior between plasma and biological systems occurring simultaneously at two interconnected levels: chemically and electrically. Detailed knowledge of the transport behavior of RONS from the gas phase into the liquid phase is crucial to understand the complex processes and interactions when organic matter is introduced in the liquid. Plasma-organic matter interactions are dynamic and co-evolve, in particular when living organisms are present. Some changes occur in real time and others develop slowly over minutes, hours, or even days later. Static measurements do not reflect the true picture, raising questions and challenges for future research: How does the cell response contribute to plasma-liquid chemistry? And how does changing chemistry feed back to the plasma discharge? Ideally, measurements of physical and chemical changes must be made in the presence of the biological target, during exposure to plasma.

In addition to the chemical feedback and coupling, there is electric coupling and potential feedback from the living target back to the plasma. Physical properties such as electric fields and surface charging affect cell properties, dielectric properties of the target material, and the transport across the interface. As the cells are reacting to the plasma treatment, they also may affect the coupling and the plasma itself.

The multifaceted interactions are explored by measuring chemical species in gas and liquid phase, as well as plasma discharge current and voltage in the presence of different targets, ranging from dielectrics to fungi, and ultimately human keratinocytes and mice. We aim to measure the electrical behavior of the complex system as one system in situ and to monitor changes in dielectric properties of materials and changes in the gap electric field during plasma treatment of targets to fully explore the dynamic and transient interactions between plasma and biological targets.

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Hydrogels in plasma medicine: focus on the chemical interaction between reactive species and biopolymers

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Hydrogels are hydrated polymer networks that are used for various biomedical applications, such as tissue modelling, drug delivery, tissue regeneration and as medical dressings. They have recently been introduced in the plasma medicine field with two main purposes: 1) as models to study the interaction of plasma-generated reactive species (RS) with soft tissues and 2) as vehicles for local RS delivery [1].
This contribution will present the primary findings of the research conducted at the PlasmaMED lab at Universitat Politècnica de Catalunya (UPC) in recent years in these two fields, with a particular emphasis on the chemical interaction between RS and biopolymers.

Hydrogels as models to study the interaction of plasma-generated RS with tissues. Comparison of results obtained using hydrogels composed of different biopolymers – ie. agarose and gelatin - and containing colorimetric probes revealed that the chemical composition plays a major role in the distribution and penetration of RS. These species were quantified in the hydrogels after plasma treatment using newly developed protocols.

Hydrogels as vehicles for local RS delivery. The effect of direct plasma treatment on various biopolymers' solutions (polysaccharide- and protein-based) was analyzed with the focus of quantifying the total amount of RS that can be stored in the solution and of assessing any eventual changes at molecular level of the biopolymers' structures. These experiments were done using chemical probes, chromatographic analysis and computational modelling. The findings highlight the direct plasma's capability to degrade and oxidize the chains of biopolymers, and the impact of these modifications on the generation and stability of RS in solution [2,3]

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Innovative Solutions in Agriculture and Biomedicine: Leveraging Electrical Discharges in Gases for Plasma Applications

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Non-thermal biocompatible pressure plasma (NBP) has emerged as a versatile tool within bioresearch, spanning disciplines such as biotechnology, food safety, agriculture, and medicine. Its capacity to treat a diverse array of biomaterials swiftly and effectively, facilitated by adaptable device setups and electrode configurations, has garnered significant attention.

In agricultural contexts, NBP finds utility in mitigating seed, soil, and land contamination caused by biomass, plastics, medical waste, and environmental factors like droughts and floods, thus contributing to environmental remediation efforts. Within biomedicine, NBP exhibits multifaceted applications, including tissue treatment, cancer therapy, wound healing, sterilization of medical instruments, viral inactivation, microbial degradation, and surface modification of microorganisms.

Recent advancements in plasma agriculture have focused on manipulating electric fields to alter genotypic and phenotypic traits. This study seeks to assess the effectiveness of Plasma treated water in disease management, seed decontamination, enhanced germination, accelerated plant growth, and microbial elimination, utilizing a nitric oxide generating plasma source. Our long-term objective is to establish a market for NBPs targeting cancer cells, plant seeds, and microbial populations.

Keywords: Non-thermal biocompatible plasma, plasma-based fertilization, disinfection, agriculture.
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The Mechanisms of Cold Atmospheric Plasma in Promoting Healing of Infectious Wounds

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Infectious wounds pose significant challenges in wound management [1-2]. Cold atmospheric plasma (CAP) has emerged as a promising therapeutic approach for enhancing wound healing [3-5]. However, a crucial question remains unanswered regarding the differential effects of CAP on microorganisms and normal tissue cells during wound treatment. This study aimed to address this question by investigating the effects of CAP on common wound infection bacteria and normal skin cells under identical treatment conditions [6].

In vitro experiments were conducted to assess the impact of CAP on bacteria and cells. Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli, common pathogens in infected wounds, were selected, along with human keratinocytes (HaCaT cells) and human skin fibroblasts (HSF). These cell types play crucial roles in wound healing processes. Plasma treatment was administered to specific areas containing bacteria and cells, with careful consideration given to their respective sizes. Bacterial inactivation and cell viability were quantitatively evaluated, alongside the secretion of type I collagen by fibroblasts.

Results indicated that CAP effectively inactivated all three bacteria within 3 minutes, with Pseudomonas aeruginosa being particularly sensitive and inactivated within 2 minutes. HaCaT cells maintained over 90% activity, and HSF cells maintained over 70% activity when treated with the same dose of plasma. Additionally, plasma treatment led to an increase in the secretion of type I collagen by HSF cells. These findings suggest that CAP can selectively target bacteria while preserving the viability and activity of normal tissue cells, thereby promoting wound healing.

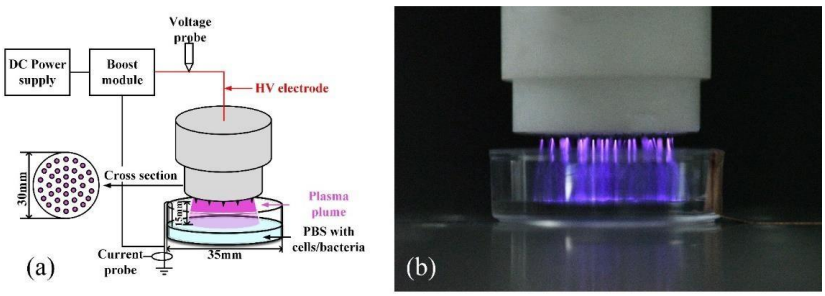

Fig. 1 Diagram and photograph of device for treating bacteria and cells with air plasma jet

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IT-9

Plasma Polymers as Surface Finishes for Tissue Engineering Materials

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One of the primary efforts in tissue engineering is to find materials suitable as tissue scaffolds supporting cell growth, i.e., tissue regeneration. High biocompatibility, low immunogenicity, appropriate biodegradation, and good mechanical properties are required, but the ideal biomaterial should also promote cell attachment, proliferation, migration, and differentiation in a desired way. Therefore, a critical factor is building a proper extracellular matrix (ECM) that contains proteins, growth factors, and other substances regulating cellular functions and determining the action and fate of the cells that it surrounds. Although the ECM is secreted by cells, the advantageous artificial scaffolds should morphologically resemble the structure of ECM [1].

Initial cell interactions with biomaterials, which are in direct contact with body fluid and cells, are influenced by surface energy, chemistry, and morphology, including the pore size in the case of porous materials. The interaction starts with wetting the surface and the adsorption of proteins from the blood or sera [2]. Synthetic biodegradable polymers, such as polycaprolactone (PCL), poly (L-lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid), are promising candidates for biomaterial development because they can be easily electrospun in the form of nanofibers resembling the structure of ECM offering more controllable nanofibrous morphology than natural polymers [3]. However, it is necessary to overcome their hydrophobicity, which is further increased by the topological effect of nanofibrous architecture [4].

Plasma polymerization provides versatile technique that can easily modify the surface of synthetic polymers by depositing a thin functional film, even if they are in the form of temperature sensitive nanofibers [5,6,7]. The gas feed to the plasma can target the deposition of either nitrogen or oxygen containing organic films [8]. The rationale behind these choices is the bioactivity of primary amines (-NH_2) or carboxyl (-COOH) groups, respectively. In this contribution, both these types of plasma polymerization processes will be discussed concerning the relation between the plasma conditions and thin film properties, cell adhesion, proliferation and cell imune reaction to PPs. We will review the findings on peculiar adhesion of cells to amine PPs [9] and compare it with comercial aminated cultivation dishes and poly-L-Lysine coatings.

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Albumin aggregation by cold atmospheric plasma

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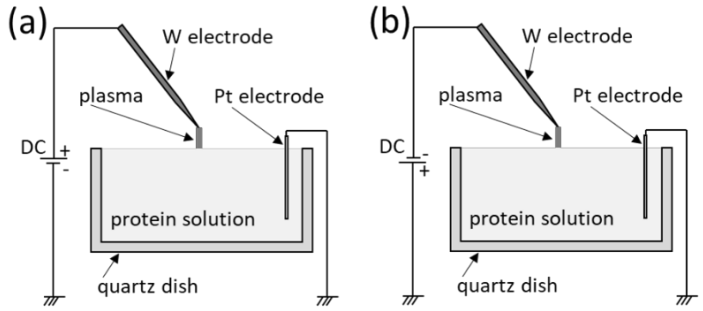
Cold atmospheric plasmas have been investigated and tested for biomedical applications such as sterilization, wound treatment, cancer treatment and bleeding control [1]. The low temperature of the plasma allows us to treat living and liquid substances without thermal damage. One example of the biomedical applications is blood coagulation by a plasma treatment using a helium plasma jet [2]. In the blood coagulation there are three major plasma-induced behaviors: aggregation of protein, hemolysis, and aggregation of platelet and fibrinogen [3]. This report presents the process of albumin (the most abundant protein in blood) aggregation by plasma treatment.

It was already reported that the albumin aggregation depended on how the charge was supplied onto the albumin solution by a plasma discharge [4,5]. The recent studies revealed that the electrical conditions of plasma discharge determined the level of aggregation on the protein solution. However, the mechanism of protein aggregation by the plasma treatment remains unclear.

The aim of this study is to understand the mechanism of albumin aggregation by the plasma treatment. Better understanding of the mechanism results in a further optimization of plasma- hemostasis process. In the presentation, the role of plasma agents such as reactive species, charged particles, UV light, on the albumin aggregation is shown. Moreover, using DC discharge plasmas as shown in Fig. 1, the relation between the aggregation and the deposited charges on the albumin solution is discussed.

Fig. 1 Plasma treatment using DC discharge plasmas for protein aggregation. (a) plasma production with



positive polarity, and (b) that with negative polarity.

Acknowledgement

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IT-11

Cold Atmospheric Pressure plasmas uses in the food sector

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Cold Atmospheric Pressure plasmas (CAP) and Plasma Activated Water (PAW) are gaining interest as innovative non-thermal methods for food and food packaging decontamination. They've shown potential in combating new pathogens and meeting changing consumer needs. Numerous studies have proven CAP's ability to deactivate harmful enzymes and microorganisms in food. However, safety concerns need addressing before widespread use. Initial tests on food matrices show promising results for decontamination, but the impact on nutritional profiles needs further study. For instance, CAP's effect on Aspergillus strains on dried tomatoes was examined, showing reduced spore germination and damage to cellular structures. Yet, the tomatoes' physicochemical properties and antioxidant activity remained unchanged.[1] Similarly, plasma exposure on pistachio kernels didn't significantly alter their lipid composition. [2] Moreover CAP effect on horseradish peroxidase (HRP) activity in phosphate buffer and sugar model systems is investigated. The results showed that sugars, especially disaccharides, reduced CAP's efficacy on enzyme inactivation. Spectroscopic analyses revealed that sugars preserved HRP's structure and hindered heme degradation. However, these findings only partially explain the protective effects of sugars on HRP. Other factors like the ability of sugars to quench plasma reactive species and stabilize proteins also play a role in reducing HRP inactivation. PAW also effectively reduced bacteria on rocket leaves with minimal impact on quality and nutrition. [4] In addition to food decontamination, cold plasma is also being recognized as a potential solution for sanitizing food packaging surfaces. This is a vital process in the food industry to maintain product safety and quality. A review was conducted to evaluate the effectiveness of plasma-assisted systems for packaging decontamination. The results showed that this sanitation technique effectively reduced the pathogen load on packaging, with an average logarithmic reduction above 4. This aligns with the standard requirements for commonly used antiseptics, making it a promising technique. Future research should aim at optimizing these processes for industrial applications. This collective research enhances our understanding of CAP's effect on food and packaging decontamination and shows that naturally occurring sugars can mitigate the inactivation and structural changes induced by CAP on enzymes [5].

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Monday, 9 September 2024

Oxidative stress of cancer cells mediated by organic molecules and plasma-modified liquids/hydrogels

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Plasma Treated (PT) liquids currently stand out in the field of cancer treatment as sources of exogenous blends of reactive oxygen and nitrogen species (RONS). Recent advancements have demonstrated that plasma treated organic molecules could exert an active role in promoting anticancer activity [1] and that phenols, as an example, due to their pro-oxidant ability could generate in vitro and in vivo RONS like O_2^-, H_2O_2 that are cytotoxic toward cancer cells [2]. Thus, combining plasma produced RONS and organic molecules, in principle, we can carry out a targeted attack on tumor cells. Yet, so far, this combination, to the best of our knowledge, is almost unexplored both in PT-liquids and PT-hydrogels. In this study, we investigated the role of phenolic side chains or amphiphilic linear molecules in promoting the formation of RONS both in plasma treated PT-liquids [3] and thermo-responsive hydrogels. The aim of the present investigation was to induce tumor toxicity and trigger apoptosis pathways. A deep chemical characterization shows a tight correlation between the produced RONS and organic molecules in PT-liquids and PT-hydrogels. Moreover, the results indicate a reduced cell viability and oxygen uptake, due to an increase in intracellular ROS levels, and activation of apoptosis pathways. These results should be related to the activation of the mitochondrial-mediated and p-JNK/caspase-3 signaling pathways. Innovative devices based on infusion solutions or PT thermo-responsive materials have been studied as potential RONS delivery systems, against head and neck (HNC) or colorectal (CRC) cancers. The observed results offer improved knowledge about the mechanisms underlying cancer treatment and a valid method to set up a prompt, adequate, and effective combined therapy in the clinic.

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Investigating the Effects of Plasma-Treated Hydrogel for Dual Oxidative Stress and Doxorubicin Delivery with an *in ovo* Sarcoma Model

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Plasma-treated hydrogels (PTH) are a novel plasma treatment modality that could enable precise and minimally invasive delivery of plasma-derived reactive species to internal tumors [1]. PTH also offer a possibility to combine plasma with hydrogel-based drug delivery and tissue engineering. Recently, we developed an injectable alginate PTH that could kill osteosarcoma cells and promote release of danger signals to enhance the uptake of cancer cells by immature dendritic cells [2]. Followingthese promising results, we investigate alginate PTH for dual delivery of plasma-derived reactive species and Doxorubicin (PTH-D). A conceivable application of PTH-D could be the treatment of surgical margins following excision of sarcomas. Such combinatorial and local treatment may help combat drug resistance and side effects, which are common for this type of drug and cancer. To test the proposed hypothesis, human osteosarcoma and liposarcoma tumors were first grown on the membrane of fertilized chicken egg (*in ovo* tumor model), enabling a more realistic and vascularized 3D environment which enhances the translatability of the research results. Tumors underwent either mono- or combinatorial treatment and were then collected for *ex vivo* analysis. Immunohistochemical stainings were performed to evaluate the effect of the treatment on multiple cellular markers associated with different types of cell death or poor prognosis. The combinatorial treatment led to a statistically significant, but modest, reduction in tumor size. More pronounced effects were visible at microscopic levels, where the combinatorial treatment could reduce the expression of glutathione peroxidase 4 (GPX4) in osteosarcoma. GPX4 is a promising target in tumor treatment, as its inhibition favors ferroptosis, which can play pivotal role in tumor suppression and reversal of drug-resistance.

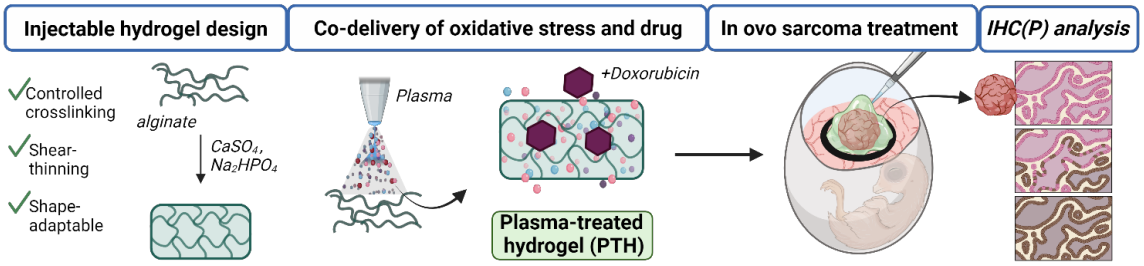

Fig. 1 Characterization of PTH-based combinatorial treatment in an *in ovo* sarcoma model.

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Synergistic Effects of Plasma-Treated Water Solutions and Doxorubicin in Head and Neck Cancer Treatment

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Head and Neck cancer (HNC) represents a significant global health challenge, constituting the 7th most prevalent cancer worldwide [1]. Conventional therapies are characterized by high morbidity rates and poor prognosis [2], highlighting the need for innovative interventions. Plasma Treated Water Solutions (PTWS) have recently emerged as a novel and effective cancer treatment approach [3]. Here, we investigated the effect of a combination of PTWS, generated by adding L-tyrosine (Tyr) to an electrolyte rehydrating III solution (SIII) treated with cold atmospheric plasma (CAP) and doxorubicin (doxo) on a HNC cell line. To determine the optimal combination, the IC50 was calculated by exposing the human epidermal keratinocytes (HaCaT) and the hypopharyngeal carcinoma cell line (FaDu) to doxo concentrations ranging from 0.01 µM to 10 µM. Then the effect of SIII, supplemented with Tyr and/or activated with air or O_2 plasma for 10 and 20min, on the proliferation inhibition was tested by MTS at 24, 48 and 72h. Subsequently, wound healing and clonogenic assays were performed. In FaDu cells exposed to doxo and SIII-Tyr alone or in combination, the proliferation inhibition was quite low. However, when the clinical solution was treated by plasma, the cytotoxicity increased significantly up to 60%. Interestingly, after 72h PTWS and doxo exerted a synergistic effect in the O_2-10 min and air-20 min samples. Additionally, air- 10min PTWS showed low cell proliferation inhibition at 48 and 72h, while the combination with doxo increased cell proliferation inhibition to over 40%. For non-tumoral HaCaT cells, cell proliferation inhibition was lower than 40% in all experimental points, with no synergistic effects found in the combination. The HaCaT wound healing assay revealed no significant differences among the various experimental points, except for the O_2-10min PTWS, where the gap remained open for 72h after treatment. Conversely, air and O_2 PTWS effectively reduced wound closure when combined with doxo, supporting the hypothesis of a synergistic effect. The clonogenic ability of FaDu cells, preserved in the SIII-Tyr compared to the control, was inhibited by treatment with doxo and PTWS, either alone or in combination. For HaCaT cells, the results were very similar to those found in tumor cells. The wound healing and clonogenic assays further support the synergistic potential of PTWS and doxo, indicating promising therapeutic outcomes for cancer treatment.

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Plasma Enhance Immunotherapy for Cancer Treatment

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Cold atmospheric plasma (CAP) is a unique form of physical plasma with great potential for cancer therapy. Our previous studies showed that local CAP treatment on residual tumor cells at the surgical cavities effectively induces cancer immunogenic cell death in situ and evokes strong T cell-mediated immune responses to combat the residual tumor cells [1-2]. CAP uses ionized gas to induce lethal oxidative stress on cancer cells, and the efficacy of CAP therapy continues to be improved. We reported a CAP-mediated ICB therapy integrated with microneedles (MN) for the transdermal delivery of ICB and an injectable Pluronic hydrogel was employed for intratumoral administration [3-4]. We found that the hollow-structured MN (hMN) patch and injectable hydrogel facilitate the transportation of CAP through the skin for enhanced ICB therapy causing tumor cell death and releasing tumor-associated antigens in situ and evoking both strong innate and adaptive, local and systemic anti-tumor immune responses, therefore, which can synergistically augment the efficacy of immune checkpoint inhibitors. In addition, we described an injectable hydrogel-mediated approach that can further enhance CAP-induced ICD by elevating the phosphorylation of eIF2α [5], CAP/trehalose therapy promoted dendritic cell (DC) maturation, initiating tumor-specific T-cell mediated anti-tumor immune responses. Overall, those treatment strategies with CAP can not only potentially minimize ICB-related systemic side effects. But also, can be extended to treat different cancer types and various diseases on cancer treatment.

Keywords: Cold atmospheric plasma, Immune checkpoint blockade, Immunogenic cell death, Cancer immunotherapy, Drug delivery

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Medical gas plasma technology synergizes with immune checkpoint blockade in the treatment of dermatologic malignancies

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Immune checkpoint inhibitors have revolutionized clinical outcomes of patients suffering from melanoma and many other malignancies in recent years. Yet, inefficient priming of an anti-tumor immune response limits therapeutic efficacy in up to 65 % of patients. Recently, several studies demonstrated increased efficacy upon combined radiotherapy, improving initiation of the cancer immunity cycle via induction of immunogenic cell death (ICD), antigen release from irradiated cells and increased tumor sensitization to T cell responses. Similar observations have been made for medical gas plasmas. Despite direct tumor-toxic effects, plasma-derived ROS have been shown to create an inflammatory-like environment able to trigger antitumor immune responses, e.g., through induction of ICD via ROS-induced ER-stress [1] or formation of neoantigens via oxidation of biomolecules [2]. Concomitant with increased leucocyte infiltration, we could recently show that gas plasma treatment induces ICD and protects mice from tumor growth after preventive vaccination [3]. In this light, we hypothesized that the bimodal action of gas plasma treatment could potentiate responses to anti-PD1 immune checkpoint blockade (ICB). Using the plasma device kINPen Med, accredited as a medical device class IIa in Europe, combinational effects were evaluated in a syngeneic model of melanocytic and non-melanocytic skin cancer *in vivo*. Tumor growth was monitored based on bioluminescent imaging, and single-cell suspensions were retrieved from each tumor to characterize tumor-infiltrating leucocytes using multi-color flow cytometry. Complemented by gene expression profiling using NanoString technology and RNA sequencing of explanted tumors our data indicate that local tumor oxidation primarily acts on antigen-presenting cells (APCs) facilitating T cell activation and infiltration of T cells in the tumor microenvironment. Antibody- mediated depletion of T lymphocytes and myeloid cells *in vivo* outlined the central role of APCs as effectors of plasma-induced immunomodulatory effects. Collectively, our results outline mechanisms and prospects of applied redox medicine in immunooncology and the treatment of dermatologic malignancies.

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Revolutionizing pancreatic cancer therapy with the combination of chemotherapy and cold atmospheric plasma using the turkey *in ovo* model

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Pancreatic ductal adenocarcinoma (PDAC) has low five-year survival rates of 2-9% and is characterized by high resistance towards chemo- and radiotherapy due to an extreme desmoplastic tissue [1, 2]. Therefore, new alternative or combinational treatment approaches are necessary to target this disease and improve survival outcome. It has been observed that CAP does not only influence cancer cells, but also the pancreatic stellate cells (PSCs) in the tumour microenvironment (TME) [3, 4]. In their activated state, PSCs play an important role in the development and survival of PDAC tumours by creating a complex TME [5]. The aim of this study is to evaluate the effect of CAP on PDAC cells in combination to chemotherapy, while considering the complex PDAC TME.
Our study utilized a turkey chorioallantoic membrane (CAM) model to investigate the efficacy of combining cold atmospheric plasma (CAP) with chemotherapy for PDAC. Using turkey eggs, which allow for a longer experimental period than chicken eggs, we mimicked a complex tumour microenvironment by seeding both PDAC tumour cells and pancreatic stellate cells onto the CAM. CAP treatment, delivered via the kINPen MED device at a flow rate of 2 slm, was combined with direct injection of chemotherapy into the CAM's blood vessels. Tumours were harvested and weighed to evaluate treatment outcomes and any potential synergistic effects. Flow cytometric analysis was used to assess treatment effects on both the cancer and stellate cell populations separately. Overall, our findings highlight the effectiveness of combining CAP with chemotherapy in targeting PDAC tumours within a complex TME. The use of the turkey CAM model offers a practical and ethical approach for studying tumour biology and therapeutic interventions. Further investigations are necessary to optimize the treatment strategy for potential clinical applications.

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Implementation of Non-Thermal Plasma as an Immunogenic Therapy Addition to the Standard-of-Care for Head and Neck Squamous Cell Carcinoma

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Patients with advanced head and neck cancer often face relapse, metastasis (R/M HNSCC), and detrimental outcomes. First-line immunotherapy, alone or with platinum-based chemotherapy (CIS), has limited benefit due to low response rates and severe side effects [1]. Therefore, well-tolerated therapeutic strategies to improve the established therapies are much required. As non-thermal plasma (NTP) has been reported as an inducer of immunogenic cell death (ICD) [2], our goal was to evaluate the immunogenicity of a novel combination of NTP with the R/M HNSCC first-line therapies. With the purpose of clinical introduction, this study was performed in advanced 3D *in vitro* and *in vivo* tumor models. All experiments were performed using a microsecond-pulsed dielectric barrier discharge plasma system. For the first phase of our study, we optimized a micro-tissue spheroid model for several HNSCC cell lines. After tumor kinetics were determined, combination treatments of NTP and CIS were analyzed for the induction of several membrane-associated and secreted ICD markers. Immunogenicity was tested functionally with dendritic cell (DC) co-culture experiments. Our data showed a significant upregulation of ecto-calreticulin, an important 'eat-me signal' for immune cells, along with two heat- shock proteins at 24h post treatment. In addition, combination therapy improved the release of both the early ICD marker ATP, as the late-stage factor HMGB1. Moreover, DC co-culture experiments demonstrated increased phagocytosis of HNSCC tumor cells after NTP-CIS application. This data is currently being supplemented with an in-depth MSD screening of immune-regulating cyto- and chemokines. In the next phase of our study, an optimized NTP-CIS combination was tested in 'patient-derived organoids' (HNSCC-PDO), to validate treatment efficacy in a state-of-the-art tumor model with strong clinical translation capacity. For the analysis of these therapeutic effects, our lab employed a novel kinetic drug screening platform – Orbits . We demonstrated that our 'multi-organoid-per-well' model can be used to evaluate PDO killing, with increased tumor cell death in the NTP-CIS regimes compared to monotherapies. Evaluation of immune engagement by our optimized treatment is ongoing. Lastly, our study will be supplemented with the golden-standard *in vivo* vaccination assay [3] to confirm bona fide ICD induction of our combination therapy in a fully immunocompetent biological system. In summary, our results underline the potential of NTP as a valuable and immunogenic therapy addition to improve established HNSCC therapy. Experiments performed in advanced cancer models are of great scientific and translational importance, as the results gathered have high potential to accelerate implementation of innovative therapies, like NTP, into the clinic.

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Cold plasma endoscopy: investigating intrabody fluid issue for bile duct cancer local treatment

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Cholangiocarcinoma (CCA) is a rare cancer of the biliary epithelium, primarily occurring in the extrahepatic bile ducts and often asymptomatic early on, making treatment challenging. Cold atmospheric plasma (CAP) is explored as a novel treatment within endoscopic technology, leveraging reactive oxygen and nitrogen species (RONS) produced by CAP to induce oxidative stress and cell death [1]. In CCA, the antitumor effect of CAP has already been demonstrated upon *in vivo* campaigns on murine models carrying subcutaneous CCA [2].

With the objective of preparing preclinical trials on live porcine models and clinical trials by 2026 on patients with CCA, we evaluate the feasibility of cold plasma catheter technology on bile duct models, focusing on host and practitioner safety [3]. The key component of our endoscopic plasma device is a catheter (3 mm outer diameter, 3 m long) comprising a metal wire ended by a dielectric barrier. It is supplied by 1 slm of helium and powered by a high voltage pulse generator (1-12 kV, 5-50 kHz). Our research aims to mitigate the electrical and thermal risks associated with the operation of the plasma catheter in two preclinical models:

(i) an artificial bile duct (ABD) model mimicking electrical response of porcine bile ducts. This constrained environment can be completed by an equivalent conductive bile (ECoBi) liquid that replicates the electrolytes and bile salts to reproduce bile's electrical conductivity ($\sigma = 10$ mS/cm).

(ii) a post-mortem digestive porcine (DiPor) model, studied at the Surgery school of Paris, with the guidance of a professional endoscopist (Pr. M. Camus).

Combinations of voltage-frequency are investigated in the ABD model to guarantee safe electrical metrics ($I_{RMS} < 100$ µA) and safe bile duct temperatures ($T_{bile\ duct} < 40$°C). This study is achieved following an optimal scenario (dry inner walls) and a worst-case scenario (inner walls totally filled with the ECoBi). Besides, plasma-derived ROS contents (H_2O_2, NO_2^-) are also quantified in ECoBi and porcine bile to assess plasma efficiency. Complementarily, experimental campaigns are achieved on the DiPor model to refine the relevance of cold plasma technology in targeting diseased biliary tissue while considering all the issues related to a human-like digestive system (device insertion, electrical compatibility between catheter and duodenoscope, electromagnetic noise, …). The issues related with the unpredictable flow of bile and cold plasma interaction are also discussed. These investigations show promising results that will enable us to begin trials on alive healthy porcine models in the near future.

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Understanding the effects of plasma species on gap junction channels in the propagation of cancer cell death

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Intercellular communication plays a crucial role in the maintenance of physiological cell functions, and failure or improper interactions result in a large variety of pathologies, such as skin disorders, cardiomyopathies, sensory defects, psychiatric disorders, and cancers [1]. One of the proteins responsible for intercellular communication is the gap junction (GJ) channel, composed of two docked hemichannels from two neighboring cells. It allows the passage of ions, signalingmolecules, and reactive oxygen and nitrogen species (RONS) from the interior of one to another cell [2]. Since cold atmospheric plasma (CAP)-generated RONS have shown great potential to eliminate several cancer cell types both *in vitro* and *in vivo* by mitochondria-mediated apoptosis and endoplasmic reticulum stress [3], we can use GJ formation to facilitate the transport of CAP-generated RONS between adjacent cells to cause cancer cell death. Indeed, GJs have proven able to activate the anti- cancer immune system and to propagate "death signals" to neighboring cells via RONS exchange [4]. However, the way how RONS are transported through GJs and how RONS-induced GJ modification affects their structure/function, and the propagation of cell death is still uncovered.

In this study, we performed molecular dynamics simulations to evaluate the effect of GJ modification (induced by CAP-generated RONS) on GJ formation/stability. We equilibrated an oxidized Cx26-composed GJ (Cx26-GJ) in a water box for 300 ns and analyzed the channel properties, and compared it with a non-oxidized Cx26-GJ system. We oxidized the 5 major amino acid residues typically modified by CAP treatment (i.e., cysteine, methionine, tryptophan, tyrosine, and phenylalanine) in the extracellular region of the GJ channel and inside the pore, with solvent accessible surface value higher than or equal to its hydrophilic surface. We chose the Cx26 protein based on its experimental relevance in cancer cells and its response to CAP treatment [5].

Our results can help to elucidate the molecular mechanism of how CAP treatment may affect the communication between cancer cells, via GJ activity. Our simulation results will be verified experimentally in three different cancer cell types (glioblastoma, melanoma, and colorectal cancer) with different Cx26 and Cx43 expression levels, treated with CAP. This outcome will determine the ability of CAP-treated cells to transfer small dye molecules through GJs upon treatment, therefore revealing the effect of CAP on GJs.

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Identification of gas plasma resistance mechanisms in human squamous cell carcinoma cells

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Although the antitumoral potential of medical gas plasma has been demonstrated in several *in vitro*, *in ovo*, and *in vivo* studies [1], clinical trials with patients suffering from advanced head and neck cancer only showed partial success. Besides promising reduction in tumor burden, 50 % of treated patients experienced cancer recurrences resulting in treatment failure [2]. The high genetic heterogeneity and mutation rates of malignant cells promote phenotypic plasticity, allowing them to adapt and survive versatile environmental conditions. To improve the therapeutic efficacy and outcomes during oncological gas plasma application, we aimed to unveil the adaption mechanisms that render cancer cells insensitive towards this technology and mediate resistances. To this end, a novel *in vitro* cell culture model was established, wherefore tumor cells were subjected to gas plasma treatment once per week in eight treatment cycles. The cellular response of squamous cell carcinoma (SCC) cells to repetitive gas plasma exposure was evaluated by flow cytometry, colorimetry assays, confocal laser scanning microscopy, and transcriptomic and proteomic analysis. A gas plasma-resistant phenotype displaying stem cell characteristics and oxidative adaption developed over the chronic treatment. Repeatedly-exposed (RE) cells were endowed with a different surface marker profile that suggested a higher tumorigenicity and lower immunogenicity. Engraftment of RE and wild-type cells on the chorioallantoic membrane of chicken embryos revealed significantly increased baseline secretion of matrix metalloprotease 2 and several immunomodulatory cytokines. In addition, investigation of the gas plasma response of wild-type and resistant SCC cells in a xenograft mouse model identified slower growth rates and less gas plasma susceptibility of multiple stressed cells.

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Large-scale transcriptomic profiling of 35 cancer cell lines reveals similar gene sets predisposing for sensitivity to plasmas and H_2O_2 but not HOCl

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Reactive oxygen species (ROS) are important mediators in physiological and pathological processes. Higher ROS levels can induce cellular distress conditions important in chronic inflammation and cancer. Nevertheless, cytotoxic ROS effects can also be harnessed for therapeutic purposes, e.g., in oncology. While the effects of various oxidant molecules in a variety of cell types were studied well in the past, less is known about *a priori* conditions, such as gene expression profiles that are associated with subsequent enhanced sensitivity towards such oxidants. To this end, we analyzed the basal gene expression of 35 cell lines from different origins (adenocarcinoma, melanoma, leukemia, and squamous cell carcinoma) using whole-transcriptome microarray panels. In parallel, we examined these cells' sensitivity based on their metabolic activity towards the well-explored ROS hydrogen peroxide (H_2O_2), the inflammation-associated hypochlorous acid (HOCl), and the therapeutically applicable gas plasma (argon plasma jet kINPen) producing a multitude of ROS such as H_2O_2, hydroxyl radical, superoxide, and reactive nitrogen species (RNS). Determined inhibitory concentrations (IC25) were correlated against relative gene expression, and significantly correlating genes were compared among the three tested oxidative stress inducers. Interestingly, when cell line sensitivity ranks were compared, we found a significant correlation between H_2O_2 and plasma sensitivity, while HOCl did not correlate with the other two oxidative stress treatments. Comparing the top 500 significantly correlating genes, a total of 182 genes matched between H_2O_2 and plasma, while for HOCl, only 6 and 3 genes matched with H2O2 and plasma, respectively. Many of the genes correlating with H2O2 and plasma sensitivity could be associated with proliferation, DNA replication, and cell cycle-related processes such as Ki-67 (MKI67), MCM complex members, denticleless protein homolog (DTL), and cyclin A2 (CCNA2). In contrast, HOCl sensitivity correlating genes were often associated with translational processes. Our results indicate that relative sensitivity to H2O2 and gas plasma-induced cytotoxicity is comparable and can be attributed to a higher basal proliferation activity. In conclusion, we identified gene transcription targets associated with tumor cell sensitivity to different oxidative stress inducers, which serve as a valuable basis to be further explored to shed more light on the underlying mechanisms of plasma treatment resistance.

Cold Plasma-based Redox Therapy for Breast-to-Bone Metastasis Tumor Growth Control

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CONTEXT: Bone, especially the spine, is a common site of metastasis for breast, lung and prostate cancers. These tumors pose a significant challenge, demanding aggressive treatments like chemotherapy and invasive surgery. To fully remove metastatic lesions, surgical procedures need to extend onto healthy tissue which reduces the probability of remaining malignant cells. Such surgery often involves the removal of healthy tissue, necessitating reconstruction and carrying a risk of infection. Cold plasma therapy, operating at temperatures below 40°C, offers a non-invasive solution by delivering reactive oxygen and nitrogen species (RONS) locally. While research shows promising results, the reaction mechanism between plasma and tissues, and proper treatment dosage and reactive species composition to reach the right effects are still topic of current research. The purpose of this project is to develop and characterize a cold plasma source and investigate its potential in mitigating bone cancer metastasis, hypothesizing its anti-tumor properties. The overall objective is to create a tissue-plasma platform for cold plasma therapy, aiming to control the metastatic spread of breast cancer cells to bone tissue.

METHODS AND RESULTS: We have developed a platform combining tailored plasma reactivity through a kHz coaxial dielectric barrier discharge source and a highly reproducible bioprinted circular bone tissue model. The bone tissue model was bioprinted (Cellink BioX) using cell-laden hydrogel. A multi-well plate was generated with identical "breast-to-bone" metastasis as a coculture model of MDA- MB-231 and human bone marrow mesenchymal stem cells (hbmMSCs). Liquid RONS are measured by UV-VIS colorimetry. For each set of plasma treatment at different parameters, metabolic activity through Alamar blue assays at day 1, 2 and 3 and live/dead measurements are used to detail the biological response of the tumor cells. Results have shown that A1G7 cell-laden hydrogel was bioprinted with reproducible results in a model of cocultured MDA-MB-231 breast cancer cells and hbmMSCs. Dose responses of plasma on cancer cells and healthy cells were assessed in 2D and 3D cultures. Furthermore, plasma showed a selective antitumoral effect on MDA-MB-231 cancer cells over hbmMSCs healthy cells in 2D and 3D cultures. Colorimetric assays have also confirmed that long-lived species (H_2O_2 and NO_2^-) can be tailored through the energy, the distance, the duration of treatment and the composition of the atmosphere around the plasma.

CONCLUSION: In essence, our platform allows us to create unique biological chemistry, observing its impact on cancerous tissue for a plasma redox-based treatment. Using a bioprinted model ensures reproducibility and precise control, enabling detailed studies of tumor migration. With a tailored plasma jet, our platform is crucial for exploring novel therapeutic approaches using exogenous reactive species.

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Numerical modeling of plasma-induced immunogenic cell death

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Immunity is one of the most important self-defense functions for humans. It is a complex system in which individual agents (cells and physiological chemicals) interact autonomously according to their respective roles. Recently, the possibility of the activation and suppression of the immune system through low-temperature plasma stimulation has been reported [1-3]. This study will benefit from mathematical simulations aimed at quantitatively understanding the effects of plasma on the immune system. However, in general, there is no universal governing equation for such a system, and it is not easy to systematically understand the whole picture or predict it quantitatively. Here, we converged conventional experimental knowledge into simple rules and modeled how collective behavior based on the cooperation of these rules naturally produces an immune effect.
Numerical simulations were performed targeting immunogenic cell death involving the actions of immune cells (macrophages and NK cells) responsible for the innate immune system and immune cells (dendritic cells and T cells) responsible for the adaptive immune system. Figure 1 shows a numerically derived two-dimensional map of immune behavior. Stress caused by plasma exposure alters the amount of cytokine secretion by immune cells and the dynamic behavior of immune cells (migration and phagocytosis). Fig. 1(a) shows that various immune cells migrate from a blood vessel (lower side) and move toward tumor cells (center). Because, the oxidative stress by plasma induces the release of damage-associated molecular patterns (DAMPs) from tumors (Fig.1(b)). Different immune cells release different cytokines, e.g. TNF-α from macrophages as shown in Fig. 1(c). Immune cells communicate with each other as they move toward their targets.

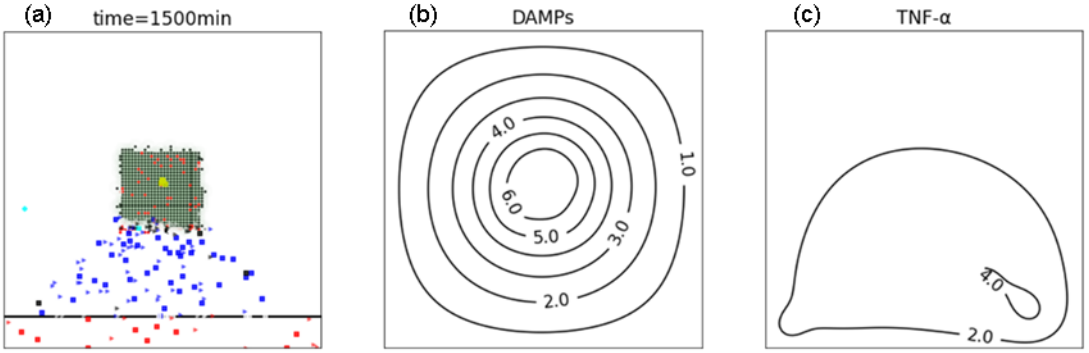

Fig. 1 (a) Tumor (center area) and immune cells, (b) DAMPs released from tumor, (c) cytokine released from immune cells.

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ICPM

PLASMA-BASED DECONTAMINATION AND STERILIZATION

Oral session (Mon - O - 2)

Monday, 9 September 2024

Plasma Treatment Effects on Destruction and Recovery of *P.gingivalis* and *S. gordonii* Dual-Species Biofilms

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Periodontitis is a common oral disease, with a prevalence of 45 to 50% in the world's population [1]. It is characterized by chronic inflammation and is associated with the development of dental plaque biofilms. Conventional mechanical scaling and root planning (SRP) can achieve a temporary reduction of periodontal bacteria colonized in the subgingival plaque [2]. However, infections often recur since plaque bacteria cannot be effectively removed from the majority of periodontal pockets by this mechanical therapy alone. Antimicrobial agents may further suppress the periodontal plaque bacteria, but usually lack effectiveness due to development of drug-resistant strains or the dampened metabolic state of the biofilm microflora. *Porphyromonas gingivalis* (*P. gingivalis*) is a well-known keystone periodontal bacterium that is frequently involved in periodontitis. In the development of pathogenic plaque biofilms, *Streptococcus gordonii* (*S. gordonii*) bacteria function as primary colonizers adhered to tooth surface's enamel pellicle and served as attachment substrates for other periodontal pathogens such as *P. gingivalis*. The binding interaction between *S. gordonii* and *P. gingivalis* is crucial for *P. gingivalis* colonization.

The aim of this study was to assess the effects of atmospheric non-thermal plasma treatment on the destruction and recovery of dual-species biofilms composed of *P. gingivalis* and *S. gordonii*. Dual- species biofilms of *P. gingivalis* and *S. gordonii* were cultured on stainless steel wafers and subjected to plasma treatment using pure argon or argon mixed with 1% oxygen for durations of 1, 2, and 5 minutes. The efficiency of the plasma treatment in disinfecting the biofilms was evaluated using CV staining and colony forming unit (CFU) counting assays. The recovery capacity of the plasma-treated biofilms under oxidative stress was assessed. Optical emission spectroscopy (OES) was employed to analyze the differences between the chemical compositions of the pure argon plasma and argon/oxygen mixture plasma. It was found that the survival rates of the bacteria within the plasma- treated biofilms were significantly lower than that within the untreated biofilms, both before and after the recovery procedure. The pure argon and argon/oxygen mixture plasma treatment led to a reduction in the bacterial load by approximately 2 and 3 log units, respectively. Under oxidative stress, the bacterial loads of the recovered plasma-treated dual-species biofilms were approximately 1.6 and 2.1 log units lower, as compared to untreated biofilm controls. The result indicates plasma treatment significantly decreased the resistance of the dual-species biofilms to oxidative stresses, suggesting that it can enhance the host's control of biofilm infections. The findings highlight the potential of plasma treatment using atmospheric non-thermal plasma treatment for inhibiting oral biofilm growth, sterilizing microorganisms, and controlling pathogenic bacteria in the context of periodontal diseases.

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Inactivation of *Escherichia coli* by CO_2-He plasma jets

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As carbon monoxide (CO) is a stable molecule and has anti-inflammatory properties, its production by plasma could be a significant advantage in the field of plasma medicine [1]. For this purpose, a small percentage of CO_2 was added in the gas mixture and was dissociated by plasma to produce CO.

This work will present a comparative study of the CO production of two different plasma jets: a MHz plasma generated by the COST jet reactor and a kHz plasma generated by a coplanar-coaxial DBD reactor [2, 3]. We will show that for the same specific energy input (SEI), the production of CO was more efficient for the kHz than the MHz-jet.

One of the most attractive features of plasma is its antibacterial properties. The aim of this work is to evaluate the antibacterial properties when CO_2 is added in this gas mixture in order to find conditions where CO is produced by plasma with no alteration of the antibacterial properties. For this purpose, we compared the inactivation of the *E. coli* strain in pure helium and with the addition of CO_2 for the two plasma jets (kHz and MHz). These comparative results will be presented and discussed. The role of CO in the antibacterial properties of plasma will be discussed, as well as the role of the others plasma components such as the reactive species, the UV, the electric field and the charged particles.

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Experimental Setup and Efficacy Assesment of a Non-Thermal Plasma System for Bioaerosol Decontamination

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The COVID-19 pandemic has highlighted the critical role of indoor air quality control and purification. To address this challenge, Cold Atmospheric plasmas (CAPs) based devices have emerged as a potential solution for mitigating aerosol transport and reducing the infectivity of airborne pathogens [1]. At the Plasma Technology Laboratory (PLT) at the University of Bologna, our research group has been investigating and utilizing a newly developed CAP source, based on a Dielectric Barrier Discharge (DBD), as an air sanitizer for enclosed environments.

The ongoing project aims to optimize the above-mentioned small-scale CAP device to reach sterilization by minimizing power consumption and the production of undesirable byproducts, such as ozone and NOx. The configuration of the prototype utilizes grid-like coated electrodes (with Rilsan® ES), powered by a 4 µs square bidirectional pulse voltage waveform with a peak voltage of 1.2 kV [2]. We have electrically characterized the discharge and estimate that the power involved is in the order of a few Watts. Additionally, we have integrated a diagnostic system to measure the ozone concentration, which can be leveraged for effective abatement processes.

To improve the chemical characterization, we used FTIR analysis at the SPC - Swiss Plasma Center - in Lausanne, supported by the COST STSM grant. Currently, at SPC, we are assessing the efficacy of these devices in reducing the microbial load by testing non-pathogenic Escherichia Coli (E. Coli). We are generating a bioaerosol, by contaminating an air stream with E. Coli, and pumping it through an enclosed tubes system within the CAP DBD reactor prototype, Fig 1.

The preliminary findings motivate further investigations to unravel the underlying mechanisms and establish a comprehensive understanding of the relationship between electrical/chemical parameters and the sterilization process.

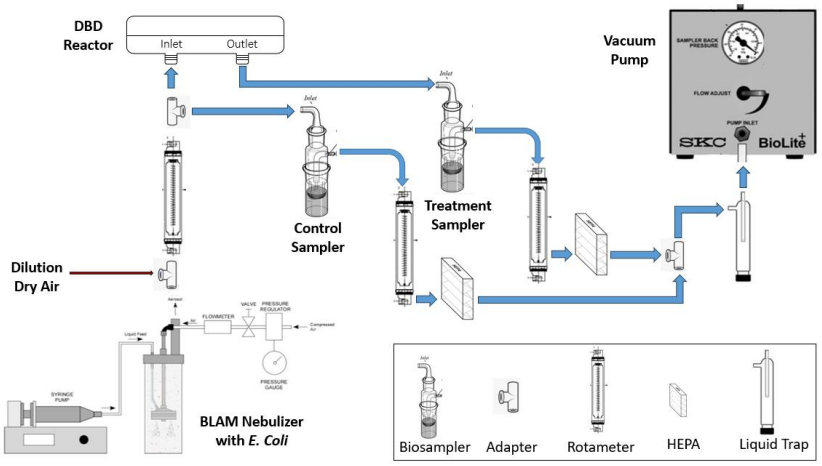

Fig. 1 - Bioaerosol Generation and Sterilization Setup

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Analysis of Cold Atmospheric Plasma Parameters for Effective Inactivation of *Aspergillus Flavus* and *Fusarium Keratoplasticum*

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Cold atmospheric pressure plasmas (CAPs), within recent years, have shown great promise in their medical applications through the life sciences in the treatment of chronic wounds [1], inactivation of bacteria [2], and in cancer treatments [3]. Few avenues though have explored the inactivation of fungal spores or biofilms. Equine fungal keratitis (EFK) is currently the leading cause of blindness in horses from all states east of the Rocky Mountains [4]. Filamentous fungi such as *Aspergillus* and *Fusarium*, which cause EFK, have become more resilient in recent years to antifungal drugs such as voriconazole, and new treatment methods are needed to combat such infections [4]. Early studies have begun to showcase the efficacy of high voltage (CAPs) in the degradation of fungal spores as well as the fungal derived toxin deoxynivalenol (DON) *in vitro* [5].

In this investigation, a microsecond-pulsed volume dielectric barrier discharge (vDBD) device was used to assess the efficacy of inactivating *Aspergillus flavus* and *Fusarium keratoplasticum* both *in vitro* and *ex vivo*. Through manipulating voltage and treatment time, optimal CAP treatments were determined for each strain based off for the CFU/mL reduction post-treatment. Gas temperature, deposited power, and reactive oxygen and nitrogen species (RONs) concentrations were quantified for each trial to indicate notable changes in chemistry after 3 minutes of treatment. This study highlights how different levels of ozone generation, deposited power, and gas temperature can impact the overall effectiveness in reduction of each fungal strains' biofilms. Preliminary results suggest that lower voltages with reduced deposited power consistently achieve over 50% reduction in both spores and biofilms when treated for more than 3 minutes.

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Plasma-mediated decontamination of animal tissue samples: a novel approach towards antibiotic-free applications

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Plasma-mediated decontamination in the field of plasma medicine offers a revolutionary approach to combat antibiotic-resistance of bacteria. We aimed at developing an innovative solution to decontaminate porcine tissue and thereby reduce the reliance on antibiotics. In this study, different plasma systems were employed to generate plasma processed liquids for treatment of porcine umbilical cords, aiming to not only eliminate bacterial contaminants but also preserve cell vitality.
Methods: Porcine umbilical cords were treated with liquids processed with diverse plasma systems under controlled conditions and using different parameters. Plasma-mediated decontamination efficacy was assessed through microbiological analyses. Cell viability and functionality were evaluated using imaging techniques and cell culture assays.
Results: We developed a protocol that demonstrates the effectiveness of plasma systems in decontaminating porcine umbilical cords, offering a viable alternative to antibiotic-based approaches. The dual functionality of the method, preserving cell vitality while eliminating contaminants, positions it as a versatile solution for various medical applications.
Conclusion: The presented research contributes to the ongoing discourse on combating antibiotic resistance by proposing a plasma-based approach to decontaminate biological tissues. This method not only aligns with global efforts to reduce antibiotic use but also introduces a preservation aspect, opening the field for diverse medical applications.
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Plasma Sterilization: Studies on Probable Mechanisms and Biochemical Actions behind Bacterial Inactivation

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Healthcare-associated infections (HAIs) are transmitted by a variety of hospital equipment used for surgical and medical procedures. Thus, in order to prevent HAIs, this equipment must be sterilized before making any such interventions. Conventional procedures (e.g., autoclaves, ultra violet (UV) radiation, and chemical treatments) have several limitations that include damage to the physical and biological performance of medical equipment, toxicity from chemicals, and the inability to sterilize temperature sensitive polymeric material due to high working temperatures. In recent times, plasma sterilization has garnered significant attention because it can overcome many of the above limitations. However, plasma interaction with microorganisms is a subject of ongoing research because the biochemical actions and mechanisms involved in plasma sterilization are still not completely understood at molecular and genetic levels. In the present work, the inactivation of pathogenic gram- positive *Staphylococcus aureus* (*S.aureus*) and gram-negative *Salmonella abony* (*S.abony*) bacteria upon exposure to microwave plasma has been investigated. After 10 min of plasma exposure, a 6-log reduction in colony-forming units (CFU) of the bacteria is established for *S.aureus* and *S.abony*. The percentage increase in reactive oxygen species (ROS), such as $^{\bullet}OH$ and H_2O_2 formed on the bacterial membrane during plasma exposure has been analyzed using a spectroflurometer. *S. aureus* and *S. abony* exhibited a significant increase in $^{\bullet}OH$ and H_2O_2 radicals on the cell membrane, respectively, which is the main cause of cell inactivation [1]. The oxidation and degradation of DNA are analyzed using an UV-visible spectrophotometer. The leakage of proteins, lipids, and nucleic acid molecules due to plasma exposure has been studied by attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR) [2]. The alteration of secondary protein structure on the bacterial cell membrane is observed using circular dichroism. Upon exposure to plasma, a secondary protein structural transition from a α-helix/β-sheet mixture to a modified β-sheet structure was observed. The bacterial morphological characteristics evaluation was done using field emission scanning electron microscopy (FE-SEM) reveals the cell membrane-blebbing, and deformation. The bacterial membrane potential is also altered due to plasma which is monitored by means of fluorescence intensities. The above studies have provided an insight into plasma sterilization processes and improved the understanding of antimicrobial approaches. Hence, combating microorganisms can have a profound impact on infection control in healthcare systems, particularly in situations where traditional sterilization methods may not be suitable.

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Sterilization Mechanisms of *Saccharomyces cerevisiae* in O_2/SF_6 Low- Pressure Plasmas

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Despite being well-established, traditional sterilization processes have limitations for disinfecting certain materials, as polymeric fabrics. Additionally, continuous scientific and technological development has driven the creation of new disinfection methodologies. Reports in the literature indicate that electronegative species, such as those generated from oxygen, fluorine and chlorine [1- 3], are effective microbial inactivation agents. Then, it was investigated the possibility of using low- pressure plasmas containing electronegative elements for the sterilization of polypropylene fabric of commercial N95 face respirators. Fabric samples were contaminated with the fungus *Saccharomyces cerevisiae* and exposed to radiofrequency plasmas (13.56 MHz, 100 W) generated from O_2/SF_6 mixtures (7.0×10^{-2} Torr). The effects of SF_6 proportion in the plasma (ranging from 0 to 100%) and the electrical configuration of the treatment (on the driven or on the grounded electrode) on fungal sterilization efficiency were evaluated in *in vitro* tests. Optical emission spectroscopy and the actinometric method were used to assess the influence of SF_6 proportion on the relative concentration of plasma species. The elemental composition, chemical structure, and morphology of the fungal cells were evaluated before and after plasma exposure using energy-dispersive spectroscopy, infrared spectroscopy, and scanning electron microscopy, respectively. Plasmas excited from pure compounds (100% SF_6 or 100% O_2) promoted a reduction of 1,000 to 10,000 times in the number of colony- forming units in the fabric, depending on whether the treatments were respectively conducted on the driven or grounded electrode. Complete fabric sterilization was attained, for both electrical configurations, when 40%, 60%, and 80% SF_6 was incorporated in the O_2 plasma. The fungus cells were also completely vanished in treatments conducted with 20% SF_6, on the grounded electrode, indicating that this configuration was the most effective for sterilization. In addition to the characteristic elements of the plasma composition (O and F), species related to plasma-fabric reactions (OH and H) as well as contaminants (N_2) were detected in the plasma phase. The O and F active species' concentrations follow different trends depending on the proportion of SF_6 incorporated into the plasma. After plasma exposure, it was observed fluorine (F) incorporation and erosion of the microorganism, which enabled the interpretation of the inactivation mechanisms based on the morphological and compositional changes induced by the plasma.

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Mon-O-2-8

Liquid laboratory biowaste sterilization by cold plasma: prospects and obstacles

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Effective laboratory sterilization is a fundamental process to ensure the safety of personnel and high standards of research practices. Sterilization is the removal or destruction of all microorganisms and other pathogens, including bacteria, spores, fungi, and viruses from an object, surface, or media. Standard sterilization methods include autoclaving (moist heat), flaming or baking (dry heat), solvents, filtration, and radiation. Among them, autoclaving is the most widely used and the most reliable method that uses heat from pressurized steam to kill microorganisms. Autoclave is convenient for heat- and water-resistant materials, effective, and non-toxic. Therefore, autoclaves are vital to daily laboratory operations. However, the constant operation of autoclaves demands enormous energy and water consumptions. One of the alternatives, cold atmospheric plasma has proven its high inactivation efficacy on a wide range of pathogens. During the last decade, plasma sterilization has continuously been implemented in medicine, agriculture, wastewater treatment, etc. [1]. As such, plasma sterilization can be potentially utilized in laboratories partially replacing autoclaves. It may reduce energy and water consumptions and provide fast effective low-cost sterilization including heat-sensitive materials.

This study seeks to address the issue of liquid laboratory biowaste sterilization by plasma. Microbial inactivation in buffered or nutrient media remains the biggest challenge for wastewater treatment. Higher inactivation is usually reached in water or non-buffered solutions [2]. To solve this, sterilization of water and nutrient media by direct plasma treatment is investigated. First, two air surface dielectric barrier discharges (SDBD) are compared in terms of power consumption and physico-chemical properties of plasma-generated PAW. SDBDs featuring different electrode patterns are used [3-4]. Concentrations of long-lived reactive species, namely nitrites $NO2^{-}$, nitrates $NO3^{-}$, and hydrogen peroxide H2O2 as well as pH, electrical conductivity, and oxidation reduction potential are evaluated. Second, the efficacy of microbial inactivation is studied on Gram-negative (*Escherichia coli*) and Gram-positive (*Micrococcus luteus*, *Bacillus thuringiensis*) bacteria and fungi (mold *Neurospora crassa*, yeast *Saccharomyces cerevisiae*) by colony counting method. Third, to elucidate the protective effect of nutrient media on bacteria during plasma treatment, the membrane damages are evaluated through membrane potential (DiOC2(3) staining) and lipid peroxidation (TBARS assay) measurements. Based on the results, approaches to efficiently inactivate both bacteria and fungi in nutrient media are proposed.

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Air Sterilisation and Treatment by Non-Thermal Atmospheric Plasma

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Air sterilisation by various non-thermal atmospheric plasma methods such as the plasma jet [1], parallel-plate dielectric barrier discharge (DBD) [2], packed bed DBD [3], and corona discharge etc. [4], has a growing interest within the scientific community since the COVID-19 pandemic. It is using the oxidative capability of reactive oxygen species generated by non-thermal atmospheric plasma (NTAP). Compared to conventional sterilisation techniques such as the ultraviolet irradiation and air filtration, NTAP offers an elegant alternative sterilisation technique as it has numerous advantages, including cost-effective operation, short treatment times [5], lower pressure drop and lower operation noise [4]. However, both the effectiveness and mechanism of bioaerosol deactivation by NTAP is not clearly identified. Previously, Fennelly et al. reported no effect of plasma treatment on airborne bacteria and fungi based on a four-week study in a hospital [6], contrasting with findings by Ruiz-Trujillo et al. who observed a 3.4 log reduction for HCoV-229E and Bacteriophage-MS2, and a 2.4 log reduction for SARS-CoV-2 at the ozone concentrations below 0.6 ppm [4]. While the role of such low ozone concentration in the virus inactivation appears to be negligible [3], Lee et al. [7] and Bisag et al. [2] showed that high concentrations of ozone (120 and 870 ppm) effectively contribute to the inactivation of coronavirus both on the solid surface or in the air. Consequently, further study is needed to investigate the efficiency and mechanism of airborne pathogen inactivation using NTAP. In this study, we employ two types of plasma sources, which are the surface DBD and volume DBD, to treat the air at different flow rates and pathogen concentrations. Their efficiencies of inactivating airborne pathogens are systematically investigated to identify the mechanism of air sterilisation by plasma. The results of this study will be used to reduce the risk of airborne infections in future NHS hospital environments through providing a safe and quite air cleaner without altering the current hospital HVAC system, called PASTA (Plasma Air Sterilisation and Treatment Apparatus).

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Advancing Bacterial Mitigation on Hospital Floors with a DBD Plasma Sheet

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This project is related to hospital floors that are commonly laden with bacteria, acting as a major source for the spreading and transmission of viruses and diseases. The current work proposed a solution to the problem identified in the research conducted by Louis Stokes Cleveland Veterans Affairs Medical Center and Case Western Reserve University School of Medicine [1]. In their study, bacteriophage MS2, a non- pathogenic, non-enveloped RNA virus was spread on the floor to examine the potential for dissemination of microorganisms from floors of isolated rooms to the hands of patients and to high-touch surfaces inside and outside the rooms. Experimental results show that the Bacteriophage MS2 spread to multiple surfaces in patient room after day 1. In addition, surfaces within 3 feet of the patient's bed had higher concentrations of MS2 compared to those more than 3 feet away. This research identified the problem but did not offer any solution to mitigate bacteria.

In the current work to solve the above problem, we designed a plasma sheet generator that produces a DBD plasma sheet (25.4 mm×25.4 mm×2.0 mm) to mitigates bacterial residing over floors. Utilizing plasma to kill bacteria is safer than using chemicals that may have detrimental impact on the room environment. In addition, a robot was designed to carry out plasma operation over the selected floor tiles. The robot has an operating speed of around 440 ft/min and carries a heavy small-size gas cylinder, a microprocessor, plasma torch stands, and gas distribution and flow meters, along with a power supply and ballet resistors for operating the plasma torches. For control purposes, the robot was equipped with multiple controllers: the TETRIS PRISM robotics controller, the MAX DC motor expansion controller, a PS4 controller, and a Tele Op control module enabling remote operation. The robot, maneuverable via a joystick, is capable of moving forwards, backwards, and sideways, which is essential for scanning the floor while the plasma torches are active. Multiple types of floor tiles traditionally used for hospital floors were selected. That included rubber and different kind of vinyl tiles. In this experiment, the DBD plasma was generated by applying a ac voltage (10kV, 30-40 kHz) across helium or argon used as a main gas. For experimental validation, standard hospital tiles were inoculated with E. Coli bacterial colonies and cultivated for 24 hours. Post-exposure to the plasma, the bacterial colonies were counted, observing a marked reduction on the treated tiles compared to the control group. Upon contact with the plasma, the reactive nitrogen and oxygen species crucially contributed to the destruction of bacterial colonies by damaging the bacteria's proteins, lipids, and DNA. In addition, the impact of adding 1% (by volume) oxygen or nitrogen on the bacteria mitigation was quantified. Results show an enormous change in the bacterial colonies as the oxygen were added to the main gas flow. This presentation will summarize our experimental results that highlights our floor bacteria mitigation with plasma for hospital indoor applications.

Table 1. Test Data

Test Data				
Plate	Control	Argon plasma	Argon plasma with nitrogen shielding gas	Argon plasma with oxygen shielding gas
3	877	781	158	
4	157	108	54	27
5	15	15	10	4
6	4	1	6	1

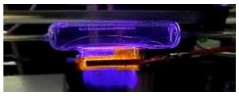

Fig 1. Plasma Sheet Generator

Fig 2. Raw data from a stage four serial dilution, from L to R: Control, argon plasma with nitrogen, and argon plasma

Fig 3. Fully operational Plasma

1. Sreelatha Koganti, Evaluation of Hospital Floors as a Potential Source of Pathogen Dissemination Using a Nonpathogenic Virus as a Surrogate Marker, Louis Stokes Cleveland Veterans Affairs Medical Center and Case Western Reserve University School of Medicine

Implementation of Atmospheric Pressure Plasma-Activated Gaseous Media into Advanced Decontamination Processes

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The plasma-activated gaseous media provides the means to decontaminate surfaces of different thermally sensitive and biological materials at remote distances. For this purpose, the multi-hollow surface dielectric barrier discharge was used for plasma activation of gaseous media to produce an atmosphere with a high ratio of hydrogen peroxide and ozone. These active species were generated in pure water vapour [1] and oxygen with an admixture of water vapour. This study aimed to determine parameters for efficient decontamination and sterilisation of thermally sensitive materials at atmospheric pressure.

The thermal and electrical properties of the used plasma source were measured. Optical emission spectroscopy was used to analyse the characteristics of the generated plasma. The reactive species in plasma-activated gas and condensed activated vapour were detected and compared for different plasma parameters, such as water vapour concentration, gas flow and power input of plasma source. The generated media was then applied to microorganisms in the form of planktonic bacteria and bacterial biofilm. Moreover, the bacterial spores were tested as they are often used as biological indicators for standard sterilisers. The germicidal efficiency of short and long exposure to plasma- activated media was evaluated by standard microbiological cultivation and fluorescence analysis using a fluorescence multi-well plate reader. The decontamination tests were repeated at different distances from the plasma source.

The decontamination efficiency of generated plasma-activated water vapour increased with the exposure time and the plasma source power input. Similar results were obtained for the decontamination by plasma-activated oxygen and oxygen with the admixture of water vapour. The longer decontamination cycles (several hours) inactivated resistant bacterial spores and affirmed the possibility of achieving efficient microorganism inactivation and surface sterilisation. The main results of this study are a decontamination chamber prototype and verified technology for constructing scalable commercial plasma devices for utilisation in medicine and bioresearch.

This research was supported by project no. LM2023039 funded by the Ministry of Education Youth and Sports of the Czech Republic. This work was also supported by project no. TJ04000329 funded by the Technology Agency of the Czech Republic. The authors would like to thank Jaromír Hašana, Eliška Kostrůnková, Lenka Smílková and Martina Valtrová from Masaryk University for their work on the project no. TJ04000329.

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Mon-O-2-12

Candida albicans biofilm eradication through cold atmospheric plasma: The impact of voltage and exposure time in the antifungal mode of action

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The escalating global trend in antifungal resistance has led to a decrease in the efficiency of conventional therapies [1]. As *Candida spp.* ranks as the fourth leading cause of healthcare-associated infections globally, there is an urgent demand for novel antifungal agents [2]. This study investigates the impact of Cold Atmospheric Plasma on *C. albicans* biofilms by analyzing their response to different parameters of CAP application. The focus of the research is on the impact of treatment duration and plasma input voltage. CAP's influence on *C. albicans* was assessed with viability, membrane integrity, and oxidative stress measurements, along with observations of biofilm chemical composition and hyphae growth post-CAP treatment.

Higher CAP input voltage corresponded to a decreased *C. albicans* cell viability. These observations were then further confirmed with a microscopic examination after BacLight staining. It is also noteworthy, that low CAP exposure may potentially induce a phenomenon known as hormesis [3], as evidenced in *C. albicans* 24 h growth measurements. Furthermore, DCFDA staining microscopy demonstrated an increased intensity of oxidative stress with prolonged treatment time. CAP treatment also influenced *C. albicans'* hyphae, which exhibited signs of contraction. The increasing of plasma voltage and exposure time also altered some of the typical constituents of fungi biofilms, such as lipids, proteins, and carbohydrates.

The study highlighted CAP's complex impact on *C. albicans* biofilms, encompassing significant reductions in cell viability and metabolic activity, inhibition of hyphal growth, and induction of oxidative stress. This shows CAP is a promising and versatile method for surface disinfection, especially in terms of combating healthcare-associated infections.

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Mon-O-2-13

Promoting the inactivation effects of non-thermal plasma on filamentous fungi using staining solutions

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Onychomycosis is a nail disease caused by dermatophyte fungi and is a significant problem across whole human population. While several treatments are available, their effectiveness is often limited, and they may carry potential side effects. Non-thermal plasma (NTP) decontamination has found its use in many fields, including agriculture, ecology, and medicine. A plasma source in a point-to-ring configuration; in the form of a very compact, user-friendly device with a power of approximately 2 W is used for the work. Previous studies have shown that NTP alone does not exhibit a sufficient impact on micromycetes in advanced stages of growth, even when considering differences between strains [1]. In this study, we investigated the use of staining solutions, such as Bengal Rose or Betadine, to enhance the inactivating effects of NTP on filamentous fungi. Experimental results, depicted in Fig. 1, demonstrate that the combination of NTP and the aforementioned staining solutions exhibits a synergistic effect. This combination effectively inhibits the growth of *Trichophyton mentagrophytes* even four days after inoculation on agar medium. Therefore, the application of NTP in conjunction with staining solutions holds great potential for improving the effectiveness of decontamination methods against filamentous fungi.

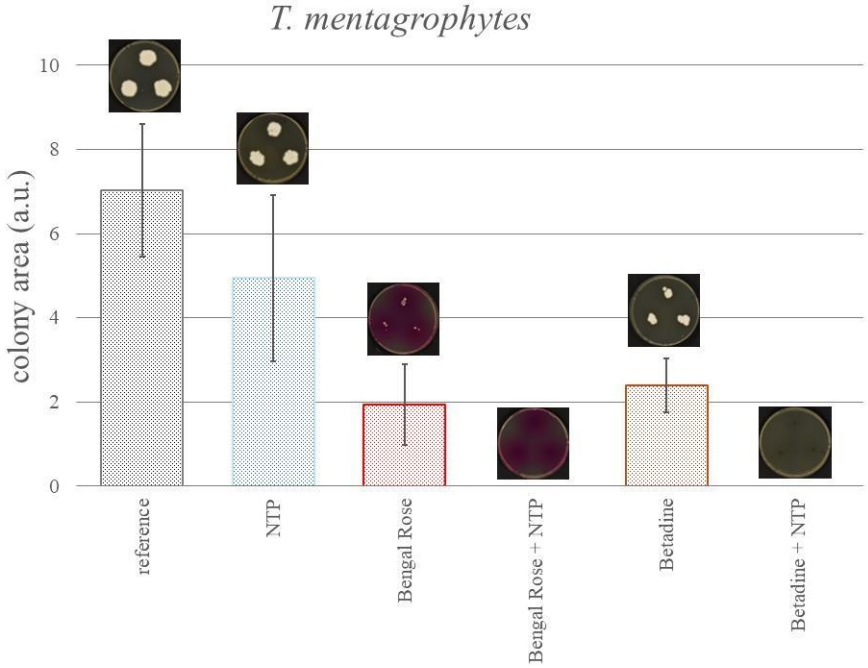

Fig. 1 Comparison of the colony area of *T. mentagrophytes* for different treatments. Treatments were applied 48 h after inoculation; evaluated at the 8th day of growth.

This work was supported from the grant of Specific university research: grant No. A2_FCHI_2024_029

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Comparison between the Antimicrobial Effect of Plasma Activated Water on Amniotic Membrane Contaminated by *Klebsiella pneumoniae* and *Escherichia coli*

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The Amniotic Membrane (AM) have a wide range of applications in the medical field, and due to the applications, there is a need to ensure its sterility, which is needed for the utilization of this material. Given the escalating bacterial resistance, there is a vital imperative to investigate innovative sterilization methods. One possible alternative method is plasma-activated water (PAW), that have studies showing its antimicrobial effect and it also eliminates the negative points of the direct use of plasma, such as temperature.
The aim of this study was to analyze the antimicrobial effect of plasma-activated reverse osmosis (RO) water on contaminated amniotic membrane using ATCC® standard strain of *Escherichia coli* (25922), *Klebsiella pneumoniae* (13883). The AM was contaminated by an inoculum at $1x10^6$ CFU/ml, which was in contact for 3 min, after which the PAW was placed in contact with the AM for 90 min. Plasma, gliding arc of argon and compressed air were used to activate two types of water, one refrigerated (4ºC) and one at room temperature (27 ºC) with an activation time of 30 min, studying the cell viability after exposing the amniotic membrane samples to the action of PAW.
The main results obtained were a cell viability of 3.01 log with the refrigerated PAW and 2.73 log with the ambient PAW for *K. pneumoniae* and 3.2 log with the refrigerated PAW and 2.98 log with the ambient PAW for *E. coli*, observing a better antimicrobial action of the refrigerated PAW. It is concluded that PAWs have a significant antimicrobial action, obtaining a reduction in cell viability of 3 logs and that *E. coli* showed a better susceptibility to the effects than *K. pneumoniae* while in contact with AM.

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001", process number 88887.649566/2021-00

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icpm 10 & iwpct 9

8 - 13 september, Portorose, Slovenia

ICPM

REGULATORY ISSUES AND STANDARDS IN PLASMA MEDICINE

Oral session (Mon - O - 3)

Monday, 9 September 2024

DIN SPEC 91315 Revised – A New Method for Risk Assessment of Reactive Gas Species Emission by CAP Sources

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Reactive oxygen and nitrogen species (RONS) are considered to be of central importance for applications in plasma medicine as mediators of processes in biological systems [1]. The range of gas species produced in CAP is enormous and the composition is specific to each plasma source. However, the availability of inexpensive, easy-to-use and non-invasive measurement equipment severely limits the characterization during operation of medical plasma sources. The assessment of health risks associated with the exposure of patients and operators to RONS focuses on the usually unintentional inhalation of RONS. Consequently, available studies address those gas species that are relevant for penetration into the respiratory tract due to sufficient lifetime, for which reliable measuring devices are available and for which guideline or target values have been formulated on the basis of occupational health assessments. These criteria usually match only for ozone and nitrogen oxides.
In 2014, the German DIN SPEC 91315 was published as the first standard to specifically address aspects relevant for therapeutic applications of CAP on humans [2]. The rationale of the approach on RONS emission was to assess the possibility of the formation of potentially harmful gases during plasma treatment. Methodologically, the concentrations of ozone and nitrogen dioxide should be measured in a sphere around the plasma source at laboratory conditions. In a recent revision of the DIN SPEC by various members of the German National Center for Plasma Medicine, the rationale was expanded to include practical aspects such as the size of the treatment room in which the plasma source is operated. As a result, a new methodology was developed that aims for determining the ozone and nitrogen oxides production rates specific for each plasma source rather than local concentrations, as the latter are strongly dependent on time and space as a result of diffusion and convection processes according to transport equation.
In brief, the new method is based on measurements of time resolved ozone as wells as nitrogen dioxide concentrations inside a stainless-steel test chamber. The gas composition inside the chamber is continually homogenized by a fan operating at elevated volume flow rate. Consequently, localized measurements inside the chamber provide data representative for the whole chamber volume. The production rate in units of µg / s can be determined from the slope of the concentration curve. The impact of the measurement technique as well as the influence of natural decay on the derived production rate of ozone is discussed. Finally, space averaged values as a function of operational time of the plasma source as well as the size of the treatment room are presented. The results from characterization of a medical plasma source based on volume dielectric barrier discharge (DBD) operated in ambient air are used as an example to illustrate the method.

This work was supported by the German Federal Ministry for Economic Affairs and Climate Action (Grant no.03TN0019C).

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By any other name: a search for optimal plasma nomenclature

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Investigators in the field of plasma medicine are challenged by a lack of standard terminology for describing non-thermal plasma. Multiple terms for the same form of plasma are used in published papers that describe plasma medicine research, including non-thermal plasma, cold plasma, and atmospheric-pressure plasma. The resulting ambiguity hampers literature searches, confuses and slows discussion, and complicates interdisciplinary collaborations. To quantitate and assess the breadth of this problem, we designed a natural language processing model (NLP) that surveyed approximately 15,000 papers identified by the query "plasma medicine" among papers indexed in PubMed between 2020-2022. Our NLP was constructed and executed using the Hugging Face transformers API and PubMed BERT pretrained model [1]. We used this model to first determine the prevalence of individual terms used to describe plasma, and then to assess the utility of each term for searching literature relevant to plasma medicine. The effectiveness of each term was measured by two parameters: precision, which is the ability to discriminate between relevant and irrelevant literature, and recall, which is the ability to retrieve as much relevant literature as possible. Finally, each term was given a combined effectiveness score on a scale of 0-1 (1 representing a term with ideal effectiveness) accounting for precision, recall, sample size, and model confidence. Our model showed that of the twelve commonly used terms analyzed, none received a combined effectiveness score over 0.025. We therefore concluded that there is currently no common term for plasma that provides a satisfactory representation of literature within the plasma medicine field [2]. These results highlight the need for standardization of terminology within plasma medicine. Once implemented, standard terminology will promote more effective collaborations, more efficient literature searches, and the establishment of common ground for discussions in the field of plasma medicine.

This work was conducted with developmental support from the Institute for Molecular Medicine and Infectious Disease and Department of Microbiology and Immunology in the Drexel University College of Medicine.

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Standardization in Plasma Medicine: From DIN Spec to IEC standards

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Over the past twenty years, the new field of interdisciplinary research - plasma medicine - successfully entered the clinics and the markets. Basic research in engineering, physics, cell- and microbiology paved the way for this new technology for the treatment of infected chronic wounds. Generating partially ionized gases at room temperature, this new type of devices – became an innovative tool for clinical application. Several spin-off companies are now producing medical devices based on this basic research results – and sell those plasma sources as medical devices class (IIa or IIb). To allow researchers and users to compare and ensure stability and safety of these plasma generators, scientists have initiated a standardization process that describes a set of basic tests in physical, chemical and biological test procedures. In 2014, a German pre-standard was published, the so called DIN Spec 91315. It is now a solid basis for the development of plasma sources with an intention for clinical application. Based on this DIN Spec 91315 approaches for a further development of national or even international standards are going on in several countries around the globe. In Germany, a follow-up process has started to revise this DIN Spec 91315 and to transform it into a regular national standard: DIN norm. Within an open process research institutes, companies and clinics involved in the production and application of plasma sources are joining forces for this ambitious goal of a DIN standard.

Recently, a newly formed working group (WG41) started in a process supported by IEC – the International Electrotechnical Commission to prepare an international standard for medical equipment. This new work group 41 started in 2021 and focusses on an IEC standard entitled: Particular requirements for the basic safety and essential performance of non-thermal plasma wound treatment equipment – which will become the international standard IEC60601-2-91. Here, we will provide a short overview on these standardization activities.

Standardized Treatment Procedures for In Vitro Analysis of Different Cold Plasma Technologies: Is It Even Possible?

Thoralf Bernhardt[1], Philipp Ficht[1], Anna Staffeld[1], Thomas Borchardt[2], Andreas Helmke[2], Mareike Meister[3], Sybille Hasse[3], Sander Bekeschus[1, 3], Steffen Emmert[1], Lars Boeckmann[1]

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The innovative field of plasma medicine emerged at the intersection of physics, chemistry, biology, and medicine. Pre-clinical and clinical studies have shown a remarkable potential of cold atmospheric plasma in various medical applications including treatment of chronic wounds and cancer. A variety of different cold plasma technologies and devices have been developed and employed in these studies. Besides the different technologies and devices there are many further parameters that may vary between different studies making it difficult to compare results.
As part of a joint research project to revise the existing German standards “General requirements for plasma sources in medicine” (DIN SPEC 91315), we aimed to identify a general experimental setup for the treatment of eukaryotic cell lines that may be used with a large variety of different cold plasma technologies and devices. We found that different technologies come with different challenges. The gas pressure of jet plasma devices may displace the liquid on top of cells during treatment and may even dry or detach adherent growing cells. Surface-microdischarge plasma devices may need special spacers to ensure that no gas can leak from the space contained by the spacer during treatment. Other devices such as volume dielectric barrier discharge plasma sources need a counter electrode at close distance for plasma generation. Therefore, depending on the geometry of the device the cell culture vessel needs to be adapted to fit the size of the plasma source. However, for many plasma sources there are no standard cell culture vessels that have the required size. Against this background, we tested an indirect approach with plasma treated liquids to allow for the generation of comparable results. We tested if using a simplified equation of “area treated” divided by the “volume of the treated liquid” times “treatment time” and treatment through a metal grid (counter electrode) would provide a way to standardize treatment for different devices. Our analyses revealed among others that large volumes required for devices with large treatment areas require very long treatment times with devices that only have small treatment areas. These long treatment times to treat the same volume of liquid bring about further challenges and make a fair comparison difficult. Therefore, we were not able to identify an experimental setup that may be used for standardized comparison of different devices. Nevertheless, the experimental setup may be standardized regarding cell lines, liquid, volume of liquid per square centimeter, and incubation time after treatment.
Taken together, indirect treatment is not a viable solution for standardized experimental setup for different devices. Therefore, direct treatment of cells with individually tailored adaptations for each device should be performed, as it incorporates all components of cold plasma.

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8 - 13 september, Portorose, Slovenia

ICPM

PLASMA LIQUID INTERACTIONS, PLASMA-ACTIVATED LIQUIDS

Oral session (Tue - O - 4)

Tuesday, 10 September 2024

Efficient Generation and Characterization of Plasma-Activated Water Using a Surface Dielectric Barrier Discharge System

Benedito Botan Neto[1], Michaela Shiotani Marcondes[1], Luan Gonçalves de Lima[1], Felipe de Souza Miranda[1,2], Douglas Marcel Leite[1], André Luis Pereira[1], Cristiane Yumi Koga-Ito[2], Rodrigo Sávio Pessoa[1]

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This study focused on designing and constructing an efficient surface dielectric barrier discharge (DBD) with mesh electrodes for generating plasma-activated water (PAW). The system was characterized by evaluating its electrical, thermal, and optical parameters, demonstrating remarkable efficiency with an average power of 20.9 W, sufficient for water activation despite a smaller activation volume. This resulted in high energy density efficiency compared to other plasma systems. The presence of primary species within the system was confirmed through optical and thermal analyses, which also revealed an increase in water temperature upon activation. The thermal flow analysis showed a rise in energy density and effective heat transfer from plasma to water, indicative of the energy supplied by the plasma initiating water activation. The PAW production process was aligned with the objectives, showing a decrease in pH due to the transport of species like HNO_2 and HNO_3 from plasma to water, making PAW acidic and oxidizing as evidenced by increased ORP. The process also resulted in higher electrical conductivity and Total Dissolved Solids (TDS) due to plasma-derived ions. Theoretical calculations for TDS and electrical conductivity were crucial in characterizing the PAW system, with *in-situ* measurements illustrating the influence of short-lived species. The quantification of nitrates and nitrites supported the increase in TDS. Examining different water volumes for PAW activation showed that smaller volumes enhanced PAW's physicochemical properties due to greater energy density, with the species density in PAW remaining consistent across volumes. The study concluded that agitation had minimal impact on PAW production, with energy density being a more critical factor. This research elucidates a reaction mechanism for chemical reactions within PAW, providing a deeper understanding of the chemical processes during plasma activation and offering insights into potential enhancements and applications in medicine, agriculture, and surface treatment. The findings contribute significantly to the understanding of plasma water activation, altering water's chemical properties to become acidic, oxidizing, and conductive. This has implications for surface disinfection, water treatment, and medical applications. Future research directions include modeling and simulation to optimize the liquid-to-plasma activation process, promising scientific and technological advancements in PAW-related fields.

This work was supported by São Paulo Research Foundation (FAPESP) under grant #2019/05856-7 and National Council for Scientific and Technological Development (CNPq) #420775/2023-4.

Exploring the Potential of Plasma-Activated Water: Impact of Gas Composition on Reactive Species Generation, Antimicrobial Efficacy, and Cytotoxicity

Felipe de Souza Miranda[1], Victoria Kelly Fonseca Tavares[1], Diego Morais da Silva[1], Noala Vicensoto Moreira Milhan[1], Nilton Franccelosi Azevedo Neto[2], Rodrigo Sávio Pessoa[2], Cristiane Yumi Koga-Ito[1]

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Plasma technology, particularly plasma-activated water (PAW), has become indispensable across various sectors, including medicine, agriculture, and electronics manufacturing. Through the creation of reactive nitrogen and oxygen species (RONS), PAW has emerged as a potent tool in anti-cancer therapies, microbial decontamination, and product functionalization [1,2]. The role of these reactive species, meticulously generated through plasma processes, is especially noteworthy in antimicrobial applications, presenting a promising avenue for combating pathogens. Moreover, ongoing efforts are dedicated to dissecting plasma chemistry, particularly understanding the spectrum of RONS generated in both aqueous and gaseous phases [2]. Recent studies also emphasize the impact of working gases such as argon, helium, and compressed air on RONS formation, heralding potential revolutions in plasma technology applications, particularly medicine. With these contexts, the present study aims to utilize a coaxial dielectric barrier discharge (C-DBD) reactor operating with three gases: argon, helium, and compressed air, targeting water activation. The water activation time was fixed at 10 min, the flow gas rate was 5 L/min (for all gases), and the samples' volume was set at 40 mL. The operational parameter of the DBD reactor was 10.6 kV (adjustable via the power supply) at a frequency of 14 kHz. The peak-to-peak current varied depending on the gas used: 102 mA for compressed air and 250 mA each for helium and argon. Therefore, the primary objective is to assess the influence of these gases on the generation of RONS and their impact on PAW; for this, the real- time RONS characterization was made using a UV-VIS spectrometer during the water activation. Also, the efficacy of produced PAW against the microbial species *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* was evaluated, in addition to its cytotoxicity to mammalian cells. Significantly, following a 10-minute exposure, the use of compressed air and argon resulted in a remarkable 99.99% reduction in *S. aureus* and *E. coli*, while reductions of 99.96% and 99.95%, respectively, were noted with helium. For *C. albicans*, reductions of 12.05% with compressed air, 22.89% with argon, and 39.76% with helium were observed. Furthermore, PAW generated with all the gases was classified as non-toxic to mammalian cells.

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Synergizing Dielectric Barrier and Gliding Arc Discharges: Advancements in Plasma-Activated Water Generation

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Plasma-activated water (PAW) has shown promise in the treatment of cancer cells, bacterial and fungal infections, and food preservation. PAW contains reactive oxygen and nitrogen species (RONS) which can cause cancer cell death, disrupt microbial membranes, and inhibit food spoilage microorganisms [1–3]. To optimize water activation, this study proposes a Hybrid Plasma Discharge (HPD) approach that combines the strengths of Dielectric Barrier Discharge (DBD) and Gliding Arc Plasma Jet (GAPJ) systems. The influence of the airflow rate and treatment time on deionized water was examined. Optical emission spectroscopy revealed that nitrogen emission lines were the majority of the spectrum, although oxygen emissions were also detected. After treatment, the physico-chemical properties of the water indicated a decrease in pH with increasing airflow and treatment time, while the oxidation-reduction potential, total dissolved solids, and conductivity showed an increase. However, stationary conditions in the physico-chemical parameters were observed after 5 L/min and 10 min of treatment. Electronic transitions of nitrite, nitrate, hydrogen peroxide, and nitrous acid in the treated water were displayed in the absorbance spectra in the UV-Vis range. Spectroscopic variations in the Raman bands associated with the librational and O–H stretching of water were correlated with changes in reactive oxygen and nitrogen species and pH in the treated water. The study demonstrates the possibility of treating activated water using a combination of DBD and GAPJ systems.

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Chemical and Bactericidal Properties of Cell Culture Media DMEM and RPMI Modified by He/O_2 Plasma Treatment

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The application of cold atmospheric plasma (CAP) in medicine is a perspective and rapidly increasing research topic. Besides direct treatment of living tissue or cells with CAP source, properties of plasma-treated biologically relevant liquids such as cell culture media gain significant interest and have been applied for various medical and biological trials. Various transient reactive oxygen and nitrogen species such as OH•, $O2^{\bullet-}$, NO• and NO2• radicals, peroxynitrite may be produced in plasma- treated liquids. These species have highly cytotoxic properties and cause biochemical and antibacterial activity of plasma-treated solutions through post-discharge processes. In addition, culture media contain a complex mixture of inorganic salts and organic compounds such as amino acids, vitamins, glucose, antibiotics, etc., significantly influencing the properties and activity of these plasma-treated liquids. Nevertheless, diagnostics of reactive species and reaction pathways responsible for biological responses caused by these multicomponent liquids are affected by many factors which significantly influence the selectivity, sensitivity, and precision of the analytical methods applicable to detect and quantify the chemical species produced by plasma in these liquids and evaluation of reaction mechanisms.
In this work, we studied He/O2 plasma chemical modifications of cell culture media DMEM and RPMI. The COST-Reference plasma jet operated at a helium flow rate of 1.4 slm with a 0.6% oxygen admixture was used as a plasma source to treat the culture media [1-5]. We performed a chemical analysis of the plasma-treated media and correlated the chemical properties with their antibacterial properties on *E. coli*. We compared the direct treatment (plasma treatment of bacterial suspensions) and indirect treatment (incubation of bacteria in post-discharge plasma-treated media) of bacteria. Chemical changes in the treated media were analyzed by various diagnostics regarding the possible interferences taking place in these complex mixtures. The contributions of the specific components in plasma-treated media to their antibacterial effects were evaluated. Special attention was paid to the role of amino acids in the plasma-induced biocidal activity of DMEM and RPMI.
Plasma-treated cell culture media have shown long-term post-discharge chemical and bactericidal activity. Formation of organic mono- and dichloramines of amino acids in DMEM/RPMI by hypochlorite produced by the reaction of plasma-generated O atoms with Cl^- ions present in culture media were the major chemical contributors to the observed biocidal activity of plasma-treated cell culture media. This behavior was attributed to the action of tertiary chemical products formed by the decay of organic dichloramines. The thiobarbituric acid assay revealed malondialdehyde formation from glucose oxidation as the major component of the media.

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Plasma-Generated Nitric Oxide Water for Biological Applications: Infection Control and Cosmetic Innovations

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This presentation aims to elucidate the research underpinning nonthermal gas plasma methodologies with significant applicability in decontamination, microbial inactivation, viral sterilization, and broader environmental considerations. Aspiring towards a pathogenic-free global milieu, our focus lies in industrial interventions targeting the neutralization of environmental contaminants, specifically microbes within soil, water, and air matrices. Our laboratory engages in foundational investigations concerning plasma-generated nitric oxide water and eco-friendly plasma-based techniques for nanomaterial synthesis, exploring their diverse biomedical applications.

Within this domain, we present an innovative approach utilizing plasma-generated nitric oxide water for the pathogenic inactivation, encompassing viruses and bacteria, fostering environmentally sustainable agriculture, and facilitating the ecologically sound synthesis of metal nanoparticles. Additionally, our endeavors extend to diverse experiments in the realm of plasma-generated nitric oxide water-based cosmetics and aesthetic applications, including anti-aging effects. These ecologically conscious methodologies epitomize cost-effectiveness, environmental responsibility, and sustainability, thereby serving as viable modalities for biological, environmental, and nanobiotechnological applications, with promising prospects as therapeutic agents and industrial commodities.

In summation, the investigation into plasma-based environmentally benign methodologies holds the potential for future advancements in agriculture, bioscience, nanotechnology, and environmental sciences.

Assessing the antiviral effect of plasma-conditioned media against herpes simplex 1 infection in polarized vaginal epithelial cells

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Genital herpes is one of the most common sexually transmitted infections in the United States, with over 18 million existing cases and over 500,000 new infections reported each year [1]. While genital herpes is most often associated with HSV-2, in recent decades HSV-1 has been the cause of up to 50% of new infections in certain populations [2,3]. Genital HSV-1 infection is currently uncurable, and standard of care antiviral drugs are limited in their effectiveness [4]. Given the prevalence of the disease and the limited available management options, new avenues of treatment are sorely needed. We propose the use of indirect application of non-thermal plasma to achieve antiviral effects in both infected vaginal epithelial cells and vaginal epithelial cells susceptible to infection. To investigate the efficacy of indirect treatment *in vitro*, we examined the effect of plasma-conditioned medium (PCM) application on both monolayer and polarized human vaginal epithelial cells (VK2 cells). Using VK2 cells cultured as a confluent monolayer, we demonstrated that PCM application to cells reduced the susceptibility of cells to subsequent HSV-1 infection. We also tested the ability of PCM to disrupt virus replication in VK2 cells already infected with HSV-1. Our experiments demonstrated that incubation with PCM reduced early viral gene expression in monolayers of HSV-1-infected VK2 cells. Furthermore, we demonstrated that application of PCM to VK2 cells induced an oxidative stress response, which we hypothesize is a contributor to the antiviral activity of PCM. These antiviral effects were not caused by detrimental effects on cell viability since we demonstrated that application of PCM was not cytotoxic to monolayer VK2 cells. Similar studies involving application of PCM to polarized VK2 cells are now underway. Our work thus far has shown that application of PCM is a promising antiviral approach for treating women with existing vaginal HSV-1 infections as well as women at risk for HSV-1 transmission.

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Plasma activated water pre-treatment substantially enhances phage activity against *Proteus mirabilis* biofilms

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Background

The integration of cold plasma technology and bacteriophage therapy is a formidable strategy against the biofilm-forming *Proteus mirabilis*, especially pertinent in urinary tract infections (UTIs) linked with long-term urinary catheter use. Our study investigates the synergistic effects of bacteriophages and PAW in targeting the biofilms of *P. mirabilis*. The study encompasses two objectives: the first is to determine the interactive dynamics between bacteriophages and ROS/RNS when applied to biofilms — whether these interactions are synergistic, enhancing the biofilm eradication, or antagonistic, detracting from their individual effectiveness. The second objective is to explore how the variations in plasma discharge parameters, such as strength and design, influence the antimicrobial efficacy of PAW. By conducting this dual-focused inquiry, we aim to shed light on the potential of leveraging bacteriophages and PAW in concert to overcome the formidable defence mechanisms of *P. mirabilis* biofilms, thereby contributing to the development of more effective strategies for infection control and antimicrobial resistance mitigation.

Methods

Generated through a specialised non-thermal or cold plasma discharge setup, PAW is composed of an array of reactive oxygen and nitrogen species (ROS/RNS), well described for their antimicrobial capabilities. Additionally, bacteriophages have gained a recent resurgence, with their bacterial strain specificity, present as viable bio-control agents. Our study explored the interaction between bacteriophages and ROS/RNS on biofilm eradication and assessed the impact of different discharge setups on the antimicrobial efficacy of PAW. The stability of phage vB_PmiS_PM-CJR in PAW, alongside the susceptibility of both planktonic and biofilm cultures to PAW, was assessed, offering critical insights for enhanced antimicrobial strategies.

Results & Conclusion

The sequential application of PAW followed by phage significantly reduced biofilm biomass and bacterial load; the reverse order (phage followed by PAW) did not show better antibacterial effects compared to using PAW or phage alone. We hypothesise that PAW can disrupt biofilm structures, thus enhancing phage penetration and bactericidal action. The synergy unveiled between cold plasma and bacteriophages offers a broader view for tackling biofilm-associated infections in clinical settings and is a promising pathway towards not only managing UTIs associated with *P. mirabilis* but also mitigating the broader antibiotic resistance quandary.

Keywords: Bacteriophage, Cold Plasma, Plasma Activated Water, *Proteus. mirabilis*, Biofilms

The effects of plasma-activated liquid on urinary tract infection and colitis in mice

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Urinary tract infections (UTI) are caused by uropathogenic bacteria which are often resistant to antibiotic treatment. Microbial dysbiosis is a crucial component of the etiopathogenesis of inflammatory bowel diseases (IBD). Cold plasma-activated water/liquids (PAL) have known antimicrobial properties with applications in disinfection and wound healing. The aim of our study is to evaluate PAL (in our study plasma-activated phosphate buffer saline) as a potential treatment of UTI *in vivo* in an animal model and to analyze the effect of rectal administration of PAL on the oxidative status and gut microbiota diversity in mice with and without induced colitis as a model of IBD.
Based on *in vitro* tests using uropathogenic *E. coli*, PAL generated in the atmospheric air glow discharge plasma had the strongest antimicrobial effect in comparison to PAL prepared by other air discharges and was further tested *in vivo*. Single transurethral PAL application had no effect on the mouse model of UTI. Upon investigating the treatment failure, we found that urine completely prevented the antimicrobial effects of PAL and PAL treatment of isolated neutrophils resulted in their reduced viability and loss of mitochondrial membrane potential [1]. Rectal application of PAL increased oxidative stress markers and this effect was more pronounced in colitis. In addition, PAL increased microbial diversity in the healthy gut and decreased it in colitis [2].
These results do not support the hypothesis that the *in vitro* antimicrobial effects of PAL can be translated to the *in vivo* model of UTI. This could be explained by the attenuating effect of urine on the antimicrobial activity of PAL and its toxicity on immune cells. The detailed mechanisms of the observed effects require further investigation.
Our results also show that PAL increases microbial diversity in healthy gut and decreases it in inflamed gut. Similar to UTI, our findings do not support the proposed therapeutic potential of PAL in IBD. We conclude that the effect of PAL is bidirectional and depends on the underlying conditions. Further research is needed to elucidate the specific reactive species composition in PAL that may lead to beneficial effects *in vivo*, since this can vary strongly depending on the plasma discharge and experimental conditions.

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Plasma Activated Water is Effective against Redoubtable Nosocomial Microorganisms

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Nosocomial microorganisms are a major source of morbidity and mortality and are the second most prevalent cause of death. In addition, the increased resistance of microorganisms to antibiotics and disinfectants and emergence of biocide-resistant strains have made their elimination difficult. Disinfectants are effective in reducing healthcare-associated infections, but the correct choice of disinfectants given their range of effectiveness against hospital pathogens is crucial in this process. Also, some yeasts and bacteria has been shown to survive on dry and moist surfaces, so environmental cleaning to eliminate a source of nosocomial infections is a challenge. Thus, the search for a new effective agent with fungicidal and bactericidal properties is still a hot topic nowadays. Non-thermal plasma-activated water (PAW) has recently emerged as a potent antimicrobial agent, but no data about its efficacy against all nosocomial pathogens are not available.

The aim of our study was to assess the possibility of using PAW as a new alternative in disinfection against nosocomial pathogens. Twenty clinical isolates and seven reference strains such as *Candida auris* (CBS 10913), methicillin-resistant *Staphylococcus aureus* (ATCC 4330 MRSA), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Burkholderia cepacia* (ATCC 10673), carbapenem-resistant *Enterobacteriaceae*, *Serratia marcescens* (ATCC 13880) were used in this experiment. PAW was prepared using distilled water and a GlidArc reactor as previously described [2]. The final parameters of PAW were as follows: conductivity 446 ± 25 µS/cm, pH 2.78 ± 0.12, oxidation reduction potential (ORP) + 1.06 V, NO_2 192 ± 10 mg/L, NO_3 1550 ± 95 mg/L, H_2O_2 2.6 ± 0.12 mg/L, and O_3 1.08 ± 0.07 mg/L.

Suspensions of yeast cells and bacterial cells (10^9 CFU/ml) were prepared from overnight cultures and subsequently treated with PAW in a ratio of 1:10 for different periods of time (1, 3, 5, 7, 10, 15, and 20 minutes). Precise volumes of the mixtures were further inoculated on Sabouraud Dextrose Agar plates, Tryptone Soy Agar plates and Bact/Alert FA Plus bottles (bioMerieux, France) in order to evaluate the reduction of microorganism's burden after each contact period. In addition, some instrumental analysis (IA) methods were used in order to assess the impact of PAW treatment on yeast cell structure: Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), Fourier Transform Infrared Spectrometry (FT-IR), and Dynamic Light Scattering (DLS). All tests were performed in triplicates.

A reduction higher than 5 $\log_{10}$ of viable yeasts and bacteria was achieved within 1-2-3 minutes depending on the species. The sterilization level (i.e., >6 $\log_{10}$ reduction) was reached after 3 to 5 minutes for the strains tested. IA clearly objectified the morphological changes in the treated yeasts compared to untreated ones.

Our research has successfully demonstrated the fungicidal and bactericidal effect of PAW against all nosocomial pathogens, opening a new field of research in the area of disinfectants.

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Inactivation pathways of *Escherichia coli* and *Staphylococcus aureus* induced by transient spark discharge in liquids

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Microbial inactivation is one of the biggest issues for medicine, the food industry, agriculture, and environmental protection. Conventional decontamination methods, such as heat or UV treatments, photochemical oxidation, pulsed electric field, ozonation, etc. have the potential to reduce pathogenic microorganisms, however there is a demand for new effective and affordable methods. Cold atmospheric pressure plasma represents an easy and environmentally friendly method that has been effectively tested in bactericidal, virucidal, sporicidal, and antiparasite activity. Moreover, plasma-treated liquids (PTLs), possess various biological effects given by the concentration of reactive oxygen and nitrogen species (RONS) in the liquids. It has demonstrated antimicrobial, anticancer, wound healing, and other interesting effects. Although the antibacterial activity of the plasma is indisputably confirmed in diverse applications, the plasma–bacteria interaction pathways and mechanisms of plasma induced bacterial inactivation are yet to be fully explored. The study contributes relevant knowledge to an elucidation of the complex plasma-bacteria interaction pathways. Existing studies usually deal with one type of bacteria, in one growth phase, and the effect of plasma is evaluated by a limited number of experimental techniques. The comprehensive analysis of plasma effects on Gram− *E. coli* and Gram+ *Staphylococcus aureus* in different growth phases confronted with chemical characterization of PTL emphasizes the originality of the presented research. The plasma-induced damages and the roles of different RONS during plasma treatment were determined. Bacterial viability, metabolic activity, cell membrane integrity, accumulation of intracellular ROS, and morphology were investigated. The physicochemical properties of PTL, including pH, conductivity, oxidation–reduction potential (ORP), and concentration of long-lived and accumulated total amount of short-lived species were monitored. Results showed a high bactericidal efficacy and induced up to 7 log and 3 log reductions within 15 min plasma treatment of *E. coli* and *S. aureus*, respectively. A slightly higher inactivation was observed for bacteria in the exp growth phase than in the stat growth phase, highlighting lower resistance of cells toward plasma during their growing phase (exp phase) with a high level of metabolic activity. Interestingly, a strong reduction of metabolic activity of S. aureus by 93% did not cause a total inactivation, whereas total inactivation of *E. coli* corresponded to only a 77% reduction. The discrepancy of bactericidal effect with metabolic activity in the case of *E. coli* might be due to viable-but-not-culturable phenomena. Plasma-generated reactive species were found to destroy the cell membrane of *E. coli* facilitating the rapid accumulation of intracellular ROS followed by a collapse of intracellular redox balance. Moreover, multiple morphological damages were confirmed for plasma-treated *E. coli*. On the contrary, *S. aureus* survived plasma treatment for even 20 min. We suggest the preservation of the cell membrane and morphological integrity provided protection from plasma-generated reactive species, leading to the survival pathway of S. aureus. Altogether, the bactericidal efficacy of discharge depends on the type of bacteria, its growth phase, and the physicochemical properties of its surrounding environment.

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Decontamination of Healthcare Textiles Using Plasma-Activated Water

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Healthcare-associated infections (HAIs) in healthcare facilities are one of the greatest challenges to healthcare systems, and according to the European Centre for Disease Prevention and Control (ECDC), more than 3.5 million people are affected every year in the European Union and the European Economic Area alone, resulting in more than 90,000 deaths [1]. The spread of pathogens between healthcare workers and patients is an important factor, and transmission via healthcare workers' clothing or healthcare textiles in general is recognized as an important route [2;3]. Washing machines are commonly used to clean textiles, requiring temperatures of around 60 °C and chemical additives to achieve sufficient decontamination. The disadvantages are using harmful substances to humans and the environment, insufficient antimicrobial efficacy, and contaminating wastewater and machines [4;5]. A new approach to decontaminating healthcare textiles could be using plasma- activated water (PAW), which has a broad spectrum of antimicrobial activity, is environmentally friendly, and does not produce process residues [6;7]. This study investigated whether PAW can be used to decontaminate textiles and how repeated treatment with PAW affects their properties. For this purpose, deionized (DI) water was activated using an Openair® plasma system, and the antimicrobial effect was tested using 100 % cotton samples contaminated with *Escherichia coli* or *Staphylococcus aureus*. Parameters such as optimal treatment time (0, 2, 5, 10, 15, 20, 25, and 30 minutes) and PAW agitation speed (0, 250, and 500 rpm) were tested, and the antimicrobial efficiency of PAW was compared with DI water as a negative control and ethanol as a positive control by counting the colony- forming units. In addition, the mechanical and chemical properties of the textiles were tested after up to 15 repeated PAW treatments for 1 hour at 500 rpm. The results showed that *Escherichia coli* was completely removed after 15 minutes and a PAW agitation of 250 rpm, while *Staphylococcus aureus* required at least 15 minutes and 500 rpm. Mechanical and chemical properties showed no changes or changes similar to those after water treatment despite repeated treatment with PAW. In conclusion, PAW can be an alternative to conventional chemical detergents for decontaminating textiles and preventing the spread of pathogens.

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Utilizing the Potential of Plasma-Treated Water for Enhanced Maize Growth and Stress Tolerance

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Cold atmospheric plasma (CAP) technology has been extensively studied for decades for its application in disinfection, wound healing, cancer therapy, seed germination and plant growth promotion, as well as for food preservation and extending shelf-life of fresh produce. CAP is considered a potential alternative to traditional agriculture with fewer adverse effects on the environment and potentially higher yields. Moreover, CAP could potentially reduce or completely replace the use of pesticides and fertilizers. The implementation of plasma-treated water (PTW) as a source of aqueous reactive oxygen and nitrogen species (RONS) acting as signal molecules and source of plant macroelements offers various beneficial aspects in agricultural applications. H_2O_2, NO_2^- and NO_3^-, are known to have significantly positive effects on stimulating seed germination rate, plant development, stress response and overall growth, however, their dose is essential. The importance of H_2O_2 and NO_3^- in PTW was previously reported and compared with various H_2O_2 or/and NO_3^- chemically equivalent solutions applied to lettuce [1].

In a previous investigation [2], the response of maize (*Zea mays* L.) to PTW treatment was examined, revealing enhancements in root and shoot growth, as well as accelerated endodermal development in roots. The influence of PTW on maize was further evaluated under arsenic stress conditions, demonstrating increased antioxidant capacities and enhanced tolerance against arsenic stress in young seedlings cultivated hydroponically. These findings collectively suggest that PTW application positively contributes to the overall survival and resilience of the plant, particularly under adverse environmental conditions such as arsenic stress.

In the present project, we studied effects of PTW generated by air transient spark (TS) discharge in a peristaltic system with continuous water inlet to plasma discharge. We described physical-chemical characteristics (e.g., pH, conductivity, H_2O_2, NO_2^-, etc.) of PTW. Plants were analyzed after a 14-day cultivation in soil. PTW was first applied only on the maize kernels (three selected hybrids) during 16-hour imbibition. Subsequently, in a chosen hybrid with the best biochemical response on PTW treatment, several treatments of foliar PTW application with respect to different doses of PTW were conducted. Growth parameters (root length, leaf area, dry mass percentage) were measured. On the biochemical level, antioxidant enzyme (guaiacol-peroxidase, catalase) activities, non-enzymatic antioxidants (carotenoids, total soluble phenols) and concentrations of photosynthetic pigments (chlorophylls) were analyzed. We also monitored and described the PTW-influenced plant response to stress factors. The results will be presented at the conference.

This work was supported by Slovak Research and Development Agency, APVV-17-0382 and APVV-22-0247.

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Microbiological and pH Assessment in Pineapple Jams and Zucchini Preserves Treated with Plasma Activated Water

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The use of high temperature combined with the control of the pH in food preserves with an addition of new technologies, are important measures to inhibit the growth of pathogenic microorganisms, thus this article aims to present the results of using Plasma Activated Water as a form of treatment for pineapple jams, zucchini preserves, performing microbiological, and pH analyzes on different homemade preparations. The comparison was made in four groups, a group control, another group using the method proposed by the SENAR (National Rural Learning Service) [1] for jams and Embrapa[2] for preserves, treatment with water activated by Plasma[3] and conventional home treatment. After three months, the bottles were opened, for microbiological analysis, total and thermotolerant coliforms were investigated for both preparations, fungal research in the jams and total mesophiles in the preserves, and the pH of all bottles was also determined. After the incubation time, colony-forming units were counted in each of the dilutions and the results were calculated as the average for both jams and preserves. For the investigation of total and thermotolerant coliforms, no growth was observed in all groups of both preparations. The results of other microbiological and pH analyzes are described in the table below.

	Treatments	*Microbiological analysis (UFC)*	*pH analysis*
Pineapple compote	Control	6.8 x 10^4	3.30
	SENAR	5.5x10^4	3.30
	PAW	0	3.10
	Home treatment	0	3.50
Canned zucchini	Control	>1.0x10^6	5.20
	Embrapa	0	3.75
	PAW	8.96x10^3	5.69
	Home treatment	0	3.59

Table 1. Average results compared in different treatments.

As expected, the group treated with Plasma Activated Water proved to be effective for fungi, as there was no growth in the pineapple jams, which can be explained by the pH of the jams in this treatment. However, for mesophilic bacteria in canned zucchini, only a reduction in the number of CFU was observed, since the microorganisms found, *B. cereus* and *S. epidermides* have resistance characteristics, which suggests that the use of PAW requires specific care that varies according to the cell concentration and the species of microorganism.

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ICPM

PLASMA SURFACE INTERACTIONS/ MODIFICATIONS FOR BIOMEDICAL APPLICATIONS

Oral session (Tue - O - 5)

Tuesday, 10 September 2024

Insights into the use of aerosolized precursors in atmospheric pressure plasma polymerization: process control and coating stability

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The use of aerosolized precursors (instead of vaporized ones) in atmospheric pressure (AP) plasma polymerization processes offers several advantages, such as the considerable simplification of the experimental set-up and the possibility to efficiently carry nano-additives into the discharge. [1,2] Nonetheless, the aerosolized form affects the nature of the interactions between the precursor and the discharge, thus typically leading to a not-trivial process control and to a production of coatings characterized by poor stability upon immersion in liquid environment. [3,4,5] In this work, a study of the validity of the Yasuda Parameter (W/FM) as controlling parameter in a polymerization process assisted by an AP single electrode plasma jet and an aerosolized organosilicon precursor is proposed and the stability upon immersion of the deposited coatings is investigated.
The chemical and physical properties of the deposited coatings are assessed by means of ATR-FTIR, XPS, WCA, and SEM analyses. Surface characterization techniques reveal the presence of the so-called energy-deficient and monomer-deficient domains as a function of W/FM, thus suggesting the validity of W/FM as a controlling parameter. In addition, the key role of W/FM in the process is further demonstrated since coatings deposited under the same W/FM exhibit similar properties, regardless of how W/FM is obtained. The analysis of the stability upon immersion in liquid environment show that the characteristics of the coatings from aerosolized precursor remain almost constant up to 28 days and are comparable with those from the same flow rate of vaporized precursor. This allows to infer that the precursor droplets are subjected to evaporation when the discharge is ignited or that, below a certain size distribution droplet, the behavior of the droplets is no longer different from the one of vapor molecules.
This work aims to provide useful insights into the use of aerosolized precursors in AP polymerization processes, focusing on two topics of high industrial relevance: the control of the process and the stability of the coatings upon immersion in liquid environment. It is demonstrated that W/FM can be a proper controlling parameter for the process and that the advantages of aerosols in terms of setup simplicity and ease in the carrying nano-additives can be combined with the typical characteristics of stability upon immersion of coatings from vaporized precursors.

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Production of reactive oxygen species and its controlled delivery by functionalized thin films deposited by plasma technology

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Nanoporous plasma polymer films (PPFs) deposited at room temperature by low-pressure plasma polymerization are excellent candidates for the functionalization of catalytic systems producing reactive oxygen species (ROS). The polymers are deposited by cyclic processes of deposition (i.e., plasma polymerization) and plasma etching, leaving connected open voids which determine the porosity [1]. By following this approach, volumetric porosities of about 25% can be obtained, as we recently reported with an experimental approach named near-plasma chemistry (NPC) [2]. Since the nanoporous PPFs derived from HMDSO have a SiOx chemistry, the polymers are resistant to highly oxidizing conditions such as those presented when ROS are produced. Moreover, the superhydrophilic wetting behavior of the polymer and its nanoporosity allows for fast diffusion of water through the polymer, as well as the delivery of reactive oxygen species (O_2^-, H_2O_2, etc.). With this contribution, we would like to share novel results regarding the functionalization of catalytic metal oxides surfaces with nanoporous PPFs. Catalytically-active metal oxides are deposited as thin films by sputtering of titanium and silver targets followed by plasma oxidation in an Ar/O_2 environment. The non-stoichiometric oxidation state of both metals attained due to the plasma process, as well as their nanostructured combination accounts for the capability to produce ROS in low light conditions without needing UV light activation. Therefore, the catalytic activity can be regenerated readily by exposing the material to daylight. Moreover, additional plasma reactivation can recover high activity levels characteristic of the as-prepared material. In this regard, both low-pressure and cold atmospheric-pressure discharges (CAP) can be used to reactivate the catalytic surface, thus also tuning the material's activity by light or plasma exposure. This concept, together with the surface functionalization using thin plasma polymer films (5-100 nm), opens up a broad range of applications (e.g., in plasma medicine), for materials in solid state producing reactive oxygen species fully fabricated by plasma technology.

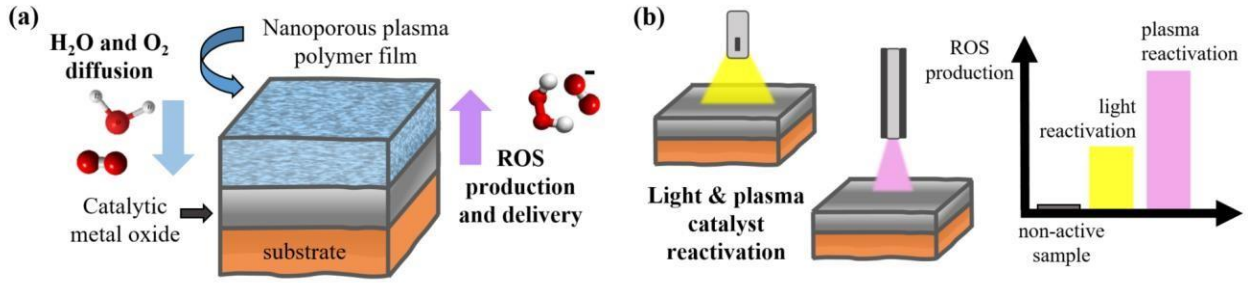

Fig. 1 – (a) Drawing showing the diffusion of water and oxygen and the delivery of ROS through the PPF. (b) Visible light exposure and plasma treatments can reactivate the catalytic surface.

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Commercial Medical Applications of Surface Treatment by Cold Plasma

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Hydrophilic surface is an advantage for different kinds of implant surfaces. Hydrophilic dental implants are known to have a shorter healing time [1]. Hydrophobic breast implants tend to accumulate a biofilm which cannot be eradicated by the immune system [2].

Nova Plasma has developed plasma surface treatment devices that are designed to be used in the operation room setting. The devices maintain sterility, and enable a simple and easy activation, with minimum interference with the physician work. The Active+ device activates the dental implant inside the original sterile package. The device uses the package as the dielectric barrier of a DBD plasma source without the need to extract the implant from its package. The implant becomes super hydrophilic after an activation of 30 seconds. An in-vitro study on activated titanium dental implant showed that MSCs and osteoblast-like cells (MG63 s) produced increased concentrations of osteo- calcin, osteopontin, and osteoprotegerin after plasma treatment [3].

The Active-Si device activates silicone breast implants and enables covering them by antiseptic liquid such as antibiotics and prevent growth of bacteria and biofilm on their surface. In an in-vitro study, a complete elimination of bacteria was seen after activation of silicone surface when comparing to a non-activated surface which accumulated a biofilm [4]. The presentation will introduce the devices and the biological and microbiological studies that were made to show their efficacy.

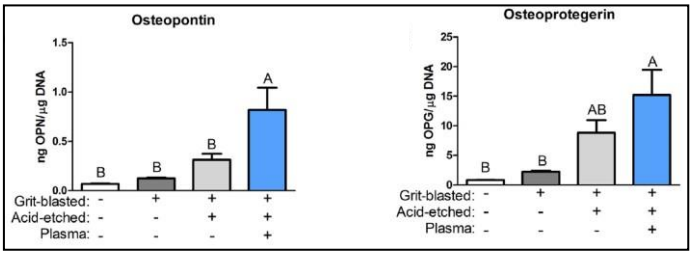

Fig. 1 MG63 Cellular response to plasma treated titanium Vs non plasma treated.

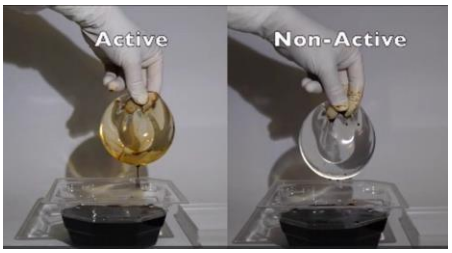

Fig. 2 Activated silicone implant immersed in iodine (left) and non-activated (right).

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Tue-O-5-4

Biomimetic surfaces for improved biological response

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Implant-associated infections (IAIs) are frequently cited as a primary cause of implant failure, leading to escalated medical expenses and posing significant risks to patient health. These infections typically arise from bacterial colonization, which subsequently fosters the formation of biofilms on the implant surface. Nanostructured surfaces have emerged as promising candidates for inhibiting bacterial adhesion, primarily attributed to their unique surface nanotopography and inherent antibacterial properties. Alterations in surface topography affect various physicochemical properties, including surface chemistry, morphology, wettability, surface charge, and may as such significantly influence biological responses. Three different Ti surfaces were developed: micro flowers, obtained by plasma induced electrochemical anodization, nanotubes obtained via electrochemical anodization, and nanocubes obtained by hydrothermal (HT) method. In addition to nanostructured surfaces low pressure gaseous plasma was used to fine tune surface chemistry for desired biological response. The nanostructured Ti surfaces were studied for its application in vascular stents or dental implants. Their morphology, surface chemistry, and wettability were evaluated by scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), and water contact angle analysis. While interaction with endothelial and smooth muscle cells was done by *in vitro* biological response, where cell proliferation was analysed by fluorescence microscopy. The antibacterial effectiveness of the synthesized nanostructures was evaluated using the *Escherichia coli* bacterial strain and multispecies oral biofilm of both Fusobacterium nucleatum ATCC 25586 and saliva microorganisms (isolated from a patient with periodontitis). The anti-biofilm activity was assessed via the Colony Forming Units (CFU) count and the Live/dead assay followed by Fluorescence Microscopy analysis. Bacterial adhesion and biofilm formation on the Ti surfaces were also observed via Scanning Electron Microscope (SEM). Our results indicate that changes titanium oxide layer as well as surface nanotopography significantly influence on specific cell-surface interaction. This may, open new insights for designing of multifunctional biomaterial surface, which can promote growth of one cell type over another and at the same time reduce bacterial infections.

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PLASMA MEDICAL APPLICATIONS – CLINICAL AND ANIMAL STUDIES

Oral session (Tue - O - 6)

Tuesday, 10 September 2024

Non-Invasive Physical Plasma (NIPP) Improves Conventional Wound Management of Cut and Bite Wounds in European Hedgehogs: Results of a Prospective, Non-Randomized Monocentric Wildlife Clinical Trial

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Background: In human medicine, non-invasive physical plasma (NIPP) has been successfully applied in wound healing for over two decades. Thus, it can be assumed that NIPP can also be used for wound management in mammals and other vertebrates.

Methods: 43 European hedgehogs (*Erinaceus europaeus*) with cut and bite wounds received veterinary care at a non-profit wildlife rescue center in North Rhine-Westphalia, Germany. 21 patients received conventional wound treatment (CWM) and were compared with 22 patients who received CWM plus 2 min NIPP treatment (CWM+NIPP). For NIPP treatment, the Plasma Care device (Terraplasma Medical, Garching, Germany) was utilized.

Fig. 1 NIPP treatment of a wild hedgehog with the Plasma Care device (Terraplasma Medical, Garching, Germany). The patient was not anaesthetized.

Results: Treatment with NIPP showed no signs of pain, stress or discomfort in any of the patients, even after seven applications. Systemic or local anesthesia was not necessary. In 76 % of CWM+NIPP patients, three or four NIPP applications were sufficient. In patients in the CWM+NIPP group, wound treatment was completed statistically significantly 6 d earlier (CWM: 19.0 d versus CWM+NIPP: 13.2 d; p=0.0008).

Conclusion: The presented clinical wildlife trial demonstrates that NIPP can be used to improve wound healing in wild hedgehogs. It is possible that NIPP therapy may also be beneficial for wound management in other injured wild animals or in injured domestic and farm animals.

Evaluation of Non-Thermal Plasma for Diabetic Wound Healing: From Preclinical Studies to Clinical Trials

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Our research aims to evaluate the effect of non-thermal plasma on diabetic wound healing. Diabetic patients frequently encounter challenges in wound healing due to complications arising from neuropathy, vascular disease, and foot deformities. The utilization of a plasma jet to ionize helium gas can generate ions and free radicals, fostering sterilization and chemical processes that aid in wound healing and tissue repair [1-2].

In our first study, a plasma jet was employed to produce helium plasma, which was then applied to induced diabetic and chronic wounds in rats. The study consisted of five groups of male rats, each comprising five individuals. Results demonstrated significant enhancements in wound healing, histological improvements, and the release of TGF-β1 cytokine compared to control groups [3]. In the subsequent study, we conducted a randomized clinical trial to assess the efficacy of Cold Atmospheric Plasma (CAP) in diabetic foot ulcer healing. Patients with diabetic wounds (n=44) were randomized into two groups: standard care (SC, n=22) and standard care with CAP applied three times a week for three consecutive weeks (SC+CAP, n=22). CAP treatment involved ionized helium gas in ambient air driven by high voltage (10 kV) and high frequency (6 kHz) power supply. The primary outcomes evaluated were wound size reduction, the proportion of cases achieving a wound size reduction of <50%, and bacterial load over three weeks of treatment. The results indicated that CAP treatment effectively reduced the wound size fraction ($p=0.02$) and significantly increased the proportion of wounds reaching a size reduction of ≤50% in the SC+CAP group compared to the SC group ($p=0.006$). The mean fraction of bacterial load counted in each session 'after CAP exposure' was significantly less than 'before exposure' measures. CAP can be an efficient method to accelerate wound healing in diabetic foot ulcers, with immediate antiseptic effects that do not seem to last long [4-5].

In conclusion, our studies highlight the potential of atmospheric pressure plasma as a promising therapeutic approach for diabetic wounds, addressing critical factors such as microcirculation, oxygenation, and nutrient supply to the tissue. Future investigations will focus on elucidating the dynamics of blood flow variation during the wound healing process using diffuse correlation spectroscopy and assessing the impact of cold plasma treatment on this procedure.

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Cold Plasma Therapy for Venous Ulcers: Early Clinical Trial Outcomes

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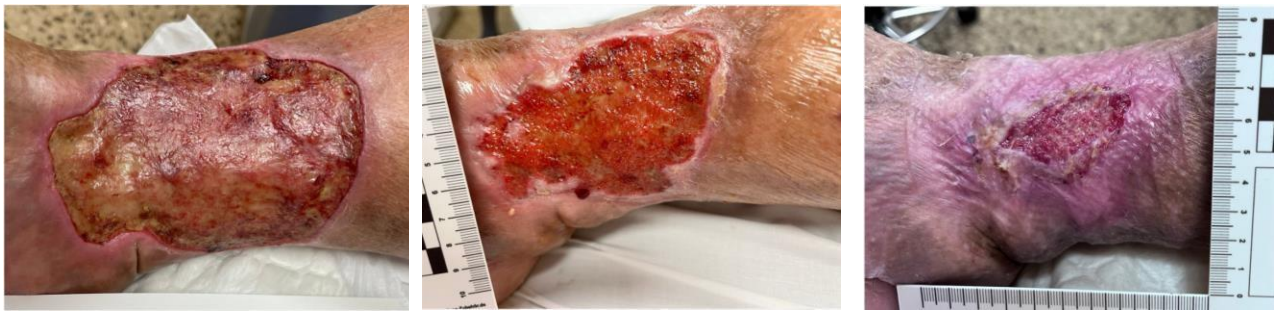
This clinical trial is investigating the efficacy of atmospheric cold air plasma jet treatment as a novel therapeutic approach to accelerate the healing of venous ulcers. The study was designed to evaluate the safety, feasibility and efficacy of this innovative treatment modality.



Week 1: start of treatment *Week 10: end of treatment* *Week 18: 8 weeks without plasma treatment*

Fig. 1 Evolution of a patient treated with cold atmospheric plasma

The study enrolled participants with active venous ulcers who were randomized into two groups: a treatment group receiving cold plasma therapy in addition to standard care with silver alginate wound dressings, and a control group receiving care with silver alginate wound dressings alone. Cold plasma treatment was administered two times a week for up to ten weeks. Primary outcome measures included ulcer healing rate, pain reduction, bacterial burden and changes in ulcer size assessed by digital image measurement. Secondary outcomes focused on assessing safety and patient satisfaction with the treatment.

Preliminary results indicate that the cold plasma treatment group experienced a significantly faster healing rate, with a significant reduction in ulcer size and pain intensity and a drastic reduction of bacterial burden compared to the control group. No adverse effects were reported, suggesting that the treatment is safe and well tolerated by patients. These findings provide promising evidence for the potential of atmospheric-pressure cold plasma therapy as an effective treatment for venous ulcers and warrant further investigation to fully elucidate its therapeutic benefits and mechanisms of action.

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Safety and efficacy study of Plasma-activated Lactate Ringer's Solution (PAL) for application on animal and human skin

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It has been demonstrated that nonequilibrium atmospheric pressure plasma (NEAPP) irradiated lactate ringer's solution (plasma-activated lactate ringer's solution: PAL) has a potential role in medical applications [1, 2]. Incorporating NEAPP into dermatology is one of the most cutting-edge advancements in medical practice. However, reports on the efficacy of plasma-activated solutions, such as PAL, in wound healing are limited. At ICPM8, held in 2020, we have already presented that PAL enhanced wound healing in the excisional wound-splinting model using diabetic mice. This time, we focused on PAL's epithelialization-promoting effects and evaluated its efficacy and safety using the same mouse model. Additionally, safety trials targeting human skin were conducted.

In the animal experiments, in addition to evaluating the epithelialization-promoting effects on wounds, safety tests were simultaneously conducted on both the wounds and normal skin. As a result, it was demonstrated that PAL diluted solution promotes healing and epithelialization compared to untreated and Lactec-treated wounds. Conversely, PAL undiluted solution showed a tendency to decrease both. We also evaluated the local effects of PAL administration on mouse wounds and normal skin and the systemic effects on organs through histological examination. We found no adverse events in either case. Having demonstrated its efficacy and safety in animal experiments, we proceeded to conduct safety trials on normal human skin.

According to the protocol approved by the ethical committee for clinical research of Nagoya University Hospital, we performed a double-blinded randomized control trial of the PAL patch test (1000-, 100-, and 10-times diluted PAL). We collected their blood and urine before and after the patch test. All participants showed no adverse event during this trial. There is no abnormal change in all blood and urine tests, but there was a statistical difference between 1000x and 10x diluted PAL groups concerning gamma-GT and lipid peroxide. There was no statistical difference among all groups with the other tests. In this trial, we demonstrated that topical application of 10 times more diluted PAL on normal human skin is safe.

This work was supported in part by JSPS KAKENHI Grant Numbers 19H05462 from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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An Innovative Method for Molecular Introduction into Fish Eggs Using Surface Discharge Treatment

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Introduction

The authors have reported that surface discharge treatment can introduce molecules into the cells within the eggs of saltwater fish, specifically Suma fish eggs [1]. This method of surface discharge is capable of mass introduction and is anticipated to replace the conventional microinjection method as a new technique for introduction. In this study, we attempted for the first time to introduce molecules into the eggs of medaka fish, a species of freshwater fish commonly used as a model organism for vertebrates.

Experimental Setup

The surface discharge treatment system has two cathode electrodes for applying high voltage and two anode electrodes on the ground (GND), for four electrodes. A solution containing fluorescent molecules (Sigma Aldrich; FITC-Dextran 10 kDa) at a concentration of 10 µg/µl was prepared, and 800 µl of this solution was dropped into a 3.5 cm dish. Medaka fish eggs were then placed in the center of the dish. Electrodes were positioned 1 mm above the solution's surface, and a half-wave rectified sinusoidal voltage of negative polarity was applied to the cathode electrode to generate a surface discharge. The medaka used was of the OK-Cab (NBRP Medaka; MT830). The distance between the electrodes and the liquid surface was maintained at 1 mm, and the discharge treatment was carried out. Two types of fluorescent molecular solutions were used: freshwater (0.03 S/m) and a solution with the same conductivity as the 50 % seawater used in the Suma fish eggs (saltwater fish) experiment (1.85 S/m). Two stages of fish eggs were used: ‘early developmental eggs’ collected immediately after egg laying, and ‘late developmental eggs’ collected just before hatching.

Results and Discussion

The results of the fluorescence observation of hatched fry following molecular introduction treatment by surface discharge in a high conductivity solution are presented in Fig. 1. The fry that underwent discharge treatment in the late egg stage of development exhibited strong fluorescence in the head and abdomen indicating the successful introduction of fluorescent molecules. In contrast, no fluorescence was observed in the fry treated with discharge during the early developmental stages. In specimens treated with discharge in freshwater with a conductivity of 0.03 S/m, minimal or no introduction was observed, regardless of whether it was early or late in ontogeny. The fact that introduction was observed in the case of the high-conductivity solution suggests that current stimulation plays a role in the molecular introduction in fish eggs and animal and plant cells. This could be attributed to the vulnerability of fish eggs to external stimuli in the early stages of development, and the stress caused by current stimulation may prevent the formation of embryoid bodies.

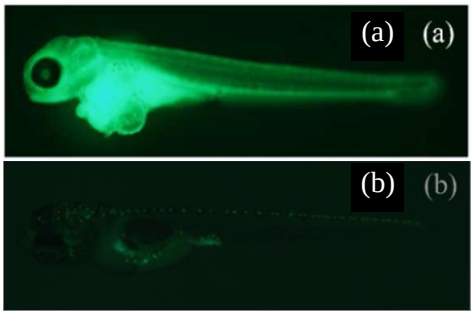

Fig.1. Fluorescent images of medaka fry treated with plasma in a high-conductivity solution(a) and freshwater (b).

Acknowledgments

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Treatment of psoriasis with an ALOE device: pilot research

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Low temperature plasmas that can be generated at atmospheric pressure and at temperatures below 40°C have in the past couple of decades opened up a new frontier in plasma applications: biomedical applications. Plasma medicine is about using low temperature atmospheric pressure plasmas to generate controllable amounts of specific chemically reactive species that are transported to react with biological targets including cells and tissues. [1]
At the Center for Plasma Physics (Minsk, Belarus), an apparatus for generating a plasma jet "ALOE" (Fig1*a*) has been developed, which is now undergoing medical examination (clinical trial stage). It was previously shown that the main mechanism of the effect of a plasma jet on biological objects is RONS [2-5]. We have also repeatedly noted the influence of a plasma jet on a wide range of museum strains of microorganisms, isolates taken from the human environment, isolates taken from the mucous membranes and sputum of patients during the period of illness of the upper respiratory tract. [2-4]. The high ability of the device we are developing to influence microorganisms allowed us to move on to more complex experiments, in particular on animal models [for example 5]

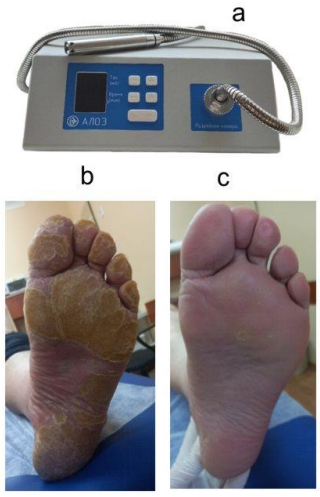

Fig. 1. ALOE device (a) and photo of a woman's foot before (b) and after (c) treatment

A 60-year-old woman with diagnosed palmoplantar psoriasis, which they have been trying to treat with medication for the last 2 years, applied to the Minsk Regional Clinical Hospital.
Psoriasis is an autoimmune disease, which complicates its treatment with traditional methods. Previously, there were references in the literature to the treatment of psoriasis using plasma medicine.[6]
At the time of treatment, the woman had tissue crusts, secondarily populated with microflora, and multiple cracks. The woman was treated with an ALOE device 4 times (once a week) for 7 minutes, photographs before and after treatment are presented in Fig. 1 (*b* and *c*). A similar picture is observed on the second foot and palms. At the moment, no relapse of the disease has been observed.
This work was supported by BRFFR no. F22SRBG-006.

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Cold atmospheric-pressure plasma and plasma treated liquids for veterinary applications

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The use of a cold atmospheric-pressure plasma (CAP) has become a very discussed topic in the last few decades, thanks to a wide range of applications in various fields of industry, agriculture, and medicine. This work deals with the applications of CAP and plasma-treated liquids (PTL) in veterinary medicine. A special attention is brought to an alternative treatment of *otitis externa* in dogs. *Otitis externa* or so-called swimmer´s ear, is a condition that causes an inflammation of the external ear canal. One of the factors causing this disease are microorganisms. An increasing resistance of microorganisms to antimicrobial drugs urges the researchers to find new alternative treatment methods. Thus, the antimicrobial effects of CAP and PTL on bacteria and yeasts were tested. With regard to a specific application, the dog ear treatment, the plasma gun [1] with its capillary design was used for the direct treatment. The use of the plasma gun with a thin capillary allows for an endoscopic treatment inside the complex structures. As model microorganisms, bacteria (gram-negative *Escherichia coli*, gram-positive *Staphylococcus epidermidis*) and a yeast *Candida glabrata* were chosen. The tests have been done on the Petri dishes and on other relevant inoculation substrates (pork skin and 3D printed models of dog ear canals). The results confirm a high efficacy of direct CAP treatment in the inhibition of bacteria and yeasts on different surfaces.
In the indirect treatment a secondary agent (usually liquid) is plasma treated and then put into contact with the targeted subject. Plasma treated liquids (PTLs) were prepared in different plasma systems. Prepared PTLs were first characterized in terms of reactive particles (hydrogen peroxide, nitrates and nitrites) and changes in specific conductivity and pH. This was followed by the microbiological assays. Bacteria from the genera *Staphylococcus* and *Pseudomonas* were selected as testing microorganisms. These microorganisms were exposed to PTL, and the effects were studied for exposure times from 1 minute to 4 hours. In addition, different standard solutions of reactive particles (hydrogen peroxide, nitrites, nitrates), as well as chemical alternatives of PTL solutions were tested. The antibiograms were also compiled to compare the effect of PTLs with the effect of antibiotics. The results of these experiments and other studies in the field, demonstrate a high antimicrobial efficacy of the plasma therapy. A use of CAP and PTL can help to minimize the use of antibiotics or antimycotics and provide new treatment possibilities and options for the inactivation of multi-resistant microorganisms. Plasma treatment thus represents an alternative treatment not only for the *otitis externa*, but also for other diseases of microbial and non-microbial origin.

Acknowledgement
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8 – 13 september, Portorose, Slovenia

POSTER SESSION

(Tue - P)

Tuesday, 10 September 2024

Plasma Jet Treatment of the Inner Wall of Montgomery Tracheal Implant (T-Tube)

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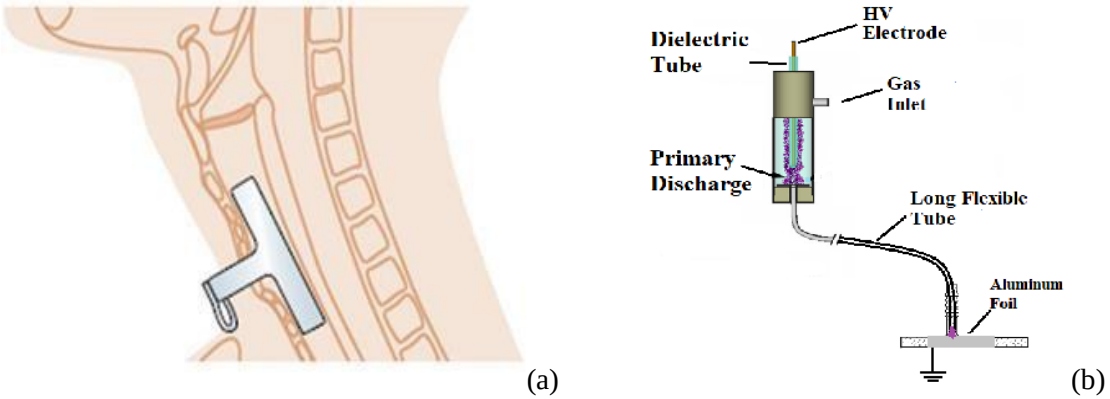
Tracheal stenosis (i.e., abnormal narrowing of the trachea) can occur due to a variety of inflammatory and, infectious processes as well as due to therapeutic procedures undertaken by the patient. The most common causes of abnormal trachea obstruction in patients have been difficult or prolonged intubations [1]. Therefore, in the wake of COVID19 pandemic a substantial increase in the number of iatrogenic tracheal stenosis is expected. Surgery is the most common option for addressing this issue, but depending on the exact location and extent of the stenosis, a tracheal stent is inserted. The Montgomery T-tube implant is a valuable tracheal stent that provides a functional airway while supporting the tracheal mucosa (see the Fig. 1(a)). However, its performance is subject to gradual deterioration due to biofilm colonization of the stent's inner wall that may explain the discomfort claimed by many patients and clinical failures.



(a) (b)

Fig. 1: (a) T-tube in place and (b) experimental setup

Recently, cold atmospheric plasmas (CAP) have emerged as alternative technology to different medical procedures, such as tissue healing, skin treatment, wound decontamination etc. [2]. Here, we study the plasma jet treatment effect on the inner wall of a T-tube implant in view of future biomedical applications. The plasma device was reported previously [3] and the experimental setup is shown in Fig. 1(b). The degree of surface modification and its extension along the inner stent surface was analyzed at different process parameters to evaluate the treatment uniformity.

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Development of a decontaminating coating activated by cold plasma for biohazard control

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The transmission of a pathogen can occur through mutual contact or indirectly through contact with a contaminated surface [1]. Therefore, surface decontamination, through chemical, physical, or combined processes, is essential to reduce the risk of spreading infectious diseases. Among the existing methods, UVC radiation devices are frequently employed due to their ability to emit radiation capable of inducing irreversible damage to the DNA structure of cells. However, devices based on mercury use involve risks to the health of the user and the environment [2].
The aim of this work is to develop a mercury-free system for surface decontamination. It is based on an excimer plasma source and a plasma activated surface including phosphors, both emitting in the UVC range. Microbe present on the surface are thus caught between the two radiations and inactivated (Figure 1).

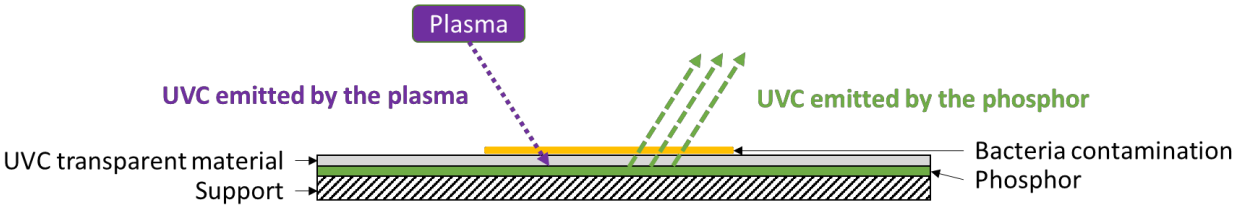

Figure 1: Schematic of the experiment.

The plasma source is a Kr-Cl excimer lamp (Oliscie) through a DBD coaxial configuration with specific dimensions (length = 200 mm; inner diameter = 20 mm; outer diameter = 50 mm) and coupled to a pulsed power supply (Power range from 20 to 70 W). Eight phosphors, distinguished by their formulation, manufacturing method and temperature processes were employed. In this study, the uniformity of the plasma discharge was observed using an intensified camera (PI-MAX1). Next, an irradiance meter (Opsytec Dr. Gröbel) equipped with a calibrated UVC detector (210 - 230 nm) was utilized to measure the irradiance distribution (mW/cm²) across a 400 x 300 mm surface with a spatial resolution of 5 mm. Then, emissions from both KrCl lamp and phosphors were examined using a monochromator (PI-HRS-750) coupled to an intensified camera (PI-MAX4). Finally, three plasma- luminophore combinations have been identified to be promising for further investigation in decontamination assays. The efficacy of germicidal activity was evaluated using *Escherichia coli* (ATCC 47076) and *Staphylococcus aureus* (ATCC 6538). The experimental log reduction results shown the interest of plasma-phosphor combination compared to plasma alone.
The authors want to thank the Research National Agency (ANR, France) for financial support.

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Cold plasma enamel surface treatment to increase fluoride varnish uptake

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Dental caries, a prevalent concern in primary school children, persists despite preventive efforts. Oral bacteria's carbohydrate metabolism lowers pH levels, causing enamel demineralization [1]. Topical fluoride forms a protective layer on enamel, yet its efficacy diminishes over time due to acid dissolution. Enhancing fluoride coating durability remains a significant research challenge. Among the available methods of enamel strengthening, fluoride varnish (FV) treatment has relatively better results [2]. On the other hand, cold plasma technology has shown promising capacities in sterilizing the environment, surface modification, and improving adhesion [3-5]. Accordingly, this study aimed to increase the adhesion of FV to the enamel surface to prolong the enamel interaction with FV with subsequently increased fluoride uptake by enamel. For this purpose, we randomly divided twenty bovine teeth into two groups: Group A (comprising four teeth) and Group B (composed of four subgroups, each containing four teeth). Samples from Group A and one specimen from each subgroup in Group B were investigated for the effect of using Helium-DBD (He-DBDJ), Argon (ArJ), and Air- DBD jet on the enamel surface. The remaining specimens in Group B were dedicated to studying the release of fluoride ions from processed enamel. Two diagnostic techniques, scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS), were utilized to examine the surface morphology and chemical composition of the samples, respectively. Additionally, the release of fluoride ions into distilled water was measured using an ion-selective electrode (ISE). SEM images revealed that ArJ and Air-DBD significantly damaged enamel hexagonal structures, while He-DBDJ only altered these structures from convex to concave. EDX analysis indicated an increase in the calcium-to-phosphorus ratio and the amount of fluoride and sodium uptake on the enamel surface layer in the group processed with He-DBDJ plasma, which helped restore the damaged parts of the enamel. However, there was no significant change in the fluoride release from FV due to plasma processing ($P \leq 0.112$). The combination of cold plasma and fluoride varnish treatment on the enamel surface might be considered a promising approach to increase enamel resistance to tooth decay.

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Evaluation of the Antimicrobial Effect of Room Temperature and Refrigerated Plasma Activated Water

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Plasma Activated water (PAW) have a lot of applications, and one of them is its antimicrobial effects, due to the creation of oxidative reactive species and its low ph. The objective of the work was to analyze the antimicrobial action of reverse osmosis (OR) water activated at room temperature and refrigerated using ATCC® standard strain of *Escherichia coli* (25922), *Klebsiella pneumoniae* (13883), *Staphylococcus aureus* (6538).

An inoculum of $1x10^8$ UFC/ml in BHI broth, was centrifuged in 4000 rpm for 15 min, where it was resuspended with PAW and incubated for 90 min. After that, a solution of 1:1 of the bacterial suspension and Trypan blue stain 2% was used to quantify cell viability using Neubauer Chamber®. The samples were sown on agar PCA after a dilution of $1:10^4$. For the activation of the water, it was used 250 ml of OR and one was activated at room temperature (27ºC), and the other one at 4ºC, where they were activated for 30 min with gliding arc plasma using Argon and compressed air, with a gas flow of 6L/min and 4L/min respectively.
Klebsiella pneumoniae showed a cell viability of 39% using room temperature PAW and 36% for refrigerated PAW, *Staphylococcus aureus* showed a cell viability of 34% for room temperature PAW and 24% for refrigerated PAW, and for *Escherichia coli* the cell viability was 35% for room temperature PAW and 25% for refrigerated PAW, the PAW pH after activation was 3.41. In conclusion, it was noted that refrigerated PAW showed a better cell viability reduction than room temperature PAW, and that *K. pneumoniae* showed a higher resistance to PAW antimicrobial effect.

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Inactivation of microorganisms on fabrics using plasma-activated nebulized mist driven by different plasma gases

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Abstract: The disinfection of fabrics is crucial in preventing the spread of infectious diseases caused by pathogenic microorganisms to maintain public health. A previous study proved that plasma- activated nebulized mist (PANM) could effectively inactivate microorganisms both in aerosol and attached to the surface. In this study, the PANM driven by different plasma gases were employed to inactivate microorganisms on diverse fabrics. The PANM could efficiently inactivate a variety of microorganisms, including bacteria, fungi, and viruses, contaminating different fabrics, and even across covering layers of different fabrics. The mites residing on the cotton fabrics both uncovered and covered with various types of fabrics were also effectively inactivated by the PANM. After 30 times repeated treatments of the PANM, notable changes were observed in the color of several fabrics while the structural integrity of the fabrics was unaffected and maintained similarly to the untreated fabrics. Additionally, only trace amounts of nitrate remained in the fabrics after the PANM treatment. Therefore, the PANM treatment supplied an efficient, broad-spectrum, and environmentally friendly strategy for industrial and household disinfection of fabrics.

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Investigation of cold plasma treatment on antimicrobial peptides as strategy for selective bacterial targeting

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Operation under ambient air conditions facilitates the generation of various reactive oxygen and nitrogen species (RONS) through the implementation of Dielectric Barrier Discharges (DBD). These reactive species show significance not only in the context of treating bacterial diseases but also in the field of chemical modification. This characteristic opens avenues for cold plasma treatments to selectively modify small biomolecules. Preliminary investigations have already shown that the treatment of glutathione (GSH) and glutathione disulphide (GSSG) with DBD leads to modifications on the sulfur moiety.[1] In the case of GSSG, the cleavage of the disulphur bridge has been identified under plasma conditions.[2] Consequently, the exploration of the potential applications of these properties in the domain of biomolecules becomes an intriguing challenge.

Small biomolecules, such as antimicrobial peptides (AMP), emerge as interesting candidates for synergistic interactions with cold plasma. Within this category, a subgroup of arginine (R) and tryptophan (W)-rich (RW)n peptides, known for their demonstrated antimicrobial properties and low cytotoxicity in studies, stands out. Although the precise mode of action still needs to be clarified, investigations suggests that the positively charged residue of arginine interacts with the negatively charged bacterial membrane through electrostatic attraction, enhancing membrane permeability. Conversely, the tryptophan residue engages with the lipid bilayer via hydrophobic interactions. The combined effects lead to the destabilization of the cell membrane, pore formation, and eventual bacterial cell death.[3]

Therefore, we designed a peptide containing (RW)3 which is prevented from interacting with the bacterial cell by a coupled antagonist with opposite charge. The 3,3-dithio-dipropionic acid linker is used to connect the two sequences as the crucial breaking point, designed to facilitate the release of the active (RW)3 peptide upon exposure to cold atmospheric plasma treatment. With this approach, we aim to take a significant step towards the manipulation of biomolecules by employing cold plasma, with the objective of attaining an elevated selectivity factor along with enhanced cytotoxicity. For this purpose, detailed investigations were carried out on the oxidation behaviour of the peptides as well as treatments of the individual amino acids. In addition, the behaviour of the target peptides treated by plasma on *B. subtilis* was analyzed and discussed.

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Reduction in bacterial survival on the surface of F9 filters after being exposed to cold atmospheric plasma treatment

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The significance of air purification technologies in ensuring a safe and clean ventilation system has been highlighted by the recent Covid-19 outbreak. Airborne illnesses, stemming from a range of pathogens like viruses, bacteria, and fungi, underscore the need for indoor air purification methods to curb the transmission of these infectious airborne agents. One promising technology for disinfection and sterilization, is the use of cold atmospheric plasma (CAP). This innovative technology has proven to successfully eradicate various microorganisms including bacteria, viruses, fungi, and algae [1].
In this study, we aimed to prove the disinfection efficacy of a CAP dielectric barrier discharge (DBD) source on artificially contaminated F9 air filter materials installed down-stream the DBD module. To replicate conditions for extended use in an indoor air purification setting, where bacterial aerosols gather on particulate matter (PM) filters, the filter surfaces were contaminated with Escherichia coli (E. coli) and subjected to an air flow containing reactive species. The experimental assessment of our proposed DBD disinfection system included evaluating the electrical power consumption, key plasma characteristics, volume flow and air flow velocity, concentrations of reactive gas species, and rates of E. coli inactivation.
Our findings indicated that the viability of bacteria significantly decreased as the exposure to DBD plasma increased. We observed that 91% of bacteria were eliminated after 5 minutes of DBD exposure, 98% after 10 minutes, and 99.95% after 15 minutes. The impressive effectiveness of the DBD plasma air system in combating E. coli survival highlights its promising application in air filter disinfection technology. However, additional research studies involving the contamination of F9 filters with different bacterial strains, spores, and microorganisms are essential to fully assess the potential of the DBD air purification system.

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Comparative Study on Bacterial Inactivation using O_2 and N_2-added He/Ar Plasma

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Plasma, the fourth state of matter, offers a promising avenue for bacterial inactivation through the generation of reactive species. Dielectric Barrier Discharge (DBD) Plasma, characterized by non-thermal, non-equilibrium conditions, produces Reactive Oxygen and Nitrogen Species (RONS) upon the excitation of gas molecules. Specifically, the Reactive Atomic Oxygen (RAO) produced within DBD plasma can react with water, yielding Hydrogen Peroxide (H_2O_2), which, when applied to bodily tissues, allows for the formation of growth factors, thereby lowering activation energies for chemical reactions. In this comparative study, we investigate the efficacy of Helium DBD plasma and Argon DBD Plasma, both with Nitrogen and Oxygen addition (0.1% by volume, each) on a diverse panel of bacterial strains. Before analyzing the results on bacteria, emission spectroscopy will be conducted on the plasma to understand the effects of Nitrogen addition and Oxygen addition on Argon plasma and Helium Plasma. The selected strains encompass various characteristics, including cell wall structure, metabolic pathways, and environmental adaptations. These strains include Staphylococcus Epidermidis and Micrococcus Luteus, common skin commensals and soil inhabitants, respectively, Neisseria Subflava and Providencia Alcalifaciens, part of normal human flora and soil microorganisms, respectively, as well as Aeromonas Sobria, Pseudomonas Aeruginosa, and Clostridium Sporogenes, known for causing opportunistic infections, Deinococcus Radiodurans (Enterobacter), and Mycobacterium Rhodochrous, characterized by extreme radiation resistance and environmental pollutant degradation abilities. Additionally, these results will be compared to previous experiments that have been done with the E. coli K-12 strain, to understand which properties of bacteria DBD plasma affects the most. The investigation aims to elucidate how DBD plasma treatment impacts bacterial inactivation across species with distinct properties. Understanding the differential responses of bacteria to plasma treatment holds significant implications for developing targeted and efficient antimicrobial strategies in diverse clinical and environmental settings. The presentation at ICPM will contain full results on all operating conditions and all bacterial strains that are employed in this research.

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The influence of non-thermal plasma on fungal growth on building materials

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Indoor mould exposure can cause allergic and respiratory diseases in humans [1]. The risk of mould growth is particularly high in buildings that are temporarily wet or have suffered water damage. It is important to at least slow the growth of fungi before the affected area dries out. The treatment of building materials to prevent mould growth has been a topic of discussion for several decades [2] and the development of non-thermal plasma (NTP) applications could potentially contribute to solving this problem.
The research aimed to evaluate the impact of NTP application on fungi growth on fibreboard and plasterboard. Building materials of 5×5 cm were inoculated by a fungal inoculum containing approximately 40 spores, including *Aspergillus brasiliensis*, *Cladosporium* sp., and *Fusarium* sp. primarily isolated from building materials. The study exposed selected samples to NTP using a direct bipolar corona discharge generated in a point-to-ring electrode system at atmospheric pressure and ambient temperature and after incubation, the growth and sporulation were evaluated by image analysis as relative surface coverage. As the results, the NTP application under certain conditions can effectively reduce the growth of micromycetes (Fig.1). This method can prevent mould growth in building materials during periods of increased moisture, particularly in cases of *Fusarium* infestation.

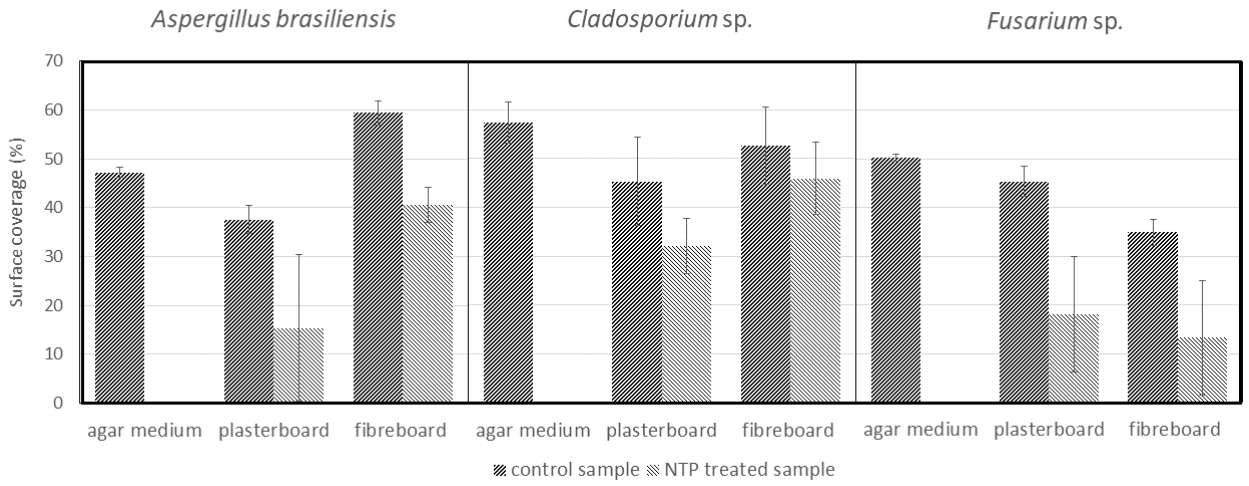

Fig. 1 Growth of *A. brasiliensis*, *Cladosporium*, and *Fusarium* on agar medium, plasterboard, and fibreboard, control and NTP treated samples

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Evaluation of Resistance Development in Bacteria Against Cold Atmospheric Plasma in Consequence of Repetitive Plasma Treatment Methods

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Bacterial resistance to antibiotics has long been known, and bacteria have been shown to develop resistance to other antimicrobial agents. Repeated and prolonged exposure to antibiotics and antimicrobial agents plays a role in the development of bacterial resistance [1]. There is insufficient information in the literature on the possible development of bacterial resistance to Cold Atmospheric Plasma (CAP) technology, which is increasingly used in biomedicine and has a good antimicrobial effect [2]. In the limited number of publications on the possible development of resistance in bacteria, it has been reported that plasma treatment does not cause bacteria to develop resistance to CAP after a few (4 and 6) repetitions of plasma treatment, but it has been reported that the development of resistance in bacteria after more plasma treatments should be investigated [3, 4]. The aim of this study is to investigate the possible development of resistance to CAP in bacterial generations obtained after repeated direct plasma treatment of *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus).* Having determined the lethal and non-lethal doses of CAP, the zone of inhibition (ZOI) values obtained in each generation as a result of multiple repeated non-lethal plasma treatments on two different bacterial strains were recorded. So far, repeated CAP treatments are underway. Upon the completion of the consecutive and repeated treatments, every fifth generation will be picked and will be compared by means of growth kinetics and responses to CAP treatments. This study will provide a better understanding of the possible development of resistance in organisms which have been exposed to a certain amount of plasma treatment after each session in multi-session applications of CAP devices for the treatment of infected wounds.

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Plasma-activated liquids reduce the viability of biofilms associated with endodontic infections

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Enterococcus faecalis and *Candida albicans* have been frequently associated to failures in endodontic treatment. Both species have the ability to form biofilms in the root canal and may persist even after instrumentation and irrigation with conventional solutions. This study aimed to evaluate the effect of plasma-activated distilled water and physiologic solution on *Enterococcus faecalis* and *Candida albicans* dual species biofilms. Also, the cytotoxicity of activated liquids against Vero cells was evaluated. Mono and dual species biofilms of *Candida albicans* (ATCC 18804) and *Enterococcus faecalis* (ATCC 29212) were formed (24 hours 37°C, aerobic conditions). Distilled water and physiologic solution (NaCl 0,9%) were activated by gliding arc plasma jet generated from compressed air (flow rate 1.5 SLM) using a kHz power supply (signal frequency 20 kHz, mean power 10 W) for 30 minutes. Biofilms were exposed to the activated liquid for 1 minute and 1 minute and 30 seconds. Subsequently, the microbial suspensions were serially diluted and plated on Sabouraud dextrose agar with chloramphenicol and M-Enterococcus agar. Negative control groups exposed to non-activated liquids were included. After 24 hours, the number of remaining viable cells was determined. The experiments were performed in triplicate at three different times (n=9). Cell counts were compared among the groups by ANOVA and Tukey's post hoc test, with a level of significance of 5%. A significant reduction ($p<0.05$) of viable cells in *C. albicans* and *E. faecalis* monospecies biofilms was observed after exposure to the activated liquids for 1 min. For the dual species biofilms, significant reduction was detected after 1 minute and 30 seconds ($p<0.05$). The cell viabilities after exposure to the activated liquids were above 70%. Exposure to cold plasma activated liquids for 1 minute and 30 seconds significantly reduced the viability dual species biofilms formed by *E. faecalis* and *C. albicans*, with low toxicity for Vero cells.

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Helium Cold Atmospheric Plasma Effectively Acts on Multispecies Cariogenic Biofilms Formed *in situ*

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Low-temperature plasma jet shows an inhibitory effect on cariogenic biofilms formed *in vitro* [1]. However, its effect on biofilms formed *in situ* is still unknown. The present study aimed to evaluate the effect of helium plasma jet on biofilms with increasing degrees of cariogenicity formed in situ. For this purpose, nine standardized bovine dentin specimens were fixed to palatal devices and received different daily supplementations with sucrose 20% for 48 hours. The protocol of the study was approved by the Institutional Ethics Committee and all the volunteers signed an informed consent form. Three groups of 3 volunteers were established: group 1, biofilms were not exposed to sucrose solution; group 2, biofilms were exposed to sucrose solution 4 times a day; and group 3, biofilms were exposed to sucrose solution 8 times a day. After 48 hours, the biofilms (n=9/group) were treated with a helium plasma jet for 7 minutes or 0.12% chlorhexidine digluconate (positive control). Non-treated controls were included. The plasma jet was generated using a previously reported experimental set up [2]. Determination of the number of viable cells, scanning electron micrographs, and fluorescence microscopy were carried out. The results of colony-forming units were compared among the groups by ANOVA and Tukey's post-hoc test, with a level of significance of 5%. A reduction greater than 2.0 log10 CFU/mL was detected, both for total microorganisms and mutans streptococci after treatment with helium plasma jet ($p<0.0001$) in all groups analyzed, independently of the degree of cariogenicity. In conclusion, helium plasma jet showed a significant inhibitory effect against cariogenic biofilms formed in situ.

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The Effect of Bisphenol a Degraded by Non-Thermal Plasma on Yeast

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Bisphenol A (BPA) is a toxic chemical compound used as a monomer in the production of plastics, especially polycarbonates and epoxy resins, which are traditionally not recycled and go to landfills [1]. BPA can leak from the materials to the environment including beverages, food etc. Bioactive levels of BPA were detected in water, soil, air and living organisms [2-5]. It is a known endocrine disruptor linked to numerous diseases in humans, including cancer, in which it can induce resistance to chemotherapeutics [6]. Slowly, BPA is being banned by regulatory agencies to produce certain items, but its production is still predicted to grow in the near future [1]. Recent studies show it is possible to degrade BPA by non-thermal plasma, which could be potentially applied in wastewater treatment plants [7-8].

We investigated the BPA degradation by plasma and the impact of the plasma-treated BPA solutions on the model organism *Saccharomyces cerevisiae* to verify whether the produced intermediates and long-lived reactive oxygen and nitrogen species are not more harmful to the cells than BPA itself. The effects of two non-thermal plasma sources, transient spark and dielectric barrier discharge, were analysed. We measured cell survival and intracellular oxidation by flow cytometry. Preliminary results indicate that immediately after the degradation of BPA, the cytotoxicity of the plasma-treated solutions is higher compared to the positive control, represented by untreated BPA solution. In the following weeks post plasma treatment, however, the tested parameters improve and can exceed the values of untreated BPA. The BPA content in the plasma-treated and untreated solutions was measured by HPLC (High-Performance Liquid Chromatography).

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Combined Antimicrobial Properties of Ultraviolet radiation and Plasma- Activated Water

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Non-equilibrium plasma discharges at atmospheric pressure are used in microelectronics, polymer deposition, light sources, medicinal applications, and environmental remediation [1–3]. Biomedical uses of cold atmospheric plasma produced in ambient air are emerging due to their unique features. Both long-lived and short-lived reactive oxygen and nitrogen species (RONS) are produced by non- equilibrium plasma in the air atmosphere. These long-lived RONS are transported into plasma- activated water (PAW) solutions, which are produced when atmospheric plasmas and water come into contact. PAW solutions are efficient for both killing microorganisms and inactivating cancer cells.

On the other hand, UV radiation (UVA 315-400 nm, UVB 280-315 nm, UVC 120-280 nm) is commonly employed for water and air purification, medical sterilization, food and beverage sector, and analytical methods [4–6].

The goal of this research is to better understand how cold air plasma interacts with bacteria by examining the impact of UVA radiation as a supporting factor on the production of specific RONS in gas and water, as well as the subsequent effects on bacteria viability. The investigation specifically examined RONS produced by transient spark (TS) discharge plasma and UV radiation in both the gaseous and liquid phases upon the interaction between plasma and water. The concentrations of the gaseous plasma species, namely H_2O_2, O_3, and $(H)NO_x$ (HNO_2, NO_2, and NO), as well as their dissolution in water as $H_2O_2(aq)$, $O_3(aq)$, nitrites $NO_2^-(aq)$, nitrates $NO_3^-(aq)$ are measured. Figure 1 shows the schematic diagram of our UV/ plasma setup. Different modes of treatment are examined including plasma alone, UV alone, plasma and UV, plasma followed by UV, and reverse.

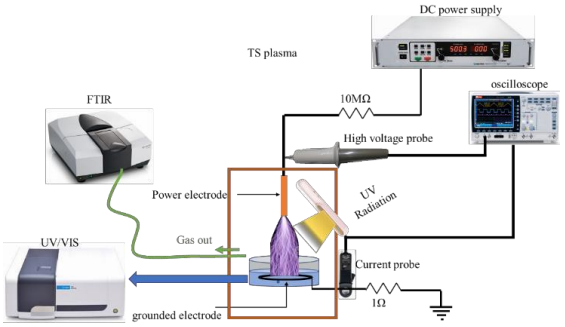

Fig. 1 schematic of UV/plasma setup

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Synergistic Enhancement of Antibiotic Efficacy Against Biofilms with Cold Plasma

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The escalation of antibiotic-resistant bacterial infections poses a severe risk to public health. Non-thermal cold atmospheric plasma presents itself as a promising adjuvant therapy, particularly against ESKAPE pathogens and biofilm-associated infections, such as those caused by methicillin-resistant *Staphylococcus aureus* (MRSA). This study explores the synergistic potential of cold plasma in combination with antibiotics, offering a novel approach to address microbial resistance.

A sub-lethal plasma regimen was applied to bacterial biofilms prior to the introduction of antibiotics, examining the combined effects through measurements of minimum inhibitory concentrations (MICs), minimum biofilm eradication concentrations (MBECs), and isothermal microcalorimetry. The study also utilized bioinformatic techniques to analyze the oxidative impacts on bacterial cell structures, gene expression changes, and the corresponding stress response.

Initial findings indicate that cold plasma pre-treatment notably increases the efficacy of antibiotic treatments, reducing MICs and MBECs significantly. The enhanced disruption of metabolic activity, implies that combined cold plasma and antibiotic therapy induces a distinctive response in biofilms, compared to either treatment alone. Gene expression analysis supports the hypothesis that plasma exposure induces an oxidative stress response, potentially disrupting outer membrane integrity and facilitating increased drug uptake.

The data supports the premise that cold plasma can act as a potent adjuvant to antibiotics, impeding biofilm resistance mechanisms and advancing the permeability and effectiveness of drug therapies. These promising results advocate for the inclusion of plasma in treatment protocols, potentially transforming the clinical management of stubborn, antibiotic-resistant infections. Moreover, the study underscores the necessity of understanding device-specific plasma interactions to fine-tune this intervention, optimizing its application against resilient biofilm-related infections.

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Role of long living species in the inactivation of bacteria: comparison of different plasma sources

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With the rapid growth of plasma medicine in recent years, many cold atmospheric plasma sources have been designed by different research groups with specific biological aims. Various layouts have been explored, involving different operating gases and power supply characteristics. The lack of a common testing protocol, however, prevents a proper comparison among the available technologies and the identification of the most efficient ones.

When directly treating a biological substrate, reactive oxygen and nitrogen species are often highlighted as having a key role in the plasma-tissue interaction. The efficiency in the production of species and the balance between them are peculiar characteristics of each source, but these properties are also affected by the treated substrate and the treatment protocols. Moreover, when during the treatment the substrate is faced to the plasma, short living species are also interacting with bacteria, as well as electric fields and radiation. This complexity obscures the role of individual actors involved in the interaction, and the full map of the biochemical pathways triggered.

In this work, multiple cold atmospheric pressure plasma jets are tested as bactericidal devices on a common setup. Helium, argon, and air are used as working gas, and the discharge is ignited with waveforms spanning from radiofrequency to micropulses. In the biological treatment 10^8 CFU/ml of *E. coli* are suspended in 6 ml of ultrapure water and directly exposed to the plasma for 10 minutes. The biological effects are estimated in terms of bacteria abatement, performing CFU counting after cultivating the treated samples. Spectrophotochemical diagnostic techniques are used, mimicking the treatment conditions, to measure the concentration of the long living species, while spectroscopical apparatus are exploited for quantifying the short living species and the fast chemistry. Comparing the direct treatment with indirect ones, finally, the time scales are decoupled.

The obtained results suggest that optimal *E. coli* abatement is not contingent on a specific compound, but rather, a well-defined balance between the involved actors; moreover, long living species appear to have a leading role in the interaction. This study contributes to the understanding at a fundamental level of the plasma-cell interaction and suggests directions for an efficient improvement of the source and an aware choice between available plasma generation technologies.

Antimicrobial Activity of SDBD Plasma Treatment on Planktonic and Biofilm Forms of *Escherichia coli*

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By using a water solution as a discharge electrode, it is possible to combine the basic features of both water discharges and surface dielectric barrier discharges (SDBD) [1]. The benefit of such a combination lies in obtaining highly oxidative plasma in contact with liquid without problems of electrode erosion. The SDBD is generated from the contact line between the liquid electrode, air, and dielectric material (tube). Such combined systems were used for plasma activations of hollow object surfaces [2], which performed splendidly in achieving uniform plasma treatment of the inner surface of fine tubes.

This study investigated several approaches to plasma interaction with bacteria. The first scenario is the effect of plasma on the liquid contaminated with planktonic bacteria *E. coli* (CCM 3954). Various power inputs and treatment times were investigated. The second approach is to determine the effect of plasma treatment on *E. coli* 48h biofilms deposited on the inner surface of polymeric tubes. In the third approach, the plasma pre-treatment of polytetrafluoroethylene (PTFE) tubes before biofilm formation was performed (Figure 1) to investigate its ability to change the microbial surface adhesion and therefore influence biofilm maturation by changing the surface properties of the PTFE tubes.

Our results show that plasma treatment reduces microbial water contamination, impedes the biofilm formation process, and decreases the biomass of the mature biofilm.

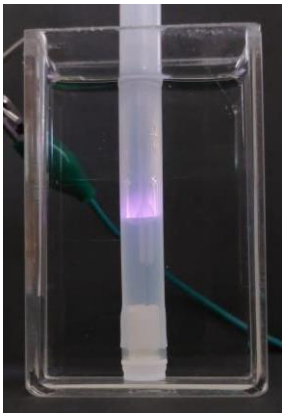

Fig. 1. A photo of the PTFE tube during plasma pre-treatment.

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Bacterial decontamination by a He plasma jet operating in different concentrations of O2

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Cold atmospheric plasma jets (CAPJ) have been widely studied as an alternative to microbial decontamination. It can prevent microbial colonization by bacteria and fungi in medicine and the food industry. Reactive oxygen and nitrogen species (RONS) and UV irradiation are described as the main factors to the CAPJ antimicrobial activity [1]. However, the parameters that generate the plasma (e.g., electrical properties, gas composition, reactor type) can influence the CAPJ properties and performance. In this study, we tested *in vitro* the influence of a mixture of He and O2 to generate the CAPJ to inhibit *Escherichia coli*. The device used to generate CAPJ consists of a DBD reactor connected to a portable power supply [2]. The first discharge is generated in a chamber and transferred through a long, flexible capillary (inner diameter of 2.0 mm) with a wire electrode at a floating potential. The plasma is reignited a few mm before the latter capillary outlet. This device was designed to be a portable and safe alternative in biomedical applications. The inhibition halo test was performed using *E. coli* strain (CIP54117). The bacterial suspension at 10^7 CFU/mL were plated on Luria-Bertani agar. The plates were treated with CAPJ using two different device configurations for 1 and 5 min. The O2 concentrations studied were 0, 0.1 and 0.16%. Figure 1 presents the inhibition halo area resulting from the treatment. The treatments performed for 5 min showed a higher inhibition area than the 1 min treatment. The addition of oxygen also increased the inhibition area after the 5-minute treatment. The 5-minute treatment had an inhibition area of 0.56, 0.91, and 2.03 cm^2 for helium plasma and the addition of 0.1% O2 and 0.16% O2, respectively. To the highest concentration of O2 and treatment time, a few colonies remained. The same was not observed in the other groups.

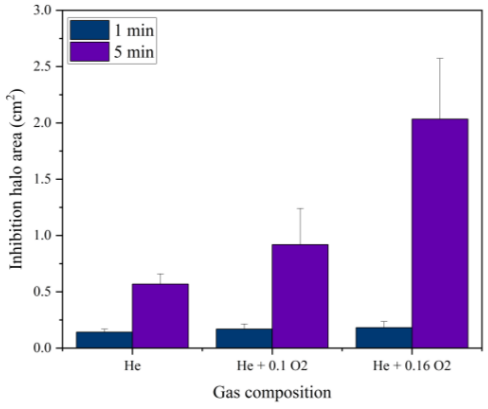

Fig. 1 Inhibition halo area after 1- and 5-min plasma treatment with different gas compositions.

This work was supported by São Paulo Research Foundation (FAPESP) grant no. 2023/02436-2, 2021/02680-5 and 2019/05856-7 and National Council for Scientific and Technological Development (CNPq) no. 309762/2021-9.

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Electrical Characterization and Efficacy Assessment of a Cold Atmospheric Plasma System for Closed Environment Sanitization

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The COVID-19 pandemic has highlighted the critical role of indoor air quality control and purification. To address this challenge, Cold Atmospheric plasmas (CAPs) based devices have emerged as a potential solution for mitigating aerosol transport and reducing the infectivity of airborne pathogens [1]. At the Plasma Technology Laboratory (PLT) at the University of Bologna, our research group has been investigating and utilizing a newly developed CAP source, based on a Dielectric Barrier Discharge (DBD), as an air sanitizer for enclosed environments.

The ongoing project aims to optimize the above-mentioned small-scale CAP device to reach sterilization by minimizing power consumption and the production of undesirable byproducts, such as ozone and NOx. The configuration of the prototype utilizes grid-like coated electrodes (with Rilsan® ES), powered by a 4 µs square bidirectional pulse voltage waveform with a peak voltage of 1.2 kV [2]. We have electrically characterized the discharge and estimate that the power involved is in the order of a few Watts. Additionally, we have integrated a diagnostic system to measure the ozone concentration, which can be leveraged for effective abatement processes.

To improve the chemical characterization, we used FTIR analysis at the SPC - Swiss Plasma Center - in Lausanne, supported by the COST STSM grant. Currently, at SPC, we are assessing the efficacy of these devices in reducing the microbial load by testing non-pathogenic Escherichia Coli (E. Coli). We are generating a bioaerosol, by contaminating an air stream with E. Coli, and pumping it through an enclosed tubes system within the CAP DBD reactor prototype, Fig 1.

The preliminary findings motivate further investigations to unravel the underlying mechanisms and establish a comprehensive understanding of the relationship between electrical/chemical parameters and the sterilization process.

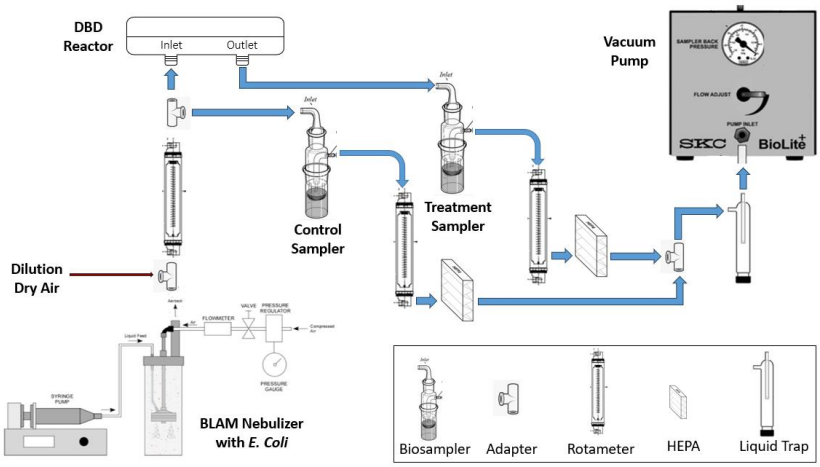

Fig. 1 - Bioaerosol Generation and Sterilization Setup

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Cold Plasma: A promising Approach to Mitigate Common Ragweed Pollen Allergens

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Allergic diseases are among the most common immune system disorders, with recent studies indicating a rise in sensitization and related conditions like allergic rhinitis and atopic asthma, affecting up to 40% of the population in developed nations [1]. Airborne allergens, particularly inhaled aeroallergens derived from various pollen types, represent one of the primary causes for the development of respiratory allergies [2]. Common ragweed (*Ambrosia artemisiifolia*), notorious for its highly allergenic nature and invasive spread, poses a significant challenge in Europe, with each plant producing over one billion pollen grains [3]. Given that the situation will worsen due to the global climate change [4], there is an urgent need for a radical new approach to fight this problem. Herein, cold atmospheric pressure plasma (CAP) was employed for the treatment of common ragweed pollen. Both indirect and direct plasma systems operating in air were applied to achieve optimal reduction of Amb a 1, the most important ragweed pollen allergen. The protein composition of CAP-treated common ragweed pollen was analysed using various techniques such as Bradford assay, enzyme-linked immunosorbent assay (ELISA), sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), and immunoblotting. Furthermore, the impact of plasma on the chemical and morphological surface characteristics of pollen grains was assessed through Fourier- transform infrared spectroscopy (FTIR), Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), and scanning electron microscopy (SEM).

CAP treatments led to the significant chemical and structural modifications of Amb a 1 and other common ragweed surface proteins, primarily through oxidation-induced chain cleavage, resulting in a marked reduction in antigenicity. Additionally, CAP treatment was shown to influence various other essential compounds present on the pollen surface, such as lipids, polysaccharides and sporopollenin. Furthermore, CAP treatment influenced the composition of other essential compounds on the pollen surface, such as lipids, polysaccharides, and sporopollenin. These findings highlight the potential of CAP technology as an innovative solution for air purification, addressing concerns related to airborne allergens and associated health issues. While our results are promising, further research is necessary to expand upon these findings, including immunological assessments of the allergenicity of CAP- treated allergens.

The research presented in this work received support from grants Z3-3210 (awarded to N.H.) and P1- 0207 (awarded to I.K.) from the Slovenian Research and Innovation Agency (ARIS), as well as grants EP/R041849/1, EP/N021347/1, and EP/S025790/1 from the UK Engineering and Physical Science Research Council (EPSRC; awarded to J.W.).

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Toxicological characterisation of BPA and BPS and their transformation products formed after degradation with cold atmospheric pressure plasma

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Bisphenols (BPs) are a group of synthetic chemicals used in the production of polycarbonate plastics, epoxy resins, and are widely used in the manufacture of numerous consumer good. Among them, BPA poses a significant risk to public health and the environmental organisms due to its known endocrine disrupting and genotoxic activities. Bisphenol S (BPS) has been used as an alternative to BPA, however, literature data on its harmful effects similar to those of BPA are accumulating. This study investigated the application of Cold Atmospheric Pressure Plasma (CAP) as a novel method for the degradation of BPA and BPS in wastewaters and the toxicity of the transformation products (TPs) formed during the degradation process. The toxicity of parent compounds (BPA and BPS) and CAP treated samples for 30, 120/240 and 480 s was evaluated in human hepatocellular carcinoma (HepG2) cell line after 24-hour treatment of cells by assessing the influence on cell viability (MTS assay), DNA damage (comet assay) and expression of selected genes (transcriptomics; Fluidigm). The results showed no effect of the studied BPs or their TPs on cell viability, except for BPA TPs formed after 480 s, where a 30% time-dependent increase in the activation of mitochondrial dehydrogenase enzymes was determined. Additionally, the study revealed that both BPA and BPS significantly increased DNA damage compared to the control, with BPA being more potent. BPA TPs showed reduced DNA damage over time, however, they still caused significant formation of DNA strand breaks after 480 s. In contrast, DNA damage related to BPS TPs was only observed after 30 s of exposure. Moreover, the genotoxic effects of BPA, BPS, and their TPs formed after 480 s of CAP exposure were further examined by analysing their influence on the expression of selected genes involved in the metabolism (phases I and II) and response to DNA damage. The results showed that BPA (0 s; no CAP treatment) up-regulated the expression of AHR, CYP1A1, CYP1B1 and UGT1A1 genes, while PXR was down-regulated. Also, BPA TPs up-regulated AHR, PXR, CAR, CYP1A1, CYP1B1 and UGT1A1, with most genes being more highly expressed than with BPA. In the case of BPS (0 s), CYP1A1, CYP1B1 and UGT1A1 were up-regulated, while AHR and PXR were down-regulated. Similarly, the expression of AHR, CAR, CYP1A1, CYP1B1 and UGT1A1 was up- regulated to a greater extent after exposure to BPS TPs than BPS alone. Furthermore, BPA, BPS, and their TPs down-regulated the expression of genes involved in DNA damage response, with only a few exceptions. BPA down-regulated the expression of TP53, MDM2, CDKN1α, and CHEK2 genes. When exposed to BPA TPs slightly more changes in gene deregulation were observed, with MDM2 and CHEK2 genes being down-regulated, while CDKN1α was up-regulated. In the case of BPS, the MDM2, CDKN1A, and CHEK2 genes were down-regulated. Additionally, BPS and its TPs down- regulated MDM2, CDKN1α, and CHEK2 to a similar extent, while TP53 was up-regulated. Toxicological characterization has demonstrated that the bisphenol TPs formed during CAP causes less adverse effects than their parent compounds BPA and BPS, indicating that CAP is an effective water treatment technology. By investigating both the degradation efficiency of CAP and the safety of formed TPs, this study aims to advance our knowledge on using CAP for environmental remediation, potentially offering safer and more sustainable solutions for reducing bisphenol pollution in wastewaters.

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The Slovenian Research Agency (ARIS): P1- 0143, P1-0417 and P1-0245; N1-0143, J7-3155, J2- 4427, L7-4422, J2-4451, J4-1770, Z1-3191 and Hellenic Foundation for Research and Innovation (HFRI) under the 4th Call for HFRI PhD Fellowships (Fellowship 11177, Project 75686) and from the UK Engineering and Physical Sciences Research Council (ESPRC): Grant Numbers EP/R041849/1, EP/NO21347/1, and EP/J005

Cold plasma for the treatment of house dust mite allergens

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House dust mites (HDM) are recognised as one of the primary sources of indoor aeroallergens around the world. Linked to the allergic diseases since 1920, they can lead to the health problems such as allergic rhinitis and conjunctivitis, atopic eczema and atopic asthma. It is estimated that regardless of geographical variations in temperature and humidity, up to 85% of patients suffering from asthma in Europe, North and South America, south-east Asia and Australia are allergic to HDM. WHO directives for mitigating such types of allergies include allergen avoidance, pharmacotherapy, allergen immunotherapy and patient education [1]. However, there is a need for new methods to improve indoor air quality as well.
Cold atmospheric pressure plasma (CAP) holds promise for decontaminating airborne pollutants. Essentially, plasma arises from the ionization, dissociation, and excitation of gaseous molecules. CAP generated from air is recognized as a highly non-equilibrium system, with electrons attaining temperatures up to 30,000 K while neutral species remain near room temperature [2]. In presented study, a surface barrier discharge system was employed, which is known to generate high concentrations of reactive oxygen and nitrogen species (RONS), crucial for CAP's decontamination efficacy [3]. Extracts from *Dermatophagoides pteronyssinus* were exposed to different CAP gaseous chemistries to determine optimal conditions for reducing its primary allergen, Der p 1. Following the treatments, the allergen content of *D. pteronyssinus* extracts was analysed using techniques such as Bradford assay, enzyme-linked immunosorbent assay (ELISA), sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), immunoblotting, mass spectrometry and Fourier-transform infrared spectroscopy (FTIR). Der p 1, known as a cysteine protease, was also tested for its enzymatic activity.
Results showed that CAP significantly reduced Der p 1 levels. The extent of reduction was contingent upon both the discharge power and the duration of exposure. These findings underscore the potential of CAP technology as an innovative solution for air purification, addressing concerns regarding different airborne allergens and associated health issues. The effectiveness of the method will be further evaluated through immunological tests involving allergic patients.

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Mitigating Bacteria from Hospital Floors: Plasma Approach

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Surface sanitation is important in medical settings to avoid contamination and infection. The goal of this research project is to investigate plasma techniques to mitigate bacteria on the floor tiles which are traditionally present in a hospital environment. In order to achieve this, a Dielectric Barrier Discharge (DBD) plasma torch was designed along with a robotic hoover that held the plasma torch, with the ability to scan floor tiles in an automated fashion. The plasma hoover can travel at a speed of 2-3 mph and can hold a load of 80 pounds including the gas cylinder, power supplies, motors, and all other necessary onboard components. Operating software was developed to allow for a preset path to be run, as well as for the robot to be remotely controlled using a PS4 controller. Rubber and vinyl tiles were used to simulate hospital flooring and were cut into 2 inches by 2-inch pieces to experiment on. To transfer the bacteria onto the tiles an imprinting method was used, in which prepared tiles were pressed onto a growth medium of LB gel in a petri dish, allowing the bacteria to imprint on it. Tiles were prepared by being put through various plasma tests. Each tile of interest was allowed to run under the operating plasma hoover, and the trial runs were conducted at various operating speeds. The plasma torch used to generate the plasma sheet to scan the bacteria over the floor tiles had dimensions of 25mm length, 50 mm width, and 2mm thickness. The trial runs were conducted at various speeds and at various plasma operating conditions, with the ranges being as follows: 10-15 standard litres per minute [slpm] Helium/Argon, 8-13 kV, 20-40 kHz. We also introduced the shielding gases oxygen and nitrogen to observe their effects on increasing plasma plume stability and reducing bacterial presence. Preliminary results, shown in figure 1, indicate a significant reduction in bacterial colonies that is strictly a function of plasma exposure time to the targeted area on the floor, with an even more dramatic reduction in bacterial colonies present when shielding gases are present. Our presentation will include the details of our experimental setup and results on this bacterial mitigation as a result of DBD cold plasma exposure.

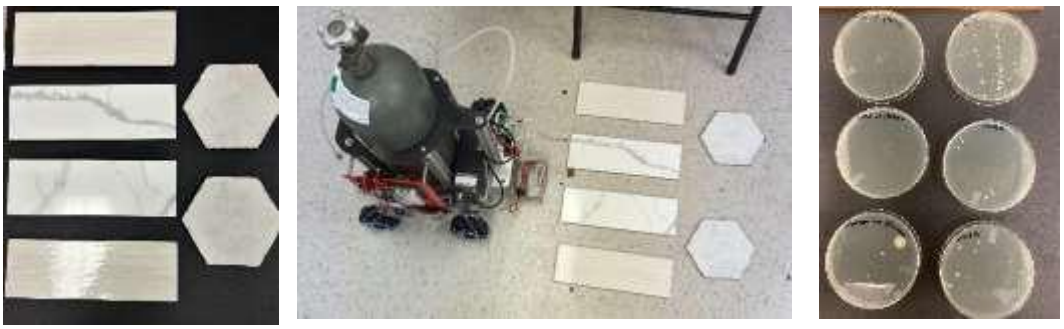

Fig1. Floor tiles, Plasma Hoover, and Bacteria colonies with and without Plasma Exposure

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Non-thermal Plasma-Activated Water Against *Candida auris* Isolates from Healthcare-Associated Infections

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Candida auris is an enigmatic yeast that continues to stimulate interest within the mycology community due to its severe infections with high mortality rates under intensive care settings. The transmission of *Candida auris* takes place in nosocomial settings, even in those implementing infection prevention and control measures. Thus, appropriate infection control measures play a major role in controlling the spread and multiplication of this pathogen. Unfortunately, there are very few data available on the effectiveness of disinfectants against resistant isolates of *C. auris*. Thus, searching for new effective fungicidal agents is still a hot topic nowadays. Non-thermal plasma- activated water (PAW) has recently emerged as a powerful antimicrobial agent, but no data about its effectiveness on resistant *C. auris* clinical isolates are available. The purpose of our study was to assess the possibility of using PAW as a fungicidal agent against resistant clinical isolates of *C. auris*. PAW was prepared using distilled water and a GlidArc reactor as previously described [1]. The final parameters of PAW were as follows: conductivity 446 ± 25 µS/cm, pH 2.78 ± 0.12, redox potential (ORP) + 1.06 V, NO_2 192 ± 10 mg/L, NO_3 1550 ± 95 mg/L, H_2O_2 2.6 ± 0.12 mg/L, and O_3 1.08 ± 0.07 mg/L.

Ten resistant clinical isolates from different hospital regions in Romania were used in this study. Suspensions of yeast cells (10^7 CFU/mL) were prepared from overnight cultures and subsequently treated with PAW in a ratio of 1:10 for different periods of time (1, 3, 5, 7, 10, 15, 20 and 30 minutes). Precise volumes of the mixtures were further inoculated on Yeast Extract Peptone Dextrose Agar plates and BacT/ALERT FA aerobic media culture system (bioMerieux, France), in order to evaluate the reduction of yeast burden after each contact period and time to detection (TTD) of *C. auris*. In addition, some instrumental analysis (IA) methods were used in order to assess the impact of PAW treatment on resistant *C. auris* cell structure: Electron paramagnetic resonance (EPR) spectroscopy Scanning Electron Microscopy (SEM), Energy-dispersive X-ray (EDX) analysis, Fourier Transform Infrared Spectrometry (FT-IR) and Dynamic Light Scattering (DLS).

A greater than 5 $\log_{10}$ reduction in viable yeast was achieved in 3 minutes. Sterilization level (i.e., > 6 $\log_{10}$ reduction) was reached after 5 minutes for all tested strains. IA clearly objectified the morphological changes in the treated yeasts compared with the untreated ones. No significant differences were recorded between each resistant strain.

Our research has successfully demonstrated the fungicidal effect of PAW against resistant isolates of *C. auris*, opening a new field of research in the area of disinfectants against this remarkable pathogenic yeast.

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Effect of NTP exposure on the electronic components

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Non-thermal plasma (NTP) is a well-known decontamination tool applicable for a wide range of microorganisms and viruses. Given the increasing requirements for the disinfection of electronic devices, the question arises as to how much NTP can damage electronics, although it is generally considered to be a very gentle agens. Therefore, the effect of NTP generated by a corona discharge in a point-to-ring device in the air [1], generated by the Plasmatico v1.0 device, was examined.
Since NTP modifies the surface, it makes sense to examine primarily electronic components (connectors, potentiometers, humidity sensors, etc.) related to the properties of the surface. For certainty, common components such as resistors and capacitors, both in THT and SMD variations, were tested. It was shown that NTP exposure does not affect them.
An interesting effect of NTP exposure was recorded on the combined humidity and temperature sensor DHT 11. While there was no measurable influence on the temperature sensor, the humidity sensor experienced an increase in signal, especially at higher humidity values.
Moreover, the effect of NTP exposure on certain types of connectors of different materials was examined. . Surprisingly, surfaces containing tin resist well to NTP, while materials containing nickel show significant oxidation by NTP resulting to increase of contact resistance. This is confirmed by EDS measurements and SEM imaging – Fig. 1.

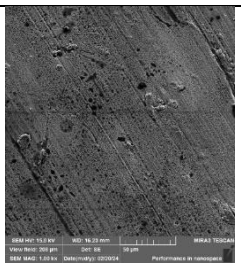
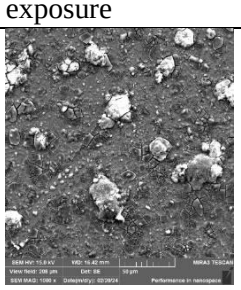
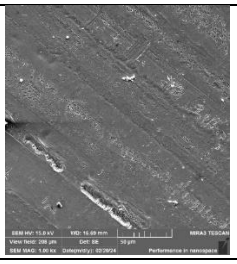
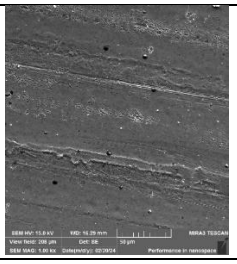
		Not exposed	After NTP exposure	EDS
header pins				Dominant - Ni Contains (Ni+Zr+Nb)
IC DIL socket				Variable ratio of Ni and SN contains: (Ni+Sn+Cu)

Fig. 1 - SEM image of samples before and after NTP exposure

This work was supported by FWF I 5293-B/GACR GF21-39019L grant.

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Establishing the role of the stringent response in non-thermal plasma treatment

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Biofilms are multicellular bacterial communities that interact and form an extracellular polymeric matrix. This extracellular matrix aids in bacterial protection against external environmental stressors produced by the host defense system, antibiotics, and antimicrobial agents, making them a major concern in chronic bacterial infections. Generally, bacterial stressors promote the generation of reactive oxygen species and subsequent microbial killing through oxidative stress mechanisms.

Within mature biofilms, nutrient and oxygen gradients are formed in relation to the core bacteria within the matrix. This nutrient deprivation activates a global stress signaling system called the stringent response forming bacterial persister cells. The stringent response is a highly conserved stress signaling pathway used to mitigate the effect of external stressors by shifting gene regulation from active division to stress survival. During starvation, the production of the alarmone (p)ppGpp by the RSH (RelA/SpoT Homologue) enzyme RelA enzyme upregulates gene transcripts associated with survival, converting bacterial cells into a metabolically quiescent state [1]. SpoT, a related regulatory enzyme, is responsible for the regulation and emergence back into a divisionally active state.

Non-thermal plasma (NTP) has been used previously for treating biofilms due to its ability to reach all parts of irregular surfaces and the reactive oxygen species-mediated degradation of the extracellular matrices [2]. While the mechanisms behind NTP's role in biofilm treatment and its effects on bacterial virulence factors are currently being studied, the connection between common bacterial stress response pathways and their role in NTP effectivity are still unknown.

This study investigates the bacterial stringent response's role in the treatment of biofilms with NTP. We use *E. coli* cf1648, and two derived mutants containing RelA and RelA/SpoT mutations. We use direct and indirect methods of NTP application to identify specific targets of NTP effectors. Understanding the role of these stress response proteins will aid in refining the application of NTP as an alternative for standard bacterial infection treatment. This knowledge will also elucidate potential combinational therapy to sensitize common stress resistance pathways for a more robust antimicrobial killing to agents such as NTP.

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Cold Atmospheric Plasma Jet with Pulsed Voltage and Gold Nanoparticles Enhance Cytotoxic Anticancer Effect

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Efficient and biologically safe mode of cold atmospheric plasma jet (CAPJ) is crucial for the development of CAPJ-based anticancer therapy. In the experiment and numerical simulations, by changing the pulse duration of a positive-pulsed (PP) voltage, we found the optimal helium CAPJ modes with a regular streamer propagation and a maximum safe discharge current at T<42°C [1,2]. These CAPJs substantially suppressed the viability of cancer cells. To enhance a cytotoxic effect of CAPJ treatment, gold nanoparticles (NPs) were added to the cells before and after the CAPJ exposure. Combination of CAPJ, generated with the PP voltage, and gold nanoparticles decreased the viability of NCI-H23 epithelial-like lung adenocarcinoma, A549 lung adenocarcinoma, BrCCh4e-134 breast adenocarcinoma and uMel1 uveal melanoma cells. Polyethylene glycol-modified nanoparticles with attached fluorescent labels were used to visualize the uptake of NPs. The treatment with optimal CAPJ modes in combination with modified NPs, bearing the cancer-addressed molecules and therapeutics may be the next strategy of strengthening the CAPJ-based antitumor approaches. We also studied the molecular basis for selectivity of the cytotoxic response of lung adenocarcinoma cells to CAPJ [3].

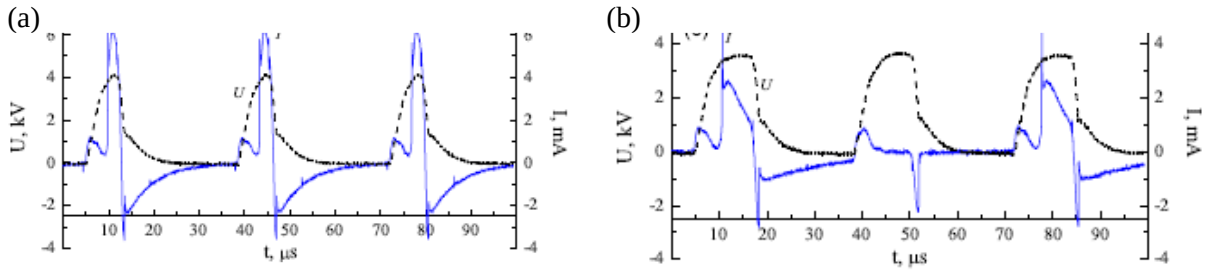

Fig 1. Voltage at U-electrode and current measured on mouse skin exposed to CAPJ with PP voltage with different τ, f =30 kHz, U = 3.8-4 kV, (a) τ = 7 μs, (b) τ = 14 μs.

For CAPJ with the PP voltage, the effect of the pulse length on streamer propagation is shown in Fig.1. The applied voltage and the current were measured on a shaved mouse skin during exposure to the PP voltage CAPJ. For a pulse length τ = 7 μs, the current I is registered in each voltage cycle and I=4.8 mA. For τ = 14 μs, the current frequency on the treated skin is a half of the voltage frequency $f_I = f/2$ and I=2.2mA (He flow, v=9 L/min, nozzle-surface gap, d=2.5 cm).

This work was supported by Russian Science Foundation Grant number 22-49-08003.

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Ferroptosis Drives Cell Death After Cold Atmospheric Plasma Treatment in Cervical Cancer Cells

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Physical plasma is known to selectively target and kill cancer cells and has shown promising effects in the treatment of several cancerous and precancerous entities [1]. Although apoptosis, necroptosis and cell cycle arrest are widely described mechanisms by which plasma mediates its efficacy, many questions remain. To advance and develop the already existing plasma treatments, it is crucial to understand the mechanism of action. Ferroptosis is a relatively new form of cell death, discovered in 2012 [3]. It is described as an iron dependent, programmed form of cell death, caused by extensive lipid peroxidation. Since physical plasma is a source of reactive species and inductor of oxidative stress, we hypothesized that ferroptosis could play an important role in plasma mediated cancer cell death.

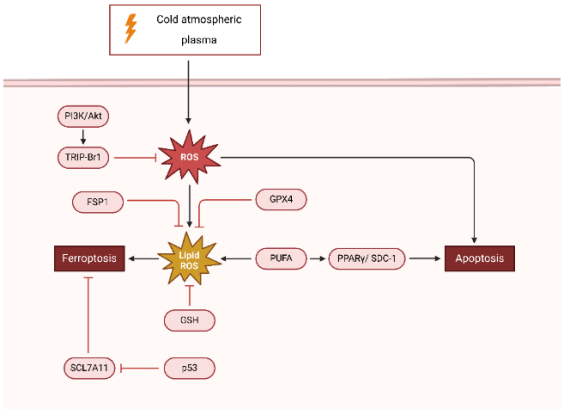

Fig. 1: Suggested ferroptosis mechanism following cold atmospheric plasma treatment. Created in BioRender, modulated from Li et al. 2020 [3]

We characterized ferroptosis related effects of plasma activated medium (PAM) on a cervical cancer cell line by affecting distinct parts of the ferroptosis signaling pathway. We modulated intracellular iron and glutathione levels before plasma application and observed the outcome by measuring cell viability as an end point. While an increase of intracellular iron and a reduction of glutathione levels increased plasma efficacy, the reduction of iron attenuated it. Additionally, we examined protein levels following plasma treatment and observed a degradation of ferroptosis suppressor protein (FSP1).
Cell death after plasma treatment is highly influenced by intracellular iron and glutathione levels. The reduction of FSP1 after treatment indicates that ferroptosis is a driver of plasma induced cancer cell death.

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Cold Plasma Deposition of Topotecan: A Novel Approach for Local Cancer Drug Delivery to Glioblastoma Cells

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Glioblastoma multiforme (GBM) is the most common, malignant and aggressive brain cancer. Despite many innovations regarding GBM treatment, the outcome is still very poor, making it necessary to develop new therapeutic approaches [1]. Cold Atmospheric Plasma (CAP) based technologies are being studied as new possible approaches against cancer, including direct plasma treatment, Plasma-Activated Liquids (PAL) [2], as well as plasma deposition (PD) of therapeutics for local delivery of oncology drugs to cancerous tissue. Possible combinatory effects with conventional therapies, such as chemotherapeutics may expand the potential of plasma-based interventions.

Topotecan (TPT), a water-soluble topoisomerase I inhibitor with major cytotoxic effects during S- phase of the cell cycle, possesses potent antitumor activity. However, systemic administration of TPT is still limited for cancer types such as Glioblastoma due to low levels of blood-brain barrier crossing [3]. For these reasons, TPT may be repurposed for local combined therapies. Previous results showed that combined treatments with Plasma Activated Water (PAW) and TPT showed a reduction of the metabolic activity and cell mass and an increase of apoptotic cell death. PAW+TPT treatments also pointed to a possible arrest of cell proliferation, also affecting the long-term survival of U-251mg [4]. The overall research aim of this study is to explore the therapeutic properties of a combination between plasma-based technologies and TPT on a human brain cancer cell line (U-251mg).

Evaluation of direct TPT plasma deposition onto U251mg cells grown in 2D or 3D culture indicated synergistic effects between the drug and the plasma treatment. Even though the cancer spheroids showed much lower sensitivity to the plasma deposited TPT than the planar cell culture, a loss of long-term repopulation capacity of the treated spheroids was observed. The direct deposition of TPT onto cancer spheroids is being investigated using metabolic assays, flow cytometry and imaging techniques.

These results can open the doors to a wide variety of new combinations and approaches to local drug application in tumor margin treatment of a range of cancers.

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Non-thermal plasma as a partner in crime for anti-cancer therapies: Optimization in the 3D spheroid tumor model for HNSCC

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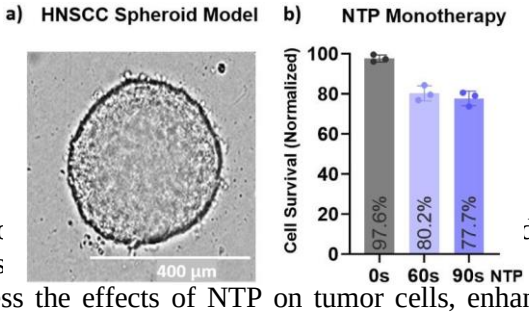
Advanced stages of head and neck squamous cell carcinoma (HNSCC) often face relapse or metastasis, with a miserable prognosis. First-line immunotherapy, with or without platinum-based chemotherapy, shows limited benefits due to low response rates and severe side effects, particularly in already weakened patients. Hence, novel therapeutic strategies are urgently needed to enhance treatment efficacy and address the shortcomings to support the patient's quality of life [1]. While cellular monolayers are routinely used in preclinical cancer research, they lack critical tumor components, hindering *in vivo* translation and clinical implementation, as evidenced by high failure rates in clinical trials [2]. In response, research has shifted to highly advanced culture techniques in order to better mimic the complex malignant environment, including extracellular matrix scaffolding, a diffusion (nutritional) gradient, and multidimensional intercellular interaction [3]. Our research group has a profound expertise in these three-dimensional (3D) cultures, including the 3D spheroid tumor model. These multi-aggregates of cancer cells have proven to be a more representative tool in oncological research, better displaying clinical drug responses [3]. Currently our lab successfully optimized protocols to establish spheroids of multiple cancer types, including HNSCC (Fig. 1a).

This research focusses on non-thermal plasma (NTP) as a valuable addition to other therapeutic modalities to synergistically enhance the killing rate of HNSCC cells.

Since our preliminary data already demonstrated the capacity of NTP to kill HNSCC cells in 2D (Fig. 1b), we continued to optimize the NTP characteristics in the HNSCC spheroid model to elicit a predefined percentage of cancer cell death, optimal to use in further combinatorial approaches. Therefore, different HNSCC cell lines were seeded at concentrations previously optimized in our lab in coated, round-bottomed 96-well ultra-low attachment (ULA) plates to promote the spheroidal shape. After 72h of incubation, the obtained spheroids were treated with a range of NTP durations and cell death was monitored by taking pictures with the Spark® Cyto every 12h for three consecutive days. All experiments are being performed using the clinically approved KINPen® MED.

Fig. 1. Assessment of cell survival after NTP in HNSCC.
a) Image of a generated HNSCC spheroid taken with the Spark® Cyto,
b) NTP monotherapy decreased the cell survival, compared to untreated



In summary, by these experiments, the most potent monc d to use in combination with other anti-cancer modalities to synergis this in the HNSCC spheroid model is an accurate approach to assess the effects of NTP on tumor cells, enhancing both biological relevance and translational applicability.

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Combination therapy of medical gas plasma and cisplatin enhances tumor toxicity and immunogenicity in human bladder cancer cells

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With about 550,000 annual new cases worldwide, carcinomas of the urinary bladder rank among the most frequently diagnosed malignancies and represent the second most common urogenital tumor after prostate cancer [1]. The stage-adapted standard therapies for bladder cancer include surgical resection (TUR-B, radical cystectomy), platinum-based chemotherapy and radiotherapy [2-4]. Compared to the chemotherapeutic side effects, local application of medical gas plasma in combination treatment with lower cisplatin concentrations leads to an increase in the tumor-toxic effect with a treatment approach that has fewer side effects. The gas plasma-cisplatin-induced tumor toxicity was investigated in various preclinical models in vitro (T24, RT-112, SCaBER) and on neo-vascularized tumors in ovo. Single and combination treatments were examined regarding their toxicity and their effects on the expression rates of selected extracellular markers and cytokine secretion were analyzed by flow cytometry and compared with each other. The combination treatment led to an increased decrease in metabolic activity from a concentration of 1 µg/ml cisplatin compared to the individual treatments in vitro. No significantly increased reduction in tumor burden and angiogenesis was observed after combination treatment of neo-vascularized urinary bladder tumors in ovo. A significantly increased expression of immunogenic cell death markers such as HSP70 on the surface of combination-treated tumor cells as well as an increased secretion of proinflammatory cytokines (e.g., IL8) compared to single gas plasma treatment indicates an increased immunogenicity of cancer cells after combination treatment. Consequently, this study summarizes the additive and synergistic effects of gas plasma-cisplatin combination treatment and supports the promising use of medical gas plasma as an additional treatment option for bladder cancer.

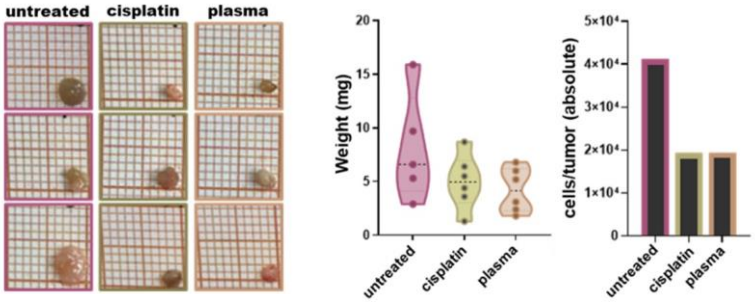

Fig. 1 Representative images of excised in ovo-treated urinary bladder tumors with subsequent determination of the tumor weight.

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Tue-P-32

Exploring the Therapeutic Potential of Surface DBD Plasma in B16F10 cells Treatment: A Comparative Study of Direct and Indirect Approaches

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The growing interest in utilizing cold atmospheric pressure plasma (CAP) for cancer therapy has recently garnered attention [1-4]. CAP has emerged as a highly promising therapeutic option for tumor treatment due to its selective ability to induce cancer cell death [3,4]. Moreover, recent findings demonstrate that plasma not only directly affects cells but also exerts an influence through indirect treatment using previously prepared plasma-activated medium (PAM) [2,3]. Therefore, this study investigates the application and characterization of surface Dielectric Barrier Discharge (DBD) plasma as a potential treatment for B16F10 cells, a widely used cell model for melanoma studies. The experiment encompasses both direct and indirect treatment approaches.

A surface DBD plasma was applied onto a 24-well plate containing B16F10 cells in RPMI culture medium, with a concentration of 6.10^4 cells/mL per well. In the direct treatment, DBD plasma was directly administered to the cell culture medium containing B16F10 cells for different exposure times: 30, 60, and 120 s. In contrast to direct application, indirect plasma treatment involves irradiating the cell culture medium, which was subsequently transferred to a culture plate containing cells. The generated DBD plasma was characterized for its physicochemical properties, including the composition of reactive species via OES and electrical characterization. The PAM was characterized using UV-Vis spectrophotometry. The effects of DBD plasma treatment on cell viability were assessed in both application methods using the MTT assay after 24 h of activation.

For the different activation times, the time in which the best results were achieved was exposing the cells in 120s, resulting in a 51% decrease in cell viability for the direct method, and 49% for the indirect method. This difference in results between direct and indirect methods can be explained because, in the direct method, at least three factors can be related: short-lived reactive species (O_3^- and OH), long-lived species (H_2O_2, NO_2^-, NO_3^- and HNO_2) and physical factors (UV radiation). On the other hand, in the indirect method only long-lived species should be considered.

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Cold gas plasma alters tyrosine kinase activity in lung cancer cells

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Albeit the antitumoral efficiency of tyrosine kinase inhibitors has been demonstrated in several anti-cancer therapies, the development of therapy resistance is still a major reason for the fatal consequences of cancer[1]. The tumor microenvironment (TME) has been suggested to correlate strongly with treatment outcomes since it is often associated with excessive production of reactive oxygen species (ROS). ROS are capable of introducing oxidative post-translational modifications (oxPTMs) to proteins targeted in cancer therapy, such as tyrosine kinases, and ROS could render their functionality[2]. Additionally, drug oxidation could have a significant impact on poor therapy outcomes. To gain a deeper understanding of the development of resistance kinases, tyrosine kinase inhibitors (TKIs), as well as cancer cells that were treated with TKIs, were exposed to plasma-induced ROS to study the impact of oxidative modifications on those targets. Since combination treatments in oncology use anticancer drugs and gas plasma treatment, we addressed this problem by testing TKI efficacy in A549 human lung cancer cells and conducted single protein oxidation experiments as model studies to unveil the mechanisms that render the therapy outcome. We performed metabolic assays to assess the drug efficiency of oxidized drugs as well as the efficiency of those drugs on prior plasma-induced ROS-stressed cells. We screened 37 drugs for a broad view and found that the majority of oxidized TKIs showed a significant decline in anticancer efficacy. Nevertheless, one compound showed slightly elevated antitumoral toxicity. In cells, which were ROS-stressed prior to the TKI application, all TKIs except one showed additive toxicity. To testify whether there is an impact on the molecular level of the protein, three kinases were treated with plasma-induced ROS and afterward evaluated by an enzymatic activity assay and analyzed by LC-MS/MS. OxPTMs were detected on amino acid residues that harbor essential structural or catalytic functions, such as the ATP binding site, but also on amino acid residues that are targets for therapeutic applications such as TKIs. The results suggest that excessive ROS concentrations potentially contribute to TK activity reduction in the TME.

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Tue-P-34

The impact of cold piezoelectric plasma on cancer cell viability: a study on cholangiocarcinoma

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Today, research into plasma-cancer treatment focuses on three main objectives: (*i*) biological mechanisms underlying cancer cell death [1]; (*ii*) combined therapies; and (*iii*) space requirements, both for the local application of internal treatments to avoid surgery [2], and for plasma generation setup. Cold Piezoelectric Plasma (CPP) sources fall within the third aim, consisting in portable devices measuring a tens of centimeters, and powered directly from the main socket thanks to resonant piezoelectric transformers with a 1000-fold amplifier ratio [3].

In this study, the piezobrush® PZ2 source from Relyon Plasma GmbH Company is utilized to generate a corona discharge (Pz-CD) and a dielectric barrier discharge (Pz-DBD), as in Fig. 1. Their therapeutic potential is investigated considering cholangiocarcinoma (CCA) cells as an *in vitro* study model of cancer. This rare and aggressive biliary tract cancer, presents high mortality rates and increasing incidence. For the study, two human CCA cell lines are treated by plasma in 6-well plates, HuCCT-1 and EGI-1, that display distinct phenotypes. Although the two sources cause a similar decrease in cell viability for the first 3 minutes of exposure for both cell lines, the Pz-CD proves more efficient thereafter. Hence, in the case of EGI-1, cell death mean value is only 37 % with Pz-DBD against 50 % with Pz-CD for a 3 min-treatment time. The uniformity of these plasma treatments is also characterized through a DAPI staining radial profile analysis of the cell monolayers, immediately and 24 hours post-treatment. Interestingly, this study reveals that the Pz-DBD induces homogeneous cell death only after 24 hours while Pz-CD provides a much more localized cell death within the well, expanding from the plasma impact point with treatment duration. Temperature measurements are confronted to cell distribution inside the well, demonstrating no correlation between cell death and the increasing temperature below Pz-CD. CPP produce reactive oxygen and nitrogen species in plasma and liquid phases, contributing to oxidative stress in CCA cells, as evidenced by the activation of DNA damage response pathway, ultimately leading to cell death. However, this effect is not counteracted, although the expression of antioxidant enzymes is increased in response to CPP.

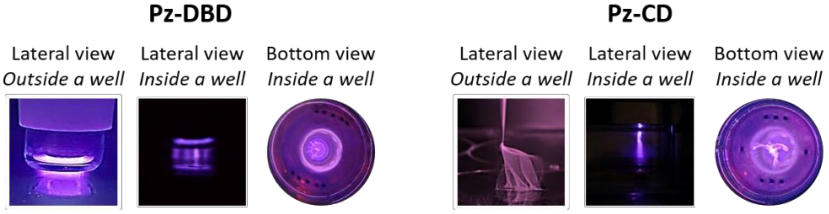

Fig. 1 Cold piezoelectric plasma photographs

Cold Piezoelectric Plasma opens new perspectives for cancer treatments, currently favorable for post- surgical procedures. Some technological increments will be required to make CPP a relevant approach in the case of cancers relying on endoscopic tools like cholangiocarcinoma.

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Combination Treatment of Head and Neck Cancer Cell Lines with Cold Plasma and Chemotherapeutics

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Ranking as the seventh most common cancer worldwide (1.1 million new cases annually) and with an incidence projected to increase by 30% by 2030, head and neck squamous cell carcinoma (HNSCC) remains a critical healthcare challenge [1]. Current five-year survival rates for HNSCC are just 50% and there is an urgent need for development of new, more effective treatments. Accumulating evidence suggests that cold plasma offers a promising new modality for cancer treatment. Encouragingly, a clinical report of palliative treatment of locally advanced HNSCC with cold plasma demonstrated lasting partial remission in a subset of patients [2].

In this study, we describe the biological effects of cold plasma when delivered in combination with chemotherapeutics. Two p53-defective HNSCC cell lines (A253 and FaDu) were co-treated with cold plasma and either cisplatin or inhibitors of the DNA damage response (DDRi). Our preliminary data show that the combination of plasma with cisplatin/DDRi treatment induces enhanced killing in both cell lines when compared to plasma or drug alone. The mechanism of this enhanced cytotoxicity will be discussed in the context of activation of the DDR (measured through detection of γH2AX) and other markers of replicative stress.

Our data suggest that the anti-proliferative effects of chemotherapeutics on HNSCC may be boosted by co-treatment with plasma, and that plasma may play a future important role in the multimodal management of this condition.

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Machine Learning Assessment of Cold Atmospheric Plasma Efficacy on Breast Cancer: Exploring Receptor Status Correlations for Optimized Treatment Strategies

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Breast cancer stands as the most prevalent form of cancer among women globally [1]. Cold atmospheric plasma (CAP) has demonstrated promising anti-cancer effects on breast cancer both *in vitro* and *in vivo* [2]. However, it's crucial to recognize that breast cancer is a heterogeneous condition with diverse subtypes based on cell surface receptors. These subtypes include Luminal-A (ER + or -
, PR + or -, and HER-2/neu -), Luminal-B (ER + or -, PR + or -, and HER-2/neu +), HER-2 positive (ER -, PR -, and HER-2/neu +), and Triple negative (ER -, PR -, and HER-2/neu -). This classification plays a pivotal role in determining clinical treatment approaches and influencing treatment outcomes [3]. Despite the acknowledged efficacy of CAP in treating breast cancer, there remains a gap in understanding its effectiveness across different receptor statuses.

This research endeavors to explore the potential correlation between CAP effectiveness and breast cancer receptor status by employing machine learning (ML) algorithms. The primary goal is to construct an ML model utilizing previously acquired data to qualitatively predict the *in vitro* anticancer effects of various plasma treatments [4]. A comprehensive literature search, mainly conducted on PubMed, yielded over 50 relevant publications. These publications provided valuable insights into parameters for ML models such as plasma type, gas, discharge gap, plasma-treated liquid, treatment volume, treatment duration, cell lines, and receptor status. The ongoing feature extraction process will be followed by preprocessing steps to refine the data, leading to the development of ML models. These models, employing classification and regression techniques, aim to predict the anticancer efficacy of plasma treatment on breast cancer.

By leveraging extensive datasets, these ML models seek to enhance the accuracy of predictions regarding the anticancer effects of plasma treatments on breast cancer. This research initiative has the potential to advance our understanding of the correlation between CAP effectiveness and receptor status, ultimately contributing to the optimization of breast cancer treatment processes.

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In Vitro Adaptive Plasma Cancer Treatment with Real-Time In-Situ Diagnostics using Impedance Measurements

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Cold atmospheric plasma (CAP) jet for cancer treatment has been studied extensively over recent years, and the effectiveness of CAP treatments has been demonstrated both *in vitro* and *in vivo* [1]. However, several difficulties are present for plasma cancer treatment to realize intended therapeutic outcomes. First, various CAP jet generation parameters and environment conditions may affect the effectiveness of the treatment. In addition, cancer cells may display diverse reactions to the same treatment conditions. In response to these challenges, the adaptive plasma cancer treatment framework was proposed [2]. The objective of the framework is to adaptively adjust the CAP treatment conditions based on actual cancer cell responses. First, from previous in-vitro plasma cancer treatment experiments, the empirical knowledge is obtained to provide initial instructions for the first treatment. Specifically, electrochemical impedance spectroscopy (EIS) is utilized to measure cell viability, as it has been illustrated that the impedance at a specific frequency is correlated with the corresponding cell viability [3]. Then, during the CAP treatment, the cancer cell responses are collected through impedance-based in-situ diagnostics, where the treatment effectiveness can be predicted so that the real-time CAP treatment adjustments can be realized.

This talk will present adaptive plasma cancer treatments with real-time in-situ diagnostics using impedance measurements. Specifically, firstly, to acquire empirical knowledge, the U-87 cancer cells were treated with various treatment time, and concurrently, impedance from a specific set of measuring frequencies was collected. Then, after 48 hours of the treatment, the cell viability was measured with the WST-8 assay. The correlation between impedance measurements and cell viability was investigated with the framework of canonical correlation analysis (CCA) which is a multivariate statistical tool to extract relation between two sets of variables. It was illustrated that the cell viability was highly correlated with impedance measurements at a particular set of frequencies, and further the regression coefficients could be found to estimate the expected cell viability, serving as the in-situ diagnostics tool. Next, adaptive CAP treatment experiments were conducted to evaluate the impedance-based real-time in-situ diagnostics. The adaptive experiments were conducted under both the conditions used for fitting the CCA framework, and the varied treatment conditions, namely the perturbed CAP treatment conditions. It has been demonstrated that utilizing the *in-situ* diagnostics, the desired treatment effectiveness, *i.e.*, cell viability, could be reached, and the capability of the impedance-based real-time in-situ diagnostics could be validated through statistical analyses.

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Evaluation of pyrazolopyrimidinones as anticancer prodrugs activated by cold atmospheric plasma

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Glioblastoma is a grade-4 IDH1/2-wildtype, aggressive brain cancer with low survival rates attributed to its poor response to conventional cancer therapies [1]. Prodrugs activated by cold atmospheric plasma (CAP) represent a promising approach for an efficient cancer therapy with minimal side effects [2-3]. CAP can be used to generate reactive oxygen and nitrogen species (RONS) locally with high accuracy, allowing the selective activation of RONS sensitive anticancer prodrugs exclusively at the cancer site [2-3]. Pyrazolopyrimidinones are nitrogen-containing heterocyclic compounds which exhibit anticancer properties and have shown potential as CAP-activatable prodrugs [3]. A novel family of pyrazolopyrimidinone-based prodrugs has been developed and its potential as a programmable cytotoxic combination therapy against glioblastoma will be evaluated. The potential of each pyrazolopyrimidinone derivative as a CAP-activatable anticancer prodrug is undergoing evaluation via an optimized screening protocol, which compares the half-maximal inhibitory concentration of the prodrug candidate after 0, 10 and 30 seconds of CAP exposure, at 48 and 144 hours; with CAP generated via the Pin-to-Plate device with the Leap100 system. Compound **10** has previously demonstrated CAP dependent prodrug activation and a 5- to 15-fold cytotoxicity increase post CAP exposure generated via the dielectric barrier discharge device [3]. Preliminary data with the Pin-to-Plate device indicates that Compound **10**, without CAP exposure, presents low cytotoxicity against the glioblastoma cells, with the cytotoxicity increasing by 7- and 20-fold, post 10 and 30 seconds of direct CAP treatment, respectively. Methodologies for direct and indirect CAP treatments using the Pin-to-Plate device are undergoing optimization, with prodrug exposure pre- and post- CAP treatment and the utilization of plasma activated liquids. Future work will focus on the biological evaluation of arising lead prodrug candidates on glioblastoma models, alongside non- cancerous models, employing CAP generated via the experimental Pin-to-Plate device to examine the prodrug candidate's applicability as a novel, minimally toxic, treatment option for glioblastoma.

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Novel combination therapy of non-thermal plasma and immune checkpoint inhibitors for melanoma

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Melanoma accounts for 65% of all skin cancer-related deaths worldwide. In 2020, approximately 325 000 global cases of melanoma patients were estimated and about 57 000 patients died from the disease. Currently, immune checkpoint inhibition (ICI) stands as the primary first line treatment for melanoma. The immune system is equipped with checkpoints to regulate and prevent excessively or unwanted immune responses. ICI prevents cancer cells from exploiting these checkpoints, improving the patient's natural immune response. ICIs as standalone therapy or in combination (i.e., anti-PD-1, CTLA-4) has resulted in response rates ranging from 45% to 60% in melanoma cases, accompanied by an overall 53.0% 3-year survival rate [1]. However, given that ICIs only benefit approximately 50% of patients, there arises a critical need for alternative treatment strategies. This brings us to non-thermal plasma (NTP), recognized for its antitumor properties attributed to the generation of reactive oxygen and nitrogen species (RONS). These RONS trigger immunogenic cell death (ICD) within the tumour, a regulated form of cell death [2]. Through the integration of the existing ICI treatment with an innovative NTP approach, two distinct steps of the cancer immunity cycle would be targeted.
The objective of this study is to devise an innovative treatment approach by integrating ICI with non-thermal plasma. We hypothesize that this novel combination therapy will synergistically enhance antitumor efficacy, overcoming resistance in non-responsive melanoma patients and enhancing outcomes in patients. I initially investigated the influence of NTP on the expression of immune checkpoint ligands in cancer cells in vitro and I am now currently validating these results in an in vivo model.

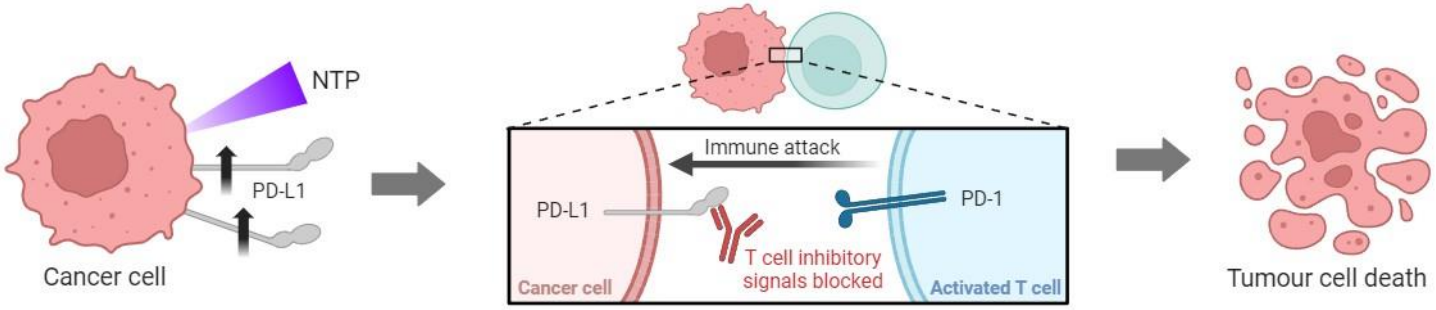

Fig. 1. Schematic figure representing the synergistic action of non-thermal plasma (left) and immune checkpoint inhibition (middle) with both therapies leading to tumour cell death (right).

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Sensitization of vulvar cancer cells with ATM kinase inhibitor prior PAL treatment

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Vulvar cancer (VC) accounts for 5% of gynecological malignancies worldwide[1]. Two types of VC are described, the first (most common in younger women) is associated with human papillomavirus (HPV) infection, and the second (in older women) is often found in patients with inflammatory dermatosis such as vulvar lichen sclerosis. The standard treatment for VC is surgical excision which may be combined with adjuvant radiotherapy or chemotherapy to prevent the risk of local recurrence. In addition to those conventional approaches, plasma treatment could be used as a specific molecular supporting therapy. In previous in-vitro and in-vivo studies, plasma-activated liquids (PAL) have shown an antiproliferative effect on neoplastic tissue[2,3]. The PAL effect is based on several molecular signaling pathways that lead to oxidative stress, cell membrane damage and DNA damage[4]. Among these, the signaling pathway via ATM, a serine protein kinase, is responsible for DNA double-strand break repair. The combination of PAL with an ATM inhibitor (ATMi) could enhance the antiproliferative effect on vulvar cancer cells and reduce the toxicity of existing therapies through synergistic effects.

In this project, SW954 cells are first sensitized with ATMi[5]. The compound is then replaced by PAL, which is generated using a VIO3/APC applicator (Erbe Elektromedizin®, Tübingen, Germany). First results show an antiproliferative effect of PAL on SW954 cells, which is enhanced by the combination with 2 M of ATMi.

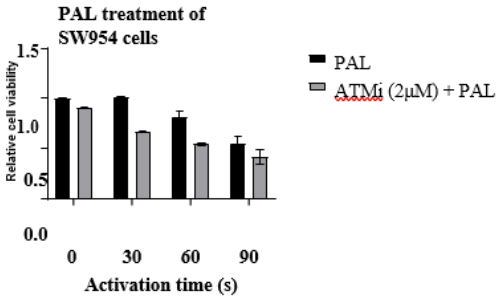

Fig. 1 Cytotoxic effect of PAL, alone or in combination with ATMi, on SW954 cells.

Conclusion: The combination of PAL with ATM inhibitor is a new promising approach for the treatment of VC lesions.

Acknowledgment: We thank Pr. Dr. Lars Zender and Athina Anastasia Moschopoulou (Department of Medical Oncology and Pneumology, Tübingen, Germany) for providing the ATM inhibitor.

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Impact of plasma-activated phosphate-buffered saline on viability of human cancer and non-cancerous cells

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This study explores the indirect application of cold plasma through plasma-activated phosphate- buffered saline (PAPBS), which contains long-lived reactive oxygen and nitrogen species (RONS). PBS activation was realized by a positive streamer corona discharge at atmospheric pressure in ambient air, using a portable plasma pen. Exposing cells to the effects of PAPBS resulted in a decrease of the quantity of viable cells across various human cancer cell lines, including both sensitive and resistant human breast adenocarcinoma cell lines (MCF-7 or MCF-7/PAX [1,2]) and a human pancreatic cancer cell line (MIAPaCa2).
The subsequent phase of our study aimed to validate the selective antineoplastic effects of PAPBS, examining its influence on a human prostate cancer cell line (PC3) in contrast to a non-cancerous human prostatic stromal myofibroblast cell line (WPMY-1). Different durations of plasma application on PBS represent varying concentrations of RONS in plasma-activated PBS [3]. The impact of PAPBS on cell viability was monitored in relation to these varying concentrations of RONS. Additionally, we investigated the effect of PAPBS based on different durations of cell cultivation in the presence of PAPBS.
Our results indicated that cancer cells exhibit a higher sensitivity to specific plasma “dose” compared to non-cancerous cells. These findings show a potential of cold plasma as a selective cancer treatment, aiming for efficient tumor removal while minimizing the therapy's adverse effects on patients' lives.

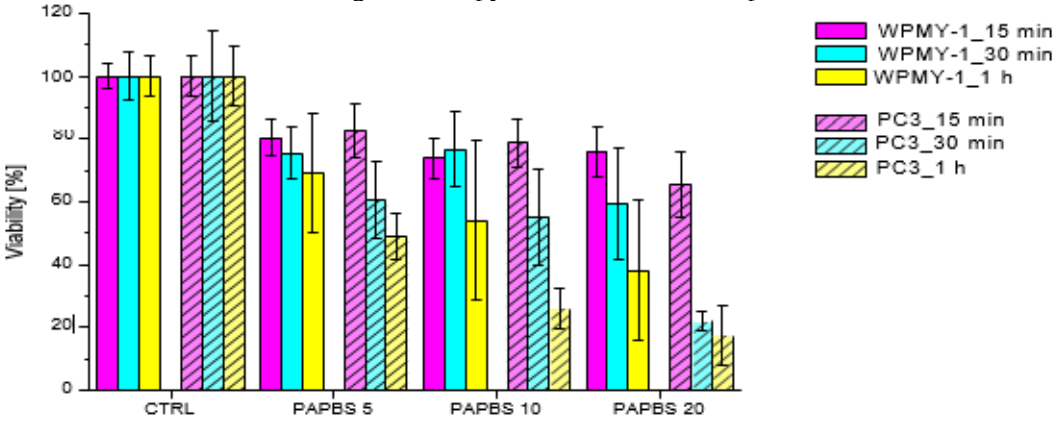

Figure 1: Effect of PAPBS treated 5, 10 and 20 min on cell viability of noncancer prostatic cell line WPMY-1 and human prostate cancer cell line PC3. The time of PAPBS action – 15, 30 min and 1 h, after which PAPBS was replaced with growth medium. Cell viabilities normalized to 100% in controls

This work was supported by Slovak Research and Development Agency, grants APVV-220247 and APVV-17-0382.

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Numerical modeling on the mechanism of plasma-induced cell death

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Recently, the effects of cold atmospheric plasma (CAP) on cells and biomolecules have been extensively investigated. Experimental studies suggests that reactive species generated by CAP influence the "survival/death" fate decisions of cancer cells [1,2]. Numerical approaches can greatly contribute to the fundamental understanding of the biophysics of CAP-cell interactions. The authors developed a zero-dimensional intracellular signalling model to quantify the effects of CAP-induced reactive species on mitochondrial redox-mediated functions and energy metabolism [3,4].

To further understand how CAP affects cell-fate determination mechanisms, the present study proposes a computational biology model focused on apoptosis induction. Figure 1 shows a schematic of the network of intracellular biochemical reaction pathways implemented in the model. This system includes death receptors, mitochondrial signalling cascade pathways, initiator/executor caspases and their regulators, anti-oxidant enzymes and nDNA. The effects of CAP irradiation are modelled as extrinsic or intrinsic stress caused by reactive oxygen species (H_2O_2, OH and O_2^-, etc.) and reactive nitrogen species (NO, NO_2, $ONOO^-$, etc.). A potential synergistic effect between multiple reactive species on apoptotic cell death has been quantitatively investigated.

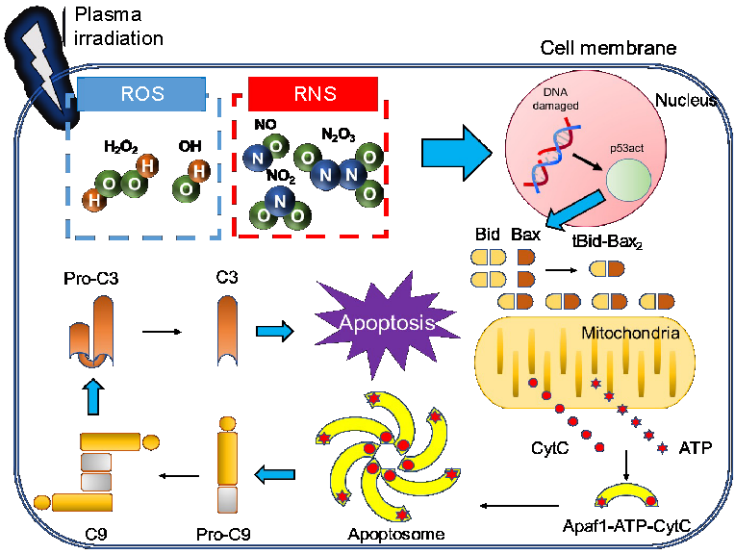

Fig. 1. Schematic diagram of the physiological and biochemical reaction system for apoptosis induction.

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The beneficial effect of successive Cold Atmospheric Plasma treatments on in vitro behaviour of human gingival fibroblasts

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While cutaneous wound healing is an extensively studied and well-characterized process, the understanding of intraoral tissue repair still presents major lacunae, an aspect which reduces the clinical translation of treatment alternatives [1]. Following injury, the oral mucosa is submitted to a cascade of biological events that culminate in the restoration of tissue homeostasis and while general similarities exist, there are stark differences in the genomics and kinetics of wound healing between the oral cavity and the cutaneous epithelium [2]. Moreover, the lack of a successful therapy for oral mucosal wounds, compelled researchers to take into consideration alternative treatments for an enhanced intraoral healing. With this in mind, in the last decade the newly found therapeutic properties of Cold Atmospheric Plasma (CAP) gave it special consideration in dental applications and numerous studies reported its beneficial effects on the intraoral wound healing process and tissue regeneration [1].

In this context, the aim of the present study has been to investigate by comparison the effects of CAP on human gingival fibroblasts (HGF-1 cell line) in culture and observe how different plasma jet exposure conditions can affect their in vitro behaviour. For this reason, the HGF-1 cells were assessed in terms of their viability/proliferation potentials, adhesion/cytoskeleton organization and fibronectin production and its subsequent arrangement into an extracellular fibrillar network. The obtained results revealed that the fibroblasts subjected to successive CAP treatments and for longer periods of time exhibited a better in vitro cellular behaviour when compared to the cells that have been exposed only once to the CAP treatment. Thus, the application of CAP for longer periods of time exerted a positive effect on cells' viability and proliferation. Likewise, the fluorescence images acquired after performing the LIVE/DEAD cell viability assay showed an increase in the cellular density with treatment length and number of treatments. In addition, the fibronectin immunolabelling revealed that the specific positive signals were better expressed at higher treatment times and after the cell culture has been submitted to successive CAP treatments suggesting their beneficial effect on the extracellular matrix formation.

Considering the above findings, the widespread application of CAP in dentistry can have a promising future, however, in depth additional studies still need to be conducted in order for the underlying mechanism of CAP in an oral environment to be fully elucidated.

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A Minimally Invasive Surgical Platform Against Pancreatic and Biliary Tract Cancers Using Cold Atmospheric Plasma

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Cancer is one of the major causes of death in Europe. Pancreatic (PC) and biliary tract (BTC) cancer are rare gastrointestinal adenocarcinomas with an increasing incidence particularly among the elderly and women in an ageing European population, and a poor prognosis poses major clinical challenges and public health burden. Cold atmospheric pressure plasma (CAP) has shown potential regressing various cancer types in the laboratory setting [1]. Owing to their properties, CAP is a unique source of high concentrations of reactive radicals, electrons, ions, UV etc. that may induce various effects in living tissue. CAP has shown selectivity in where cancer cells are treated with minimal effect to healthy ones; this renders plasma suitable to treat carcinomas in very sensitive areas or organs where an unmet need to minimise damage/side-effects exists. CAP can be delivered through dielectric tubes of variable length, which makes it ideal for minimally invasive and precise laparoscopic and endoscopic operations. Despite the great promise and potential of this concept, there is no single laparoscopic or endoscopic medical platform in the market today based on CAP to treat carcinomas. This project proposes a novel solution using cutting-edge plasma technology, in-silico models, and a system-level approach [2]. It will combine plasma with tumour multiscale simulations and will take input from pre-surgery diagnostics for in-silico model initialisation and CAP operational window determination. The developed technology will be demonstrated through in-vivo experiments, while the foundations for clinical trials and market introduction for CAP-based care of PC/BTCs will be laid out in this project. During the meeting, preliminary results and the platform architecture will be presented.

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Effects of Cold Atmospheric Argon Plasma Jet Treatment on the Biological Activity of Human Gingival Fibroblasts

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Soft tissue regeneration plays a crucial role after oral surgery, since the successful healing of the soft tissue is a primary indicator of an effective intervention. Recently, cold atmospheric plasma (CAP) has emerged as a promising therapeutic procedure, leading to notable effects on cell migration and proliferation [1,2]. Despite its potential, the application of CAP in dentistry remains underexplored. In the present work, the impact of CAP activated media on human gingival fibroblast responses was evaluated, for future wound healing strategies. The human gingival fibroblasts were exposed to complete DMEM medium (without sodium piruvate) previously activated with a cold atmospheric argon plasma jet device for distances of 2, 5, 7, and 9 mm, and with treatment times of 15, 60, 120, 180, and 300 s for 1, 2 and 3 days of culture. The cell viability was evaluated using resazurin-based method, while wound healing dynamics was assessed via the scratch assay technique using phase- contrast microscopy. The cell morphology was characterized through fluorescence microscopy using propidium iodide and phaloidin staining, complemented by scanning electron microscopy. The results revealed that treatment distance and exposure time may be influenced by cell concentration. Specially, in this study, prolonged exposures of 300 s to the CAP device resulted in a decrease of cell viability. The best results were observed with a cell concentration of $1x10^4$ cells/well compared to the other concentrations tested. For the ideal cell concentration, the treatment distance of 9 mm appears to improve human gingival fibroblast viability, while a distance of 2 mm did not significantly affect fibroblasts cells behaviour. The treatment time did not seem to be a significant factor for indirect CAP application, as both 15 s and 180 s did not statistically affect human gingival fibroblast viability.

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Safety evaluation of plasma activated water on skin and breathing: an in-vivo study

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Plasma-activated water (PAW) obtained by plasma devices containing richly reactive oxygen and nitrogen species (RONS) has been reported for agricultural and medical purposes. For instance, in agricultural applications, it is required to confirm the non-toxicity of PAW on farmers.
This research was designed and implemented in order to evaluate the possible toxic impacts of active water on the skin and breathing. The acute inhalation toxicity and eye irritation on mice by plasma-treated tap water (PTW) were investigated in accordance with OECD Guideline for Testing of Chemicals No. 403, 436, 39, OECD and OECD Guideline for Testing of Chemicals No. 405, respectively. In this study, the two experimental groups based on five NMRI mice were exposed one time and monitored daily, in the same time interval, for 1 h, 24 h, 48 h, 72 h, and 7, 14, 21 days. 1st and 2nd groups were treated using PTW and vehicle, respectively. The results indicated that there were no significant changes in body weight and survival status of mice after plasma-activated water (PAW) treatment for 3 weeks. None of acute inhalation toxicity or eye irritation was exhibited during the test period.
The results of the evaluation demonstrated the safety of plasma-activated water as none of acute inhalation toxicity and eye irritation were exhibited in the course of study.

Keywords: cold atmospheric plasma, plasma-activated water, safety evaluation, acute inhalation toxicity, eye irritation.

Anti-yeast activity of transient spark PAW combined with UV-A and natural phenolics on planktonic yeast *Wickerhamomyces anomalus*

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Plasma-activated water (PAW) has emerged as a potent antimicrobial tool with the ability to control microbial growth [1]. PAW is produced by treating water with non-thermal plasma, introducing various reactive oxygen and nitrogen species (RONS) into the water thus making the treated water possess antimicrobial properties [1]. Phenolic compounds, predominantly found in plants, have demonstrated significant potential as anti-yeast agents by disrupting cellular processes [2]. The combination of phenolics and PAW presents a promising anti-yeast strategy with potential application in medical, food safety, agriculture, and water treatment settings [3]. The synergistic effect of combining phenolics with PAW is attributed to their complementary mechanisms of action, which may include a wider spectrum of antimicrobial activity, reduced antimicrobial resistance, increased oxidative stress and enhanced membrane disruption [4].
This study involved the use of 1 kHz transient spark (TS) discharge PAW generated in the presence of UV-A and combined with cinnamic acid, vanillin, gallic acid and *p*-coumaric acid separately to create phenolics concentration of 2 or 1 mg/mL, and incubated with 106 CFU/mL of yeast *W. anomalus* SZMC 8061Mo for 24 hours at 30 oC. In addition, 106 CFU/mL of *W. anomalus* in sterile tap water was directly treated with TS for 10 min in the presence of UV-A and incubated with each of the four natural compounds under similar conditions. The efficacy of the PAW/UV-A radiation combined with natural phenolics was evaluated by assessing the growth extent after 24 hours' incubation through agar plated colony counts and compared to the untreated control samples. The findings of this research indicate that the combination of PAW/UV-A with natural phenolics represents a viable anti-yeast strategy.

Acknowledgment: This work was supported by Slovak Research and Development Agency APVV- 22-0247. We thank Dr. Miklos Takó for providing us with *Wickerhamomyces anomalus* (formerly, *Pichia anomala*) SZMC 8061Mo from the University of Szeged Microbiological Collection.

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8 - 13 september, Portorose, Slovenia

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FUNDAMENTALS OF ATMOSPHERIC PLASMA

Oral session (Wed - O - 7)

Wednesday, 11 September 2024

Online diagnostics of the production of reactive species in a micropulsed plasma jet

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Following the large growth of plasma medicine in recent years, cold atmospheric plasma sources have been used in different biological applications. From disinfection to cancer treatment, the field is experiencing a rapid development.

The interaction of a plasma with a substrate involves a wide spectrum of phenomena, from highly energetic electrons to reactive oxygen and nitrogen species, to radiation and electric fields. This variety, together with the complexity of biological substrates, prevents a full modelling of the system. Moreover, most of the plasma sources are based on non-equilibrium conditions, which makes the behaviour of the plasma itself hard to fully predict and subject to instabilities. Aiming towards a wide use of the devices, even in clinical environments, it is fundamental to develop real-time diagnostic for immediate feedback to the operator.

Of particular interest in characterizing the source effects are the reactive oxygen and nitrogen species; however, standard methods used for they evaluation, like FTIR or chemical fluorescence, are invasive and not suitable for online implementations. In this work, the possibility of using electrical and optical emission spectroscopy data as a probe for the production of species is discussed. Using a very simple source, a micropulsed plasma jet, and varying both the plasma and the substrate characteristics, the behaviour of our diagnostic is described, and its efficiency evaluated. Data from other diagnostics and for previous studies are compared to evaluate its coherence and estimate predicting power.

The results here reported can act as a proof of concept to perform further studies and improve the described technology.

Establishing a set of measurements to monitor plasma source duplicates in collaborative groups through systematic investigation and correlation of parameters

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Plasma medical research is carried out in interdisciplinary environments. This usually requires a large number of partners, with distances between sites ranging from a few floors to hundreds of kilometers. As a result, the devices under investigation are possibly distributed by different duplicates for use with different partners under conditions that are as reproducible as possible. For our study of anti-viral efficacy, multiple plasma sources were distributed across three institutes and up to three workgroups within one institute [1, 2]. In order to establish a quality control procedure as well as a set of tests that can be performed on plasma sources, a study was conducted to compare a series of plasma sources. One of the requirements was that the test must be easy to perform for scientists from all fields of science who study plasma sources and their effects. In addition, these tests must be relatively quick to perform and reliable. In this study, various measurements were performed to cover multi-parametric characterization, including energy, power, temperature, leakage current, effluent length, relative and absolute radiation, as well as reactive species concentration in the plasma treated liquid. It was found, that long term operation should be included in a selected set of parameters for a better control. By performing a correlation test, two parameters were identified that can serve as monitoring parameters for the reliable performance of the plasma source. One of the parameters is representative of the electrical behavior of the plasma source and the other is related to the effectiveness of the plasma source for biological treatment. The initial study was performed with a miniaturized neon plasma jet [3], the same tests were also suitable for the operation the same plasma jet with helium as feed gas and tested for a dielectric barrier discharge in synthetic air.

The work was funded by the German Federal Ministry of Education and Research (BMBF, Grant No. 03COV06A).

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Development of A Long Tube Plasma Jet for Treating Pipeline-shape Structures and Its Affecting FactorsStudy

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Low-temperature plasma jet containing rich chemical substances, e.g., reactive nitrogen and oxygen species, and physical factors, e.g. ultraviolet and electric field, which could induce biomedical effects when interacting with tissue, has been widely used in the biomedical field. However, for the special tube structure in the body (stomach, intestine, lung), the conventional plasma jet makes it difficult to achieve the target point. Therefore, it is necessary to develop a plasma device that could transfer the discharge plasma from the generation area to the treatment area. In this study, we developed a long- tube plasma jet and investigated the effects of electrode structures, operation parameters, and the material of the transmission hose on the length of the plasma plume inside the tube. It is found that the higher applied voltage and gas flow rate could induce a longer plume propagation in the hose. When the voltage is relatively low (about 10kV, microsecond pulses), the effect of frequency is not obvious. With the increase of the voltage level, the lower frequency could have a longer plasma propagation in the hose. The propagation distance of the plasma plume for single needle discharge is longer than it of needle-ring structure, when the other operation parameters are the same. Polyvinyl chloride (PVC), polyethylene (PE), polytetrafluoroethylene (PTFE) and polyurethane (PU) are selected to investigate the effect of the hose material, and it is found that the plasma propagation in PU tube is the longest. The permittivity of the four materials were measured and PU has the lowest value which would affect the charge and electric field distribution. The emission spectrum shows that the intensity of He (704.4nm) at different locations had little change and slightly attenuates, which would be beneficial for activating reactive species to induce bioeffects. By optimizing the operation parameters, electrode structure and transmission hose, the device which could generate a 90cm- 100cm plasma plume inside the whole tube was developed, which met the length requirement of general gastroscopy tube (about 80cm). The relatively uniform plasma distribution in the tube can realize the purpose of self-cleaning of the inner wall of the tube.

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Identification of relevant plasma components in plasma-induced microcirculation enhancement

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Plasma therapy has been used for years in clinics and by general practitioners to support the wound therapy of predominantly chronic wounds. One of the therapeutic effects is the plasma-induced increase in blood flow through the capillaries, known as microcirculation. The group around Heuer et al. achieved the first evidence of this in healthy subjects in 2015 and hypothesized that the reactive species formed by the plasma treatment (especially NO) penetrate the stratum corneum into the skin and are responsible for the increase in blood circulation [1].
Since then, various studies on healthy subjects have investigated different modalities (different treatment times, repetitive plasma treatment, etc.) of plasma-induced microcirculation enhancement, but the investigation of fundamental mechanistic effects was not the primary focus [2-5].
Additionally, there are studies that have been able to confirm the microcirculation-enhancing effects following plasma therapy in acute and chronic wounds [6-10]. However, to this day, the mechanistic effect of plasma-induced microcirculation enhancement remains unclear.
The presentation will feature yet unpublished studies on healthy subjects that aim to contribute to the mechanistic elucidation of plasma-induced microcirculation enhancement. This involves isolating individual plasma components to ensure the treatment of healthy subjects with individual plasma components or the exclusion of certain plasma components. The subsequent determination of microcirculation using hyperspectral imaging will be compared to standard plasma therapy. This allows the influence of certain plasma components on microcirculation to be viewed directly or indirectly, thus providing a contribution to the mechanistic elucidation.

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PLASMA-CELL AND PLASMA-TISSUE INTERACTIONS – BIOLOGICAL AND BIOCHEMICAL REACTIONS

Oral session (Wed - O - 8)

Wednesday, 11 September 2024

Combining Plasma, Micelles, and Chitosan into PCCM-125: Long-Term Nitric Oxide Delivery and Angiogenesis-Independent Wound Healing in Human Corneal Epithelial Cell Model

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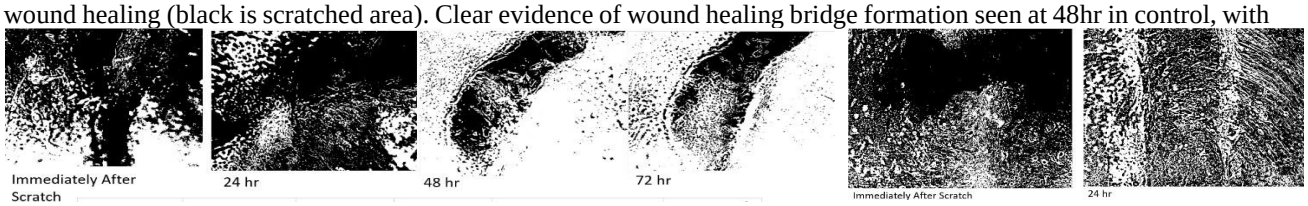
Purpose: Nitric oxide (NO) has recognized potential in significant disease processes. However, NO therapeutics are limited by rapid NO depletion due to their short half-lives. Using gliding arc plasma, micelles, and chitosan, we created a long term NO delivery vehicle, which we have termed PCCM-125. Our initial studies demonstrated PCCM-125's long term NO release in Synthetic Tear Fluid (STF) and its ability to enhance corneal regeneration, an angiogenesis independent wound healing process.

Methods: PCCM-125 was synthesized by combining gliding arc plasma-treated oil with similarly treated tween80 to form micelles, which are then coated using a chitosan solution. NO release was evaluated for PCCM-125 (1 mL in 10 mL STF), utilizing diazotization and dimethylphenol colorimetric assays for nitrite and nitrate (control was untreated micelle solution). Corneal scratch assay using 25 uL of PCCM-125 in MATTEK Epi Corneal Model.

Results: Nitrate concentration in STF was observed to steadily increase at a linear rate over 48 hours, while nitrite was shown to initially increase rapidly in the first 10 hours and remain constant at a maximal level (0.3 mg/L) until 36 hours when it starts to slowly decline. MATTEK Epi Corneal Scratch assay analyzed with Image J technology showed that wound healing in samples treated with PCCM-125 was accomplished within 24 hours, while the control samples in phosphate buffered solutions took 72 hours to fully heal.

Fig. 1 MATTEK Epicorneal Scratch assay analyzed with Image J technology. First four images on left are of a control sample PBS wound healing (black is scratched area). Clear evidence of wound healing bridge formation seen at 48hr in control, with



72hr for more significant healing. The two right -most are of an experimental sample with PCCM-125. Total scratch healing occurs in 24 hr.

Treatment	Healing Rate um2/hr	Area Healed, um2	Percent Healed
Control - 48hr	479.2	22,999.574	36.0%
Control- 72hr	554.4	39,919.977	62.5%
PCCM-125-24hr	3882.6	93,182.4	100%
Percent Increased Healing Rate with PCCM-125			700.2%-810.3%

Table 1. By using Image J, healing rate over time was shown to be 7-8 faster in the PCCM-125 treated samples than the control samples.

Discussion: PCCM-125 markedly enhances NO delivery, showcasing a sustained release profile in STF, evidenced by a steady nitrite level and a gradual nitrate increase over 48 hours. Therapeutic effect of PCCM-125 was evaluated in the MATTEK Human Epicorneal model and was found to be significantly faster than control samples. The cornea is avascular and healing here is evidence of direct cellular regeneration harnessed by PCCM-125, presumably due to the plasma-generated NO stored within them.

Using Principal Component Analysis to Investigate the Morphological Changes in Cells Exposed to Non-Thermal Plasma

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Principal component analysis (PCA) is a multivariate statistical technique used to analyze large and complex datasets. In cellular analyses, large datasets that involve numerous variables can be challenging to visualize graphically and summarize to draw conclusions. PCA reduces the dimensionality of large datasets, allowing for easier visualization and interpretation of key variables. For instance, clusters and outliers can be more easily distinguished graphically, and correlations between these clusters and the variables can help form conclusions. PCA transforms the dataset into a smaller set of uncorrelated variables called principal components, which attempts to explain key features and accounts for maximum variance in the original data. This type of analysis is already used in various fields, including machine learning, image processing, and genome research [1]. We believe PCA can be used to form conclusions about the biological effects of non-thermal plasma (NTP) on cellular targets. NTP is used in many biomedical applications, including wound healing, cancer treatment, and in infections. NTP exposure changes cell morphology (e.g., shape and size), but the mechanisms of interaction between NTP and biological targets are not fully understood. The goal of our study was to investigate the morphological changes by PCA in immune cells following their exposure to NTP. This study utilized Jurkat T lymphocytes, a human leukemia cell line. Following NTP exposure (microsecond floating electrode dielectric barrier discharge (FE-DBD)), Jurkat cells were imaged immediately after and up to 24 hours post-NTP application. Image analysis revealed changes in cell morphology (size, area, perimeter, and circularity) that varied over time in response to NTP exposure. Using PCA, this data generated plots that allowed us to discover trends to discern between morphology and NTP exposure times. From these trends, the relationship between changes in cell morphology and changes in conductivity can also be investigated [2,3]. Our results help further our understanding of plasma dose and the mechanism of plasma action.

This work was supported by the Institute for Molecular Medicine and Infectious Disease and the Department of Microbiology and Immunology in the Drexel University College of Medicine. Research reported in this publication was supported by the National Institute of Biomedical Imaging and Bioengineering of the National Institutes of Health under Award Number R01EB029705. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Plasma-treated hydrogels: manufacturing therapeutic 3D scaffolds for tissue regeneration

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The therapeutic delivery of stem cells has demonstrated great potential as an effective therapeutic approach to repair and regenerate diseased, damaged, or aged tissues. However, key challenges in advancing stem cell therapeutics arise before and after cell transplantation concerning the poor survival and retention of cells, the uncontrolled cell differentiation and the insufficient integration with the target host tissue [1]. To overcome those limitations, plasma-treated hydrogels (PTHs) can be used as cell carriers, providing a protective environment that mimics tissue microenvironments, and integrating biochemical cues that support cell survival, while fostering these cells to restore or replace the function of the target tissues. Currently, 3D bioprinting is emerging as a novel technology for the advanced manufacturing of cell-laden constructs in tissue engineering and regenerative medicine applications. One of the greatest challenges in 3D bioprinting is the elaboration and optimization of printable biocompatible hydrogel formulations [2].

In this regard, the purpose of this study is to develop biocompatible PTHs suitable for the 3D fabrication of therapeutic scaffolds as plasma-activated cells carriers to improve the physiological outcome of stem cell transplantation for regenerative purposes. In this work, we assessed the compatibility of Pluronic/Hyaluronic acid/Alginate composite hydrogels with plasma treatment, cell embedding and 3D bioprinting processes. To this end, we measured the rheological properties (viscosity and recovery capacity) of the hydrogels to adjust the concentration of each component to obtain printable formulations. Here, a rheometer AR100 (TA Instruments) was employed to evaluate the viscosity profile through shear rate sweep from 0.1 to 100s-1, and the recovery capacity through three-step oscillation measurements (strain 1%-500%-1%) at 1Hz during 60s. Then we evaluated the effect of plasma treatment of the formulations in terms of chemical composition (quantification of plasma-generated RONS) and printability (rheological properties). Our results indicate that the Pluronic/Hyaluronic acid/Alginate composite PTHs can be enriched with plasma-derived RONS, while maintaining great viscoelastic properties for successful fabrication of scaffolds through 3D bioprinting process.

Acknowledgements

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Novel skin model for plasma medicine and cosmetics

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Cold atmospheric pressure plasma (ionized gas) is being researched worldwide as an innovative instrument for medicine and cosmetic applications. Most plasma-assisted treatments are mediated by the mutual interaction between the plasma and the patient skin. Thus from a certain point of view, cold plasma could be assimilated to a transdermal drug delivery device. This project aims at verifying if synthetic skin models developed for testing transdermal drugs could be adapted to plasma medicine/cosmetics. The final objective is to validate a synthetic model that would prove simple to use, reproducible and representative of the actual skin-plasma interaction. Thus, skin models (i.e. synthetic and *ex vivo* pork skin) were mounted on classical Franz diffusion cells modified to include an electrical compensation circuit mimicking the impedance of a human body [1]. The result is a model with surface composition, temperature and electrical impedance as similar as possible to that of human skin. This novel model was used to investigate the transdermal diffusion of caffeine and fluorescein after plasma exposure to a helium plasma jet. Electrical measurements and OES analysis were performed to confirm that the plasma generated in contact with the model was very close to the one generated on a human patient. Comparison with previous results obtained on skin cell monolayers, 3D models and skin explants [2] demonstrates the validity of the proposed models to study transdermal diffusion.

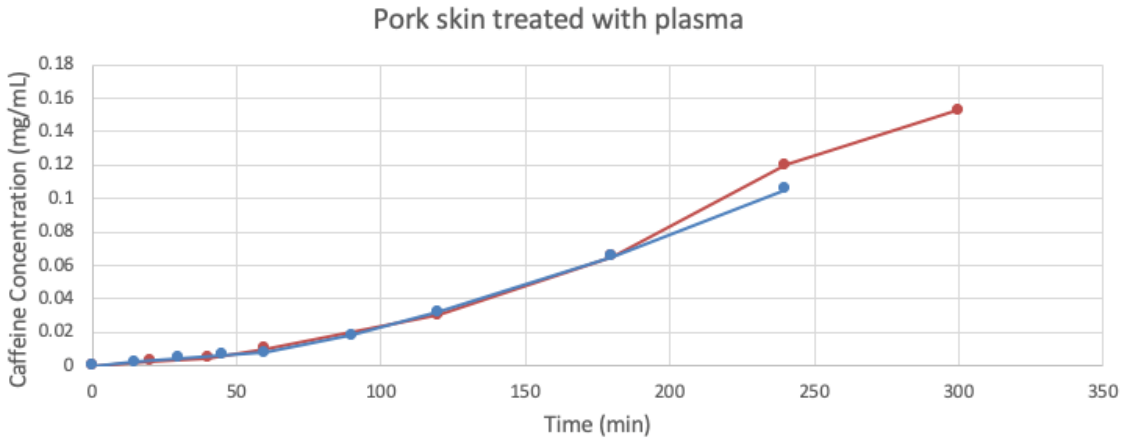

Fig. 1 Transdermal diffusion of caffeine on pork skin model treated for 1 min for two different set of parameters: (blue) 20 kHz, 10 kV, 1.5 cm; (red) 20 kHz, 14 kV, 1 cm

This work was supported by the ANR-23-CE04-0003 "PLASMASOL" and ARD COSMETOSCIENCES MINIONS

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Multipectrode DBD Plasma Torch Design with Drug Delivery Option for Wound Healing and Sterilization

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Dielectric Barrier Discharge (DBD) plasma is widely known for its non-equilibrium/ non-thermal conditions. That makes this plasma suitable for medical applications, especially in accelerating wound healing and sterilization. For this purpose, the DBD plasma jets are generated by using a dielectric tube that contains an inner electrode along with an outer electrode that is wrapped around the outer surface of the dielectric tube. Due to their relatively low ionization potential, helium or argon gasses are usually employed as the working gas and high voltages (up 5-10 kV, 20 -50 kHz) are applied to ionize the gas inside the plasma torch that ejects the plasma jet that mixes with the ambient air as it ejects from the torch. The plasma jet entrains air and generates oxygen and nitrogen radicals that play a significant role in the wound healing process. Our work shows that RONS radicals can be altered without altering the operating conditions by selecting the multiple outer electrodes mounted on the plasma tube. This particular feature was adopted in our multi electrode torch design. In addition, any inclusion of oxygen or nitrogen (between 0.1 to 0.2 slpm in helium 16 slpm flow) can impact various emission lines (nitrogen second positive system, OH radicals, atomic oxygen lines, etc). This was observed by monitoring the emission spectrum of the plasma while introducing oxygen or nitrogen into the main flow. This method allows us to optimize the plasma conditions suitable for wound healing and sterilization. The research presented in this work also addresses the issue of adding medication that may spread across the wound surface as the wound is exposed to the plasma jet. The ways to introduce liquid drops into the plasma were investigated. Preliminary work found that bigger liquid drops can extinguish the plasma momentarily and can discontinue the plasma jet. To overcome this problem, smaller diameter drops (250 to 500 microns) were generated inside the plasma. Imaging shows the drops propagating through the plasma until they impinge on the surface. During the liquid drops entering the plasma, observations were made to identify any changes in plasma characteristics like reduction in plasma luminosity, plasma emission spectrum, and plasma temperatures (gas temperatures). This research provides us a new avenue to design plasma torches with the option of adding liquid medicine to spread across the wound surface. Details on this new plasma torch design and its operation with and without liquid drops will be described. In addition, methods to have better control and precision in drug delivery will also be added in the final poster.

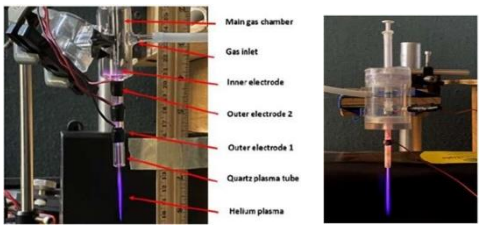

Fig. 1 Various Plasma Troches Developed for this Research

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ICPM

PLASMA-CELL AND PLASMA-TISSUE INTERACTIONS – BIOLOGICAL AND BIOCHEMICAL REACTIONS

Oral session (Thu - O - 8)

Thursday, 12 September 2024

Plasma-activated biogel for dermatological treatment

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Cold atmospheric plasma is a fledgling therapeutic technique for dermatological treatment with noninvasiveness but clinical adoption has been stifled by the insufficient production and delivery of plasma-generated reactive oxygen and nitrogen species (RONS). [1] Herein, plasma-activated biogel (PAB) is proposed for treatment of various skin diseases as an alternative to direct plasma irradiation treatment. By regulating the air discharge plasma to work successively in O_3 mode and NO_x mode, abundant high-valence RONS are produced and loaded into PAB through the complex gas-gas and gas-liquid reactions. [2] *In vitro* data show that the PAB possesses excellent storage capability, allows for slow release of plasma-generated RONS, and exhibits good antibacterial or anticancer effects. [3] Our results further indicate that the topical application of PAB to skin disease location has significant anti-inflammatory and therapeutic actions to acne, ulcers and dermatitis *in vivo*, and the implant of PAB under skin of melanoma-bearing mice could inhibit tumor progression effectively. Moreover, the preliminarily clinical research also verified that PAB is an effective clinical intervention to treat facial acne, psoriasis, ulcer, folliculitis and dermatitis in addition to excellent biosafety and absence of toxic side-effects (as shown in Fig. 1). It is demonstrated that the PAB could regulate oxidative stress in skin lesion tissues, while efficiently reduce the bacterial load and inhibit the release of inflammatory factors. Therefore, PAB is highly likely to become a safe and effective new method for dermatological treatment.

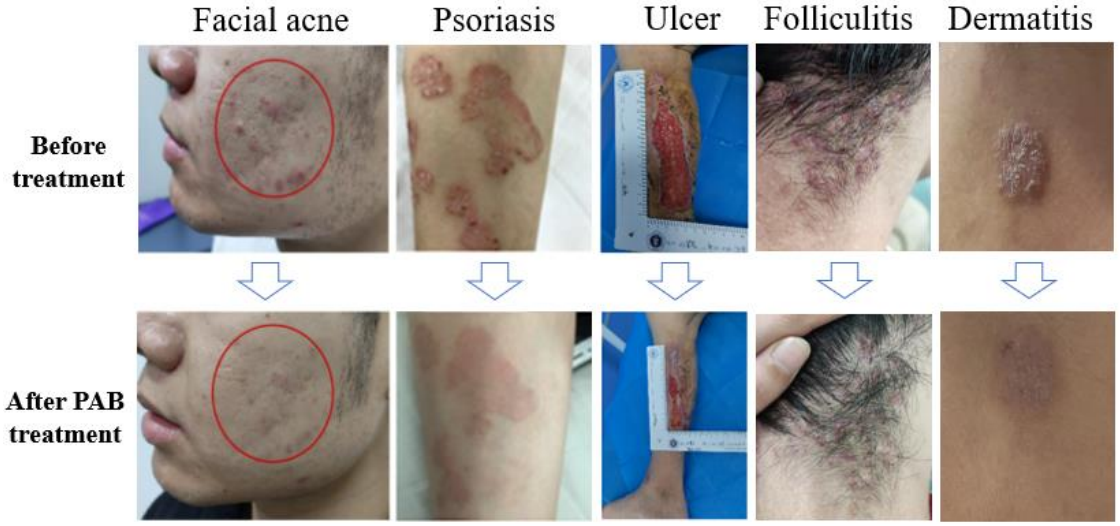

Fig. 1 Clinical treatment of facial acne, psoriasis, chronic ulcer, folliculitis and dermatitis by PAB.

This work was supported by National Science Foundation of China (No. 12175175).

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A Cold Atmospheric Plasma Jet – Composite Hydrogel Dressing for Controlled delivery of Platinum based Drugs into the Epithelium

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A composite hydrogel consisting of particles of drug load sodium polyacrylate particles (PAA) dispersed in a carrier gel of cryo-cross-linked poly-vinyl alcohol (PVA) has recently been developed by our group (figure 1) and its efficacy in delivering the antibiotic gentamicin for control of microbial infection demonstrated. [1]. The basis of the technology is the entrapment of small molecules, notably with amine functional groups in the PAA particle by Coulombic interaction. Application of a Cold Atmospheric Plasma (CAP) argon jet onto the drug loaded gel effects-controlled release of the drug from the composite hydrogel. We have demonstrated the technology works with antimicrobial drugs: gentamicin, octenidene, polymyxin B; ionic silver; amine functionalized b-cyclodextrin and recently platinum complexes used as chemotherapeutic drugs.

Platinum based anticancer drugs are some of the oldest chemotherapeutic drugs, with cis-platin first licensed for medical use in 1978. The platinum-based drugs are effective, but all are quite cytotoxic, especially when used systemically. Topical application has been explored, but the difficultly has been ensuring sufficient tissue penetration. In this presentation we will show that cis-platin can be loaded into a composite hydrogel and delivered on demand by application of the CAP to the composite hydrogel. Recent histological studies on ex-vivo porcine skin with a model cationic drug, rhodamine B, suggest using CAP allows delivery of the molecule across the epidermis into the deeper tissue (figure 2).

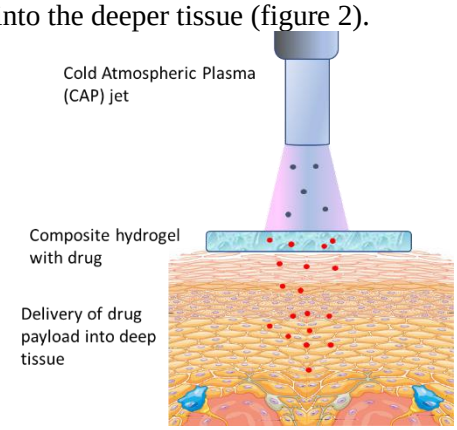

Figure 1: Schematic of CAP jet and composite hydrogel drug delivery system

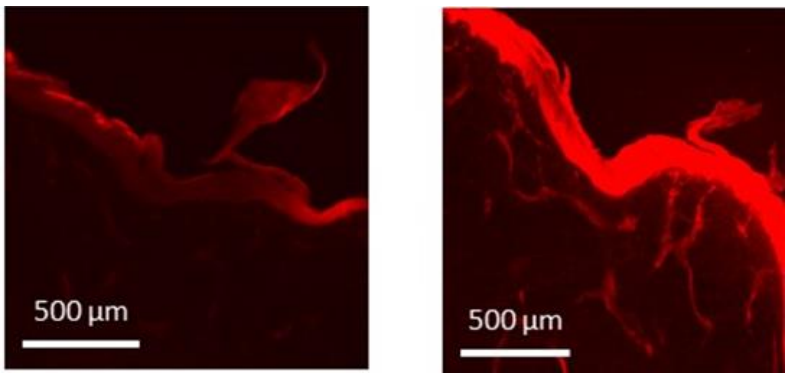

Figure 2: Histology of porcine skin biopsies showing CAP aided delivery of model drug, rhodamine 6G from a composite hydrogel deep into subcutaneous tissue (right) vs non-plasma activated control (left)

This work was supported by EPSRC grant: EP/V00462X/1

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CAP impact on the primary teeth enamel

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The outcomes related to the application of low-temperature plasma generated in a dielectric barrier discharge jet reactor [1] on specific characteristics of primary teeth enamel are discussed. Substrate gas was mixture of helium and oxygen with flowrates of 1.667 dm^3/min and 0.013 dm^3/min, respectively. Following exposure to the plasma for specified durations, parameters including color, surface roughness and topography, water contact angles (WCA) and elemental composition were assessed.

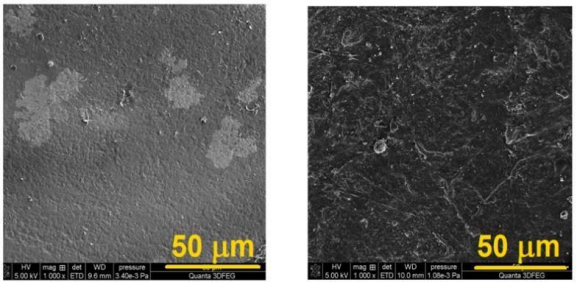

Fig. 1 Micrographs of teeth surfaces before and after 10 min CAP treatment

Treament time	Before treatment (1 s after putting a drop)	Immediatly after treatment (22 ms after putting a drop)	96 hours after treatment (1 s after putting a drop)
10 min.			

Fig. 2 Photos of drops during WCA measurement before and after plasma treatment

Cold atmospheric pressure plasma (CAP) caused an increase in the surface microroughness (Fig.1). Extended treatment time leaded to morphological alterations. An immediate enhancement in the wettability of the enamel surface (Fig. 2) and lighter color (L* parameter) of the surface compared to the control were observed.

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Direct Cold Atmospheric Plasma Treatment vs Plasma-activated Hydrogels in Wound Treatment

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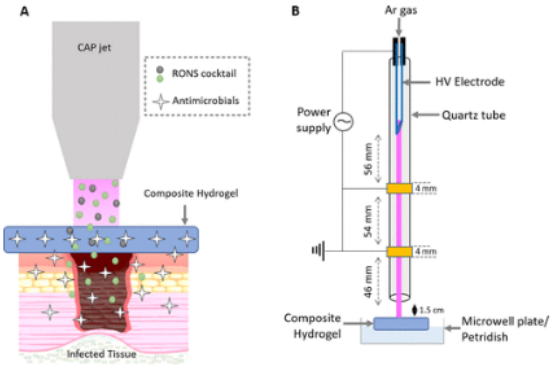

Figure 1. (A) Illustration of the concept of CAP-activated composite hydrogel therapy for an infected tissue. (B) Schematic of the Ar CAP jet operated at a gas flow rate of 1 SLPM, peak-to-peak voltage of 7 kV, and frequency of 23.5 kHz. The composite hydrogel is placed on a target (aqueous solution in a microwell plate or microbes in a Petri dish). The schematic is for illustrative purposes only and does not represent the exact laboratory treatment conditions.

Plasma activated hydrogel therapy (PAHT) is a very recent development in the use of cold atmospheric plasma (CAP). To the best of our knowledge the first description of the concept appeared in the patent US2023028285630A. Over the past 8 years, PAHT has been developed for a variety of diseases including fungal infections, vitiligo, and cancers [1]. In this presentation we will show several specific advantages of PAHT in wound decontamination and healing. Direct application of CAP is unfiltered and, in this context, may be more disruptive of biofilms and effective in killing of embedded microorganisms. However, direct CAP also has the potential to dehydrate the wound, either from the gas flow (in some devices several litres/minute) or through localized heating. The direct gas flow in CAP can also de-oxygenate the wound site through sparging. In PAHT, as shown in Fig 1 [2], the hydrogel protects the wound from the more reactive CAP components, provides a reservoir for localized and sustained delivery of reactive oxygen and nitrogen species (RONS), and can be used to co-deliver a wide range of antimicrobials (e.g. silver, octenidine dihydrochloride, PHMB, and iodophores). Using the set up in Fig 1b, employing a range of analytical techniques (electrical, optical) and sensors (humidity, oxygen, pH and temperature) we show how PAHT, through manipulation of the CAP device and hydrogel, can be used to maintain a moist wound environment optimal for wound healing, whilst simultaneously oxygenating the wound and providing RONS and/or antimicrobials, at above the MICs/MBECs of most common pathogens, and in robust biofilm models reducing bacterial loads by 99.9%.

This work supported by EPSRC grant: EP/V00462X/1

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Absorption of FD-150 into White Blood cells by Microplasma

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Introducing drugs into white blood cells is one option for targeted therapy in medicine. These cells possess several advantages that make them ideal delivery carriers, including systemic circulation, high fluidity, natural delivery mechanisms, and the ability to pass through bloodstream or biological membranes.
In our study, a dielectric barrier discharge was generated using a thin-film electrode. Atmospheric air plasma was sustained by a positive saw waveform at a voltage of 4300 V (0-p) and a frequency of 5 kHz. We studied a Fluorescein Isothiocyanate-Dextran solution (FD-150 with a molecular weight of 150 kDa) at a concentration of 3 µl in 1 ml of growth medium applied to cells. Treatment times ranging from 1 to 5 minutes at distances of 1 mm and 3 mm from the cell medium were investigated. Additionally, we explored three incubation times with plasma-treated medium: 0 hours, 1 hour, and 2 hours. The absorption of FD-150 by cells was evaluated using a fluorescent microplate reader or flow cytometer, and the number of live/dead cells was determined using a commercially available kit with fluorescence dye to stain dead cells.
Due to the small dimensions of the plasma, direct treatment of the medium by the plasma did not occur. The number of cells absorbing FD-150 increased with treatment time, along with the number of dead cells. Prolonged incubation time with plasma-treated medium also adversely affected cell viability. Fig. 1A depict live non-treated cells and their fluorescence for FD-150 and Fig 1B shows increased fluorescence after 4 minutes of microplasma treatment. Fig. 1A depicts live non-treated cells and their fluorescence for FD-150, while Fig. 1B shows increased fluorescence after 4 minutes of microplasma treatment (measured by flow cytometry). Microplasma irradiation could enhance drug delivery into white blood cells, facilitating cell drug preparation.

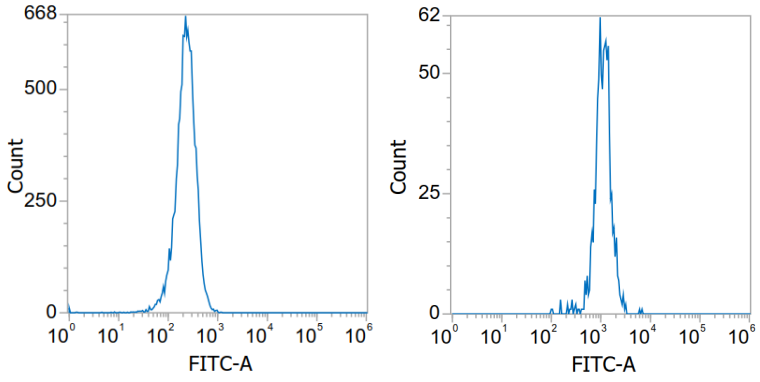

Fig.1: A. Non-treated cells with low fluorescence of FD-150 (lower than 10^3, fluorescence is on X-axis). B. White blood cells treated for 5 minutes shows high fluorescence (higher than 10^3, fluorescence is on X-axis).

Pores and Filaments – The quest for a reliable and reproducibly patterned plasma-permeabilization of the skin

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The transport of active pharmaceutical or cosmetic ingredients through skin permeabilized with atmospheric plasma has been achieved with a diverse range of plasma sources within the last 13 years in the field of plasma medicine. Several plasma jet systems, microplasma, arc discharges and volume discharges (direct dielectric barrier discharge – dDBD) have been tested [1,2] and both an electroporation-like mechanism as well as well as a mechanism based on (bio-)chemical modifications by plasma-species have been discussed.
DDBD sources have been shown to be highly effective in improving the transepithelial transport of hydrophilic and lipophilic model drug molecules [3] as well as that of cosmetically interesting molecules such as caffeine and hyaluronan. In our current research we aim to design an electrode that helps to create a structured filamentary discharge in order to improve reproducibility of electroporation-like permeabilization. Several imaging methods, (electro-)chemical and spectroscopic, permit us to visualize and quantify the pores, or permeabilized regions, created by a dDBD treatment both during the treatment and afterwards (Fig. 1).

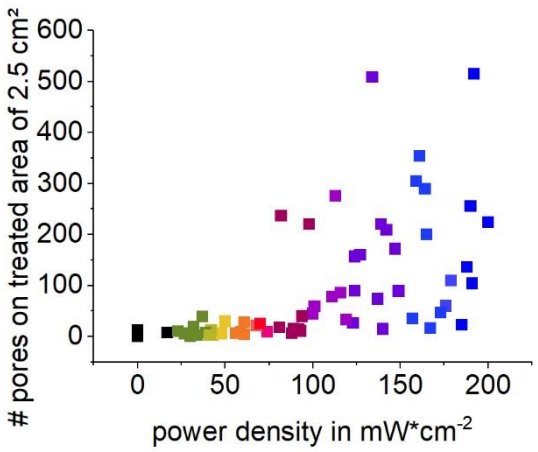

Fig. 2 The density of pores created in the dDBD treated area in relation to the power density measured during treatment.

Furthermore, dDBDs also pose great potential for the therapy of inflammatory skin disorders where a further damaging of the skin barrier should be avoided. Thus, we are investigating the alteration of skin barrier function by dDBD treatment both using *ex vivo* skin samples employing a wide range of power densities (Fig. 1) as well as treating healthy volunteers with dDBD sources certified as medical products.

This work is supported by the German Federal Ministry of Education and Research, Grant no. 03FHP179.

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Effects of Cold Atmospheric Argon Plasma Jet Treatment on the Biological Activity of Human Gingival Fibroblasts

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Soft tissue regeneration plays a crucial role after oral surgery, since the successful healing of the soft tissue is a primary indicator of an effective intervention. Recently, cold atmospheric plasma (CAP) has emerged as a promising therapeutic procedure, leading to notable effects on cell migration and proliferation [1,2]. Despite its potential, the application of CAP in dentistry remains underexplored. In the present work, the impact of CAP activated media on human gingival fibroblast responses was evaluated, for future wound healing strategies. The human gingival fibroblasts were exposed to complete DMEM medium (without sodium piruvate) previously activated with a cold atmospheric argon plasma jet device for distances of 2, 5, 7, and 9 mm, and with treatment times of 15, 60, 120, 180, and 300 s for 1, 2 and 3 days of culture. The cell viability was evaluated using resazurin-based method, while wound healing dynamics was assessed via the scratch assay technique using phase- contrast microscopy. The cell morphology was characterized through fluorescence microscopy using propidium iodide and phaloidin staining, complemented by scanning electron microscopy. The results revealed that treatment distance and exposure time may be influenced by cell concentration. Specially, in this study, prolonged exposures of 300 s to the CAP device resulted in a decrease of cell viability. The best results were observed with a cell concentration of $1x10^4$ cells/well compared to the other concentrations tested. For the ideal cell concentration, the treatment distance of 9 mm appears to improve human gingival fibroblast viability, while a distance of 2 mm did not significantly affect fibroblasts cells behaviour. The treatment time did not seem to be a significant factor for indirect CAP application, as both 15 s and 180 s did not statistically affect human gingival fibroblast viability.

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Modification of Cellular Receptors as a Potential Antiviral Mechanism of Non-Thermal Plasma

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The antiviral activity of non-thermal plasma (NTP) against cell-free viruses involves NTP-mediated damage to the virus structure, resulting in compromised virus entry into uninfected cells [1]. Recently, we demonstrated that NTP reduces the susceptibility of uninfected human keratinocytes (HaCaT cells) to herpes simplex virus type 1 (HSV-1) infection [2]. We speculated that delivery of reactive oxygen and nitrogen species (RONS) by NTP chemically compromises cell surface molecules involved in virus entry much like RONS damage virus particles and compromise their infectivity. Specifically, we hypothesized that NTP interferes with the first steps in infection by chemically modifying cellular proteoglycans involved in HSV-1 attachment and entry into uninfected cells. This hypothesis was supported by observations of reduced affinity of keratinocytes for antibodies specific to cell surface proteoglycans following NTP exposure. Additionally, we observed changes in glycan residues as measured by a lectin fluorescence-linked immunosorbent assay (FLISA) after NTP application to HaCaT cells. Cellular proteoglycans are also involved in the regulation of immune responses [3] and their modification may contribute to NTP-enhanced immunomodulation [4]. Collectively, our studies suggest that multiple mechanisms of action underlie the antiviral activity of NTP. The anticipated therapeutic effects of NTP, including interference with virus replication in infected cells, prevention of virus spread, and enhancement of host antiviral immune responses, indicate the potential of NTP as the basis of a treatment for HSV-1 infection [5].

This work was supported by an award from the Drexel-Coulter Translational Research Partnership Program and developmental support from the Institute for Molecular Medicine and Infectious Disease and the Department of Microbiology and Immunology in the Drexel University College of Medicine.

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Plasma Patch Device for Skin Disease Therapy

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Low-temperature plasmas have been applied to cure skin disease. Surface dielectric barrier discharge (SDBD) could be suitable method to make patchable plasma devices for bio-medical applications. In this presentation, we will report on the plasma patch device and the applications for human skin therapy. The plasma patch device was the isolated air discharge system in room temperature. The concentration of reactive oxygen and nitrogen species in the patch device were measured by optical absorption spectroscopy. Main species were reactive oxygen species due to room temperature operation. And, we applied the plasma patch to cure the skin diseases such as psoriasis. In animal experiment, the skin diseases were cured through recovery of ion channels, and there was no toxicity when treated for up to 10 minutes a day for a week.

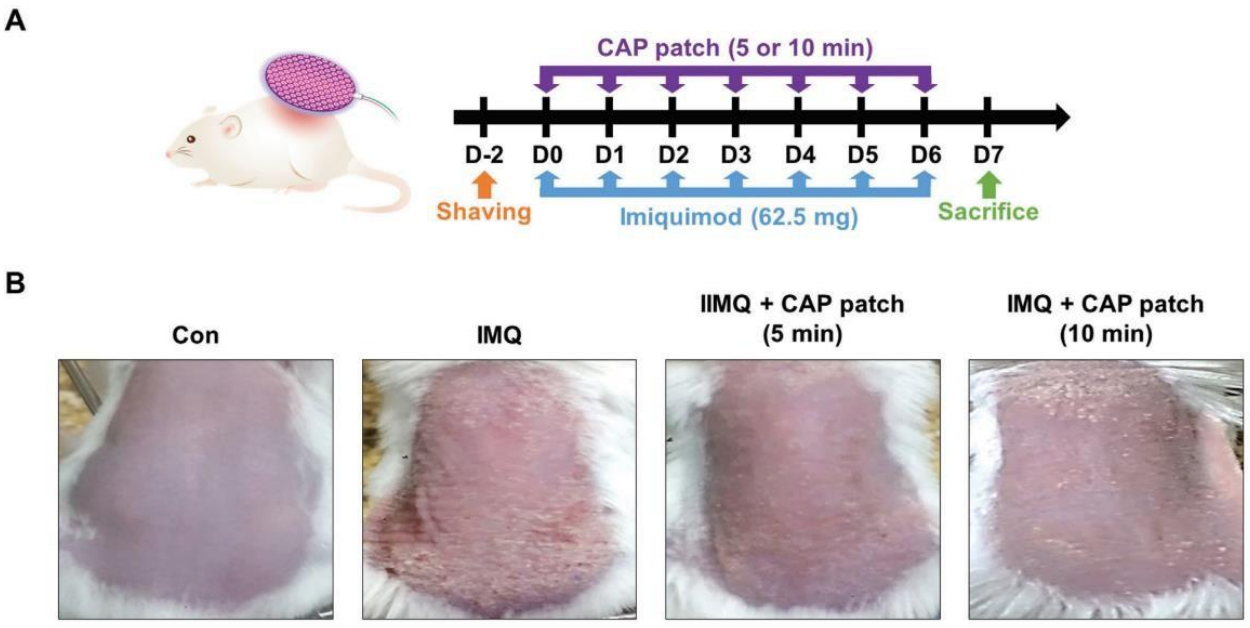

Fig. 1 CAP patch alleviated the psoriatic characteristics. A) The experimental scheme of CAP patch effect in IMQ-induced model. B) Phenotypic observations of dorsal skin.[1]

This work was supported by the Technology Innovation Program (20023929) funded by the Ministry of Trade, Industry & Energy (MOTIE, Korea)

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Thu-O-8-10

The Effect of Cold Atmospheric Plasma (CAP) and Plasma Activated Liquid (PAL) on the Proliferation of Mesenchymal Stem Cells (MSCs)

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Mesenchymal Stem Cells (MSCs) are multipotent cells that have the ability self-renew and to differentiate into multiple lineages. Due to their unique capabilities, MSCs have been intensely researched as a potential regenerative therapeutic agent [1]. Cold Atmospheric Plasma (CAP), which constitutes of ionized gas that contains electrons, electromagnetic fields, UV, charged and neutral molecules as well as Reactive Oxygen and Nitrogen Species (RONS) has wide applications in medicine, which include wound healing, cytotoxicity towards cancer cells, bactericidal effects as well as its ability to promote cell proliferation [2]. In addition to CAP, studies have also reported that Plasma-Activated Liquid (PAL) provides a more stable and easily applicable alternative to direct CAP[3]. This study aims to investigate the effect of CAP/PAL on Human Adipose-Derived Mesenchymal Stem Cell (AD-MSC) proliferation. Experiments are conducted using AD-MSCs obtained from commercial suppliers and cultured as per supplier's protocol. Plasma treatment is conducted using a helium-based Atmospheric Pressure Plasma Jet (APPJ) device (J-Plasma, Apyx Medical). For direct CAP experiments, cells are exposed directly to plasma at predefined parameters. For PAL treatment, liquids are exposed to direct CAP for different exposure times and reactive species are characterized using colorimetric assays for determination of hydrogen peroxide, nitrites and nitrates, following which cells are treated with PAL. Changes in cell metabolic activity are measured using Resazurin colorimetric analysis and changes in cellular mass are measured using Crystal Violet staining. The results of this study delineate direct and indirect plasma parameters at which AD-MSCs do not show signs of cytotoxicity. At lower CAP/PAL exposure times, which is associated with lower RONS concentrations, AD-MSCs retain their metabolic activities and cellular mass. This work determines safe CAP/PAL parameters at which further investigations of cellular changes can be conducted to further understand how CAP/PAL-generated RONS induce cellular proliferation, which could potentially be used to optimize and enhance the use of MSCs in regenerative and cellular treatments.

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Thu-O-8-11

Plasma jet characteristics and treatment consequences depend on target properties of several hydrogel and vertebrate tissue models

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As we know, physical cold plasma has been proven efficient in promoting wound healing [1] and helping neutralize cancer [2]. Nevertheless, the heterogeneity of those conditions still represents a challenge [3] as it may be translated by endurance upon plasma treatment over time. It was suggested that target properties such as electrical conductivity have an impact on the outcome in the case of conductive treatment [4]. Conductive treatment interestingly showed advanced performances on tumor toxicity compared to the non-conductive mode [5]. This study aims to better understand the effect of the target features, such as water content, elastic modulus, or conductivity, on ROS production and on-site temperature during conductive plasma treatment. To draw a parallel with clinical studies, we used the certified and well-characterized plasma jet kINPen MED operating with argon. Target properties were tuned by using simplified agarose-based hydrogel models in comparison with genuine tissues such as mouse organs or human skin. Hydrogels are materials of choice for tissue engineering. Assets such as biocompatibility/degradability, tunable features, and high availability support their use as tissue surrogates for countless applications in the bio-medical field, including plasma medicine research. Investigating the target temperature with infrared thermal imaging, we found a strong correlation between water content and the final hydrogel temperature. However, the correlation with tissues was not obvious, letting suggest additional involved properties such as conductivity. ROS production in the gas phase was investigated via Optical Emission Spectroscopy (OES). We observed significant differences in the ROS generation among the wide range of tissue types. This was supported by Principal Component Analysis (PCA) by the clustering or isolation of specific tissues.

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Thu-O-8-12

Oxidative damage in nucleic acids in human lung cancer cells irradiated with cold atmospheric pressure plasma

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Cold atmospheric pressure plasma (CAP) has emerged as a novel tool in medicine and life science research. For example, CAP irradiation has been proposed as a novel method in cancer therapy, wound healing, and infectious disease prevention. The biological effects are mainly due to CAP- induced reactive oxygen and nitrogen species (RONS). For example, CAP-delivered RONS in liquids increases intracellular RONS levels and stimulates various cellular responses.

Intracellular RONS can cause damage to biological molecules. For example, RONS induce oxidative damage to nucleic acids, including strand breaks and base modifications. CAP-induced DNA damage is a potential trigger for apoptotic cell death. We have reported that CAP treatment induced genomic DNA strand breaks and the formation of 8-oxoguanine (8-oxoG), a representative oxidized form of a base [1]. In addition, we observed that the 8-oxoG level decreased in living cells after CAP irradiation [2]. We also reported the influence of CAP treatment on 8-oxoG formation in mitochondrial DNA (mtDNA). Although 8-oxoG formation in DNA induced by CAP irradiation has been studied, there are few studies on RNA damage. This study investigated the damage of intracellular RNA after CAP irradiation.

An atmospheric pressure plasma jet (APPJ) was used in this study. We used A549 human lung carcinoma cells. Cell suspension prepared with D-PBS (-) in one well of a 24-well tissue culture plate was irradiated with APPJ. After CAP irradiation, immunofluorescence staining was performed to detect 8-oxoG. Fig. 1(a) showed that the fluorescence intensity was significantly increased in the CAP-irradiated cells compared to the untreated ctrl. In addition, RNA was extracted from the CAP- irradiated cells, and a slot blot assay was performed. Fig. 1(b) shows the results of the slot blot assay. Compared to RNA extracted from untreated cells, RNA extracted from CAP-irradiated cells showed higher signal intensity. It is concluded that CAP irradiation to cells induced base modification in intracellular RNA.

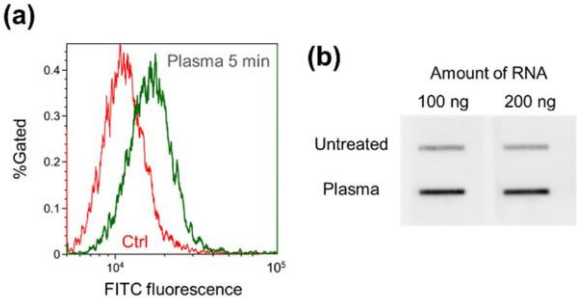

Fig. 1 Detection of 8-oxoG formation in CAP-treated A549 cells.
(a) Immunofluorescence staining (b) A slot blot assay

This work was partly supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS) and Amano Institute of Technology.

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Synergistic Enhancement of Antibiotic Efficacy Against Biofilms with Cold Plasma

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The escalation of antibiotic-resistant bacterial infections poses a severe risk to public health. Non-thermal cold atmospheric plasma presents itself as a promising adjuvant therapy, particularly against ESKAPE pathogens and biofilm-associated infections, such as those caused by methicillin-resistant *Staphylococcus aureus* (MRSA). This study explores the synergistic potential of cold plasma in combination with antibiotics, offering a novel approach to address microbial resistance.

A sub-lethal plasma regimen was applied to bacterial biofilms prior to the introduction of antibiotics, examining the combined effects through measurements of minimum inhibitory concentrations (MICs), minimum biofilm eradication concentrations (MBECs), and isothermal microcalorimetry. The study also utilized bioinformatic techniques to analyze the oxidative impacts on bacterial cell structures, gene expression changes, and the corresponding stress response.

Initial findings indicate that cold plasma pre-treatment notably increases the efficacy of antibiotic treatments, reducing MICs and MBECs significantly. The enhanced disruption of metabolic activity, implies that combined cold plasma and antibiotic therapy induces a distinctive response in biofilms, compared to either treatment alone. Gene expression analysis supports the hypothesis that plasma exposure induces an oxidative stress response, potentially disrupting outer membrane integrity and facilitating increased drug uptake.

The data supports the premise that cold plasma can act as a potent adjuvant to antibiotics, impeding biofilm resistance mechanisms and advancing the permeability and effectiveness of drug therapies. These promising results advocate for the inclusion of plasma in treatment protocols, potentially transforming the clinical management of stubborn, antibiotic-resistant infections. Moreover, the study underscores the necessity of understanding device-specific plasma interactions to fine-tune this intervention, optimizing its application against resilient biofilm-related infections.

This work was supported by National Institutes of Health (NIH) under award numbers RO1 AR076941 (NH and TF) and the Northern Ireland HSC Research & Development Division, Public Health Agency award STL/5350/17.

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Oral session (Thu - O - 7)

Thursday, 12 September 2024

Portable and affordable cold air plasma source for biomedical applications

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The low-cost handheld source of a cold air plasma intended for biomedical applications that can be made by anyone is reported. The plasma source based on our previous work [1] employs a DC discharge in the needle-to-cone electrode configuration and is an extremely simple device, consisting basically of two electrodes and a cheap power supply. To achieve the best bactericidal effect, the plasma source has been optimized on *Escherichia coli*. The bactericidal ability of the plasma source was further tested on a wide range of microorganisms: *Staphylococcus aureus* as a representative of gram-positive bacteria, *Pseudomonas aeruginosa* as gram-negative bacteria, *Candida albicans* as yeasts, *Trichophyton interdigitale* as microfungi, and *Deinococcus radiodurans* as a representative of extremophilic bacteria resistant to many DNA-damaging agents, including ultraviolet and ionizing radiation. The testing showed that the plasma source inactivates all the microorganisms tested, proving its effectiveness against a wide spectrum of pathogens, in particular microfungi, yeasts, gram-positive and gram-negative bacteria. To explore the performance of the plasma source, its diagnostics and characterization were carried out. Studies of long-lived reactive species such as ozone, nitrogen oxides, hydrogen peroxide, nitrite, and nitrate revealed a strong correlation between ROS and the bactericidal effect. Detailed technical information and a step-by-step guide for creating the NTP source are provided.

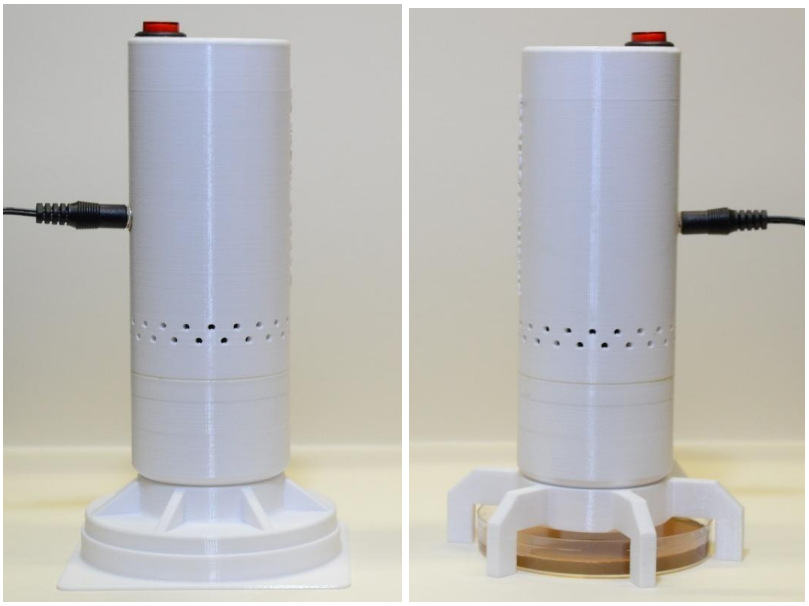

Fig. 1 The NTP source used in the closed-volume (left) and in the open-air mode (right).

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Cold plasma systems for bioaerosol decontamination: scale-up of a portable room-scale device

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The propagation of infectious diseases due to airborne transmission within indoor environments, particularly healthcare facilities, presents a significant public health concern with far-reaching societal and economic ramifications [1]. Cold atmospheric plasma (CAP) is a promising technology that can contrast airborne transmission. In literature, its efficacy in inactivating microorganisms stems from its capacity to generate many biocidal components, including reactive oxygen and nitrogen species (RONS), electric fields, and ultraviolet radiation [2][3][4]. Nevertheless, using CAPs to contrast airborne transmission of microorganisms and its associated problems is still in development and requires more research. More efforts are needed to improve the efficiency and scalability of CAP treatments for large areas and to investigate the specific mechanisms of CAP's antimicrobial action, as they are not yet fully understood.

This work presents the development of two new CAP devices for room air decontamination. The first is a small-scale CAP device that uses a particular dielectric barrier discharge (DBD) source architecture, and it is called Rotating DBD (RDBD). Considering various operational parameters, the investigation evaluates its antimicrobial efficacy against *Staphylococcus epidermidis* bioaerosol within a small stainless-steel airtight test chamber (0,09 m^3). Moreover, the ozone concentration generated by the RDBD plasma source within the chamber was quantitatively monitored employing optical absorption spectroscopy (OAS). The RDBD plasma source demonstrated a substantial logarithmic reduction in *S. epidermidis* exceeding Log 3.6. This correlation between the inactivation levels of *S. epidermidis* and the corresponding ozone concentrations produced within the test chamber suggests that ozone plays a critical role in achieving a high degree of bacterial inactivation. Furthermore, the microbial inactivation results obtained under various operating conditions indicate that the applied voltage does not directly influence the inactivation process. Finally, a scale-up system (InDuct Plasma Source) is proposed for treating air inside ducts (100 – 300 m^3/h). Preliminary results highlighted the InDuct Plasma Source's ability to inactivate *S. epidermidis* bioaerosol in a single pass through the plasma discharge zone. The study demonstrates the potential of plasma technology for air decontamination in various settings. The room-scale device represents a portable solution for air purification in residential and working environments. The duct system offers an innovative option for air treatment in ventilation and air conditioning systems.

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From Idea to Prototype – Clinic plasma treatment on-demand – the potential of the new portable device MOBIPLAS

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In today's world, where technological development is advancing relentlessly, it is crucial that we continuously search for new solutions to meet the challenges of our time. The different perspectives or promising fields of application in plasma medicine play a decisive role in the implementation of new ideas and the development of new prototypes [1]. The kINPen MED, which has already made its way into everyday clinical practice and is currently being used above all in wound healing [2], is just one of the significant successes that have already been achieved. This achievement is proof of the effectiveness and potential of new technologies in healthcare [3].
Our new prototype, the MOBIPLAS, builds on these successes and represents another step forward in medical plasma device development. Here, we will not only present the prototype but also provide an insight into the underlying plasma device development process, which has focused intensively on the possibilities and limitations of plasma technologies including the requirements of medical device development. Moreover, the device was studied for its static and dynamic optical emission and temperature profiles in presence and absence of a target along with patient leackage current analysis. In addition, the biological activity of this novel portable plasma device was investigated. Using human keratinocytes, the viability and metabolic activity along with mobility and migration of these cells upon plasma treatment was analyzed. Besides a basic characterization of intracellular and extracellular ROS production, inflammatory profiling (chemokine and cytokine release) was assessed as well. Finally, the irritation potential of the new plasma source was tested in the hen's egg test on chorioallantoic membrane (HET-CAM) as a prerequisite for safe application.

Fig. 1 Prototype MOBIPLAS

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Electrical, Optical and Thermal Characterization of Argon–Hydrogen Plasma Jets for Medical and Biomedical Applications

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Atmospheric pressure plasmas (APP) have been successfully employed in plasma medicine applications along the last two decades. There is a large number of devices that employ different working gases for producing the APPs, with argon (Ar), helium (He) and air being the most reported in the literature.

In this work we report on atmospheric pressure plasma jet (APPJ) operating with an argon–hydrogen (Ar–H_2) gas mixture (Ar = 96.5%, H_2 = 3.5%). The plasma jet was generated by a low-cost, flexible device reported previously [1]. Optical emission spectroscopy (OES) shows that under the same working conditions, the species excited in Ar and Ar–H_2 plasma jets are the same in both cases. However, the amount of the reactive oxygen species changes for each gas, with more OH being produced using Ar than with Ar–H_2, as it can be seen in Fig. 1. On the other hand, when the Ar–H_2 admixture was employed, a larger amount of excited hydrogen atoms was observed, from which it could be inferred that the production of hydrogen ions tends to be higher in this case. For a plasma source to be suitable for medical applications, the patient leakage current (*PLC*) produced when the plasma jet is in contact with the target must be lower than 100 µA–AC and the gas temperature (T_{gas}) needs to be lower than 40 °C. From Fig. 2 it can be seen that the Ar–H_2 plasma jet tends to present lower *PLC* and T_{gas} values than the Ar APPJ.

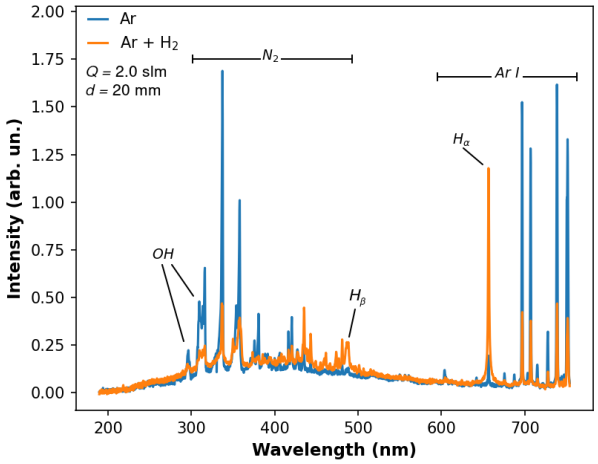

Fig. 1 Spectra overview

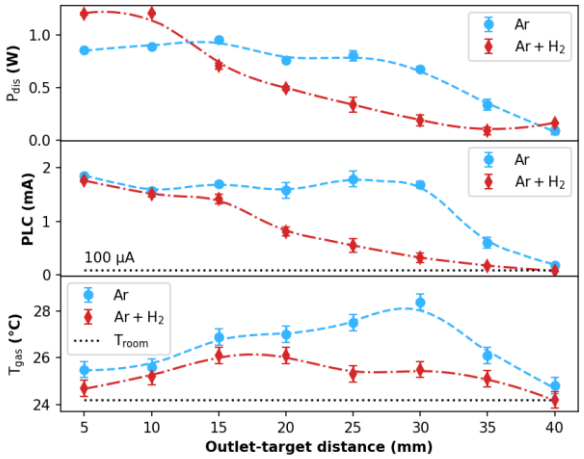

Fig. 2 P_{dis}, *PLC* and T_{gas} vs *d*

Based on the results in Figs. 1 and 2, the Ar–H_2 gas admixture appears suitable for biomedical applications such as microbial decontamination. However, under the tested conditions, it is not recommended for medical applications due to *PLC* values exceeding 100 µA.

This work was supported by the São Paulo Research Foundation–FAPESP under grants 2019/05856-7 and 2020/09481-5.

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System for In Situ Measurements During Skin Wound Treatment by Cold Atmospheric Plasma

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The use of cold atmospheric plasma (CAP) has gained increasing attention for the treatment of skin wounds. Initially, CAP was envisioned as a method for decontamination of wound surfaces, which would result in decreased risk of invasive infection and faster healing. However, evidence has accumulated that CAP may also be beneficial to wound healing, through mechanisms that are yet poorly understood, but may involve CAP-generated free radicals and short-lived reactive species. The outcomes of CAP treatment on wound healing are highly variable, partly due to the heterogeneity in wound etiology and presentation. Standardization of CAP treatment is also a major challenge, because CAP dose is dependent upon many factors, including the device and power characteristics used to generate the plasma, the distance between the CAP source and the wound surface, the composition of the gas and humidity surrounding the plasma generator, as well as the duration of treatment. In addition, the effects of CAP treatment may not be seen for several days to weeks post-treatment, and there is no way to adjust treatment dose in real-time. For further adoption of CAP, there is a critical need to develop methods that enable both (1) measuring the actual plasma "dose" received at the wound site, and (2) predicting the healing response as a function of administered dose.

In preliminary studies, we observed that CAP administered to excisional skin wounds in mice led to different responses depending upon CAP dose. There was a dose range within which a trend towards faster wound closure was observed compared to untreated control wounds. Below that range, no effect was observed, while above it, additional injury from the CAP treatment slowed wound healing. Thus, this mouse wound model was deemed appropriate for further studies that would correlate CAP dose with wound healing outcome. For this purpose, a set up was created that allows sensors into the treatment area that can be activated post CAP for rapid measurement of wound fluid concentrations (Fig. 1). The effect of CAP dose on intact skin as well as wounded skin (with wound located inside the silicone ring area) have been collected to establish the safe dose range in this modified system. Extracellular ATP measurements have been collected to optimize sensor location and establish feasibility of the measurement technique.

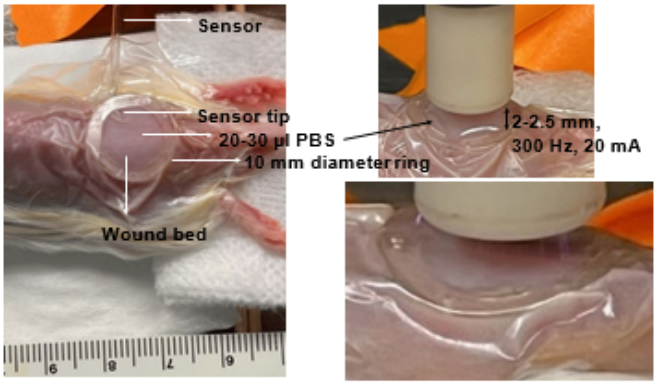

Fig. 1: CAP application on mouse skin. Skin was shaved and depilated one day before plasma treatment. A silicone ring was placed on the skin to hold a small quantity of PBS that allowed for sensors to detect species released into the environment by the CAP treatment. Distant between skin and plasma generator was 2 to 2.5 mm. Streamers were visible during CAP treatment.

This work was supported by National Institutes of Health Grant no. R01EB029705.

Computational study of a pulsed atmospheric pressure dielectric barrier discharge interacting with liquid

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An important plasma source within the plasma medicine field is the dielectric barrier discharge (DBD), as it can operate at low plasma temperatures and is resistant to arcing [1]. Various configurations of the DBD are feasible; however, the plasma medicine field frequently incorporates a liquid medium as a second dielectric barrier covering the ground electrode. Consequently, the liquid is directly exposed to the reactive species created by the plasma. There is substantial interaction between the plasma and the liquid, due to the physical and chemical processes that take place at the plasma-liquid interphase, including evaporation of water into the plasma region and solvation of ions and neutral species [2].

The transition from the gas phase to the liquid phase is particularly interesting to study, as various physical and chemical processes take place there. The inclusion of this liquid phase presents a challenge to the existing models, as the liquid component contributes to power depletion and alteration of the properties of the plasma over time. Additionally, the liquid undergoes vaporization, leading to increased humidity in the vicinity of the liquid phase, thereby altering the plasma characteristics [3]. The humidity of the air can have a significant effect on the reactive species that are formed, so it is important to take this into account. Furthermore, plasma treatment alters the pH value of the water, consequently affecting its conductivity [4].

Very few works have computationally investigated the interaction between pulsed DBD plasma and water surfaces [3, 5]. In this work, we constructed a one-dimensional model of a pulsed DBD operating in air at atmospheric pressure using COMSOL Multiphysics 6.2. The DBD is operated with multiple pulses of approximately 26 kV amplitude at a frequency of 500 Hz, as was measured during experiments [6]. The effect of the water on the plasma properties was captured in a self-consistent manner, revealing the underlying mechanisms driving the fundamental plasma processes.

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Computational modelling of *in-vitro* treatment with a plasma jet: elucidating the effects of the treatment setup

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Experimental research into the treatment of biological systems with cold atmospheric plasma (CAP) is still most often performed *in-vitro*, where cells are treated in well plates, usually covered by a liquid such as cell medium. In this system, many different phenomena are at play, including the chemical reactions occurring in the plasma itself, the interaction of the plasma with the liquid, and the effective dissolution of the plasma-produced species into the liquid [1], which together determine the outcome of the treatment. When the plasma is generated using an atmospheric pressure plasma jet (APPJ), such as the kINPen®, the treatment is additionally influenced by the mixing of the plasma effluent with both the surrounding gas and the evaporating liquid [2]. Because these phenomena happen simultaneously, and affect each other, it is not straightforward to investigate their individual influence on the treatment.

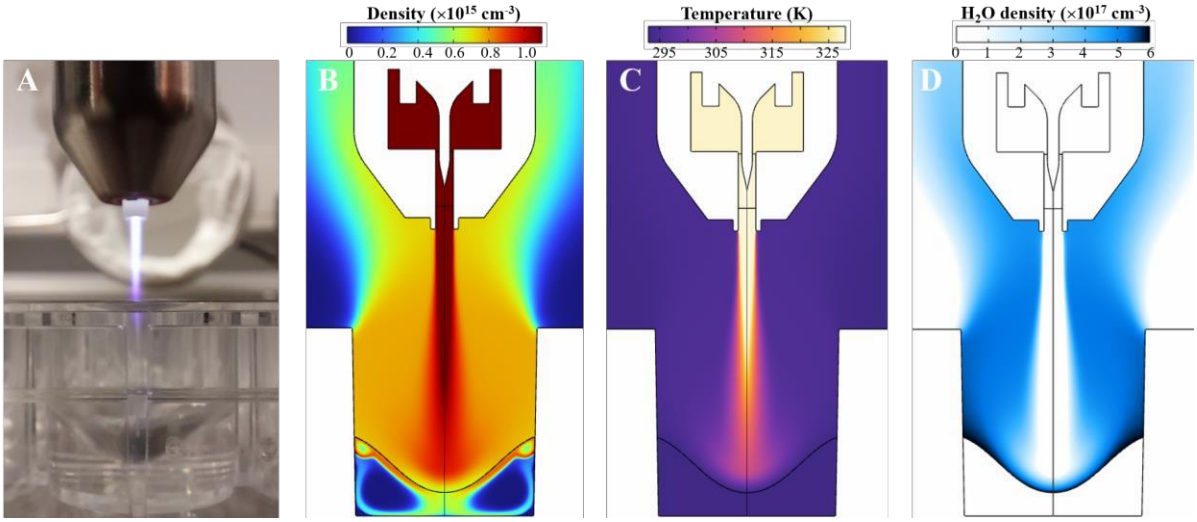

Fig. 1 Representative experimental setup (A), with RONS dissolution (B), temperature (C) and water evaporation (D) in the system as calculated by the model.

Computational modelling provides a valuable tool for elucidating the different processes that affect the plasma treatment. To this end, we developed a 2D-axisymmetric model of the kINPen plasma jet above a well filled with liquid. With this model, we investigate the various physical and chemical phenomena that occur during CAP-treatment. We show how the choice of treatment setup, such as the well size, can affect the treatment itself, and investigate the effectiveness of a shielding gas for different setup geometries. In addition, we investigate in detail the dissolution of plasma-produced RONS into the treated liquid. Our findings provide a deeper understanding of how the chosen setup geometry can influence the plasma treatment, even when all other operating parameters are unchanged. This understanding is especially important for interpretation of experimental results, and standardization in the field.

This work was supported by the Fund for Scientific Research (FWO) Flanders (Grant ID 1100421N).

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Developing a conductive treatment method for pathological specimens using plasma for the diagnosis using electron microscopy

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Thin sections of formalin-fixed paraffin-embedded (FFPE) tissues on glass slides provide crucial morphological information for life science research and are commonly used for pathological diagnosis. On the other hand, with the advent of spatial transcriptome analysis that carries on FFEP thin sections on slide glasses in recent years, there is now a growing demand for high-resolution observation against the spatial transcriptome analysed samples using scanning electron microscopy (SEM) and atomic force microscopy. However, as both thin sections of FFPE and glass slides are dielectric and can quickly become charged under observation by SEM, it is even challenging to take high-resolution morphological information. Moreover, though energy-dispersive X-ray analysis (EDS) may obtain elemental images as the results of antibody reactions, it is difficult to show the presence and distribution of protein molecules the same as fluorescence microscopy due to the interfering with the analysis by charging up of slide-glass under observation.

This presentation will show the establishment and use of a plasma treatment technique for processing glass slide FFPE specimens with OsO4 and aniline as materials. The plasma-formed Os76 film-forming method has enabled SEM observation of lung tissue from mouse models of asthma and analysis of skin ulcers from patients with Werner's Syndrome, a genetic disorder. In particular, we revealed that calcium phosphate deposits cause ulceration in Werner's syndrome [1, 2]. On the other hand, the visualisation of virus particles, which requires a high magnification of more than 10,000 times, has been achieved by establishing a conductive treatment method in which carbon plasma is generated at high voltage and high pulse. Figure 1 shows the visualisation of the presence and spread of virus particles budding in the tunica media of the pulmonary vascular wall using antibody staining against the SARS-CoV-2 spike protein. Thus, the use and sophistication of plasma technology have achieved the high-resolution observation required for morphological studies.

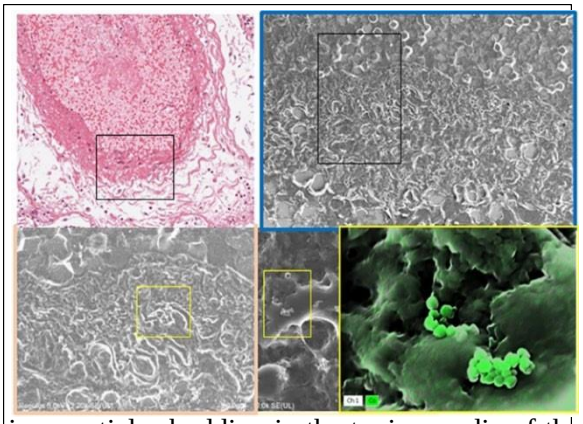

Figure 1 shows the visualisation of the presence and spread of virus particles budding in the tunica media of the pulmonary vascular wall using antibody staining against the SARS-CoV-2 spike protein.

This work was supported by the JST-Moonshot R&D (No. JPMJMS2025) and Grants-in-Aid for Scientific Research (20K08351, 23K07455), AMED-CREST (No. JP20gm1210003)

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Characterization of 3-pin Atmospheric Pressure Plasma Jet used for production of Plasma Activated Water by ICCD imaging

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The gas phase plasma chemistry at atmospheric pressure is a complex and rich environment containing enormous amounts of radicals as well as ions, metastables, UV rays etc. When in contact with liquid the reactions and chemical processes become even more complex with many short living and long living species being deposited in the liquid [1]. This plays a crucial role in applications of atmospheric pressure plasmas (APP) in medicine and agriculture [2, 3]. In order to understand the processes that lead to production of Plasma Activated Water (PAW) it is necessary to characterize the APPs in greater detail and try to find a most suitable diagnostic method which will give an indication of plasma efficiency without disturbing the treatment process. One of these diagnostic methos is electrical characterization i.e., more precisely determination of power deposited in the discharge in contact with liquid [4]. The second one can be optical emission spectroscopy (OES) and/or ICCD imaging. Like electrical characterization it does not disturb the discharge and does not interfere with treatment of liquid target (water).

Here we will present results of OES and ICCD imaging of multi-needle (3-pin) electrodes-APPJ. Three syringe needles were placed coaxially inside the glass tubes and distanced 20 mm apart. The needle tip was retracted 7 mm with respect to the end of each. The distance between the tip of the needle and the sample was fixed to 15 mm for each needle. The copper tape was used as a ground electrode by wrapping it around the bottom of the sample vessel containing 15 ml of distilled water. We have used argon gas with a total flow rate of 2 slm and 3 slm. For plasma imaging, an ICCD camera was used to obtain the necessary temporal and spatial resolution for imaging the plasma jet. The ICCD camera's lens was positioned side-on to the plasma jet observing the total length of the streamer between the electrode tip and the water surface. Band pass filters (BPF) were placed in front of the objective to single-out the spatial emission pattern at selected wavelengths, i.e., coming from different excited species (e.g., $HO^{\bullet}$, $O^{\bullet}$, $H^{\bullet}$, N_2, Ar). The highest $HO^{\bullet}$ and N_2 (SPS) emissions were detected close to the liquid surface which was expected. In this case higher intensity was observed for lower argon flow rate. Similarly, emission of H_α was more visible for lower argon flow, in lower part of the streamer and it became less intense at higher argon flow rates. On the other hand, the higher intensity of $O^{\bullet}$ and excited argon was visible in the central part. The emission profile of excited argon lines was highest in the vicinity of the needle electrode tips with reducing intensity when approaching the liquid surface for both flow rates [5].

This research was supported by the Science Fund of the Republic of Serbia, 7739780, APPerTAin- BIOM project, MSTDI-451-03-68/2022-14/200024 and Project Nowelties.

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Multimodal linking of biological imaging data in plasma medicine

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Research in plasma medicine is a multidisciplinary field dedicated to investigating the impacts of cold physical plasma on biological systems for medical purposes [1]. As the complexity and volume of data grow, arising not just from the plasma side utilized in wound healing and cancer treatment but also from diverse imaging modalities – including microscopy, spectroscopy, and tomography – within plasma medicine research, the availability and use of robust Research Data Management (RDM) tools have become essential. The incorporation of plasma parameters and treatment procedures for investigating the impact of plasma properties on treated targets (*in vitro* and *in vivo*) requires the utilization of RDM solutions interlinking metadata and datasets from various measurements. This leads to efforts focused on creating image objects adhering to the FAIR principles [2] (Findable, Accessible, Interoperable, and Reusable), tailored for linking multimodal data. This contribution introduces activities aiming at the standardization of bioimaging data in plasma using the OMERO platform [3], and the electronic lab notebook (eLabFTW) integration [4]. This framework enhances communication and interoperability across domains, benefiting fields such as cancer medicine and plasma medicine. OMERO serves as an RDM tool streamlining access to stored image data. In phase **I**, REMBI-compatible metadata requirements along with the ISA metadata standard were implemented throughout the metadata collection (Fig. 1). The domain-specific (plasma medicine) and generic/method-specific (bioimaging) RDM tools such as INPTDAT [5], Micro Meta App [6], and Adamant [7] will be used to collect related enriched metadata. The collected metadata will be incorporated into OMERO (phase **II**) as key-value pairs to describe the bioimage along with the associated study details. The enriched metadata in OMERO will also be linked with their specific metadata in plasma medicine by interlinking with the eLabFTW. During phase **III**, post-processing efforts are directed towards enhancing the FAIR attributes of image metadata within the OMERO platform.

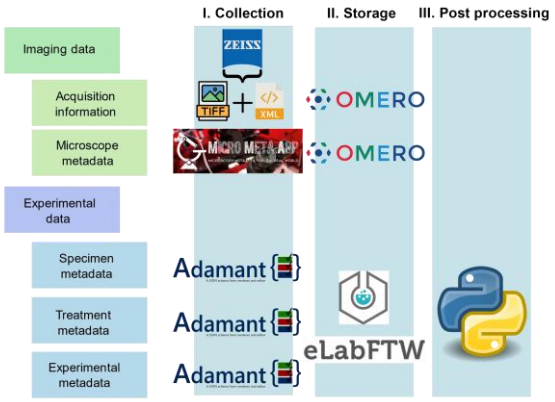

Fig. 1. RDM procedure for implementing REMBI-compatible metadata into OMERO.

The work is funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under the National Research Data Infrastructure – [NFDI46/1] – 501864659.

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icpm 10 & iwpct 9

8 - 13 september, Portorose, Slovenia

ICPM

PLASMA FOR PHARMACEUTICAL APPLICATIONS, BIOCHEMICAL AND BIOMOLECULAR ENGINEERING

Oral session (Thu - O - 9)

Thursday, 12 September 2024

Effects of OH radicals on Plasma Gene/Molecular Transfection

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We have developed novel plasma gene delivery methods using gas discharges [1]. It has been shown that the combined action of electrical and chemical stimuli induces endocytosis, a biological response, and transport of external macromolecules such as genes into the cell and that the chemical stimuli required for transduction are related to reactive oxygen species (ROS) from previous studies [2]. However, several kinds of ROS are generated by plasma treatment, and it is necessary to clarify which ROS influence gene transfer was investigated. In this study, the effect of hydroxyl radicals (·OH), which have exceptionally high reactivity among ROS, on gene transfer was investigated.

L-929 cells, which are mouse fibroblasts, were used as target cells. NAC (N-Acetyl-L-cysteine), which inhibits ROS, and L-cysteine hydrochloride monohydrate, an ·OH inhibitor, were used. Gene transfer was performed by dropping GFP plasmid solution on the cultured cells, and they were treated by micro-discharge plasma. Twenty-four hours after the control plasma treatment, cells were stained with Hoechst 33342, a nuclear staining reagent, and fluorescence observation and photography were performed. The numbers of expressing cells and viable cells were counted from the fluorescence images.

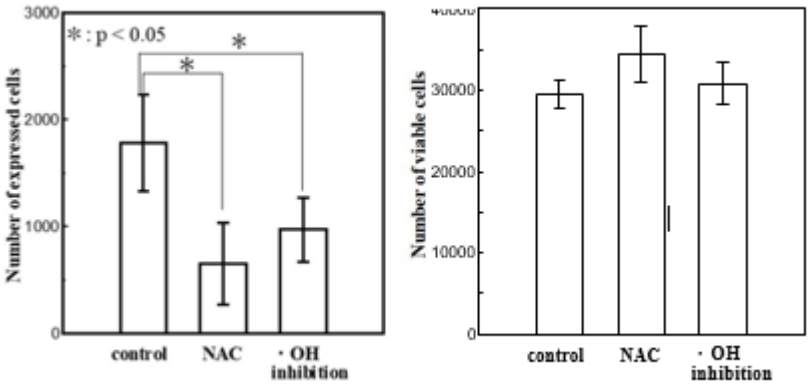

Fig. 1 Number of expressed cells (left) and Number of viable cells (right)

Fig. 1 shows the number of expressed and viable cells. When ROS was inhibited using NAC, the number of expressed cells was reduced by 63% compared to control (plasma treatment only). ·OH inhibition resulted in a 46% reduction in expressed cells compared to control. Significant difference tests against control showed $p < 0.05$ for each.

When ·OH was inhibited, the number of expressing cells showed a reduction similar to that of NAC. This suggests that ·OH is critical for triggering gene transfer among the several types of ROS generated by the plasma. Oxidation of the plasma membrane is known to be an effect of ·OH on cells. It has also been reported that oxidation of cell membranes decreases membrane tension. Therefore, it seems that ·OH generated by plasma treatment causes oxidation of the cell membrane, which in turn reduces membrane tension, thereby promoting endocytosis. Consequently, it is suggested that inhibition of ·OH inhibits this mechanism of inducing endocytosis, resulting in a decrease in the number of cells expressing.

Part of this work was supported by JSPS KAKENHI Grant Numbers 17H01068, 21H04455.

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Plasma deposition of anti-proliferative drugs for vascular stents

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The interactions between plasma and pharmaceutical actives can vary dramatically in response to process parameters. Complete degradation of the active agent can be achieved using high plasma powers and oxidative gases, whereas low power plasma systems using inert gases can have minimal impact on the drug purity. In this work, a low power inert gas plasma was used to deposit sirolimus and everolimus, which are pharmaceutical agents widely applied to drug eluting stents to overcome restenosis and other issues. They achieve this by controlling cellular proliferation and tissue responses around the implanted stents. In this study, low power plasma deposition was compared with traditional wet spray coating techniques for the preparation of coatings on a titanium substrate.
The surface topography of the substrate and the deposited materials was examined using a combination of AFM and optical microscopy. HPLC and FTIR analyses of the coatings was undertaken to determine if the deposited pharmaceutical agents were degraded by the plasma. Cell culture studies were undertaken to monitor the ability of the deposited materials to inhibit cellular proliferation. The impact of the plasma on the active agents are described and future directions are discussed.
Topic area: Plasma for pharmaceutical applications, biochemical and biomolecular engineering

This research was funded in part by the Irish Research Council Government of Ireland Postgraduate Scholarship under Project ID: EBPPG/2023/1150.

Plasma-activated Hydrogels: A Versatile Drug Delivery Platform for Multiple Clinical Indications

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We have developed a unique 'plasma-materials' platform aimed to deliver a range of therapeutic drugs effectively and plasma species safely into the diseased human tissue. The novel material is composite hydrogel composed of drug-loaded sodium polyacrylate (PAA) particles, dispersed within a polyvinyl alcohol (PVA) hydrogel matrix (Fig. 1a) [1]. The composite material, when activated using a plasma device, undergoes changes in pH and ionic strength causing the collapse of PAA particles and release of the drug deep into the tissue. As shown in Fig 1b, plasma-activated hydrogels (PAH) allow an 'on-demand' release of a range of proven antimicrobials (gentamicin, PHMB, octenidine) and carrier molecules such as dendrimers for up to a week. The release of multiple boluses of drug over a period of time is beneficial in clinical indications, such as pediatric burns, where frequent change of dressings can hamper reepithelization, slow healing and is painful. PAH can deliver antimicrobials significantly above the minimum inhibitory concentrations and achieve effective microbial killing in multi-species biofilm models.
This novel PAH platform is compatible with a range of therapeutics such as anticancer drugs (cisplatin) and immune checkpoint inhibitors (JAK inhibitors), and can be activated by different kinds of plasma devices. This highlights the versatility of PAH technology and its potential applications in a range of clinical indications including infections, onychomychosis, autoimmune skin conditions (psoriasis, vitiligo, etc.) and cancers.

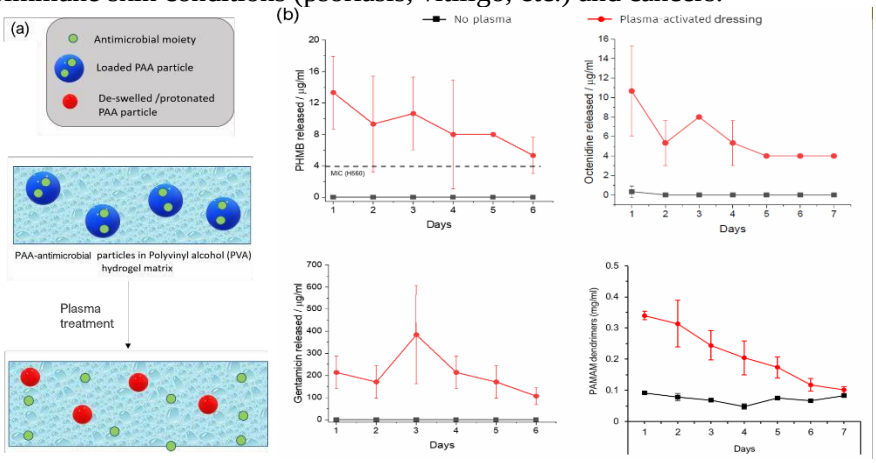

Fig. 1 (a) Schematic of the composite hydrogel; (b) On-demand release of various moieties from PAH upon daily plasma treatment for upto 6-7 days.

This work was supported by EPSRC grants EP/V00462X/1, EP/R003939/1 and an IAA award.

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Plasma-activated cryomicroneedles for transdermal drug delivery

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Malignant skin diseases such as melanoma pose a major threat to human health [1]. As a promising anti-cancer agent, plasma-activated water (PAW) rich in reactive oxygen and nitrogen species (RONS) has shown significant potential for melanoma treatment [2]. However, rapid decay of RONS and inefficient delivery of PAW in conventional injection methods limit its practical applications. To address this issue, here we report a new approach for the production of plasma-activated cryomicroneedles (PA-CMNs) patches using custom-designed plasma devices and processes. Our innovation is to incorporate PAW into the PA-CMNs patches that are fabricated using a fast cryogenic micro-molding method. It is demonstrated that PA-CMNs can be easily inserted into skin to release RONS and slow the decay of RONS thereby prolonging their bioactivity and effectiveness. The new insights into the effective melanoma treatment suggest that the rich mixture of RONS within PA-CMNs prepared by the custom-developed hybrid plasma-assisted configuration induces a synergistic combination of ferroptosis and apoptosis to effectively and selectively kill tumor cells. A significant inhibition of subcutaneous A375 melanoma growth was observed in PA-CMNs-treated tumor-bearing nude mice without any signs of systemic toxicity. The new approach based on the PA-CMNs patches may potentially open new avenues for a broader range of disease treatments that rely on transdermal drug delivery.

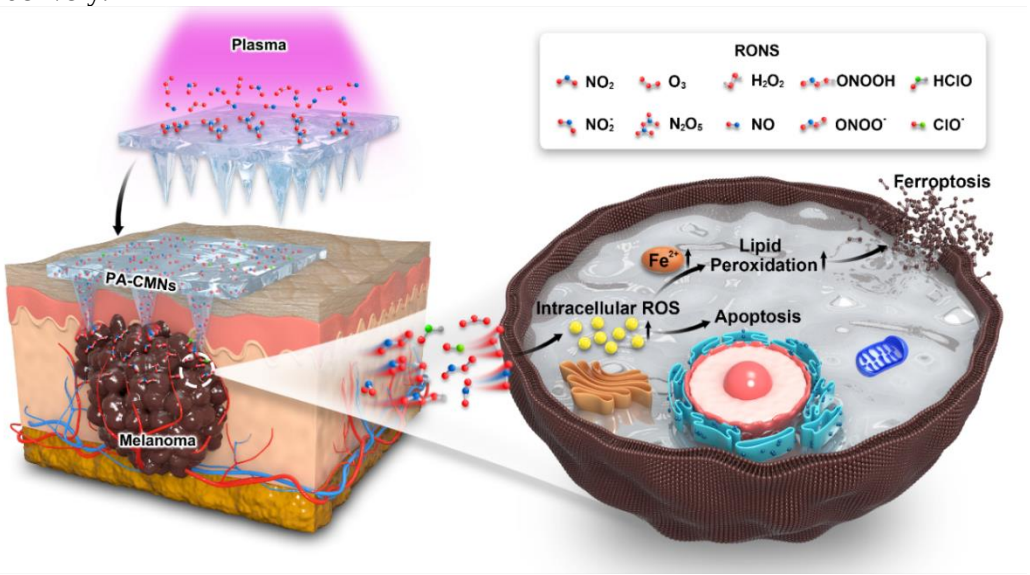

Fig. 1 Conceptual design of PA-CMNs application for melanoma treatment.

This work was supported by National Science Foundation of China (No. 52307257).

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Effect of low level of oxidation on protein structures and their function

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Over the past decade, cold atmospheric plasma (CAP) has found numerous applications in biological systems, including the treatment of viral infections [1] including SARS-CoV-2, and cancer treatment. It is hypothesized that CAP's anti-cancer and anti-viral effects result from oxidative stress, which alters proteins and consequently impacts their functions. In CAP treatment of biological systems, determining the right dosage is crucial for optimal treatment outcomes with minimal side effects. Hence, considering that methionine (Met) and cysteine (Cys) are the amino acids primarily susceptible to oxidation at low CAP doses [2], we have investigated the impact of their oxidation on protein function using molecular dynamics simulations.

We have investigated the voltage-dependent anion channel 1 (VDAC1) in the mitochondrial membrane, critical for pyruvic acid (Pyr) translocation. Inhibiting Pyr uptake through VDAC1 could potentially hinder cancer cell proliferation [3]. Our primary aim is to analyze Pyr movement across native and oxidized forms of VDAC1. In viral inactivation, we analyzed the impact of oxidizing cysteine (Cys) in the receptor binding domain (RBD) of the SARS-CoV-2 spike protein. This oxidation influences both its binding to cell receptors (e.g., ACE2 and GRP78) [4] and its conformational transition from an inaccessible to an accessible form.

Our results reveal that the free energy difference in the attraction between RBD and ACE2 upon Cys oxidation (ΔGG) obtained by the slow growth free energy simulation (see Fig.1) is equal to -55.1±31.5 kJ/mol, indicating that the oxidized RBD-ACE2 complex is more stable than its native counterpart. Conversely, the interaction energy between RBD and GRP78 shows a small reduction of 27 kJ/mol after oxidation. These findings have significant implications for controlling and treating SARS-CoV-2 infections and the role of reactive oxygen and nitrogen species (RONS) in the viral pathogenesis.

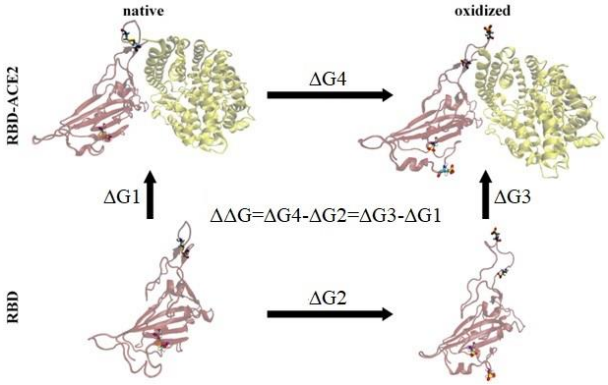

Fig.1. Schematic representation of the thermodynamic cycle for calculating changes in free energy of RBD attachment to ACE2 upon Cys oxidation (ΔΔG). ΔG1 and ΔG3 denote the attachment free energy of a native and oxidized RBD and ACE2. ΔG2 and ΔG4 represent the free energy of the alchemical transformation of the native RBD into the oxidized one and for the RBD-ACE2 complex.

Acknowledgement: This project has received funding from the European Union – NextGenerationEU instrument and is funded by the Academy of Finland under grant number 353298.

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icpm 10 & IWPCT 9

8 - 13 september, Portorose, Slovenia

POSTER SESSION

(Thu - P)

Thursday, 12 September 2024

Atmospheric Pressure Plasma Synthesis of Gold Nanoparticles for SERS Recognition of Bacterial DNA

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Atmospheric pressure plasma was used for synthesis of gold nanoparticles via a single-step reduction of vaporized ionic gold precursor. In the experiment, a $HAuCl_4$ x H_2O solution was transferred to a nebulizer connected to an atmospheric pressure plasma jet system. The setup involved two vertically aligned quartz tubes, with a helium plasma ignited in the larger tube using a gold-coated copper wire. Helium flowed at 290 sccm with the plasma operating at 25 W and 21.2 kHz, while argon gas at 1000 sccm carried vaporized microdroplets of a $HAuCl_4 \times H_2O$ solution into contact with the plasma as presented in Figure 1. Gold nanoparticles were deposited on the substate, which was used for Surface Enhanced Raman Spectroscopy (SERS) in order to identify bacterial strains based on their DNA composition. For SERS analysis, extracted genomic DNA from four different bacterial species was pipetted on the substrate. Raman signals related to base-pair vibrations were deconvoluted to estimate nucleotide content. The genomic composition (percentage of AT and GC pairs) was verified with nanopore sequencing, demonstrating that SERS can be used to specify bioentities through a discriminative principal-component statistical approach.

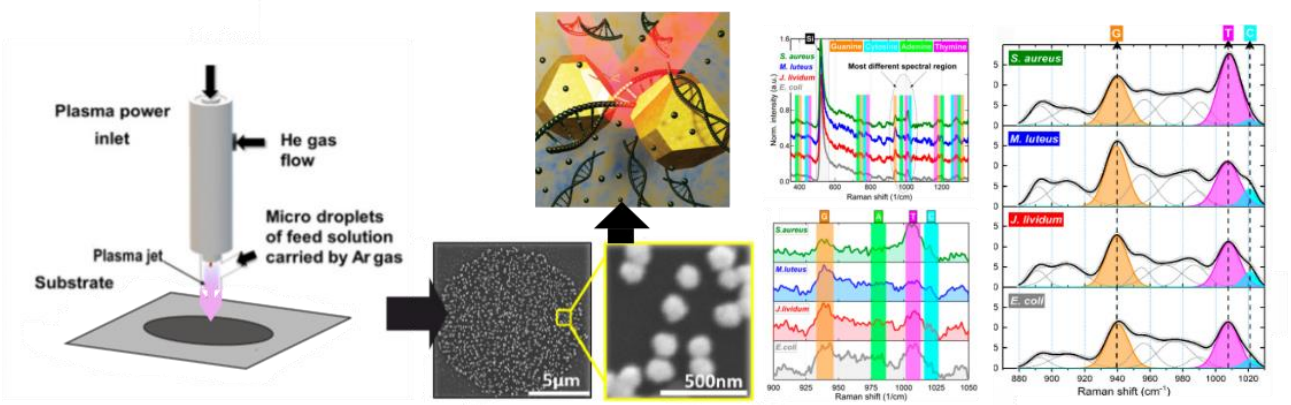

Fig. 1 Visual abstract of plasma assisted gold nanoparticles production used for SERS analysis of bacterial DNA

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Label-Free SERS Detection of Carcinogens by Plasma-Made Nanostructures

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The widespread increase of carcinogenic toxins in agriculture and food industries is a major concern and a serious threat to the environment and living organisms, including human health. Among various toxins, mycotoxins are one of the significant chemical entities severely affecting the food industry, causing cancer growth and immune deficiency. Majorly, such hazardous substances are produced by different fungi species, which contaminate food products and can be found mainly in spices, nuts, cereals, corn, etc. Even though mycotoxins are widespread, detection at low concentrations remains a critical challenge. Therefore, significant efforts have been put forward to develop a fast and easy technique to detect mycotoxins at low concentrations. One such method is surface-enhanced Raman spectroscopy (SERS), a surface-sensitive technique that enhances Raman scattering by plasmonic effects induced by nanostructures [1]. SERS techniques could provide the enhancement signal as a factor of 10^{10} to 10^{11}, indicating the possibility of detecting single molecules. Therefore, in this research, a SERS technique has been successfully demonstrated to detect mycotoxins using a high-performing nanocarbon-based plasmonic substrate, which has an analytical enhancement factor = 5×10^7. The plasmonic vertical nanocarbon substrates were designed using a plasma-enhanced deposition process, in which the vertical carbon nanotubes (VCN) were deposited directly on the metallic substrate. The plasma deposition was conducted in a low-pressure radiofrequency system at a plasma power of 800W, precursor gas methane with a flow of 20 sccm and pressure 30 Pa and deposition time was varied from 1-30 min [2]. The designed VCNs, additionally covered with Au film, were used for the detection of mycotoxins in the ppb range, including aflatoxin B1, zearalenone, alternariol, and fumonisin B1 via a fast SERS process, which has a detection time scale in seconds. The method incorporating SERS+PCA (principal component analysis) shows excellent distinguishing capability of different components, opening up a new roadmap for time-efficient and low-concentration detection of hazardous materials that exist in food and agriculture fields.

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The use of atmospheric pressure plasmas for the control of antibiotic- resistant bacteria in the agri-food sector

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Fresh cut fruits and vegetables such as leafy greens are associated with an increased risk in terms of food safety as they are most often consumed raw. Possible sources of microbial contamination of leafy vegetables include soil, wash water, and food contact equipment. More recently, several research groups demonstrated that leafy greens can harbor antibiotic resistance genes (ARGs) associated with their microbiome [1-2]. While standard cleaning processes can lower the bacterial load in these products, the degradation of genetic material such as the ARGs remains a challenge [3]. In the last decades, cold plasma technology showed promising results as a green technology for the decontamination in agri-food industry [4].
This study aims to explore the potential of atmospheric pressure plasma in the decontamination of leafy vegetables with a specific focus on its impact on antibiotic-resistant bacteria (ARB). The emissions of plasma discharge obtained using a dielectric barrier discharge (DBD) source were characterized using a monochromator (PI-HRS-750) coupled to an intensified camera (PI-MAX4). In the same time, the production of reactive oxygen and nitrogen species RONS was examined using KI – starch assay.
Salad and spinach were used in this study as leafy green models. The native bacterial communities associated with these products were characterized by classical microbiology techniques and metagenomic analysis. Furthermore, the characterization of their antibiotic resistance profiles was made using microbiological and molecular techniques. Plasma effect on the bacterial load and the antibiotic resistance profiles was assessed after treating the vegetal material with the DBD plasma in air produced inside a closed reaction chamber. For the evaluation of the bacterial load, plasma treated and untreated samples will be analyzed by classical microbiological methods (colony counting) but also by molecular methods to examine the cell membrane permeability, lipid peroxidation or metabolic activity.

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Treatment of fresh and marine water microalgae by combined gliding arc discharge plasma and pulsed electric field

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The main objective of this research was to investigate the effects of combined plasma and pulsed electric field treatment on the freshwater microalgae *Chlorella vulgaris* and the marine microalgae *Isochrysis galbana*. The study also analyzed the influence of the plasma discharge parameters on the physicochemical properties of the treated algal growth media. Concentrated algal suspensions (10 ml) were affected by gliding arc discharge (GAD) plasma with compressed air at atmospheric pressure. During experiments the output voltages of the plasma generator varied from 90 V to 250 V, keeping the treatment duration of 300 s. The PEF treatment consisted of 10 μs pulses, varying from 1 to 10 pulses, with a repetition rate of 1 Hz and electric field strength of 24-25 kV/cm. Subsequently, changes in composition of the generated air plasma, pH, electrical conductivity, cell permeability, DNA leakage and the release of a valuable compound (proteins and carbohydrates) were assessed.

Emission spectroscopy analysis of the air plasma revealed that the predominant particles were N_2, N_2^+, N^+, NO, and O species. The increase of output voltage or reduction of air flow rate stipulated the increase of concentrations of molecular nitrogen ions, atomic oxygen and exited nitrogen molecules in plasma. The pH, conductivity, nitrate, nitrite and hydrogen peroxide concentrations in the medium showed a dependence on the plasma discharge conditions. As the output voltage increased, the pH of the medium decreased, conductivity increased, and radical concentrations increased. Physiological changes in microalgae after plasma treatment were dependent on both plasma discharge conditions and microalgae species. Cell permeabilization in *C. vulgaris* was induced only at discharge output voltages above 210 V, with significantly reduced DNA and protein leakage compared to PEF treatment alone, where permeability and protein release increased proportionally with the number of pulses applied. The combination of plasma (at voltages below 170 V) and PEF treatment, showed similar effects as PEF treatment alone inducing cell permeability, DNA and protein leakage. However, above 210 V voltages, the combined treatment increased cell permeability with reduced DNA and protein leakage.

In contrast, the marine microalgae *I. galbana* showed cell permeabilization at a plasma output voltage as low as 130 V, resulting in the release of DNA and carbohydrates. Subsequently, increasing the output voltage accelerated both the permeabilization of *I. galbana* cells and the release of carbohydrates. To induce cell permeabilization by PEF, a special marine algae treatment cuvette was constructed, and high-voltage PEF pulses were applied to compensate the influence of the extremely low resistance of the marine algae medium. However, none of these techniques had any effect on cell permeabilization. It was therefore necessary to suspend the algae cells in an electroporation buffer with a lower electrical conductivity prior to PEF treatment. Overall, this study provides the foundation for further investigation and optimization of combined plasma and PEF treatment as a sustainable and environmentally friendly method for processing microalgae.

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Characterizing Reactive Species Distribution in Plasma-Based Surface Decontamination for Enhanced Food Safety

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Foodborne diseases pose a significant public health concern, often stemming from microbial contamination of food products and packaging. A promising solution is plasma-assisted decontamination [1]. A large-area system was developed, comprising a surface dielectric barrier discharge (SDBD) plasma source and two interchangeable treatment chambers, with capacities of 18.5 L and 0.9 L, respectively. The plasma source features four high-voltage electrodes, a mica dielectric layer, and four perforated plates serving as ground electrodes [2]. A comprehensive characterisation of the system was conducted, focusing on the chemical composition of the atmosphere within the treatment chamber. The kinetics of ozone and nitrogen dioxide, chosen for their known antimicrobial efficacy, were analysed by Optical Absorption Spectroscopy (OAS) in nine different positions (fig. 1a) over a 30-minute plasma generation period. Results indicated uniform concentration distributions of reactive species within the entire treatment volume (fig. 1b), suggesting that three-dimensional objects can undergo effective decontamination using the developed system. Biological testing further confirmed the efficacy of the system, with *Staphylococcus epidermidis*-contaminated polymer samples exposed to treatment yielding consistent results across different chamber positions. Additionally, experiments with a thinner treatment chamber demonstrated enhanced antimicrobial activity, also assessed against gram-positive bacteria (*Acinetobacter baumannii*), achieving a 2.8-Log reduction in bacterial load in 10 minutes. While this chamber offers superior performance, its design makes it better suited for film treatments rather than 3D objects.
In conclusion, the presented surface decontamination system shows promise in mitigating foodborne disease risks by effectively neutralising microbial contaminants on various surfaces, offering potential applications in food packaging and processing industries.

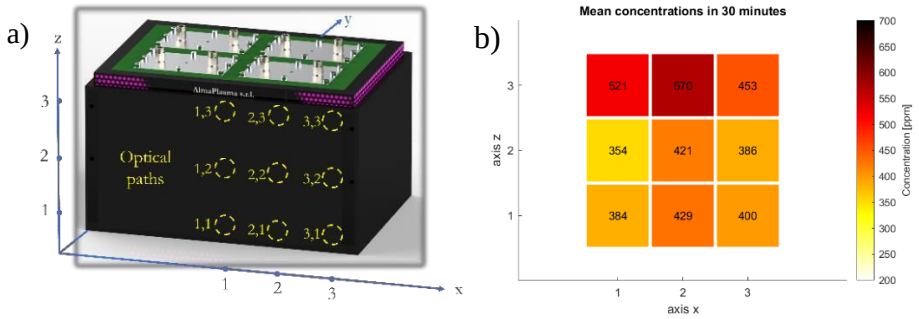

Fig. 1 a) Optical paths for OAS tests; b) Mean ozone concentrations [ppm] over 30-minute treatments

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Cold atmospheric microplasma mediated drug delivery through blood-brain barrier

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The blood-brain barrier (BBB) serves as a protective shield within the brain, impeding the entry of most drugs. The BBB is composed of endothelial cell surrounded by pericytes and astrocytes. Constructed as a continuous layer of endothelial cells, the BBB relies on tight junctions, adherent junctions, and gap junctions. Among these, tight junctions play a pivotal role in upholding the barrier's resilience and managing the transit of drugs. In recent times, cold atmospheric plasma (CAP) has gained popularity in the field of medical science, finding applications in wound treatment, drug delivery, surface sterilization, and even cancer therapy. CAP produces reactive oxygen and nitrogen species (RONS) such as nitric oxide (NO), superoxide radical, peroxy-nitrite anion, and nitric oxide radical. Our research focuses on disruption of tight junctions of the BBB to facilitate drug delivery into brain and subsequently repair the tight junction.

The bEnd.3 cell line was purchased from ATCC (USA) and cultured using DMEM medium on 6-transwell plate at 37ºC with 5% CO2. When trans-endothelial electrical resistance (TEER) reached >120 ohm-cm^2, fluorescein isothiocyanate dextran (FD-4) drug was added to the apical part (insert) of trans-well plate, and plasma was applied to the culture as follow (irradiation time: 2 min, irradiation distance: 2 mm, voltage: 4.0kVpp, frequency: 5kHz). The dielectric barrier discharge (DBD) plasma was prepared by winding a ground electrode around a dielectric barrier coating covering high voltage electrode. After plasma treatment with 1 hour incubation, florescence intensity was measured from the basolateral side and TEER was measured again. The TEER value was reduced in plasma-treated cells compared to non-treated cells. It indicates that this plasma condition produced reactive species (RONS) that are in-volved in breaking proteins of intercellular junction. Because the lower the TEER value, the more permeability in the intercellular junction. (Fig. 1). The higher florescence intensity was detected in plasma treated cells compared to non-treated cells. The higher florescence intensity indicates the higher amount of drug in basal sites of the plates (Fig. 2).

The reduced TEER and increased florescence intensity in our study confirms successful drug delivery. This result may provide a valuable insight into a new strategy for brain drug delivery.

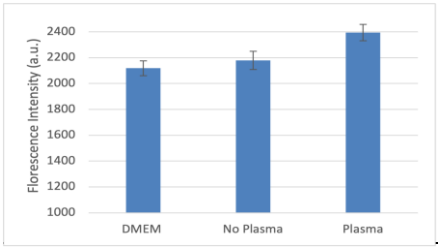

Fig 1. Florescence intensity of plasma treated cells compared to cells without plasma and DMEM having no cells.

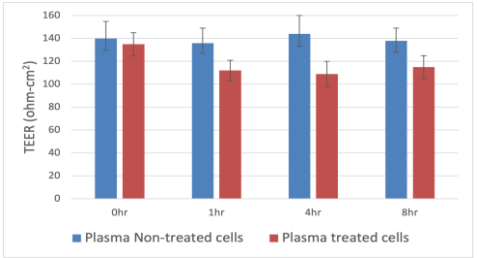

Fig 2. TEER of plasma treated cells (red) compared to cells without plasma (blue).

Study on the changes of cell membrane lipids for delivering large molecules drug by using microplasma irradiation

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Microplasma (MP) irradiation showed the capability of delivering high molecular weight molecules, including fluorescein isothiocyanate-dextran (FD-150 and FD- 2000; molecular weight:150 kDa & 2000 kDa, respectively) into rat intestinal epithelial cells (IEC6) [1]. This technique of drug delivery is painless, reduces doses and adverse effects of drugs, increases efficacy and stability of drugs. Plasma components such as reactive oxygen species and reactive nitrogen species interact with cell membrane, thus enabling the penetration of drugs into cells. In this study, we aim at studying the changes in structural lipids of cell membranes after MP irradiation. We have treated IEC6 cells with MP at optimized the condition (4 kV, 5 kHz). Air plasma discharge and a thin film electrode were used in this study. We extracted lipids using Bligh and Dyer methods (slightly modified) followed by analysis using liquid chromatography-mass spectrometry (LC/MS) technique.

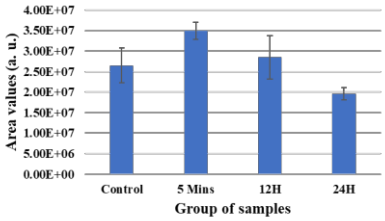

Fig. 1: Changes of total plasma membrane lipids until 24 h after MP

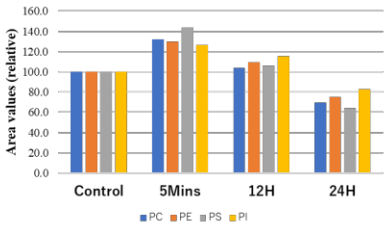

Fig. 2: Changes of glycerophospholipids until 24 h after MP

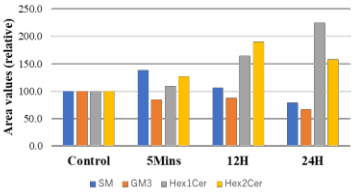

Fig. 3: Changes of spingolipids until 24 h after MP

The total cell membrane lipid decreased significantly (p <0.05) after 24h of MP irradiation compared to the control (Figure 1). All of the glycerophospholipids classes that include phosphatidylcholine (PE), phospatidylethanolamine (PE), phosphatidyl-serine (PS), and phosphatidylinositol (PI) showed similar trends— they increased within 5 min in post-irradiated cells, returned to the normal levels in 12H post-irradiated cells, and then further decreased in 24H post-irradiated cells (Figure 2). On the other hand, sphingolipid classes which include sphingomyelin (SM), gangliosides (GM3), hexosylceramide (Hex1Cer), dihexosylceramide (Hex2Cer) showed variability in their changes (Figure 3). This study showed that microplasma irradiation has profound effect on the cell membrane lipids. The lipids were rapidly increased just after microplasma irradiation and then returned to the normal level.

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Boosting transdermal drug penetration by cold plasma – insights on the molecular level

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The skin, representing 15 % of the body mass, is undisputedly the largest organ of the body and provides protection against chemical, physical, and biological threats. Its structure and chemical composition form an efficient barrier, preventing the penetration of pharmaceutical drugs significantly. Among other strategies, cold plasma offers an option to improve the penetration. How this is achieved remains a matter of debate – does the oxidation of biomolecules contribute [1,2]?

To investigate this approach, we employed a porcine ear model that perfectly resembles the human skin and is regarded as a highly valuable pre-clinical model. We investigated the impact of cold plasma (kINPen, 1 to 5 min cm^{-2}) on the penetration of two selected model compounds, curcumin, a natural compound from turmeric and ibuprofen, a widespread painkiller. HE stained tissue sections and confocal microscopy were used to determine the impact on morphology and curcumin penetration. Individual layers of the treated skin were collected (tape stripping) and further prepared for a) an absolute quantification of ibuprofen along the depth profile via targeted LC-MS and b) a layer-wise mapping the of the lipid profile by bottom-up UHPLC-HRMS (lipidomics) with a special emphasis on lipid oxidation products.

As a result, we observed changes to skin micromorphology that remained negligible within the clinically recommended treatment time (1 min cm^{-2}). Accompanying, an increase of the penetration of curcumin (+ 160 %) or ibuprofen (+ 40 %) was determined. The differences between the two model compounds are explained by their differing physico-chemical properties (water-octanol distribution coefficient). Lipid profiling showed the expected composition of epidermal lipids (ceramides, free fatty acids, cholesterol/-esters) and sebum lipids (triacyglycerols, wax esters, terpenoids). Following cold plasma treatment (1 min cm^{-2}), a limited hydrolysis and oxidation of sebum triacyglycerols and epidermal hexosylceramides was observed yielding to a reduced barrier function of the skin and explains the observed penetration increase. A more detailed analysis of lipid oxidation products is in progress.

In conclusion, the study reveals a strong impact of cold plasma on the penetration of substances into the skin and provides insights on underlying mechanism on the level of the epidermal/sebum lipids.

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Development of a Cold Atmospheric Plasma Responsive Polyacrylate Hydrogel System for On-Demand Drug Delivery

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Whilst wound decontamination can be achieved via the direct application of Cold Atmospheric Plasma (CAP), this can dehydrate the wound, thus potentially slowing down the healing process. Our work has explored the possibility of using a polyacrylate hydrogel film, loaded with cationic antimicrobial molecules including gentamicin, silver and polymyxin B [1]. Application of the CAP jet triggers the release of the antimicrobials into the wound, as well as hydrogen peroxide and oxygen, meanwhile keeping the wound moist. This means optimum wound healing conditions can be created.

Sodium polyacrylate hydrogel films were loaded with polymyxin B and other cationic drugs. To trigger drug release, drug-loaded hydrogel discs were treated for a short duration (5 minutes) using an argon CAP jet, and the release of polymyxin B (and other drugs) was quantified. Results showed that release of antimicrobials can be triggered from the hydrogel following CAP treatment, based on the inhibition of key bacteria species present in wounds, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

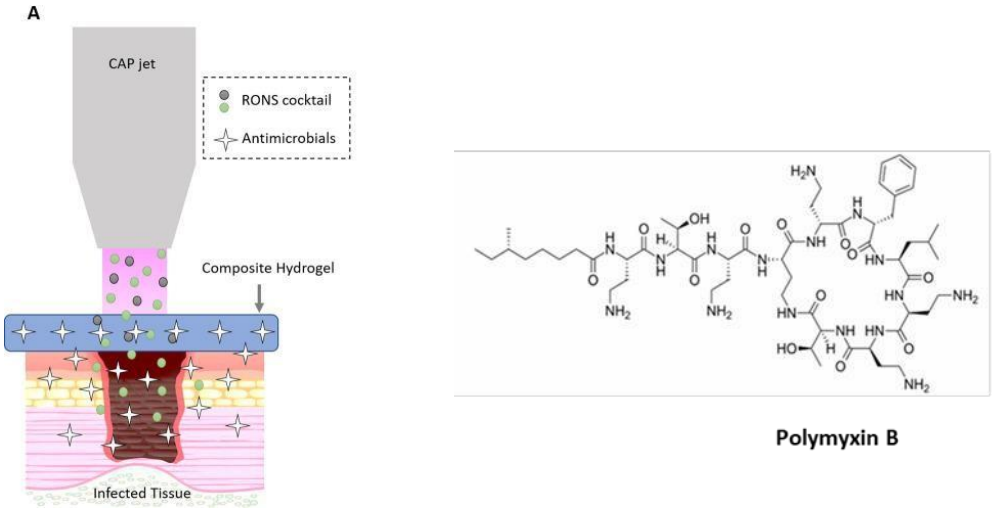

Fig. 1. Schematic showing application of the CAP jet onto an infected wound, covered with the hydrogel sheet. Antimicrobial molecules and reactive oxygen and nitrogen species (RONS) are seen to be released from the dressing and delivered to the wound site [1]. The structure of antimicrobial peptide, Polymyxin B, is also shown.

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Plasma-Designed Nanosensors for SERS Detection of Hazardous Chemicals

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Surface-enhanced Raman spectroscopy (SERS) is emerging as an advanced optical sensing method, enabling the rapid detection of analytes at concentrations on the order of parts per billion (ppb) or even lower. Its strong analytical features enable the monitoring of toxic substances with ultrafast data acquisition and high accuracy [1,2]. Successful implementation of SERS is based on the development and utilization of suitable nanostructured surfaces, which allow for the enhancement of Raman signals by several orders of magnitude due to the field confinement effect. In this regard, plasma-assisted methods stand out as ideal tools for the fabrication of SERS substrates as they offer fast and effective modification and fabrication of optically functional surfaces. In the present study, a microwave plasma system was employed to synthesize tree-like branched copper oxide structures on metallic copper film for the fabrication of nanostructured SERS substrates for the rapid detection of hazardous organic chemicals. The mechanism of nanostructure growth was investigated, discussed, and theoretically described. Plasma parameters were optimized to achieve substrate morphology that yielded the best performance for SERS detection of various explosives standard compounds as examples of hazardous molecules. For this task, the nanostructured surfaces were coated with a thin layer of silver, which was subsequently topped with a protective 10nm Au film. The synthesized nanostructures greatly enhanced the Raman signal, and the sensors demonstrated excellent response with an enhancement factor $>10^6$ and a low detection limit reaching the ppb range. The sensors were able to detect and identify a wide range of organic molecules, showing the potential for implementation in universal detectors. This research aims to advance the field of plasma-assisted synthesis of nanostructured materials and contribute to the development of materials that will shape the plasmonic sensors of the future.

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Rapid Plasma-Driven Fabrication of Gas Sensors for Safety Against Hazardous Gases

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The rising environmental pollution and safety concerns call for advanced gas sensors that can detect toxic and harmful gases even in minute concentrations. Among various nanomaterials used for gas sensing applications, graphene oxide (GO) has emerged as a promising candidate due to its simple large-scale production and ease of surface modification [1, 2]. However, the insulating nature of GO is a major limitation for practical gas sensing applications [1, 3]. This study proposes to use a plasma technique for GO reduction for enabling efficient ammonia detection at room temperature. The proposed method involves exposing GO solution-casted sensors to hydrogen plasma for controlled surface modification. Varied plasma treatment durations yield different reduction levels, thereby influencing sensor response to low concentrations of ammonia. Prolonged plasma reduction treatments resulted in sensors with good stability and recovery but lower sensitivity, whereas short plasma reduction times resulted in the opposite. The sensitivity and recovery trade-offs are attributed to a proposed chemisorption-physisorption interaction mechanism, supported by structural and chemical analyses. Such a potentially scalable, environmentally friendly and room-temperature H2- plasma reduction process enables one of the fastest approaches for designing advanced rGO sensors with tunable sensing capabilities towards ammonia.

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Synergistic Application of Cold Atmospheric Plasma and Vancomycin in MRSA biofilms

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Methicillin-resistant *Staphylococcus aureus* (MRSA) biofilms are a major contributor to the severity and persistence of hospital-acquired infections, often demonstrating formidable resistance to standard antibiotic therapies. This study explores a novel synergistic approach, combining Cold Atmospheric Plasma (CAP) and Vancomycin, to enhance the eradication of these challenging biofilms.

Employing an *in vitro* biofilm model, we assessed the efficacy of CAP, Vancomycin, and their concurrent application. Quantitative analysis of biofilm formation employed crystal violet staining, with bacterial viability assessed via Miles and Misra drop counts and the Alamar Blue Assay.

Results demonstrated that the co-treatment significantly exceeded the performance of individual treatments, with a notable reduction in biofilm biomass and a decline in bacterial viability—evidenced by a statistically significant decrease in CFU counts ($p < 0.05$). Additionally, ATP leakage assays suggest that CAP may compromise the biofilm matrix, thereby bolstering the penetration and effectiveness of Vancomycin.

The synergy observed offers an innovative pathway for combatting antibiotic-resistant infections and fills a critical void in contemporary treatment strategies. The potential for this method to significantly influence the management of biofilm-associated infections is profound, particularly in environments burdened by antibiotic resistance. Understanding the precise interactions between the reactive species generated by CAP and the biofilm structures could forge targeted strategies to disrupt biofilms, thus enhancing antibiotic efficacy.

In summary, the amalgamation of CAP with Vancomycin represents a promising strategy to surmount MRSA biofilm-related challenges. This study not only underscores the potential of CAP as an adjuvant therapy but also catalyses the exploration of combined treatments, potentially revolutionising the approach to antibiotic-resistant infections.

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Comparative Studies of two Low Temperature Microwave Plasma Devices at Atmospheric Pressure for Medical Applications

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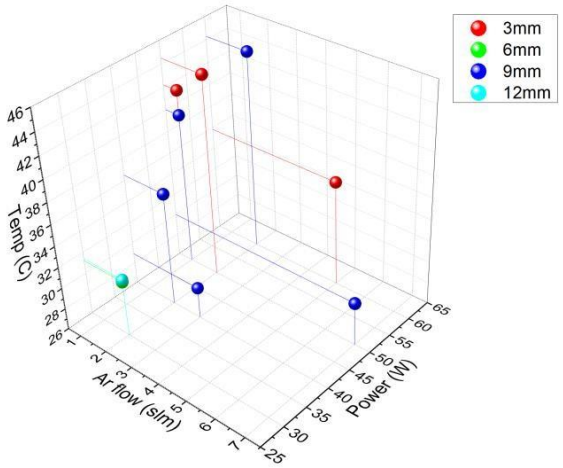
Efforts to develop low temperature atmospheric plasma sources at atmospheric pressure, particularly the surface-wave-sustained discharge (SWD) at 2.45 GHz, have gained traction for applications in biology, medicine, agriculture, and food industries. There are no electrodes, so the contamination of the plasma with metal nanoparticles is avoided. Adjusting parameters (power, gas flow, quartz tube diameter) optimizes operation while minimizing temperature impact (1). Exposure of the sample to plasma can be direct or indirect. Indirect treatments mean that plasma is used to produce plasma activated water (PAW) or plasma activated medium (PAM) that later comes into contact with the biological sample. Similar to our system, Microwave-Excited Atmospheric Pressure Plasma Jet, creating PAM in combination with cisplatin, has been demonstrated to act synergistically against cancer cell growth in vitro (2).

In our work two types of microwave plasma devices at atmospheric pressure were compared – inductively coupled Sairem S-Wave Launcher and capacitively coupled home-made S-wave Launcher. Both devices were characterized in details through optical emission spectroscopy (OES) by varying the applied power, argon gas flow rate and length of the discharge tube. As expected, emission spectra show predominantly Ar atomic lines, alongside the presence of NOγ, NOβ and OH bands, atomic oxygen lines, such as those at 777 nm or 844 nm, and N_2 bands. We have correlated the intensities of main oxygen and nitrogen bands/lines with the generation of reactive oxygen and nitrogen species (RONS) produced in PAW for both devices. Another important measured parameter is the temperature of water during and after treatment. The temperature of water after 10 min treatment by capacitively coupled S-Wave Launcher under different conditions are shown in Fig. 1.

Fig1. The temperature of the plasma-treated 32 ml water, in relation to the argon flow rate , the input power and the length of the discharge quartz tube.



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Electrical and optical characterization of a plasma source for the biological decontamination of waste water in volume

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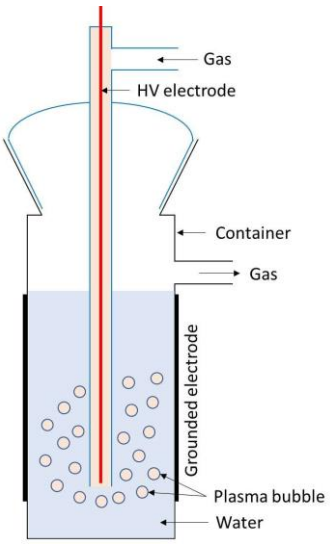
Effluents are typically decontaminated through a range of physical, chemical, and biological methods. However, each of these approaches has its limitations, including cost, feasibility, environmental impact, and the potential for generating chemical by-products [1]. As a result, the search for sustainable, environmentally-friendly methods that pose fewer risks to both the environment and human health remains a significant challenge.

The aim of the project is to characterize and optimize a modifiable wastewater decontamination system, conceived and built in our laboratory (Figure 1). The system is based on the creation of an immersed plasma at atmospheric pressure that should reduce the bacterial and viral load within a reasonable treatment time. The experimental setup primarily consists of a glass container, containing the water to be treated. Immersed within the water is a tube through which gas is injected. The injected gas exits from the other end of the tube, creating gas bubbles in the water. Inserted into the tube, a high voltage electrode is connected to a pulsed power supply that generates pulses with widths less than 3 µs. The voltage amplitude ranges from 1 to 20 kV with a fixed repetition frequency of 20 kHz. A grounded counter electrode is wrapped around the water container. This system can be considered as a complex form of a coaxial DBD configuration. (Figure 1 - Schematic of the experimental setup)



First, employing an equivalent electrical circuit, the energy deposited into the plasma discharge during a single pulse was estimated. The total current supplied by the power supply was measured using a current monitor (PEARSON^{TM}), while the applied voltage was monitored with a voltage probe (Tektronix P6015A). Both the applied voltage and total current waveforms are recorded by a digital oscilloscope (ROHDE & SCHWARZ RTE1204). Then, optical measurements were conducted with and without water in the container. ICCD Camera (Princeton Instruments PI-MAX1) was used for imaging the plasma propagation inside the tube and the container during a single pulse. Optical emission spectroscopy (PI-HRS-750 coupled to an ICCD camera PI-MAX4) was performed to identify the reactive species generated by the plasma.

This work was supported by the research grant program of Occitanie Region, France.

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Thu-P-15

Comparative bio-application study of various non-thermal plasma generating devices using reference protocol

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The biological applications of non-thermal plasma (NTP), especially its antimicrobial properties are of a great interest of many scientific groups. Thanks to the great expansion of this field of research, there exist many types of NTP generating devices with different technical specifications and antimicrobial efficiencies, therefore also with different suitable applications. However, the results presented in numerous studies are basically impossible to compared between each other due to the variation in species of microorganisms, sample preparations, treatment conditions, etc. Thus, in this work a potential solution was proposed and the comparison of 7 different NTP generating devices regarding their technical parameters and sporocidal properties was made. For this purpose, the robust and reproducible standard protocol based on previous research [1,2] was used to prepare and process the samples. It is based on the well-defined samples of *Bacillus subtilis* spores prepared by filtration on porous membrane. Inhibition zones on a sample as well as the overall inhibition of viable spores numbers after the NTP treatment were compared.

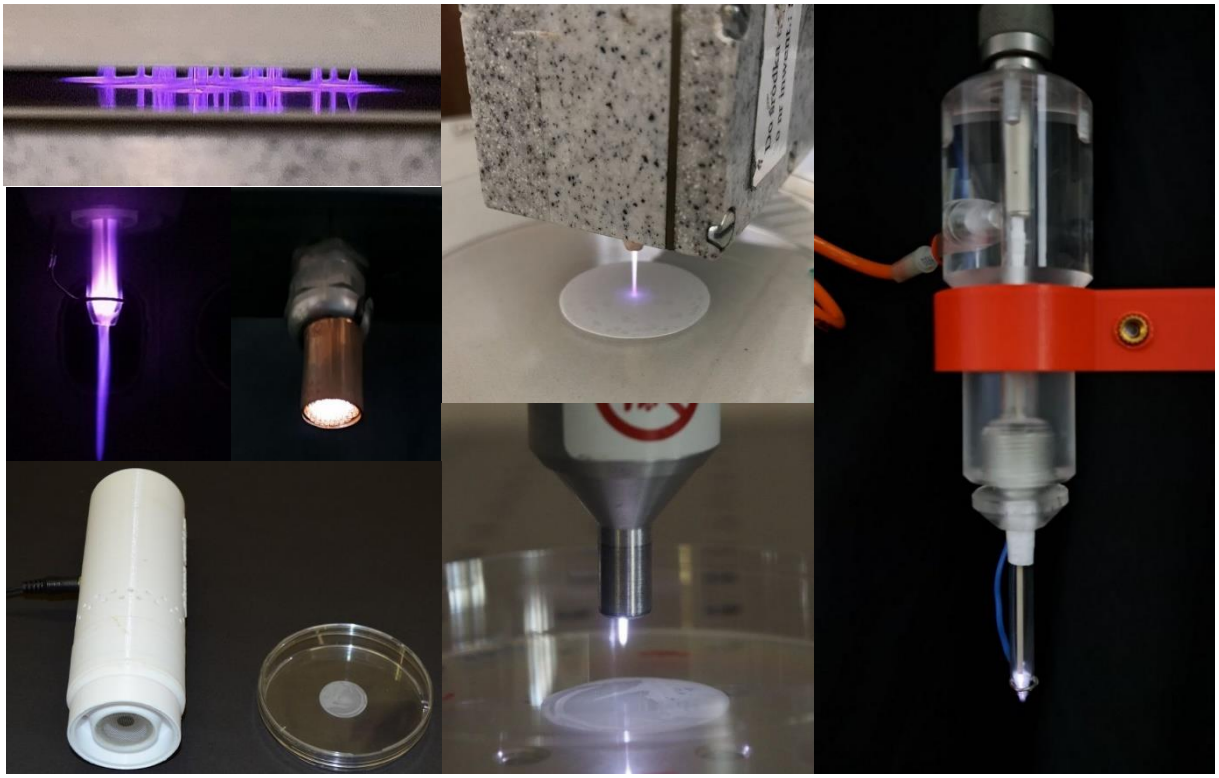

Fig. 1 The comparison of 7 various plasma generating devices was made

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The equivalent electrical circuit for describing the dielectric barrier discharge plasma jet

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The electrical characterization of an atmospheric pressure dielectric barrier discharge (DBD) complemented by the model of equivalent electrical circuit can significantly contribute to a better understanding of plasma behavior. The modeled DBD plasma jet operates with He gas and has two copper electrodes wrapped around a 20 cm long glass tube. The electrodes are 15 mm wide and separated 15 mm from each other. The lower electrode is 15 mm away from the tube end and is powered with high-voltage sinusoidal signal at 30 kHz frequency. The upper electrode and the target holder beneath the jet are grounded. The voltages measured on the R=100 kΩ resistors are used for monitoring the currents through grounded electrode and the target ($i_{ge}(t)$ and $i_{te}(t)$, respectively). Three targets with different electrical characteristics are analyzed: Cu, PET and H_2O.

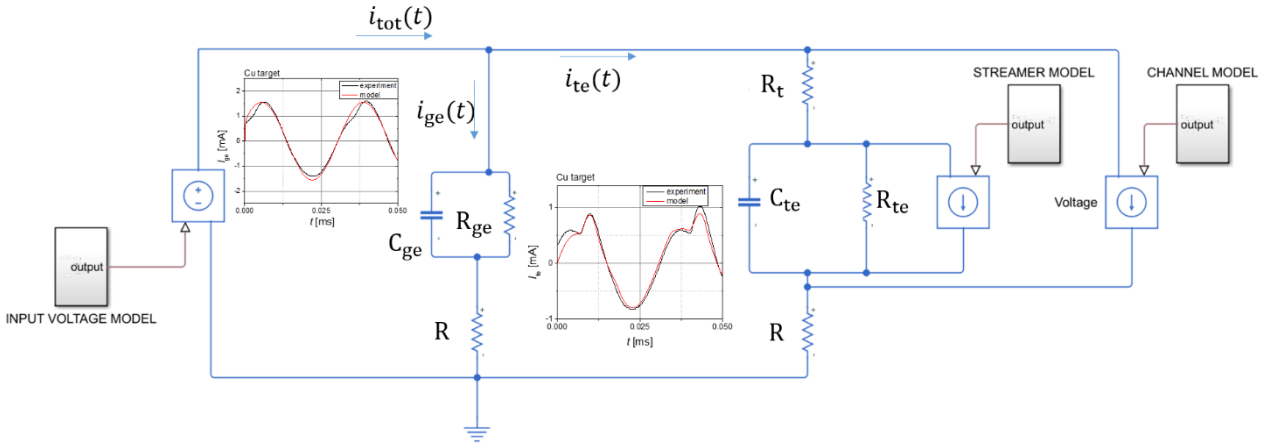

Fig. 1 Schematics of the equivalent electrical circuit representing DBD plasma jet when plasma is on. Waveforms inserted in the picture present comparison of the measured currents and modeled for copper target: left-hand side $i_{ge}(t)$ and right-hand side $i_{te}(t)$.

The model of the equivalent electrical circuit, when plasma is ignited, is presented in Fig.1. Circuit consists of the grounded branch and the target branch. Both have equivalent impedances that correspond to the recorded currents $i_{ge}(t)$ and $i_{te}(t)$, respectively (inserted waveform plots in Fig.1). The shape of the current $i_{te}(t)$ shows a peak appearing only in the last quarter of the positive part of the input voltage amplitude. The peak in the current waveform stem from streamer (bullet) formation and its width corresponds to the third harmonic, oscillating at 90 kHz, with some delay compared to the voltage waveform. That streamer is modeled with controlled current source. Its input port is connected to the mathematical sinusoidal signal with frequency of 90 kHz and predetermined delay. Also, there is a channel appearing only in the negative part of the target current affecting the waveform by increasing the current amplitude. The channel is added in the circuit model as a controlled current source with input port connected to the mathematical sinusoidal signal at 30 kHz with no delay compared to the voltage.

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Experimental characterization of a flat DBD plasma source

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Nowadays, dielectric barrier discharges (DBDs) are widely studied as plasma sources for surface decontamination. The species generated by non-thermal plasma from these sources can be brought into contact with different types of surfaces. The potential applications are therefore very numerous, particularly in the agri-food [1] and medical fields [2].
The aim of the main project is to design, characterize and optimize an original flat DBD source for food decontamination. This plasma source will be used to explore the potential of atmospheric pressure plasma in the decontamination of leafy vegetables. In this preliminary work, the principle of such a structure has been experimentally evaluated and the corresponding results will be presented.
The source consists of a dielectric disk whose diameter is large compared to its thickness. On one side of the disc, the ground electrode completely covers the surface of the dielectric, on the other side the electrode connected to the potential does not cover the entire surface. Patterns can be used (holes, lines). In the case of this preliminary work, the conductive part is just pierced with one hole revealing the dielectric. A pulsed generator is used to create the plasma that occurs in the hole surface. The pulse repetition frequency can vary between 20 kHz and 80 kHz. The current peak can be adjusted between 1 A and 10 A. The plasma is generated at atmospheric pressure in ambient air. For optical spectrometry, a high-speed HR2000+ miniature fiber optic spectrometer (Ocean Optics) was used. It offers an optical resolution of 0.5 nm. The HR2000+ allows you to acquire emission spectra between 200 nm - 1100 nm. ICCD Camera (Princeton Instruments PI-MAX1) was employed to determine the plasma distribution on the source surface. For mass spectrometry, time-of-flight mass spectrometer (TOF-MS, TOFWERK, Switzerland) was used. It is a compact analyzer coupled to an atmospheric pressure interface with differential pumping which adapts the pressure difference between the ion source and the analyzer, while efficiently transporting ions from the source to the TOF-MS.
In this work, we carried out a parametric study. The parameters were as follows: permittivity of the dielectric, dielectric thickness, current intensity, and pulse frequency. Typical emission spectrum, mass spectrum and ICCD measurement will be presented in the reference conditions. Next the influence of the parameters on these characteristics will be shown and discussed.

This work was supported by the research grant program of Toulouse Tech Transfert (France) in 2021.

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Thrust force generated by surface dielectric barrier discharge

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Atmospheric plasma generated by surface dielectric barrier discharge (SDBD) is promising for sterilizing medical surfaces and equipment. This study explores creating cold atmospheric plasma with SDBD, where applying a high-voltage electric field between electrodes separated by a dielectric lead to ionization. Collisions between charged particles and neutral gas molecules above an ionized cloud produce a thrust force. We focus on how the dielectric's thickness and the geometry of electrodes influence this force, particularly at voltage frequencies starting from 1 kHz and above 20 kHz, an area that is still largely unexplored. Using an analytical balance precise to 1 mg, we examined how the design of the discharge setup affects the thrust force and its changes over time. The findings show that reducing the dielectric thickness and the diameter of the wire electrode increases the thrust. However, if the insulated electrode is too narrow, the force stops increasing beyond a certain voltage, a problem that does not occur with wider electrodes. We found that the thrust is strongest with isolated and exposed electrodes offset by about 1 mm, regardless of the voltage, electrode width, or voltage frequency. The force direction stays roughly 10° from the dielectric surface, not affected by changes in voltage level, electrode size, or frequency. We observed that over time, especially at higher power, the force decreases, which we linked to the air warming above the dielectric. That was confirmed by infrared temperature checks and oscilloscope discharge measurements. These results help improve airflow control in various settings, including cleaning and sterilizing in medical contexts, by fine-tuning the use of SDBD-produced atmospheric plasma.

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Thermal Stress and its Role in Plasma Bacterial Inactivation: High resolution Characterization

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In the past few years, the field of plasma medicine has been widely investigated. The efficiency of the cold atmospheric pressure plasma (CAPP) for bacterial decontamination or cancer cells treatment is nowadays established. For bacterial decontamination, dielectric barrier discharge plasma jet has been deeply studied for their important oxygen and nitrogen reactive species creation rate [1]. However, there are only few relevant temperature measurements of the thermal stress induced by the plasma jet [2]. In the perspective of an accurate characterization of this thermal stress on a target, we used a high-speed high-resolution thermal camera (*Infratec ImageIR® 9400)* [3]. It provides a unique temporal and spatial resolution compared to the conventional temperature sensors. For typical plasma jet exposure settings, previous diagnostics using common device of lower resolution indicated an averaged temperature on the agar surface below 40°C. The new method allows us to detect distinct thermal peaks exceeding 80°C for the same configuration. In order to understand the role of the thermal stress on bacterial inactivation, three different diagnostics were compared. We assess and compare the thermal stress spatial expansion, the plasma jet-target interface and the bacterial inactivation surface following either static or a dynamic CAPP treatment.

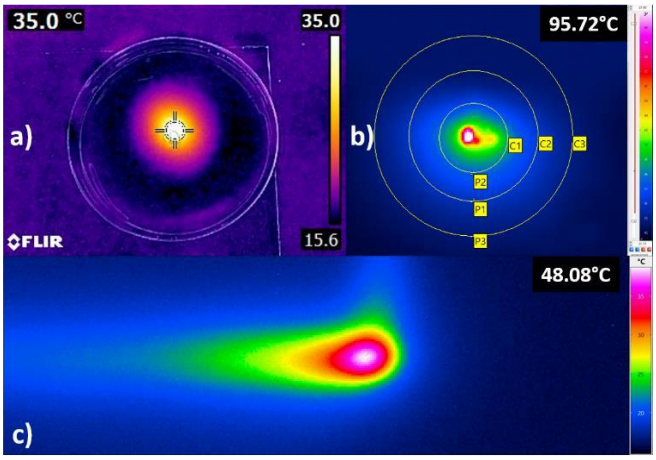

Fig. 1: 60s static plasma treatment, thermal acquisition: low-resolution camera (a), high-speed high- resolution thermal camera (b). Dynamic plasma treatment high-resolution thermal acquisition (c)

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Scale-up of non-thermal plasma and photocatalysis for bacteria inactivation andVOCs removal

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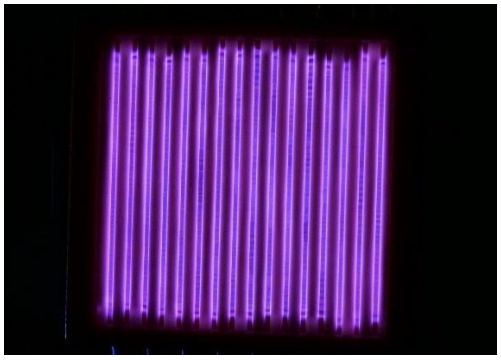
Indoor air contains many harmful components (chemical pollutants, bacteria, pathogenic aerosols, tobacco smoke, etc.) that can cause diseases under long-term exposure. Hospitals-acquired infections are also spread through air contaminants [1]. Finding an innovative technology that would efficiently remove all kinds of airborne pollutants without producing harmful by-products and with a low energy cost would be not only a major advance for public health but would also help preventing the spread of airborne pathogens such as in the case of the recent COVID-19 pandemic. The goal of this work is to assess the efficacy of non-thermal plasma (NTP) combined with photocatalysis for the removal of VOCs and inactivation of aerosol-borne bacteria at a high gas flow rate. NTP and photocatalysis have proven their capabilities to decompose or inactivate a broad range of harmful compounds present in indoor air. Moreover, combining these two techniques may offer a very effective hybrid air decontamination device, as studies suggest a synergetic effect [2].

We designed an indoor air decontamination device that combines a Dielectric Barrier Discharge (DBD) for the NTP generation (Fig. 1), and a TiO2 coating which is activated by UV-A LEDs. Despite a very short residence time of the pollutant in the reactor (gas flow rate was set above 300 L/min) and the use of a single-pass method, our results show the removal efficiency of about 40% of the formaldehyde concentration. The obtained results of the decontamination of bio-aerosols are also very promising since we reached a 3,73 and 3,32 log reduction for the inactivation of *Escherichia coli* and *Staphylococcus aureus* respectively. We also monitored the concentration of ozone generated by the DBD, which is not desired for human exposure, and we observed its decomposition by the photocatalytic process.

Fig. 1 Photo of the DBD module



This work was supported by Slovak Research and Development Agency APVV-17-0382, APVV-22- 0247, and APVV-20-0566, and a postdoctoral support of Comenius University Bratislava.

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Characterization of a cold atmospheric air plasma jet supplied by microsecond voltage pulses

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In last two decades, atmospheric pressure argon and helium plasma jets have been extensively exploited for surface modifications as well as for decontamination/sterilization purposes, skin treatments, and cancer cells abatement. These devices present several advantages like a homogeneous plasma plume, moderate applied voltages and the possibility to accurately treat small areas. The main effects of plasma jets are related to the creation of Reactive Oxygen and Nitrogen Species (RONS) produced in the air discharge. The use of noble gases as carrier gas is required to obtain a homogeneous and stable discharge. Unfortunately, these gases are expensive and require bulky tanks, thus limiting the employability of plasma jets in real-life applications and their future advancements. Recently, atmospheric plasma jets using exclusively air as working gas have been investigated and tested for biomedical applications [1].

Our research group developed an atmospheric pressure cold plasma jet fed by synthetic air. The reactor chamber is constituted by a metallic needle connected to a high voltage terminal and positioned inside a cylindrical grounded metallic cavity (Fig. 1). A homemade high voltage power source [2] feeds the discharge with either positive or negative microsecond pulses with an adjustable amplitude between 3 and 5 kV and a FWHM of 10 μs (Fig. 2). Different voltages, pulse repetition rates and gas flow rates have been investigated. The average power has been found to range between 0.4÷1.2 W. This quantity increases with the gas flow rate and applied voltage, and it can be easily adjusted by varying the time interval between pulses. The plasma plume is not strongly affected by the input parameters, ranging between 5÷6 mm in length for all tested conditions (Fig. 1).

A metallic plate, which can be either electrically floating or grounded, has been used as a first target. When touching the target surface, discharge average power was not varied in a consistent way with respect the free flow condition. This result agrees with the absence of charges measured over a second insulating target impinged by the plasma jet, demonstrating the negligible electrical coupling between the proposed reactor and a treated surface.

The target temperature has been measured by means of an infrared camera, showing values in the range 32÷42 °C when gas flow rate was varied between 6 and 2 l/min respectively.

This preliminary experimental campaign shows that this cost-effective air plasma jet device allows an easy adjustment of the discharge average power, guaranteeing a limited thermal stress to the target surface. This homogeneous plasma plume can thus be exploited for biomedical applications.

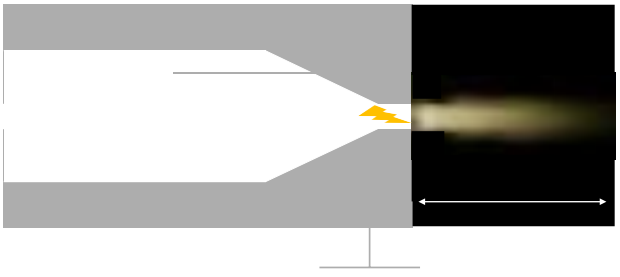

Fig. 1 Air plasma jet scheme and plume

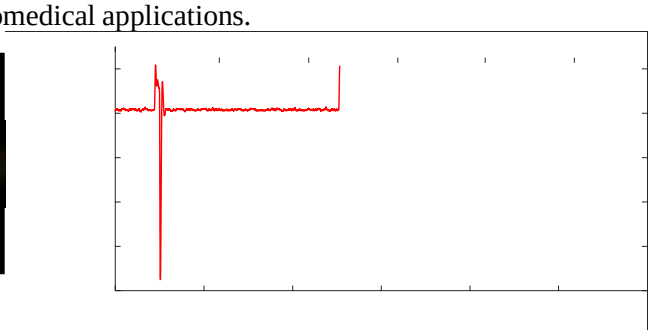

Fig. 2 Example of negative voltage pulses

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Thu-P-22

Evaluating Antimicrobial Effects of Plasma-Activated Liquids Using Surface-Wave Microwave Plasma Jet: A Comparative Analysis of Deionized Water and Saline Solution

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Abstract

The utilization of non-thermal plasma for liquid activation results in plasma-activated liquids (PAL), a substance that has garnered significant attention across multiple sectors, such as agriculture, environmental studies, sterilization, and healthcare [1,2]. Notably, in the medical and dental domains, PAL serves as an effective antimicrobial agent [2]. The objective of this research is to perform a microbiological evaluation of two plasma-activated liquids: deionized water and 0.9% saline solution. The PALs were produced using a surface wave microwave plasma jet, targeting *S. aureus*, *E. coli* and *C. albicans*.

The study used a surface-wave microwave plasma jet operating at 2.45 GHz with continuous argon gas flow. The Microwave power up to 200 W was supplied by a solid-state source, with experiments conducted at 70 W. Deionized water (DI) and 0.9% saline solution (SS) were activated in volumes of 40 mL at 10- and 30-min intervals. The antimicrobial efficacy was evaluated in groups: DI (control), SS (control), DI activated for 10 min, DI activated for 30 min, SS activated for 10 min, and SS activated for 30 min. A standardized microbial suspension (750 µL) containing 10^6 cells/mL was meticulously prepared in sterile saline solution (0.9% NaCl) using a spectrophotometer (AJX- 1600, Micronal, São Paulo, SP, Brazil) to ensure uniformity. Subsequently, the prepared microbial suspension was mixed with 1250 µL of PAL and incubated for 10- and 30-min. Serial dilutions were made in sterile saline and 100 µL aliquots were spread on the appropriate culture media. The same procedure was repeated for SS. The plates were then incubated aerobically at 37°C for 24 hours, after which CFU/mL were determined based on colony counts.

Microbiological tests revealed a notable reduction in E. coli and S. aureus with activated DI, particularly at the 40-minute mark. However, this effect was not as pronounced for the C. albicans fungus or with the plasma-activated 0.9% saline solution. The research suggests that the low pH of the samples is a key factor in their antimicrobial activity [2]. Therefore, it is evident that activated DI is an effective method for bacterial inactivation at a low cost, but it does not have the same effect on fungi under the current conditions. In contrast, the 0.9% saline solution did not show similar effectiveness against bacteria and showed less pH reduction compared to activated DI.

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Underwater Discharge Plasma for in situ Generation of Micro-nanobubbles

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For most biomedical applications of plasma activated water (PAW), the liquid-phase reactive species are the key factor determining the application effect [1], and the study of plasma-liquid interactions, including the mass transfer, transformation, and the control of reactive species in the gas-liquid interface, is one of the major challenges facing the plasma discipline at present [2]. In this work, we introduce a novel underwater discharge reactor to produce micro-nano bubbles (MNBs) in-situ to enhance the interface reaction between plasma and liquid. The size of microbubbles and nanobubbles range from 40~60μm and 200~400nm, respectively. MNBs guided the establishment of spark discharges and help prepare 100mL PAW in 5min with low energy consumption. The smaller specific surface area, slower rise rate, and surface properties of the bubbles enhance the solubilization and generationof some species including O_3, H_2O_2, and $ONOO^-/O_2^-$. Correspondingly, the micro-nano-bubble plasma activated water (M-PAW) has also shown excellent performance in sterilization, with strong inactivation of both *Staphylococcus aureus* and *Saccharomyces cerevisiae*. Adjustment of the reactor parameters can change bubblw size and the concentration of RONS, which is expected to be a effective way to regulate and promote the PAW activity.

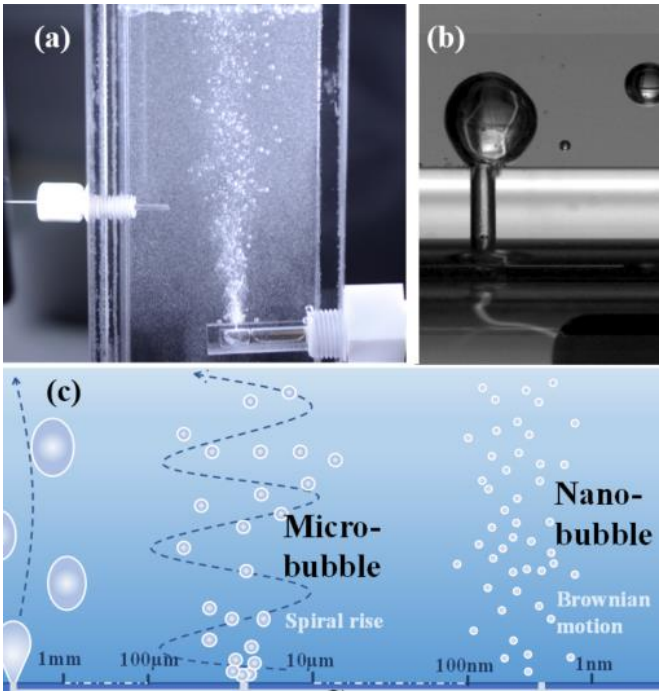

Fig. 1 The generation and characters of MNBs during discharge

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Application of plasma-activated water on oral pathogens

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Cold atmospheric-pressure plasma (CAP) can efficiently inactivate microbial cells and could be developed into an effective disinfection strategy for medical fields. Plasma-activated saline, as a derivative form of plasma, also exhibits strong bactericidal activity. Oral diseases stemming from oral pathogenic bacteria pose a significant global health concern and plasma-activated water can be used for this issue. In our previous study, the plasma-activated water prepared by the NO_x-gas, O_3-gas, and Mixed-gas that from the mixture of NO_x-gas and O_3-gas, and plasma-activated water prepared by the Mixed-gas exhibited the strongest inactivation effect. Then the plasma-activated water prepared by the Mixed-gas was applied to inactivate oral pathogenic bacteria, including *Streptococcus mutans* and *Porphyromonas gingivalis*. The plasma-activated water could reduce more than 6.1-$\log_{10}$ planktonic bacteria and 4.1-$\log_{10}$ bacteria within biofilm, respectively. Plasma-activated water treatment of planktonic bacteria also effectively inhibited biofilm formation. The treatment of plasma-activated water caused deformation and damage to the integrity. Compared to chlorhexidine, a widely used oral disinfectant, plasma-activated water exhibited superior inactivation effects in both planktonic bacteria and biofilm. Based on these results, plasma-activated water presented a potent strategy for bacteria eradication to reduce the incidence of oral diseases.

This work was supported by National Natural Science Foundation of China (Grant No. 51977174).

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Plasma-activated medium for intraoperative adhesion prophylaxis

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Peritoneal adhesions as a result of tissue trauma during surgery are a major clinical problem, affecting around 93% of all patients. Tissues that are not normally fused together, such as the intestine and the peritoneum, stick together due to abnormal wound healing during the healing process. This can lead to pain, secondary infertility or even ileus, which in the worst case can lead to death. The prevention of adhesions is therefore a vastly underestimated field that should urgently receive more attention in research. Prevention methods already in use, such as barrier methods, have not yet shown any clear reliable effect [1]. Since the antiproliferative effects of plasma-activated media (PAM) are known, this is a possible starting point for preventing the development of peritoneal adhesions [2].

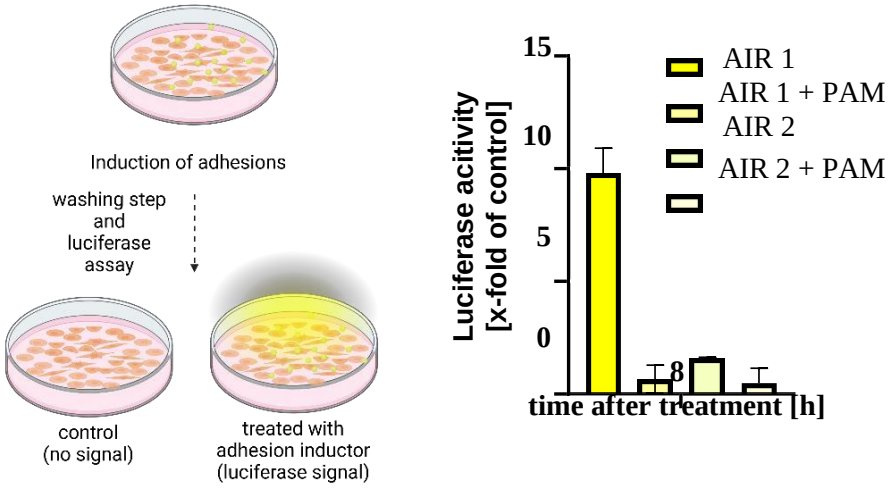

Fig. 1: 2D i*n vitro* Adhesion Assay and effect of PAM on adhesion model. AIR= adhesion inducing reagent.

Methods and Findings

We found an antiproliferative effect when cells were treated with different PAM and further identified effects on cytoskeletal regulation, cell cycle control, apoptosis and various intracellular signaling pathways via protein analysis, which differ in mesothelial cells and fibroblasts. To simulate adhesions an adhesion model was established by adhesion inducers. Treatment with PAM showed that adhesions can be prevented in the 2D *in vitro* model by treating them with PAM.

Conclusions

PAM treatment leads to various cellular effects that can be made therapeutically useful. Treatment with PAM leads to the prevention of adhesion formation in the *in vitro* adhesion model. Futher research will expand our results towards “in-vivo-like” patient-derived peritoneum on chip models.

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Thu-P-26

Proteomics and single-cell microscopy analysis for the investigation of plasma-activated water inactivation mechanism of *Escherichia coli*

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Investigating the inactivation mechanism of plasma-activated water (PAW) holds great importance in optimizing plasma treatment parameters, enhancing our comprehension of plasma-bacteria interactions, ensuring precise application, evaluating safety and efficacy, and fostering novel applications. This line of research serves to propel advancements in PAW-based antimicrobial technologies across diverse domains such as healthcare and food safety. Within this context, the interaction between two distinct samples of plasma-activated water (PAW-10 and PAW-20) and *Escherichia coli* has been examined via proteomics analysis. Notably, the two PAW variants exhibit differential effects on *E. coli* due to their different chemical properties. The PAW samples, produced by a reactor developed in our laboratory [1], have been fully characterized in terms of long-lived species (NO_2^-, NO_3^-, H_2O_2), pH, oxidation-reduction potential and electrical conductivity. The milder treatment, employing PAW-10, results in approximately a 1-log reduction in bacterial cell count, whereas the more potent inactivation is achieved with PAW-20, yielding a 5-log reduction in the initial bacterial population. Analysis of protein differential expression across the various PAW treated samples allows the identification of upregulated and downregulated proteins. Changes in protein abundance are statistically analyzed and used for the interpretation of biological pathways and mechanisms induced by PAW exposure.
Concurrently, single-cell microfluidic experiments have been conducted on *E.coli* cells, enabling time-lapse microscopy of bacteria in real-time exposure to PAW. Bacteria viability and duplication is first observed in cells provided with growing media. Subsequently, bacteria are exposed to a PAW flow for a controlled duration. Finally, an 8-hour growing media rewash is performed. The effect of PAW on bacterial size and duplication rate is assessed. The results of proteomics and single-cell microscopy are finally integrated with the objective to improve the understanding of PAW interaction mechanisms with bacteria.

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Thu-P-27

Development of a Plasma Functionalised Liquid for prevention and control of MRSA biofilm device-associated infections.

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Orthopaedic implant infections impose a huge burden on healthcare systems globally. Current treatments include wound debridement, antibiotic treatment and if necessary, removal of the implant via revision surgery. Treatment of orthopaedic implant infections is further challenged by antibiotic- resistant pathogens such as *Staphylococcus aureus* and their ability to form treatment resistant biofilms. There is a need for alternative therapies to control and mitigate these infections. Cold atmospheric plasma (CAP) and plasma functionalised liquids (PFLs) have demonstrated useful antimicrobial efficacy and wound healing properties [1] and have potential to be applied in sequence as a viable new treatment for these infections.

In this study the efficacy of PFLs generated from an in-house pin discharge to liquid plasma system known as the reactive species specificity system [2] in both Spark and Glow configurations and a microwave discharge - the MidiPlexc system [3] against methicillin-resistant *S. aureus* (MRSA) biofilms was compared. The predominant reactive Nitrogen and Oxygen species within the liquids were established using colorimetric and ion chromatographic methods. The PFLs generated from the three systems had distinct chemistries. The PFLs were shown to be more efficacious against planktonic *Pseudomonas aeruginosa* and methicillin-susceptible *S. aureus* strains compared to the MRSA strains. MRSA biofilms were grown on abiotic and biotic material for 24-72h. MidiPlexc and RSS Spark PFLs were effective at reducing bacterial biofilm numbers, but that this was a function of biofilm maturity, where 72h biofilms were more tolerant to PFL treatment compared to 24h biofilms. This study aims to assess the efficacy of PFLs in combination with direct medically approved CAP devices.

This work was funded by Science Foundation Ireland and the Health Research Board under the tri- partite US-Ireland programme under the grant agreement 20/US/3678

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Addition of ROS scavengers provides an insight into the antimicrobial effect of plasma treated liquids

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The antimicrobial effect of plasma treated liquids (PTL) is a well-established fact. However, attempts to manifest antimicrobial effects in buffered solutions, which do not acidify when treated with plasma, had been futile so far [1][2], implying that a low pH environment is an important factor contributing to the antimicrobial effect.
By utilizing a DBD with a concentric electrode setup, we can however generate a very potent antimicrobial Tris-HCl buffer solution, achieving a reduction of log 6, while maintaining a neutral pH.
To verify the results obtained by CFU counts, a fluorescence-based live-dead assay was performed. The fluorescent dyes stain bacteria red or green, depending on whether their membrane is perforated or intact. While the results show that close to 100 % of the bacteria had a damaged membrane, thus confirming the CFU counts, it became obvious that the plasma species also affect the performance of the fluorescent dyes. To alleviate this Thiosulfate was added, after the bacteria had been exposed to the PTL for 30 min, but prior to the addition of the fluorescent dyes. Curiously, while this measure did protect the fluorescent dyes, it also was found that the addition of the thiosulfate seems to reverse the effect of the plasma, i.e., a significant portion of the bacteria were shown to have intact membranes. Furthermore, when plating bacteria that had been supplied with thiosulfate after exposure to PTL, a considerable number of CFUs were observed, whereas there were zero CFU if thiosulfate was not added.

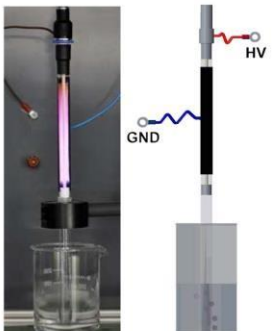

Fig. 1 Photo of the plasma source demo version and schematic

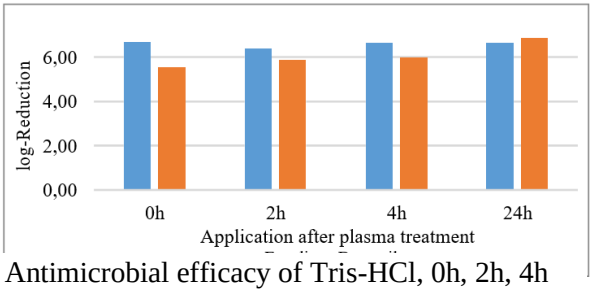

Fig. 2 Antimicrobial efficacy of Tris-HCl, 0h, 2h, 4h

These data show that the elimination of bacteria by plasma generated species in PTL does not happen instantly, but is instead a time dependent process. It seems that the plasma generated species do not kill the bacteria directly, rather they trigger a cascade reaction which leads to their death and the initial damage caused by the plasma species can be reversed to a degree.

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Thu-P-29

Disinfection of *Enterococcus faecalis* on baby bottle nipples using atmospheric low-temperature plasma bubbled-up water

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Introduction

It is estimated that 10^{6-8} CFU of bacteria live in the human oral cavity per mL of saliva. Although rinses with disinfection effects are commercially available to keep the oral cavity clean, they contain alcohols and strong irritants that can damage oral mucous membranes and tissues [1]. In particular, since infants have low immunity and are highly irritated by drugs, there is a need for a safe and reliable method of sterilizing baby bottles, but the conventional sterilization method as boiling takes time and labor.

Recently, atmospheric low-temperature plasma has been attracting attention as a new disinfection method without heat or discharge damage. The disinfection is performed by the reactive species generated in the plasma. In our group multi-gas plasma jet has been developed that can generate plasma with molecular gases such as oxygen and nitrogen or mixtures of these gases. It has become clear that the type and amount of reactive species produced depends on the gas types used [2]. For example, oxygen-derived reactive species have a high bactericidal effect. In our previous study, it has been clarified that plasma-treated water, which has disinfection effect, can be produced in a short time by bubbling the oxygen plasma and introducing reactive species into the liquid through a porous filter. We call this plasma bubbled-up water (PBW) and it is a kind of plasma-treated water.

In this study, bactericidal effect of PBW using oxygen plasma, which can produce a large amount of oxygen-derived reactive species, was evaluated against *E. faecalis* suspension and *E. faecalis* attached to baby bottle nipples.

Experiment method

As the target of this study, *E. faecalis*, which cause various diseases and are found on teeth, were used. The concentration of *E. faecalis* was prepared at approximately 10^7 CFU/mL. Plasma was generated by introducing 3 L/min of oxygen into a multi-gas plasma jet and applying a high AC voltage. PBW was generated by bubbling plasma into 50 mL of purified water through a porous glass filter for 2 min. After PBW generation, the PBWs were allowed to stand for 0, 1, 5, 10, and 30 min, and 900 µL of PBW was collected at each time and mixed with 100 µL of *E. faecalis* suspension. Immediately after the mixing, the mixture was agitated for 5 s, and 20 µL was dropped onto agar medium after step dilution. In the case of the disinfection of baby bottles nipples with *E. faecalis*, approximately 10^7 CFU of *E. faecalis* were attached to the baby bottles nipples and immersed in PBW. Thereafter, 100 µL were collected every exposure time and dropped onto 20 µL agar medium after step dilution. The bacteria were incubated at 37°C for about 24 hours, and the number of viable bacteria was evaluated using the colony count method.

Result and discussion

When oxygen PBW was mixed with E. faecalis suspension immediately after generation, the number of viable bacteria decreased by more than two orders. However, the bactericidal effect decreased with time when PBW was mixed with the bacteria, and after 30 minutes, oxygen PBW showed little bactericidal effect. Results of the bactericidal effect against *E. faecalis* on baby bottle nipples will also be reported in the presentation.

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Developing a Chicken Juice-Based Infection Model to Assess the Antimicrobial Effects, Mechanisms of Actions, and Modes of Delivery of Plasma Functionalized Liquid.

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Plasma Functionalized Water (PFW) is a flexible form of cold plasma (CP) intervention with the potential to mitigate microbiological concerns across a range of biological systems and abiotic environments. The present study characterized the key process parameters that govern the chemical and antimicrobial profiles of two custom system setups, namely the reactive species specificity (RSS) [1] and Microwave Induced Plasma (Midi-Plex) [2], which were employed to generate PFW. The chemical profiles of PFWs were identified in terms of pH, conductivity, and RONS concentration. An infection chicken juice model was developed in liquid and solid formats to assess different delivery forms of PFWs to control pathogens including *Salmonella* Typhimurium, *Campylobacter jejuni and Staphylococcal spp.* The target pathogens were tested in different phenotypic forms, namely planktonic cells in suspension or attached to biotic or abiotic surfaces and biofilms attached to different surface materials. The antimicrobial efficiency of PFWs was identified using microbial recovery and metabolic activity tests. The associated mechanisms of action were investigated by employing Live Cell Imaging, Scanning Electron Microscopy, Atomic Force Microscopy and Confocal Laser Scanning Microscopy. Results illustrated that the chemistry and efficiency varied largely with the plasma generation time, system configuration, contact time, target pathogen and physiological stage. The electrode-based system (RSS) generated ROS and RNS depending on generation time and discharge configuration. The electrodeless system (Midi-Plex) generated only RNS, specifically NO_3^- and NO_2^-. The PFWs enabled pathogen reduction of between 7 and 9 $\log_{10}$ CFU/ml within 15 sec. Intracellular oxidative stress, membrane deterioration, cell deformation and leakage, rupture of biofilm structure and topography were all induced by PFW treatments. The antimicrobial efficacy varied significantly with the mode of PFW delivery. PFW is a promising and scalable approach to ensure rapid and sustainable sanitary conditions due to its irreversible damage induced within bacteria cells; regardless of the phenotype or the environment.

This work was funded by the Department of Agriculture, Food and Marine under grant number 17/F/275 under the Food Institutional Research Measure (FIRM) and Science Foundation Ireland and the Health Research Board under the tripartite US-Ireland program under the grant agreement 20/US/3678

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Thu-P-31

Antibacterial effect of Plasma Activated Water against L. *monocytogenes* and E. *faecalis*

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Nosocomial infections have emerged as a global menace, resulting in thousands annual deaths owing to the proliferation of resistant bacterial strains. In this sense, *Listeria monocytogenes* (*L. monocytogenes*) as a typical foodborne pathogen and *Enterococcus faecalis* (*E. faecalis*) as the cause of life-threatening infections in human beings were studied. Over the last years, plasma activated water (PAW) has demonstrated its antibacterial effect and can be used as an alternative antibacteria l solution. PAW was generated by an atmospheric pressure cold plasma jet system with dielectr ic barrier discharge in a bubble configuration reactor where plasma gas is bubbled through water, letting greater transfer of reactive species into the water.

PAW generated with four different air flows (60, 80, 100 and 120 slm) were tested against *L. monocytogenes* and *E. faecalis*. The contact times between PAW and 10^8 CFU/mL of bacteria were 10 min, 20 min, 30 min, 1 h, 2 h and 4 h and after those, microbiological analysis was conducted. Physico-chemical parameters of PAW were determined such as nitrates, nitrites, and different reactive oxygen and nitrogen species (NO_2•, NO•, OH•, HNO_2, NO_2^-, $ONOO^-$). Furthermore, oxidation-reduction potential (ORP), electrical conductivity (EC) and pH were also measured. Finally, in order to understand inactivation results, computational fluid dynamic (CFD) simulations were performed.

In relation to *L. monocytogenes*, total inactivation was achieved after 2 and 4 hours of contact PAW-bacterium regardless of the PAW tested. On the other hand, for E. *faecalis* total inactivat ion was only achieved after 4 hours of contact PAW-bacterium with the PAW generated with the highest air flow. Then, it is proposed that the higher the air flow when generating PAW, the stronger the antibacterial effect. Similarly, when comparing contact times for one PAW, the inactivation rate increases when the contact time does.

Based on the simulation results, it can be suggested that an air flow of 120 slm enhances bubble distribution throughout the water bulk and provoked a larger air-water interface area. This indicates a more favorable scenario for improving dissolution and mass transfer of reactive species in water, potentially contributing to the production of greater bactericidal PAW, as verified by microbiologica l tests.

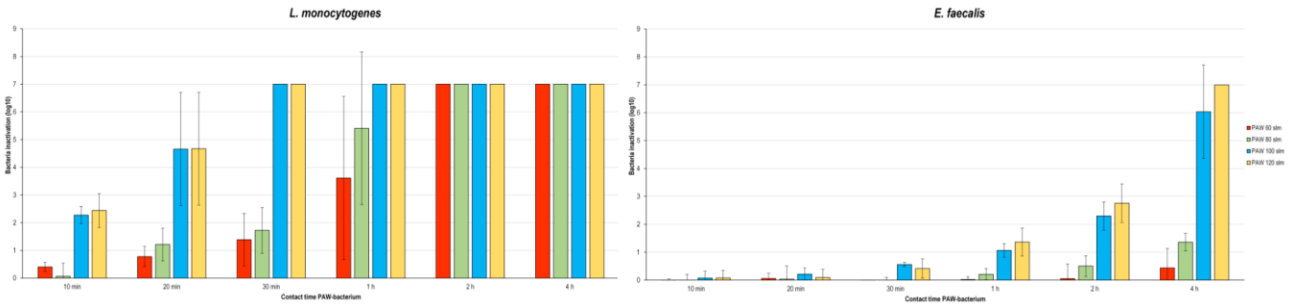

Fig. 1. Bacteria inactivation rate against *L. monocytogenes* and *E. faecalis* after each PAW contact time.

Thu-P-32

Evaluation of Plasma Activated Liquids for Decontamination of Flexible Endoscopes

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Flexible endoscopes have become a prevalent feature of modern clinical practice with demand continuing to increase, however the inner channel systems of the reusable devices are complex and provide an ideal environment for biofilm formation following repeated use. Endoscope reprocessing involves multiple stages: including pre-cleaning, manual cleaning, high-level disinfection, and drying. Despite improvements to endoscope reprocessing over the years, the continued presence of build-up biofilm contamination within "patient-ready" reprocessed flexible endoscopes highlights our need for a new approach to disinfection.

In this study, an evaluation of the efficacy of plasma activated liquids for disinfection of endoscope associated biofilm contamination was carried out. Teflon endoscope surrogate test pieces of 2.0- or 6.0- mm diameter were contaminated with 1% human serum and clinically relevant single and mixed species biofilms. Cold atmospheric pressure plasma was used to activate liquids, including water and a commercially available pH buffered peracetic acid-based disinfectant. Each disinfection method was circulated through endoscopic test pieces containing biofilm contamination, and their efficacy compared against a widely used commercial disinfectant. To investigate potential surface damage resulting from the decontamination process, the surface composition and morphology were examined using FTIR, XPS and AFM.

Plasma activation was found to increase the antibiofilm capabilities of pH buffered peracetic acid by introducing a selection of reactive species into the solution. In comparison, disinfection of endoscopic test pieces with plasma activated disinfectant resulted in complete removal of culturable biofilm contamination in 5 min, surpassing the 4.39 $\log_{10}$ reduction observed with the currently used endoscope disinfection method. Results showed a reduced regrowth and recolonization of the surface of the endoscopic test pieces following plasma-based disinfection; however, it is suggested that plasma activated liquids would not increase likelihood of any potential surface damage over time.

Acknowledgements

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Investigation of toxicity effects of Plasma Activated Water on a water model plant *Lemna Minor*

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Non-thermal atmospheric pressure plasmas play a crucial role in biomedical applications due to the highly reactive chemistry of Reactive Oxygen and Nitrogen Species (RONS) they produce in gas phase [1, 2]. When treating water, RONS produced in the gas phase interact with the liquid interface, creating Plasma Activated Water (PAW). The RONS concentration can be tailored by changing plasma chemistry in the gas phase, water type, sample vessel properties etc. In recent years PAW is being developed as part of a new field of plasma agricultural applications. When PAW is used as a watering agent it results in a better yield of plants and has a positive effect on germination percentage [3,4]. However, the toxicity of PAW and its impact on the environment if it is released in underground or fresh water has not yet been investigated in detail.

In this work, we used a plasma jet with pin electrode operating at atmospheric pressure to obtain PAW by treating deionized and tap water. The plasma jet was powered by a kHz sine signal and used Ar as a working gas, with a total gas flow of 1 slm. To investigate the toxicity of PAW, the primary goal was to produce a liquid with the highest amount of the measured reactive species (H_2O_2, NO_3^-, NO_2^-). Deionized water treatments have demonstrated that, independent of the amount of treated sample, the pH of the water decreases significantly, which can be hazardous to plants. To eliminate the possible toxicity of PAW due to acidity, we selected to treat tap water, which retained its pH value even after treatment. Finally, PAW was obtained by treating 15 ml of tap water for 10 minutes.

The selected PAW was combined with two different matrices, namely tap water and Danube (river) water which were introduced by making PAW dilutions. Four different dilutions (100%, 60%, 30%, and 10%) were prepared for both matrices to assess their impact on *Lemna minor* samples. The plants were cultivated in beakers containing 25 ml of liquid, maintaining controlled conditions, such as temperature, humidity and daylight cycle, for a period of 17 days. The root lengths, leaf surface area, and chlorophyll percentage were then measured. Each experimental set was carried out in triplicate. The results showed that a smaller amount of PAW had a beneficial impact, whereas greater percentages were completely toxic. In all PAW samples, root lengths were reduced but leaf surface area increased. Notably, PAW diluted to 10% and 30% in either tap water or Danube water resulted in a higher chlorophyll percentage than the control samples.

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Effect of plasma-activated water produced in an Air-Operated DBD Coaxial Reactor on *Candida albicans* Biofilms

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Candida albicans is an opportunistic fungal species that is frequently related to persistent endodontic infection, due to its remarkable ability of forming biofilms withing the root canals associated to the resistance to conventional irrigants and medications. This study aimed to evaluate the effect of plasma-activated deionized water produced in an Air-operated DBD Coaxial reactor on *Candida albicans* biofilms. Mono species biofilms of *Candida albicans* (ATCC 18804) were formed for 24 hours, at37°C, and aerobic conditions. Deionized water was activated by using a coaxial DBD reactor using compressed air as working gas (flow rate 5 L/min) under the following parameters: signal frequency 14 kHz and voltage 10.6 kV for 10 minutes. Biofilms were exposed to the activated water for 1, 2 and 3 cycles of 1.5 min exposure. Subsequently, the biofilms were recovered, and the fungal suspensions were serially diluted and plated on Sabouraud dextrose agar. Non-activated deionized water was the negative control. Then, the number of colonies were counted. The experiments were performed in triplicate at three different occasions (n=9). Cell counts were compared among the groups by paired Mann-Whitney test, with a level of significance of 5%. The percentual reduction in relation to negative control was also determined. A significant reduction of viable cells in *C. albicans* biofilms were observed after exposed to plasma-activated deionized water for 1, 2 or 3 cycles of 1.5 min exposure, with reductions of 67.57% (p=0.01), 95.29% (p=0.00) and 94.38% (p=0.00), respectively compared to negative control. Plasma- activated deionized water reduced the *C. albicans* biofilm viability more than 90%, suggesting a promising application in Endodontics.

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Thu-P-35

The influence of Cold Atmospheric Plasma treatment on patient-derived 3- dimensional organoids

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Although traditional 2-dimensional cell culture lines are broadly used in cancer research, they do not accurately represent the complex interactions between cells and their microenvironment. Therefore, we present the use of 3-dimensional patient-derived organoids in plasma medicine, a more representative model of real tumours that preserves heterogeneity and morphology of the tumours in patients, as well as the phenotype and genotype of the original tumors. [1]

The aim of this study is to develop a comprehensive protocol to investigate the impact of plasma treatment on organoids. The initial step involves the creation of organoids (pancreatic ductal adenocarcinoma and head and neck squamous cell carcinoma), utilizing a protocol optimized [2] in our laboratory (Fig. 1 A). Afterwards, the seeding rates, use of different types of well plates, coating optimization, and various analytical approaches, were studied in the context of plasma treatment. Using this newly adapted protocol, we studied the kinetics and growth rates of organoids, both with and without plasma treatment with the kINPen MED, using Cytotox green to fluorescently label the dying cells. Furthermore, we are assessing the concentration of reactive species after treatment, investigating the induction of immunogenic cell death, and conducting pathway analysis. Our results show sustained and reproducible reduction of tumour organoids over time in a dose-dependent way (Fig. 1 B).

Altogether, this study will provide a streamlined protocol for the investigation of the influence of cold atmospheric plasma on organoids, valuable for the exploration of single and combinatory therapies against cancer in patient-derived 3-dimensional tumours *in vitro*.

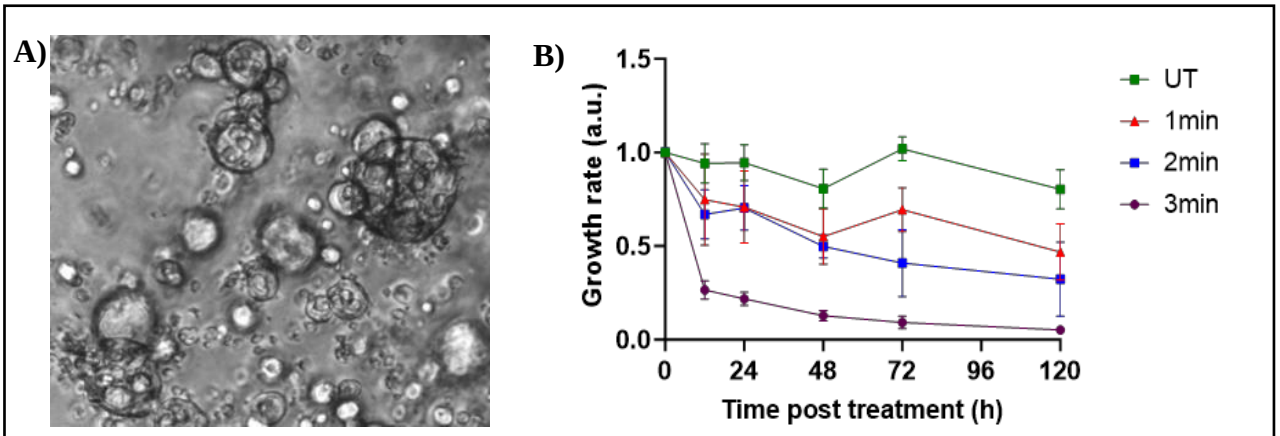

Fig.1. (**A)** Representative image of PDAC organoids, protocol optimized in our lab. (**B)** Growth rate of organoids followed up for 5 days after plasma treatment with different conditions, normalized to untreated (UT) control. Mean +/- SD.

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Thu-P-36

Cold atmospheric plasma treatment of chronic wounds—investigation of the effects of reactive oxygen and nitrogen species on fibrosis-related cellular signaling pathways

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A novel treatment for chronic wounds is cold atmospheric plasma. Non-equilibrium plasmas generate highly reactive species at low temperatures. This allows treatment of human tissue in-vivo and can induce locally confined redox-chemistry in biological organisms. Reactive oxygen and nitrogen species are known to play a vital role in cell signaling and influence a range of mechanisms implicated in all phases of wound healing—inflammation, vascular formation, proliferation, remodeling of scar tissue [1]. Plasma gives us a tool for controlling and modulating dosage of the redox species cocktail deposited on the wound bed—by modifying plasma environment and the mixture in the feed gas [2]. By better understanding the effect of reactive species on cells, we can tailor the plasma reactivity to supply the adapted treatment to the tissue. A special focus is put on fibrosis, a form of disrupted wound healing. Reactive species have dual functions depending on the healing phase, their concentrations, etc. This is why a good characterisation of the plasma composition is essential**.** Plasma composition was simplified to the hypothesis of 2 regimes which could have dual effects on fibrosis—oxygen regime vs nitrogen regime. Reactive oxygen species are known to be pro-inflammatory by increasing the oxidative stress in the environment of the cells, and reactive nitrogen species have anti-inflammatory behavior [3]. Spectroscopy techniques are used to measure key species density in the plasma, informing us on which regime is at play e.g., UV-absorption spectroscopy at 254nm is used for quantifying ozone. Biological experiments are conducted with each regime, analyzing cell behavior as a response to modulated plasma treatment. Tailoring the plasma reactivity to biological needs, to reach a bio-chemical effect on imbalanced healing environment in tissue models will deepen our comprehension on the physiology of chronic wounds. It will also pave the way to a personalized plasma treatment technology greatly beneficing the health sector.
This work was supported by NSERC and TransMedTech Institute grants.

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Hemostasis by plasma treatment shortens the wound healing duration by bypassing the inflammatory response and proliferation of bone marrow- derived fibroblasts.

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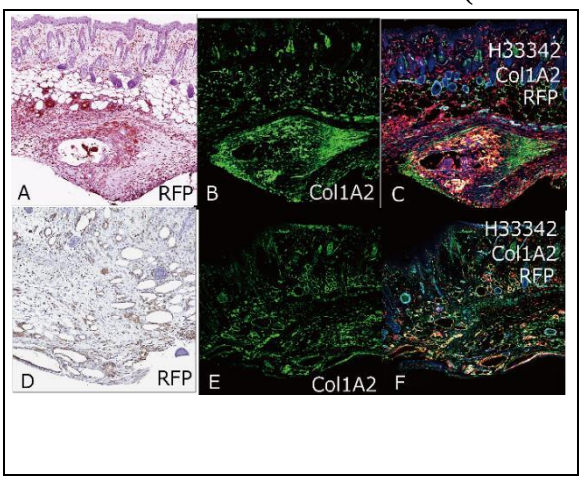
Introduction: A hemostasis method using non-equilibrium plasma at atmospheric pressure (NEPAP) covers the disrupted vascular wall with the induced serum protein coagulation by the supplied charged molecules and stops bleeding [1, 2]. On the other hand, high-frequency electric coagulators (HFEC) provide heat to shrink the tissue containing the disrupted vascular wall to stop bleeding. Therefore, HFEC treatment is a cause for triggering pathological repair such as hypertrophic scar (HS) formation and overproducing collagens in postoperative disorders [3, 4]. In this study, by determining if HS formation occurred after HFEC use through the accumulation of bone marrow- derived cells (BMCs), we sought to demonstrate that plasma treatment shortens the wound healing duration by bypassing the inflammatory response and proliferation of BMCs.

Methods: In the transplanted B6 mice with the bone marrow from RosamTmG mouse (all cells expressing the red fluorescent protein (RFP)), we traced BMCs in HS formation that appeared after the cauterisation with HFEC, referring to the tissue in NEPAP treatment. Bleedings were from the injured subcutaneous vessels between the thorax and abdomen, and either NEPAP or HFEC was applied to the injury for hemostasis. The pathological repairs were histologically analysed, and multi-color immunofluorescence analysis using confocal microscopic was applied to identify the type of cells and the collagens produced.



Results in HS formation where collagens overproduce appeared after hemostasis with HFEC. In the HS, there was a large number of RFP+ BMCs that produced type I (Fig.1 A - C) and type III collagen.
On the other hand, in the tissue after the hemostasis with NEPAP, neither increment of RFP+BMCs nor collagen overproduction occurred (Fig.1 D - F).

Discussion & Conclusion This was the first study to identify that hemostasis by plasma treatment bypassed the inflammatory response and proliferation of BM-derived fibroblasts. In particular, plasma hemostasis effectively shortens the duration of wound healing and prevents HS formation.

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Thu-P-38

Enhancing Wound Healing with Bioprinted Chitosan Hydrogels Treated by Cold Atmospheric Plasma Jets

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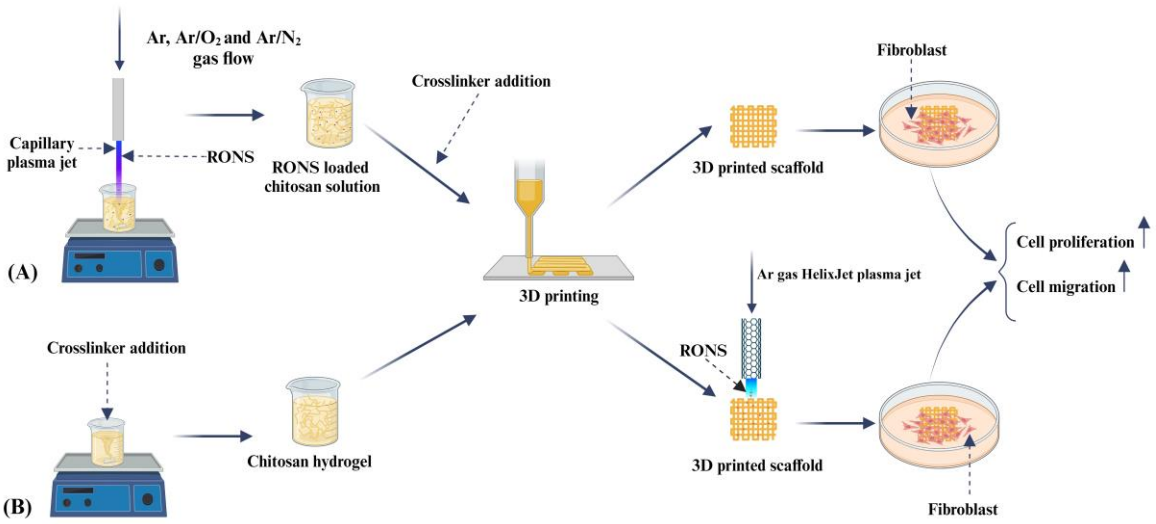
Uncontrolled bleeding in many kinds of injuries and accidents is a major factor leading to death. Severe blood loss can result in hypothermia, coagulopathy, and multiorgan failure, while bacterial infections can further complicate the recovery process [1]. Addressing this, we investigate the ap- plication of plasma treatment to improve the antibacterial characteristics of chitosan (CS) hydrogels and augment their suitability for bioprinting applications. We employ 3D bioprinting to fabricate CS hydrogel scaffolds because it can closely mimic the architecture of human tissues [2]. The 3D printing precision allows the fabrication of specific supports and improved transfer of essential nu- trients, which is crucial in severe bleeding where conventional hydrogels and dressings may not be adequate [3]. Plasma activation enhances hydrogels by introducing oxygen functionalities, including reactive oxygen and nitrogen species (RONS), providing a novel approach to wound healing [4]. We investigated two approaches that use cold atmospheric pressure plasma jets (Fig. 1): (A) plasma treat- ment of uncrosslinked CS solutions and (B) surface modification of printed CS hydrogel scaffolds. Our initial findings suggested that the plasma-treated hydrogel scaffolds exhibit higher hydrophilic- ity, i. e. reduction of water contact angle. Cell viability experiments showed that plasma-treated hydrogels significantly enhanced fibroblast cell morphology and growth. Our findings indicate that plasma-treated CS hydrogels may find applications in wound dressings.

Fig. 1: Two approaches for enhancing chitosan hydrogel scaffolds by plasma treatment



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Thu-P-39

Using atmospheric-pressure plasma for precise and less invasive suture wounds

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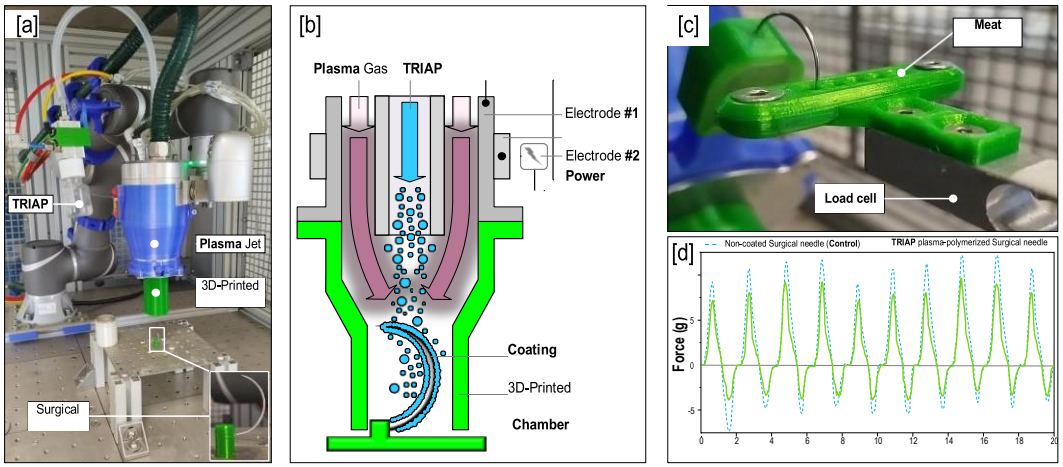
Atmospheric-pressure plasma polymerization coatings have been deposited over surgical needles with the aim of reducing the insertion and extraction forces during wound healing. The high forces that are experienced during suturing procedures not only affect the patient´s pain and vaccination rates, but also cause needle bending and tissue displacement, which decreases the accuracy and effectiveness of the methods and increases bleeding and recovery times [1].

Currently, the manufacturers of needles apply plasma technology in a multi-step procedure to reduce friction forces. Firstly, a plasma treatment is applied to activate the surface of the needles. Then, the needles are dipped in silicone solutions to reduce the friction forces and, finally, the silicone is cured in an oven for a long time (2h-6h) and at high temperatures (150-200ºC) [2].

In this work, a plasma-polymerized coating based on N1-(3-trimethoxysilylpropyl) diethylenetriamine (TRIAP) that was applied at room temperature for 3 minutes of treatment and in a single step reduced the friction forces by around 30% when compared with currently commercialized suture needles. The insertion forces were evaluated by inserting coated and non-coated needles 10 times into meat samples; following the ASTM F3014-14 standard test method. The durability and toxicity of the coatings are critical for wound suturing applications. The coatings that come off the surface of the needles during the suturing procedure increase the force exerted along the successive insertions into the skin and remain inside the human body, being able to cause skin lesions such as granulomas and allergies. In this regard, the needles were subjected to a non-targeted analysis by Gas chromatography-Mass Spectrometry (GC-MS) to determine the volatile and semi volatile compounds. SEM, XPS and toxicity results showed the potential application of plasma- polymerized coatings for the wound suturing industry.

Fig. 1. [a] Plasma Jet equipment, [b] Plasma-polymerization scheme, [c] close view of the insertion test, and [d] force results



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The *plasmACT* European Doctoral Network (2024-2027) – Plasma Medicine Against Actinic Keratosis

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The quality of human (and veterinary) health care systems substantially depends on key innovations. Often, these were driven by the field of physics, followed by interdisciplinary and inter-sectorial actions in engineering, chemistry, biology, and medicine, such as X-rays in medical diagnostics, ionizing radiation in cancer treatment, and femtosecond lasers for precision surgery. Medical gas plasma technology was introduced to human health care a decade ago. Today, accredited medical plasma devices are in daily operation in dozens of dermatology centers in Central Europe to improve wound healing. In addition, physical plasmas were shown to inactivate cancerous cells. Actinic Keratosis is a premalignant skin disease affecting millions of Europeans and making them prone to invasive and deadly skin cancer. Many of the available treatment options are associated with low efficacy, pain, risks, and/or high costs. Medical gas plasma technology is operated at body temperature and applied painlessly, cost-effectively, and without notable side effects. Gas plasma has been suggested to be active on high-grade cancer cells, but its activity against premalignant cells, as in Actinic Keratosis, is unknown. By using beyond state-of-the-art plasma multijet technology, the primary technical objective of PlasmACT – Plasma against Actinic Keratosis – is to support skin cancer prevention by medical gas plasma therapy of Actinic Keratosis. PlasmACT does so by educating a new generation of application-oriented scientists that are exposed to questions and findings from different scientific fields (interdisciplinary from physics over chemistry and biology to medicine) and capable of addressing questions in view of both academic as well as business needs (inter-sectoral) while incorporated in a vivid and productive environment across borders and cultures (international). The *plasmACT* MSCA-DN is the first Plasma Medicine-focused European Doctoral Training School and an overview of the scientific network, goals, partners, and eight Ph.D. programs will be presented.

Fig. 1. *plasmACT* project: www.plasmact.eu

Non-thermal Plasma Induces Oxidative Stress Markers Relevant to Wound Healing

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The wound healing process is complex and regulated, involving interactions between various types of cells, extracellular matrix, and reactive oxygen and nitrogen species (RONS) [1,2]. RONS are derivatives of oxygen and nitrogen that are produced during mitochondrial ATP production and act as signaling molecules in tissue repair. The optimal levels of RONS are necessary for normal wound healing, and deviations from these levels can result in oxidative damage and cell apoptosis [3].

Non-thermal plasma (NTP) is partially ionized gas that generates RONS, including OH^-, O_2^-, H_2O_2, NO, and NO derivatives. These molecules are similar to those produced during normal wound healing but are short-lived and present at very high, localized concentrations during NTP exposure. This makes NTP a potentially effective and safe therapeutic approach for wound healing [4,5].

To evaluate the effect of NTP on wound healing, a study was conducted using cultured, wounded keratinocytes. Human keratinocytes (HaCaT cells) were exposed to suboptimal, optimal, and excessive doses of NTP and assessed for wound closure rate and markers for cell migration, proliferation, and oxidative stress. The results showed that NTP treatment increased the levels of RONS and antioxidant activity, promoting cellular proliferation and migration, which are essential components of wound healing.

Overall, this study highlights the potential of NTP as a promising therapeutic approach for enhancing wound healing by modulating oxidative stress pathways and promoting cellular activities crucial for tissue regeneration.

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The beneficial effect of successive Cold Atmospheric Plasma treatments on in vitro behaviour of human gingival fibroblasts

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While cutaneous wound healing is an extensively studied and well-characterized process, the understanding of intraoral tissue repair still presents major lacunae, an aspect which reduces the clinical translation of treatment alternatives [1]. Following injury, the oral mucosa is submitted to a cascade of biological events that culminate in the restoration of tissue homeostasis and while general similarities exist, there are stark differences in the genomics and kinetics of wound healing between the oral cavity and the cutaneous epithelium [2]. Moreover, the lack of a successful therapy for oral mucosal wounds, compelled researchers to take into consideration alternative treatments for an enhanced intraoral healing. With this in mind, in the last decade the newly found therapeutic properties of Cold Atmospheric Plasma (CAP) gave it special consideration in dental applications and numerous studies reported its beneficial effects on the intraoral wound healing process and tissue regeneration [1].

In this context, the aim of the present study has been to investigate by comparison the effects of CAP on human gingival fibroblasts (HGF-1 cell line) in culture and observe how different plasma jet exposure conditions can affect their in vitro behaviour. For this reason, the HGF-1 cells were assessed in terms of their viability/proliferation potentials, adhesion/cytoskeleton organization and fibronectin production and its subsequent arrangement into an extracellular fibrillar network. The obtained results revealed that the fibroblasts subjected to successive CAP treatments and for longer periods of time exhibited a better in vitro cellular behaviour when compared to the cells that have been exposed only once to the CAP treatment. Thus, the application of CAP for longer periods of time exerted a positive effect on cells' viability and proliferation. Likewise, the fluorescence images acquired after performing the LIVE/DEAD cell viability assay showed an increase in the cellular density with treatment length and number of treatments. In addition, the fibronectin immunolabelling revealed that the specific positive signals were better expressed at higher treatment times and after the cell culture has been submitted to successive CAP treatments suggesting their beneficial effect on the extracellular matrix formation.

Considering the above findings, the widespread application of CAP in dentistry can have a promising future, however, in depth additional studies still need to be conducted in order for the underlying mechanism of CAP in an oral environment to be fully elucidated.

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Mouse Skin Response to Cold Plasma

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This research explores the potential benefits of using an atmospheric pressure plasma jet for mouse skin treatment, focusing on reducing skin damage. While previous studies primarily analysed *in vitro* mouse skin cells, understanding the effects of plasma on living systems has been challenging. Therefore, this study conducts an *in vivo* assessment of the effects of different plasma gases and jet orientations on mouse skin damage. The analysis indicates that a perpendicular He plasma jet significantly reduces skin damage. Additionally, the study evaluates three damage mitigation strategies: incorporating a liquid interface, adding N_2 to the gas flow, and regulating flow dynamics with a funnel. Ethanol proves highly effective in mitigating skin damage, while the inclusion of N_2 further enhances damage reduction. The addition of a funnel to the jet's glass tube markedly limits plasma plume interaction with ambient gas, further reducing skin damage. Overall, this study identifies promising approaches for minimizing skin damage due to plasma jet treatment, paving the way for safer clinical applications.

Thu-P-44

Effect of plasma activated ringer lactate and saline solutions on the migration and proliferation of murine melanoma cells (B16F10)

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Although melanoma corresponds to only 4% of all cases of skin cancer, it is responsible for more than 80% of the skin cancer deaths and up to 2% of all cancer deaths due to its high ability to spread to lymphatic tissue and blood vessels. Melanoma incidence is more important in fairy skinned populations and its incidence has been continuously increasing worldwide. In this work, it has been investigated the effect of incorporating species into liquid solutions with atmospheric pressure plasmas (APP) and whether the presence of such species interferes with the survival rate of melanoma cells in *in vitro* experiments. For this purpose, saline (0.9% NaCl) and Ringer lactate solutions were exposed to argon plasmas for 30, 45, and 60 minutes. Murine melanoma B16F10 cells, with a concentration of $1x10^5$ cells/ml, were distributed in Petri dishes and after adhesion the cells were submerged for 30, 60, and 120 minutes in the treated solutions. After the predetermined time, the liquids were replaced with culture medium and the cells were incubated for 24 hours. To evaluate the effect of the activated liquids on healthy cells, the same procedure was applied to murine fibroblast L929 cells. Cell viability and morphology and gene expression for cytokines were analyzed after 24 and 48 hours. It has been observed that both treated solutions have a significant effect in reducing the viability of B16F10 cells when compared with pristine liquids. Treated samples have also been stored at -20ºC and more than 75 days after the treatments, the effects of the plasma exposure could still be observed. According to results of flow cytometry assays, all the treated solutions induced apoptosis and necrosis of the tumor cells in different degrees depending on the exposure time of the solutions to the APP and the time of contact with cells. Furthermore, while the contact with pristine solutions have note affected the cell morphology, the immersion in plasma activated solutions modified the cell structure and decreases the migration of B16F10 cells. The results suggested that plasma activated liquids can be considered as promising adjuvants in cancer therapy

This work was supported by Fapesp (grant 2020/0667-8), CNPq (grant no 303777/2021-4) and Prope/Unesp (call 06/2024).

Plasma Controlled Nanogold for Detection and Recognition of Explosives with Machine Learning Assisted SERS

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The contamination of soil and surface waters by nonbiodegradable chemicals used in military explosives and ammunition poses a significant risk to human health and ecosystems. These contaminants, including metals such as lead (Pb), antimony (Sb), and uranium (U), as well as organic explosives like trinitrotoluene (TNT), and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), are persistent in the environment and resistant to biological degradation. As these substances accumulate in terrestrial ecosystems, especially in areas with frequent military activity, they can be absorbed by living organisms, leading to adverse health effects. The detection and identification of these hazardous compounds at trace levels is crucial for environmental monitoring and public health protection. [1]

This study introduces a novel, simple, and highly effective sensor for the detection of aromatic and aliphatic nitro explosives, utilizing surface-enhanced Raman scattering (SERS) assisted by machine learning (ML). The SERS sensor leverages the unique optical properties of gold nanoparticles (AuNPs), which were synthesized using a single-step plasma-liquid redox method. This approach allowed for precise control over the size and shape of the AuNPs, which were then deposited onto gold-coated CPU pins to create an optimized sensing platform.

The resulting nanogold structures exhibited highly efficient plasmonic hotspots, which significantly amplified the Raman signals of explosive analytes, including TNT, TNB, picric acid, RDX, PETN, and HMX. The sensor demonstrated a remarkable detection limit (sub 10^{-7}M), which is nearly three orders of magnitude more sensitive than conventional UV-Vis absorbance methods.

To enhance the accuracy and reliability of the SERS-based detection, machine learning techniques were employed. Specifically, principal component analysis (PCA) combined with k-nearest neighbors (k-NN) classification was used to analyze the complex SERS spectra. This ML-assisted approach enabled the rapid and precise identification of different NO-containing explosives, achieving a prediction accuracy of over 99%. Such high accuracy is essential for real-world applications, where quick and accurate detection is necessary to mitigate the risks posed by these hazardous substances.

This work was supported by Republic of Slovenia (ARIS), projects J2-4490, J2-50066, J2-50066, J2-4451, and N2-0213, NATO NOOSE “Nanomaterials for Explosive Trac-es Detection with SERS” grant no. G5814. The UK EPSRC, project EP/S025790/1 and EP/N021347/1.

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The influence of extracellular DNA on the sensibility of antibiotic resistant bacteria to cold atmospheric plasma

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The over-consumption of antibiotics made antibiotic resistance a global environmental problem. Recently, antibiotic resistant bacteria (ARBs) and antibiotic resistance genes (ARGs) have been classified as emerging contaminants in the environment. *Enterobacteriaceae* is a large heterogeneous group of Gram-negative rod-shaped bacteria that are naturally found in the mammalian gut and are considered as the largest reservoir of ARGs. They contribute largely to the dissemination of antibiotic resistance in the environment [1].

Decontamination processes such as chlorination and ozonation are widely applied to guarantee the safety of the aquatic environment. However, these processes alter the bacterial cell membranes without affecting the DNA integrity. Therefore, ARGs harbored by ARB can be released in the environment, become extracellular ARGs and can be transferred to other microorganisms by transformation [2]. Recently, several studies showed that plasma exposure can degrade bacterial DNA and inhibits the conjugative transfer of antibiotic resistance genes [3].

This study investigates the effect of cold plasma on the ability of *E. coli* to integrate exogenous DNA, as well as the impact of the inclusion of plasmid DNA in the bacteria susceptibility to plasma treatment. The plasma discharge obtained using an APP multi-jet in He:O_2 was characterized by optical emission spectroscopy. The reactive oxygen and nitrogen species (RONS) formed in the treated liquid were quantified by spectrophotometry. *E. coli* DH5α competent cells were exposed to plasma and their ability to integrate extracellular DNA was examined using bacterial transformation assays. Furthermore, bacterial cells with and without extrachromosomal DNA containing ARGs were exposed to plasma and the viability, metabolic activity, membrane integrity and intracellular ROS levels were examined. The results showed that the inclusion of the extracellular DNA increases the sensibility of the bacteria to plasma treatment and their intracellular ROS production. Finally, RT-qPCR was used to analyze the differences in gene expression between the two cell types after plasma patterns, namely for oxidative stress response (*kat*E, *kat*G, *sod*B, *sod*C), DNA repair system (*rec*A, *rec*B) and cell membrane permeability genes (*omp*F and *omp*C).

This work was supported by the research grant program of Occitanie Region, France.

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Thu-P-47

Reconstructed Epidermis Permeation Studies Using a Plasma Jet and Spray Device

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As plasma treated liquids have been shown to have applications in the plasma medicine field, their effects on skin for cosmetic purposes are also of interest as they could provide an alternative way to treat skin without the electric hazards correlated with the use of a direct plasma device. Our previous work on human skin explants showed an increased transdermal diffusion of cosmetic ingredients (caffeine and hyaluronic acid) after a direct plasma jet treatment [1]. While the efficiency of such methods was demonstrated, they still involve some toxicity. We, thus, set up a plasma spray device to nebulize plasma-treated liquids on different biological targets [2]. In this project, we studied both skin cell membrane permeabilization and reconstructed human epidermis (RHE) permeation of a fluorescent probe (fluorescein) after a plasma spray treatment in comparison with a plasma jet. Our results showed that both plasma jet and plasma spray treatments displayed an enhancement of permeation kinetics of fluorescein (Fig. 1). Plasma spray treatments showed an efficiency similar to that of certain plasma jet conditions. We also investigated RHE electrical resistance profiles after plasma jet and spray treatments, our results demonstrated a specific time window for each conditions.

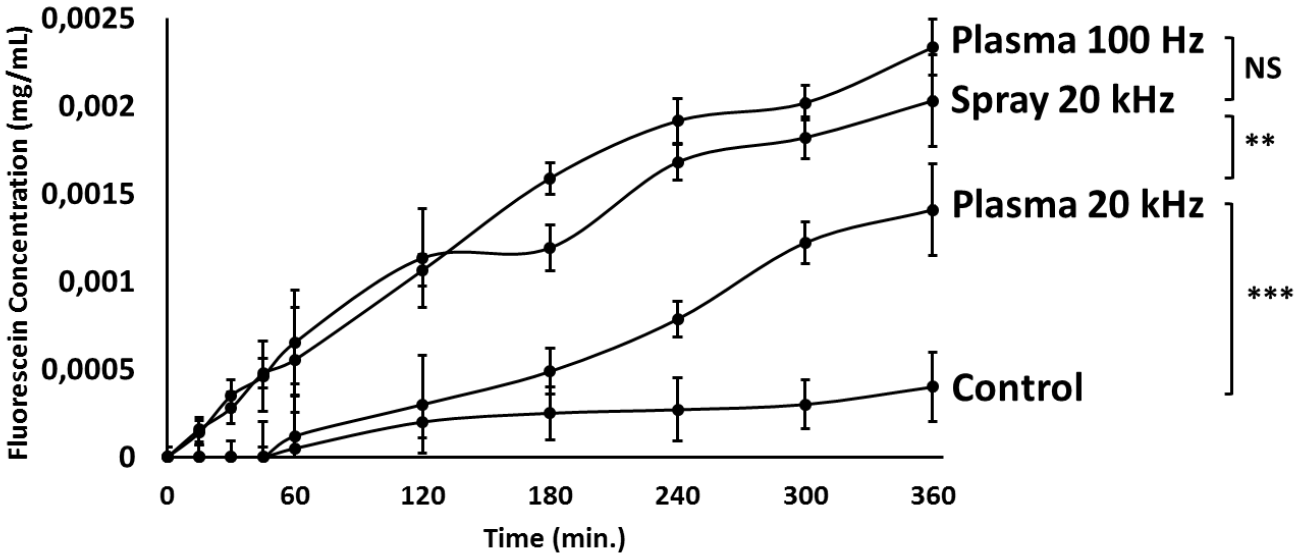

Fig. 1 RHE permeation kinetics of fluorescein following direct plasma jet treatments of either 20 kHz or 100 Hz and spray treatments of 20 kHz

This work was supported by the ARD COSMETOSCIENCES MINIONS and the ANR "PLASMASOL" ANR-23-CE04-0003

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Role of plasma and the carrier gas on CO binding with human hemoglobin

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Carbon monoxide (CO) is a very attractive molecule in medicine and can be easily produced by plasma [1–3]. This molecule has a broad spectrum of biological activities such as anti-inflammatory, vasodilatory, anti-apoptotic, and anti-proliferative effects [4]. It is a stable molecule in the gas phase and *in vivo* and does not produce byproducts, which means that it can exert its signaling properties in stress conditions.

Although plasma has achieved great results in numerous medical applications, its role on inflammation remains unclear [5]. To better control inflammation, the production of CO by plasma represents an excellent alternative. For this purpose, in this work, we designed a plasma reactor able to produced CO from the dissociation of CO_2.

First, we will present a comparative study of CO production by an external rings DBD plasma jet fed with two different gas mixtures: helium/CO_2 and argon/CO_2. We will show that the CO concentration can be controlled from a couple of ppm to thousands of ppm, which is typically the range used in clinical application with CO inhalation [4]. We will present that, surprisingly, the use of helium and argon as carrier gases is equivalent in terms of CO production, and that the CO concentration depended on the specific energy input (SEI) and the ratio of CO_2 in the gas mixture.

Secondly, we will present a study on the interaction of this plasma with human hemoglobin. In the presence of hemoglobin, CO binds to it and forms carboxyhemoglobin (COHb), which can be quantified by light absorption. We will show that the amount of COHb formed depends on the carrier gas (helium or argon) and not on the CO concentration produced by the plasma.

This work was supported by the French Research Agency, ANR (MediCO-Plasma|ANR-21- CE19-0005).

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8 - 13 september, Portorose, Slovenia

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PLASMA AGRICULTURAL APPLICATIONS

Oral session (Fri - O - 10)

Friday, 13 September 2024

Bactericidal and Plant-Growth Effects of Amino-Acid Solutions Irradiated by Electrically-Neutral OxygenRadicals

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Recently, plasma-activated solutions are attracting a lot of attention owing to their unique advantages such as bactericidal effects. Previously, we reported that oxygen-radical treatment of solutions containing organic compounds with a benzene ring such as L-phenylalanine (L-Phe) can kill bacteria such as *Escherichia coli* (*E. coli*).[1] Considering L-Phe and other benzyl compounds are contained in organic fertilizers, this sterilization technique is expected to be used in agricultural systems such as hydroponics and plant factories. However, the killing rate of the L-Phe solution was less sufficient, 2-log reduction of *E. coli* after 24h reaction. To improve the bactericidal effect, a compound with another type of organic ring was such as L-tryptophan (L-Trp), which is also a fertilizer component, used in this study.

The survival number of alive *E. coli* or *Staphylococcus aureus* (*S. aureus*) in L-Trp solution after oxygen-radical exposure decreased as the exposure time increased, and reached the detection limit of colony forming unit assay by 1-min or 5-min exposure, respectively. To identify essential chemical structure to produce the rapid bactericidal effects of oxygen-radical irradiation to L-Trp, *E. coli* was suspended into PB solutions (pH 6.3) containing benzene, pyrrole or indole. The concentrations of benzene, pyrrole or indole was set at 1 mM. Indole is a combined structures of benzene and pyrrole rings. 3 mL of the *E. coli* suspension in a φ38-mm sterile dish was treated using the oxygen-radical generator for 0 or 5 min. 6-log reduction of *E. coli* survival number was achieved only in the cases of pyrrole or indole solutions. Therefore, oxygen-radical irradiation to pyrrolic compounds was suggested as a key to produce the rapid bactericidal effect.

Moreover, for evaluating the temperature dependence of L-Trp solution on the bactericidal effect, the temperature of the solutions was regulated from -5 to 20 ℃ using a Perche device. Only L-Trp solutions were treated for 1min. After the treatment, *E. coli* was suspended to the solutions within 30 s and incubated for 1 min on the Perche device under the same temperature. After the incubation, the survival number of *E. coli* was evaluated. 6-log reduction was achieved from -5 to 10 ℃ whereas 2- log and less than 1-log reductions were obtained at 15 and 20℃, respectively.

The concentrations of H_2O_2 in the treated L-Trp solutions were measured using HPLC, and it was found that of 0.41, 1.10, 1.50 mM of H_2O_2 was generated via 1, 3 and 5-min treatment, respectively.
E. coli was suspended to H_2O_2 solutions (0.41, 1.10, 1.50 mM) with reaction of 1, 3, 5 min, respectively, and then the survival number was evaluated. As a result, almost no death of *E. coli* was confirmed.

These results suggested that the bactericidal factor generated from L-Trp is likely to be a short- lived organic reactive species at room temperatures. Moreover, LC-MS, NMR and ESR analyses revealed that the bactericidal species is the tryptophan radical [2] and suggested that plant-growth species is not the tryptophan radical but *N*'-formylkynurenine with L-tryptophan.

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Modeling of *Aspergillus brasiliensis* growth and inhibition by non-thermal plasma

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Aspergillus brasiliensis belongs to the Nigri section of the *Aspergillus* genus. It is a common contaminant found in cosmetics, food, and building materials. Although infrequently, it can lead to aspergillosis, i.e. an infection primarily affecting the lower respiratory tract, caused by inhaling spores of the filamentous fungus *Aspergillus* from the environment. Hence, it is imperative to explore novel methods to inhibit its growth, such as use of non-thermal plasma (NTP).

Our study involves modeling the growth on agar medium with and without NTP, a direct bipolar corona discharge generated in a point-to-ring electrode system under atmospheric pressure and ambient temperature conditions was used. The model was constructed based on the mathematical representation of microorganisms' growth on the surface, resulting in a nonlinear logistic equation [1]. Experiments were conducted using *A. brasiliensis* under various conditions, including different microbial concentrations and culture temperatures. The collected data were instrumental in creating the model. The model's functionality was validated by predicting the growth of micromycetes under conditions distinct from those during its development. The accuracy of these predictions was subsequently confirmed through experimental data, both with and without NTP treatment.

NTP exposures were conducted at 24-hour intervals, with each exposure lasting 10 minutes. The findings indicate that a single treatment partially suppresses growth, with the degree of suppression depending on the initial concentration of micromycetes. Repeated treatments, using our plasma source, may lead to a decline or complete inhibition of micromycetes growth rate (see Fig. 1).

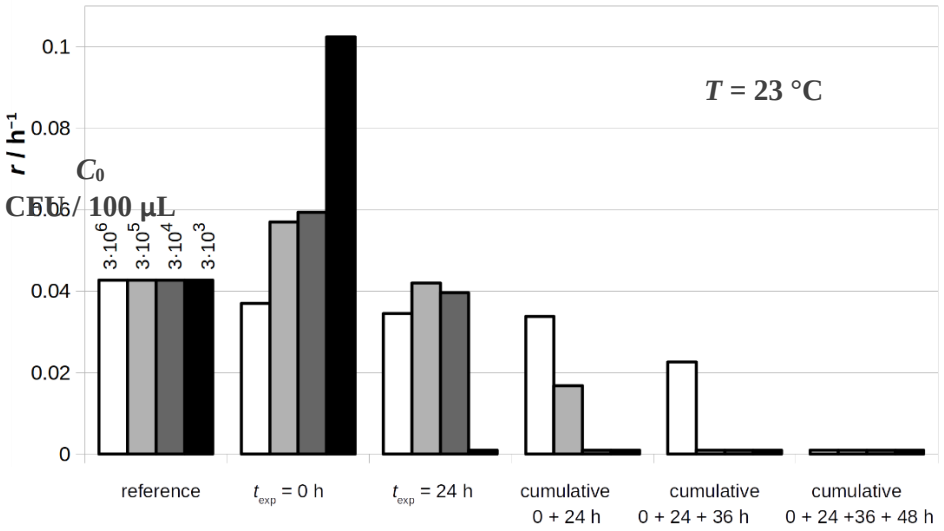

Fig. 1 The parameters of the growth rate *r* for the reference (untreated) samples and for the samples exposed to NTP at time t_{exp} = 0 h and/or t_{exp} = 24 h or cumulative, after inoculation.

This study was supported by the Grant Agency of the Czech Republic project No. 22-06621S.

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Potential of non-thermal plasma in the induction of adaptive response in plant seeds

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Plants are sessile organisms that are exposed to abiotic and biotic stressors on a daily basis. These stressors include e.g., heavy metals, temperature fluctuations, soil salinization, drought, pests, pathogens and herbivores [1]. Plants have developed various strategies to recognize stress and adapt to adverse environmental conditions, but due to climate change, global population growth and shrinking arable land, yield losses in agricultural crop are increasing [2].

An adaptive response is the ability of cells or organisms to better resist harmful factors after being pre-treated with a lower dose of the same or another stressor, and is a widespread phenomenon observed from prokaryotes to mammals [3]. The adaptive response is triggered by a variety of agents and factors that induce changes in the gene expression of various defense proteins during the adaptive response, thus ensuring the protection of cells and plants from subsequent high doses of the stressor [3-4]. Since the adaptive response can be induced by different agents and stressors, non-thermal plasma could represent an effective and ecological alternative to the initial stressor for its induction.

In our study, we focused on the effect of non-thermal plasma generated in air by the diffuse coplanar surface barrier discharge on pea and barley seeds with the aim of inducing an adaptive response and investigating the mechanisms of its action. We used plasma as a potential inducer and different agents such as heavy metals, drought, radiomimetic substances or environmental pollutants as stressors in the next phase. Our preliminary results show that plasma can be used at low exposures as an inducer of an adaptive response only in pea seeds to the adverse effects of zeocin (a radiomimetic) and bisphenol A (an environmental pollutant). In contrast, it was not possible to induce an adaptive response in barley seeds to the effects of cadmium oxide or polyethylene glycol (drought). The mechanisms by which plasma can elicit an adaptive response include primarily the activation and expression of specific defence proteins and antioxidant enzymes, such as superoxide dismutase, catalase and peroxidase, which we have demonstrated by quantitative PCR and western blotting. The parameters for which plasma-treated seeds showed better properties than non-plasma-treated ones include, in particular, increased germination, improved growth parameters and reduced DNA damage. The results of our study indicate the potential of using non-thermal plasma in agricultural practice for sowing and cultivation of the seeds of some crops even in inhospitable conditions.

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PLASMA FOR PHARMACEUTICAL APPLICATIONS, BIOCHEMICAL AND BIOMOLECULAR ENGINEERING

Oral session (Fri - O - 9)

Friday, 13 September 2024

Plasma-oxidized proteins cause alterations in antigen-presenting cell maturation

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Reactive oxygen species (ROS) are naturally generated in cells due to redox stress or produced by immune cells during an inflammatory response. Consequently, structural changes and oxidatively posttranslational modifications (oxPTMs) in proteins appear, which can lead to altered activity, function and recognition by other cells. Changes in immunogenicity are driven by altered recognition and uptake by antigen-presenting cells (APC) and different cytokine secretion, leading to enhanced or decreased T-cell activation. Using cold physical gas plasma technology enables investigations of ROS-modified proteins, and different gases generate different ROS, leading to various oxidized protein variants. We previously found that plasma-modified ovalbumin is preferred to be taken up by APCs and increase T cell activity [1], but studies with other proteins and cellular mechanisms are lacking.
Proteins that are relevant to chronic inflammation, infection, and cancer, such as insulin, Epstein–Barr virus, Chemokine (C-X-C Motif), Ligand 1, and others, were exposed to gas plasma and given to human THP-1 monocytes or primary monocyte-derived cells (moDCs). Oxidized proteins caused maturation phenotype alterations in moDCs concerning surface marker expression and chemokine and cytokine secretion profiles. Interestingly, oxidized insulin and CXCL1 showed concentration-independent effects and concentration-matched H_2O_2-treated proteins did not recapitulate the effects of gas plasma, suggesting sufficiently short diffusion distances for the short-lived reactive species to modify proteins. Investigation of the cellular mechanism using protein kinase phospho-array showed enhanced AKT and RSK2 phosphorylation in monocyte-derived cells pulsed oxidized proteins. Our data provide evidence of dendric cell maturation and activation upon exposure to gas plasma- but not H_2O_2-modified model proteins. The biological consequences of these findings need to be elucidated in future inflammation and disease models.

This work was supported by the German Federal Ministry of Education and Research (BMBF), grant numbers 03Z22DN11 and 03Z22Di1.

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Modelling by Electrical Equivalent Circuit Network of Cell Death by Plasma Irradiation

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To understand the mechanism of the plasma gene transfection, we have created an equivalent circuit network model of the whole system that includes cells, DNA solutions, and a 96-well plate [1]. This model suggests that intracellular currents trigger endocytosis. In this study, the authors used equivalent network analysis to investigate the relationship between the injected electrical power into cells and cell death. A 96-well plate was placed on a grounded electrode plate, and the cell and DNA solutions were processed with discharge plasma for 2-50 ms. The plasma was generated by applying a 20 kHz sinusoidal voltage to a very fine electrode placed 0.5 mm above the liquid surface in the center of the well. The applied voltage was 20 kVpp. After 24 h of incubation, the green fluorescence expressed by GFP plasmid transfection was observed.

As shown in Fig. 1, many cells died in the concentric circular area with the center of the well, and no transfection-induced fluorescence was observed in this area. The radius of this circular region (cell death circle) was measured as an index of the cell death region. Assuming that there is a threshold power P_{th} at which the cell feels the electric stimulation, the accumulation of the sensed electric stimulation (instantaneous power P) can be regarded as the amount of electric power (energy density). This is defined as the amount of effective injected power W. We assume that there is a threshold W_{th} at which W causes cell death. The electric power P per volume injected into the cell and W per volume was calculated from the equivalent circuit network analysis. W was obtained by integrating P while P exceeds P_{th}. If cell death occurs when W exceeds W_{th}, the relationship between the plasma processing time and the radius of the cell death circle at which the cell death occurs was obtained. P_{th} and W_{th} were set to obtain the result closest to the experimental result. The relationship between r of the cell death circle finally obtained and the plasma processing time is shown in Fig. 2. The experimental results show that r is linearly related to the logarithm of the plasma processing time, and the calculated results show good agreement with the experimental results. If the cell death was caused by necrosis, r should have relied on the injected energy, and r should have increased with the processing time. However, r is linearly related to the logarithm of plasma processing time, and cell death by apoptosis as a physiological response seems dominant. Therefore, cell death by plasma seems to be caused by apoptosis induced by electrical stimulation.

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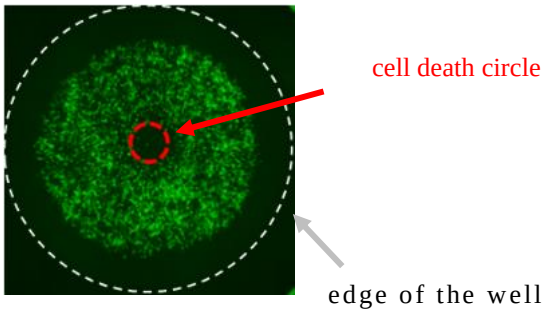

Fig. 1. Fluorescence of GFP

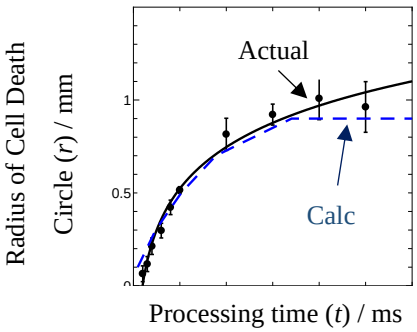

Fig. 2. The radius of cell death circle VS processing time

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Genome Content Sensitive DNA Optical Analysis with Nanoplasmonics

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Exploiting the unique electrochemical properties of plasma, this research focuses on advancing the fabrication and design of functional nanomaterials to contribute to the development of novel sensing technologies. The primary challenge addressed in this study is the customization and adaptation of functionality to optimize the performance of optically based detection methods. In the field of optoelectronics, plasmonic sensing is proving to be a powerful instrument for investigating molecular properties, often associated with techniques such as SERS, SEIRA and SEF spectroscopy. In particular, Surface-Enhanced Raman Scattering (SERS) is an important method for studying analytes on a smaller scale using nanostructured signal amplifiers. This technique is particularly important for the rapid and precise analysis of biologically significant samples, including DNA and RNA, which are crucial in modern nanomedicine, vaccines and functional genetic engineering. While PCR-related methods are currently at the forefront of molecularly sensitive assays at the DNA level, optical techniques such as SERS offer untapped potential in terms of detection time, simplicity of measurements and variety of analytes. In this study, a nanoplasmonic sensor was fabricated by a plasma-mediated electrochemical reduction process supported by a He/Ar plasma jet at atmospheric pressure and kHz operating frequencies. Reactive species and electrons via liquid gas electrochemistry facilitated the reduction of Au(3+)-containing microdroplets, resulting in the formation of circular aggregates of truncated gold nanoparticles (AuNPs). These nanoparticles enabled optical confinement and a high electric field, resulting in an analytical enhencement factor of about 10^7. This substrate enabled the acquisition of Raman spectral fingerprints of bacterial DNA fragments (M. luteus, S. aureus, E. coli, J. lividum) at nanovolume sample quantities within seconds. In addition, principal component analysis has been used to reliably classify bacterial strains based on extracted molecular DNA vibrational signatures in conjunction with nucleobase content [1]. This research focuses on the development of innovative functional plasmonic nanomaterials that will improve the diagnostics of genomic materials.

The work was conducted under financial support from NATO Grant 417 G5814 – NOOSE and Slovenian ARIS-J2-4490 national project.

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Plasmonic Sensors for small and large entities from Molecules to Monitoring of Biofilm Growth on Surfaces and Their Plasma Cleaning

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Plasmonic sensors are based on the excitation of surface plasmon resonance (SPR), an evanescent optical wave propagating at the interface between the metallic surface and analyte medium with a field penetration depth of a few hundred nm. The extended SPR is highly sensitive to the concentration of the analyte and can easily measure fractions of nm/ml [1,2]. When the metallic surface is at the nanoscale the plasmon is localized (LSPR) and so also its evanescent field becomes tightly localized within a range of a few tens of nm from the surface. With this localization, however, the field intensity gets enhanced drastically causing molecular emission and absorption to get enhanced as is the case with surface-enhanced spectroscopies (SERS, SEF, SEIRA) [2].

During the last decade, we have been working with various concepts to improve the performance of these sensors and applied them to sensing biological entities such as viruses and bacteria, as well as molecules such as pesticides, explosives, proteins, DNA, and different biomarkers [1,2]. Lately, we have also been able to monitor the growth of biofilms on the surface as well as their cleaning with plasma using an insulator-metal-insulator SPR structure which enhances the wave penetration depth up to 10 mm or more [3]. Plasma was found to be able to clean surfaces from bacteria efficiently hence addressing the importance of this methodology and the long penetration depth SPR for monitoring it, for the medical devices industry.

In this presentation, we will review some of the main concepts developed including SPR, LSPR, and the coupling between the two as a methodology to enhance further the local field and hence SERS and SEF, as well as the enhanced penetration depth IMI SPR chip.

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Oxidative modification of proteins – a general concept in plasma medicine?

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Cold plasmas have entered the medical stage as an alternative means to combat inflammatory disorders, including (chronic) wounds and (pre-) malignant conditions or tumors. It can be argued that the major driver of cold plasma-driven effects is the formation of a multi-ROS environment, whose composition can be modulated in a wide range. While it is accepted that long-lived species such as hydrogen peroxide can penetrate into tissues, the mode of action of short-lived species (atomic oxygen, singlet oxygen) remains controversial. We hypothesize that the oxidation of biomolecules, especially proteins and lipids, contribute to downstream physiologic processes triggered by cold plasma. In pilot experiments we determined distinctive oxidation products of amino acids and model peptides by high-resolution mass spectrometry and found a site- and sub-structure specificity of plasma-derived reactive species. Aromatic and Sulphur-containing amino acid residues were preferentially attacked [1]. Using heavy oxygen isotopes, we confirmed the incorporation of atoms from both the gas and the liquid phase into dissolved target molecules, showing a substantial contribution of gas-liquid interface reactions.

Along these lines we currently investigate the relevance of hypochlorite ions, a prominent secondary species generated from atomic oxygen and chloride ions, in the oxidation of the filamentary matrix protein fibronectin (FN) that is involved in the pathophysiology of cardiovascular diseases. Highresolution mass spectrometry serves as analytical tool and will be combined with cell adhesion and antibody binding assays. The newly introduced functional groups identified so far are dominated by oxygen (hydroxyl, oxo groups), and – depending on the discharge parameters – chlorination, nitration, or ring opening reactions (tryptophane). Of note, FN oxidation varies significantly when access to the gas-liquid interface is possible. Subsequently, modified FN changes in functionality and cellular or molecular recognition as can be shown by antibody binding. The results will be discussed in relation to *in vitro*-findings on albumin, phospholipase A_2, and oxidoreductase oxidation by cold plasma. We seek to confirm the findings by the detection of the identical oxidative modifications in cold plasmatreated *ex vivo*-patient samples (human thrombocytes).

Taking the findings so far into account it can be acknowledged that the oxidative modification of proteins is part of pro-oxidant treatment regimens including cold plasma and contribute to the observed biological or clinical impact. With respect to medical plasma applications, further proof and exploitation of the concept is needed and in progress.

Acknowledgement

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Expanding plasma-driven biocatalysis using unspecific peroxygenase from *Collariella virescens* and a capillary plasma jet

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Plasma is a complex mixture of many components, including electrons, ions, radicals, neutrals, and excited species. Especially, reactive oxygen and nitrogen species (RONS) are of interest for biological applications. RONS can be distinguished based on their lifetimes. One of the long-living species is hydrogen peroxide (H_2O_2), which we utilize in biocatalysis applications to convert substrates into more valuable products using suitable enzymes. Enzymes are of great importance as catalysts in different industries, converting organic and biological substances. Biocatalysis is thought to present an environmentally friendly and sustainable alternative to chemical catalysis. The enzyme class of peroxygenases carries out one-electron oxidation reactions and stereoselective oxyfunctionalizations using H_2O_2, which results in various chemical groups such as hydroxyl or epoxide moieties. Their industrial application and the use of hydrogen peroxide in biocatalysis is very challenging, since high concentrations of hydrogen peroxide result in enzyme inactivation (suicide inactivation).

We previously reported a non-invasive approach for in situ H_2O_2 production for biocatalysis using non-thermal atmospheric pressure plasma [1]. Plasma-driven biocatalysis enables the tuning of H_2O_2 formation to meet the specific needs of the respective enzyme without a requirement for additional components. It has already been shown that plasma-driven biocatalysis with unspecific peroxygenase from *Agrocybe aegerita* (*Aae*UPO) is possible using the microscale atmospheric pressure plasma jet (µAPPJ) as plasma source and immobilization of the enzyme as a protection strategy. The immobilization of enzymes describes their attachment to functional groups of a carrier material. This allows to place the enzymes at a distance from the liquid surface, creating a buffer zone in which the short-living species react [2]. This protection strategy at the same time facilitates the collecting of the enzyme for reuse.

For biotechnological use, it is necessary to produce enzymes in large quantities in a cost-effective manner. *Escherichia coli* (*E. coli*) is a commonly used host organism for overproduction since it can be handled safely and multiplies quickly. At this time, *Aae*UPO cannot be produced in *E. coli*. Therefore, it appears attractive to employ other enzymes to perform such oxidation reactions. The unspecific peroxygenase from *Collariella virescens* (*Cvi*UPO) can be overproduced in *E. coli* [3] and could thus present an alternative. Here, we present the expansion of plasma-driven biocatalysis to *Cvi*UPO produced heterologously in *E. coli*. We were able to maintain the plasma-driven biocatalysis with helium or argon as feed gas for up to 390 min. Additionally, we expand plasma-driven biocatalysis by establishing the conversion of unsaturated fatty acids into epoxides, which are versatile components for the pharmaceutical, flavoring, and polymer industries.

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Local plasma jet application in the oral cavity to combat respiratory virus infections

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Cold atmospheric plasma (CAP) is a well-studied antimicrobial tool against various bacteria and fungi, including bacterial biofilms and spores. Several *in vitro* studies suggest the antiviral effects of CAP. The SARS-CoV-2 pandemic highlighted the importance of respiratory virus infections as well as the role of the lung as the primary organ of virus entry, replication and spread from person-to-person. Consequently, we propose CAP to locally reduce viral loads in the upper respiratory tract mucosa to reduce patient infectivity [1]. A flexible, miniaturized, catheter-shaped neon-driven plasma jet was evaluated [2]. After comprehensive multi-parametric jet characterization [3], a Phi6-bacteriophage test model with *Pseudomonas sp.* as host microorganism and a murine hepatitis virus (MHV-A59- GFP) test model with 17Cl-1 murine fibroblast cells as host cells were used to investigate the antiviral activity. Virus titers could be reduced substantially by CAP treatment, whereby conductive plasma jet treatment was more effective compared to non-conductive (remote gas) treatment [4]. To investigate the antiviral plasma efficacy in a more practically relevant *in vitro* approach, an air-liquid interface (ALI) lung mucosa model of human airway epithelial cells (Calu-3) was infected with human coronavirus HCoV-NL63. Again, a reduction of virus load was observed. However, due to lack of regenerative capacity of the *in vitro* ALI mucosa cell culture, a plasma-caused damage of the infected cells could not be completely ruled out. Consequently, as a meaningful pre-clinical model, an influenza A virus (2009 pH1N1 IAV) infection model in Syrian golden hamster was used for a first proof-of principle of virus burden reduction *in vivo*. First results indicate reduced viral loads in plasma-treated animals as estimated by virus titer determination in the upper and lower respiratory tract as well as low toxicity as determined by animal weight monitoring, cytokine measurement and histopathological investigation of parts of the respiratory tract. These initial results demonstrate that local plasma treatment of virus-infected mucosal tissue of the oral cavity not only results in a local reduction of virus titers, but seems to have also a positive effect on the course of the infection in general. This may open up new ways of therapeutic plasma application in the upper respiratory tract.

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Oral session (Fri - O - 4)

Friday, 13 September 2024

Metabolic Disorders in *E. coli* Induced by Electrically Neutral Oxygen Radicals Irradiation of Tryptophane-Containing Solutions

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We reported previously bactericidal effect of the O-radical treatments of *Escherichia coli* (*E. coli*) suspension contained L-phenylalanine (L-Phe).[1] Very recently, we have extensively studied with a focus of L-tryptophan (L-Trp) containing solutions. These amino acids are useful for organic fertilizers in hydroponics and plant factories, and providing a function of sterilization at the same time. Yet the bactericidal effect has not been clarified yet. In this study, we have investigated the mechanisms with respect to radical generations in liquid phase and cellular metabolic modifications.

The bactericidal factor generated from L-Trp is likely to be a short-lived organic reactive species at room temperatures. Analyses using liquid chromatography mass spectrometry (LC-MS), nuclear magnetic resonance (NMR), and electron spin resonance (ESR), revealed in situ generation of short-lived tryptophan radical as bactericidal species just during the O-radical irradiation [2]. Immediately, tryptophan radical varies *N*'-formyl kynurenine and kynurenine [2].

Metabolites of *E. coli* present in solution were compared between oxygen radical-irradiated and non-irradiated *E. coli* in solution without L-Trp. Among the glycolysis metabolites, the levels of 3- phosphoglyceric acid (3-PG), 2-phosphoglyceric acid (2-PG), and phosphoenolpyruvic acid (PEP), were significantly reduced by the O-radical irradiation with solutions containing L-Trp (50 mM). This indicates that O-radical irradiation affects central carbon metabolism in *E. coli* suspended in solutions containing L-Trp.

Furthermore, enzymes involved in glycolysis and the tricarboxylic acid (TCA) cycle, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and aconitase, are widely distributed in microorganisms. The activities of GAPDH and aconitase in cell extracts prepared from *E. coli* cultured in O-radical-irradiated solution with L-Trp were significantly reduced by O-radical irradiation compared to those in the unirradiated solution even no significant expression of protein patterns in *E. coli*. Thus, O-radical irradiation of the L-Trp solution diminished the activity of these enzymes in *E. coli*.

Consequently, we conclude that lethal metabolic disorders of glycolysis and TCA cycle are induced in *E. coli* by O-radical irradiation in the presence of L-Trp. The activities of GAPDH in glycolysis and aconitase in the TCA cycle were significantly reduced by O-radical irradiation in the presence of L-Trp. These metabolic disorders, which occurred via enzyme deactivation, are considered the main bactericidal mechanisms of O-radical irradiation in an L-Trp-containing solution.

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Mode transition of underwater air bubble discharge for the inactivation of *Saccharomyces cerevisiae*

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The plasma-liquid interface is critical for mass transfer and the formation of reactive oxygen and nitrogen species (RONS) in the liquid. The use of non-thermal plasma (NTP) in a bubble reactor[1,2], where plasma-activated air is delivered directly into the water, can significantly improve the water activation efficiency. The high mass transfer efficiency and RONS yield of bubble discharge result in effective microbial inactivation. In bubble reactors, the electrode structure can be modified to a shift in discharge between DBD and spark modes within a single reactor configuration, resulting in different fungal inactivation performances. This work reports on the discharge characteristics, liquid-phase chemistry, and inactivation effects towards *Saccharomyces cerevisiae* in a bubble reactor with different discharge modes. The detection of aqueous RONS showed that DBD discharge mostly produces O_3, while spark discharge produces mainly NO_x^- in liquid. In reference to the cell flow analysis, this paper highlights that the short-lived ROS in the online treatment may be the primary cause for the more efficient induction of apoptosis in the DBD mode. Additionally, the spark mode demonstrates a more intensely destructive effect on the cells, with the breakage of the cell membrane and the elevation of the intracellular ROS.

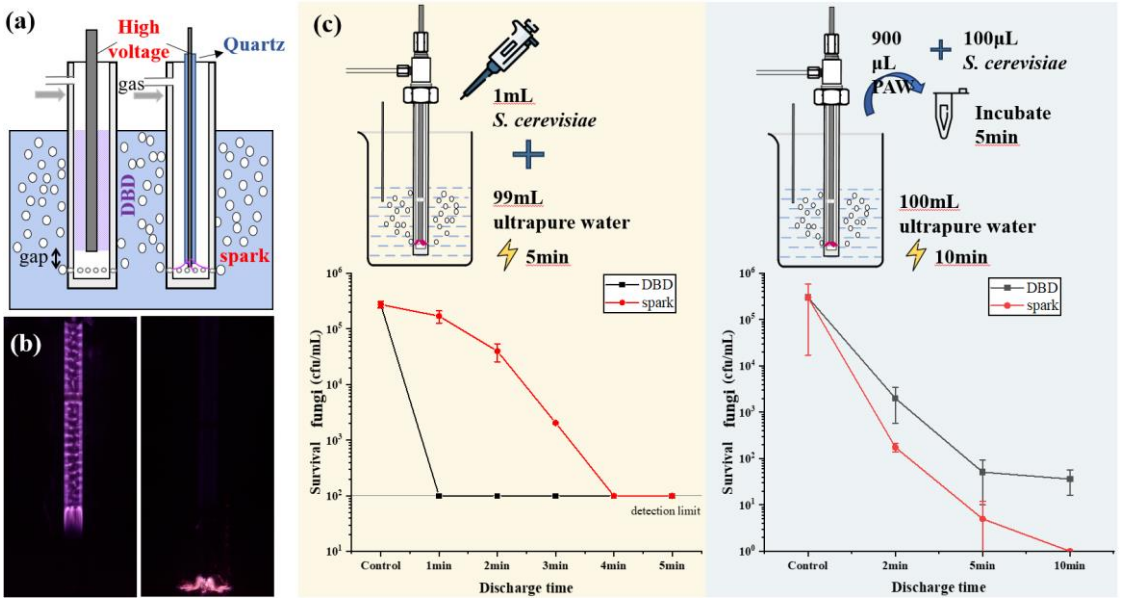

Fig. 1 Design, images and fungal inactivation comparison of different discharge modes

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Atmospheric pressure plasma – a chance for wastewater management in an industrial context?

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Along with the effects of climate change, water will become an increasingly scarce and precious resource in the coming decades. Transparency and sustainability of water use are therefore becoming more and more important and thus companies are getting encouraged to monitor their so-called "water footprint". The term includes the total amount of water used for the production process of goods and services in order to identify potential savings and reduce ecological impacts. With this value “governments can define limits for water consumption and water pollution”, as proposed by the non- profit Water Footprint Network [1]. Such limits will be a major challenge, especially for smaller companies. In the search for alternative water treatment methods, non-thermal atmospheric pressure plasma is becoming the focus of interest. While its potential for inactivating bacteria in aqueous environments has already been proven many times, this research work is now focusing on upscaling from the proven µl range for medical technology applications to an industrially relevant size in the liter range [2-5]. To achieve this, a commercially available plasma system was used that operates on the basis of a pulsed low-current high-voltage discharge. Such systems can be regarded as efficient for this type of application [6]. For the experiments, the plasma parameters were kept constant and compressed air was used as plasma gas. The experimental setup used is schematically shown in Figure 1. Different water qualities were tested, with a sample volume of 5 liters for each test run. The results were assessed by measuring the pH value, the electrical conductivity, the oxidation reduction potential value and live cell count of a test germ Pseudomonas aeruginosa ATCC 15442.

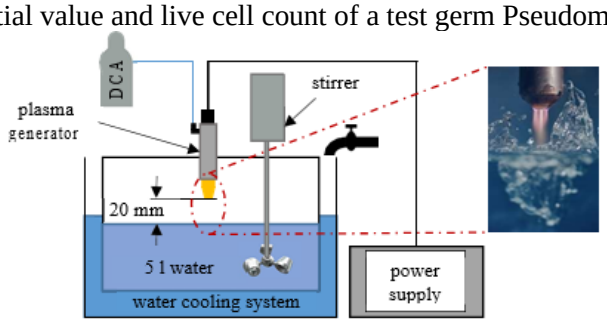

Fig. 1: Schematic diagram of the experimental setup.

When treating 5 liters of deionized water, a reduction in the live cell count of at least 6 log levels was detected after 20 minutes of plasma treatment. Using tap water, the samples had to be treated with plasma for 30 minutes in order to achieve a reduction in the bacterial count of ~ 3 log levels. In addition, differences in water hardness as well as differences in contamination and turbidity inhibited the antibacterial effect of the plasma. Detailed experimental results will be presented and the feasibility of using such a COTS device for the purpose of efficient water treatment will be discussed.

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Combining sub-lethal doses of cold atmospheric plasma with vancomycin-loaded liposomes to eradicate MRSA biofilms

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MRSA, the main causative organism of chronic osteomyelitis, persists extensive antibiotic treatment through biofilm formation on implants[1]. Cold Atmospheric Plasma (CAP) has been shown to rapidly decrease the viability of bacterial biofilms and act in synergy with conventional antibiotics[2], however, specific effects of combination treatment of CAP and antibiotic-loaded liposomes has not been described previously and may hold the potential to resolve chronic bone infections.
Liposomes were characterised using a zetasizer and HPLC. Sub-lethal dosage of the J-plasma Precise device against USA300 biofilms was determined using a time-kill curve. Minimum Biofilm Eradication Concentrations (MBEC) were determined for both vancomycin and vancomycin-loaded liposomes, with or without a sub-lethal dose of CAP. For confocal imaging, biofilms were grown on Ti sheets before treatment with CAP, followed by the addition of live/dead stains to investigate biofilm structure and cell death.
Liposomes showed a high vancomycin encapsulation efficiency of 83.5%. To determine sub-lethal CAP exposures the viability of USA300 biofilms exposed to cold plasma was shown to be reduced as the exposure time increased, with a sub-lethal exposure time being determined at 60 seconds (1.51- log reduction). Pre-treatment of biofilms with CAP resulted in significant reduction of MBEC values for both vancomycin and vancomycin-loaded liposomes (2-fold and 8-fold, respectively). Confocal microscopy provided insights into how CAP interacted with the extracellular matrix of both untreated and vancomycin/liposome treated USA300 biofilms.
The J-Plasma Precise cold plasma jet produces a CAP which enhances the efficacy of vancomycin and vancomycin-loaded liposomes against MRSA biofilms. Fluorescent microscopy shows evidence of both disruption of ECM and greater proportion of dead cells following CAP exposure.

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