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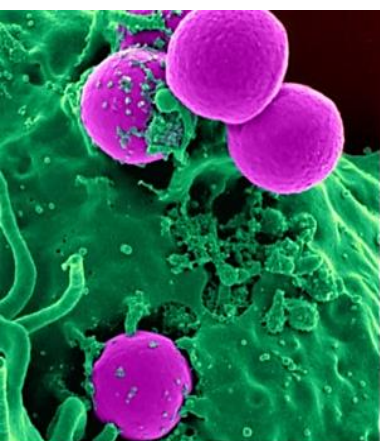
PlasTHER

THERAPEUTICAL APPLICATIONS OF COLD PLASMAS



CA19110

Plasma applications
for smart and
sustainable agriculture



2nd Training School

Cold plasmas to fight microorganisms, viruses & toxins for medical and agricultural applications

13th - 16th February, 2023. Bari (Italy)

Book of abstracts



CA20114



CA19110

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The **PlasTHER COST action** deals with Therapeutical Applications of Cold Plasmas , with the participation of 24 countries as partners. This Action is concerned with the **medical and biomedical** applications of **cold atmospheric plasmas**. The **main aim** of the **PlasTHER COST Action** is to exploit the **unprecedented possibilities of atmospheric pressure plasmas in medicine** to share, develop and consolidate suitable **therapies currently under investigation to make Europe's science and healthcare world leaders in this field**. In this sense, PlasTHER COST Action aims at establishing a synergistic network that articulates researchers, the medical community, industry and patient associations, among others, and coordinate the European activity in this domain to foster the leadership of Europe in this emerging field.



CA19110
Plasma applications
for smart and
sustainable agriculture

The **PLAGRI COST action** aims to investigate the potential of low-temperature plasmas (cold plasmas) as a green alternative to conventional chemicals in agriculture to improve yields, increase plants' size and robustness, and reduce (or eliminate) the need for antifungal agents. It will break the classical field boundaries for a new dimension in sustainable agriculture with a lower chemical impact. The Action will address **the use of plasmas for treating food and packaging**. The Action aims to combine numerous European scientific communities dealing with plasma, biology, agriculture, and food processing to identify and develop food production applications. Transfer of plasma technology to the industry will be based on understanding plasma's most essential processes with further considerations, including (Novel food) legislations, energy consumption, food safety, and quality. The Action will help define a new field in science by a coordinated, joint effort across Europe and broader, through exchange and better use of resources and by intensive study of the basic mechanisms within the context of the well thought out present or future applications.

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Preface

The 2nd Training School “Cold plasmas to fight microorganisms, viruses & toxins for medical and agricultural applications”, is jointly organized by the [PlasTHER CA20114](#) and [PIAGri CA19110](#) COST Actions. The hybrid modality chosen for the event is aimed to include as many participants and instructors as possible to generate A GOOD NETWORK of SCIENTISTS AT EACH LEVEL, according to the main guidelines of the COST Actions, interactive and stimulating.

The 2nd training school presents both basic level and state-of-the art information on cold atmospheric pressure plasmas and plasma treated liquids investigated to fight microorganisms, viruses and toxins in the fields of medicine and agri-food. An outstanding line of invited instructors from different scientific fields has been assembled to present the various topics related to the applications of plasma in medicine and agri-food. The students, moreover, are allowed to present their research activity in the form of pitch sessions and onsite posters, to share their results, receive inputs, and practice their scientific presentation skills in public.

Special care towards gender equality and students from Inclusiveness Target Countries (ITC) has been scrupulously applied in the selection process of the students to be funded by the two COST actions.

The idea behind the organization of this School is to: i) establish a structured link between theoretical and practical interdisciplinary knowledge allowing participants, students, young researchers, and innovators and all the other colleagues, ii) to bridge the gap between theory and experiments in Plasma Applications for Medicine and Agriculture, and iii) to promote collaborations and networking between researchers in different fields and different groups. This will hopefully positively impact their future careers and prepare them for future academic and applicative activities.

The school is focused on integrating fundamental knowledge in these multidisciplinary fields and understanding specific aspects in topics such as:

- Plasma physics/chemistry;
- Fundamentals of microbiology in medicine and agrifood;
- Medical sciences;
- Plasma treatment of materials/cells/tissues/soil/seeds/plants;
- Chemical analyses of plasma treated liquids.

Some general topics will also be introduced to give early stage researchers insights on how to apply for EU project calls, basic information on how to plan and organize plasma and microbiology experiments, and how to properly use statistical analyses of data.

These types of events are also meeting places where collaboration projects and researchers exchanges can emerge from the interactions among the participants. To encourage networking and exchange of ideas for future collaborations, the program of the 2nd Training School includes also an afternoon visit altogether in the Old City of “Bari Vecchia” to enjoy the sunset in a historical contest.

Forewords and acknowledgements

As of today, after having ignited many other practical applications in several disciplines through decades, Cold Plasmas are certainly at the highest level of interest for academic, clinic and industrial scientists active in the fields of Clinical Therapeutics and Sustainable Agriculture. The newer disciplines of Plasma Medicine and Plasma Agriculture, indeed, are well developing innovative strategies of Wound Decontamination-Healing and Cancer Treatments, as well as sustainable approaches to Seeds, Food and Plants Decontamination and Growth Enhancement.

This Training School on “cold plasmas to fight microorganisms, viruses & toxins for medical and agricultural applications” is the second Training School planned for the participants to both COST Actions involved, namely, PlasTher CA20114 (Therapeutical Applications of Cold Plasmas) and PlAgri CA19110 (Plasma Applications for Smart and Sustainable Agriculture). We expect about one hundred of participants, mostly students and Early Stage Researchers, from about 20 European countries. We sincerely hope that all participants will remember the School for the scientific quality of the contributions, for the discussions, for the sharp organization, and for the warmth of the organizers. This is, at least, what we are strongly trying to offer.

We like to warmly thank all members of the Core Groups and Managing Groups of the PlasTher and PlAgri COST Actions, lead respectively by Cristina Canal (Barcelona, ESP) and Nevena Puač (Belgrade, SRB), all members of the Scientific Committee and of our Local Organizing Committee, Noelia Aparicio and Nikola Skoro for all their efforts, enthusiasm and competence, and all participants to the school. Special thanks go to the Director of the Department of Chemistry of the University of Bari, Gerardo Palazzo, and to Mario Colonna and Gianni Liano of the staff at the venue of school, who helped us in several occasions.

We wish you all a fruitful Training School and an enjoyable stay in Bari.

Bari, February 1st, 2023



Committees

Organizing Committee:

Pietro Favia (University of Bari, Italy)
Eloisa Sardella (CNR-NANOTEC, Italy)
Fabio Palumbo (CNR-NANOTEC, Italy)

Additional Local Organisers:

Roberto Gristina (CNR-NANOTEC, Italy)
Francesco Fracassi (University of Bari, Italy)
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Anton Nikiforov (Gent University, Belgium)
Matteo Gherardi (Alma Mater Studiorum – Università di Bologna, Italy)
Joanna Pawlat (Lublin University of Technology, Poland)
Zdenko Machala (Comenius University in Bratislava, Slovakia)
Tomislava Vukusic Pavicic (Faculty of Food Technology and Biotechnology, Croatia)
Franta Krcma (Brno University of Technology, Czech Republic)
Cristina Canal (Universitat Politècnica de Catalunya, Spain)
Sarah Cousty (CHU Toulouse, France)
Abraham Lin (University of Antwerp, Belgium)
Romolo Laurita (Alma Mater Studiorum- Bologna University, Italy)
Eloisa Sardella (CNR-NANOTEC, Italy)
Sander Bekeschus (Leibniz Institute for Plasma Science and Technology, Germany)
Joanna Sadowska (Royal College of Surgeons in Ireland, Ireland)
Sara Laurencin-Dalicioux (Paul Sabatier University, France)
Susana Serio (FCT/UNL, Portugal)
Nikola Skoro (Institute of Physics Belgrade, Serbia)

Venue

The 2nd Training School in Cold plasmas to fight microorganisms, viruses & toxins for medical and agricultural applications is taking place as a **HYBRID EVENT** at the **Ex. Palazzo delle Poste e Telegrafi (Centro polifunzionale Studenti), Piazza Cesare Battisti, Bari (Italy)**.



Scientific Program

TRAINING SCHOOL: Cold Plasmas to fight microorganisms, viruses & toxins for medical and agricultural applications				
	Monday 13 th February	Tuesday 14 th February	Wednesday 15 th February	Thursday 16 th February
9:00-10:30		Microbiology in medicine Daniela BOEHM	Use of plasma technologies for medical and agricultural applications Ita JUNKAR	Foodborne pathogens and their influence on human health: a microbiology perspective Dragana VUKOVIC
10:30-11:00		Coffee break	Coffee break	Coffee break
11:00-12:00		Introduction to microbiology: from Soil to Seeds and Plants Jure MRAVLJE	Difficult to treat infections in clinical practice Davide BAVARO	Toxigenic Fungi and related mycotoxins in cereals Antonio MORETTI
12:00-13:30		Student POSTER/PITCH presentations (12:30-13:30)	Considerations for non-thermal plasma for inclusion as a therapeutic for patients with bacterial and viral infections Vandana MILLER	Prospects of plasma decontamination processes in agriculture and food industry - how to implement plasma technology efficiently Romolo LAURITA
13:30-14:30	OPENING (14:00-14:15)	Lunch break	ONLINE/ONSITE GROUP PHOTO	Lunch break
14:30-15:30	Plasmas, Plasma Sources and Decontamination/Desinfection Jean-Michel POUVESLE	EU Opportunities for early career investigators Cristina CANAL/Joanna SADOWSKA	Free time	Hands-on: planning plasma and microbiology experiments Judit BUXADERA
15:30-16:00	Coffee break	Coffee break	SOCIAL ACTIVITIES	Coffee break
16:00-17:00	Single-molecule reliable bioelectronic large area sensors for biomedical and food applications Luisa TORSI	Plasma gas and liquid chemistry – emphasis on bioactive compounds and their interaction with microorganisms relevant for agriculture and food processing Zdenko MACHALA		The use of plasma activated water (PAW) to improve health and fitness of agricultural crops Assunta BERTACCINI/Nicoletta CONTALDO
17:00-17:30	Student POSTER/PITCH presentations (17:00-18:30)	Student POSTER/PITCH presentations (17:30-18:30)		If on a winter's night a scientist encounters data Roberto GRISTINA
17:30-18:30				
20:00			SOCIAL DINNER	

Trainers - Short Biographies

Jean-Michel Pouvesle

Jean-Michel Pouvesle received the Ph.D and the Doctorat d'Etat degrees in plasma physics from the University of Orléans, France, in 1981 and 1986 respectively. He is currently a CNRS Emeritus Scientist working in GREMI, joint laboratory from the CNRS and University of Orléans, of which he has been Director from 2003 to 2011. His research interest includes fast electrical discharges, non equilibrium atmospheric pressure plasmas and related plasma kinetics, reactive species production, and biological applications of plasma. He is Fellow of the International Plasma Chemistry Society. He received the Plasma Medicine Award from the International Society for Plasma Medicine in 2016.

Luisa Torsi

ORCID.0000-0002-0798-0780,

<https://www.linkedin.com/in/luisa-torsi-75b8bb34/>

<https://scholar.google.it/citations?user=4vNWapMAAAAJ&hl=it>

- IUPAC 2019 Distinguished Women in Chemistry or Chemical Engineering <https://iupac.org/iupac-2019-distinguished-women/>

- Coordinator of the SiMBiT project: <http://simbit-h2020.eu>

- Adjunct Professor - The Faculty of Science and Engineering - Åbo Akademi University - Finland

- Member of the H2020 Programme Committee of the Italian Ministry for Education and Research

Luisa Torsi is professor of chemistry at the University of Bari and adjunct professor at Abo Academy University. She received her laurea degree in Physics and the PhD in Chemical Sciences from UNIBA and was post-doctoral fellow at Bell Labs in USA. She is the past-president of the European Material Research Society, being the first women to hold this role. She is also the only women awarded with the H.E. Merck prize and the recipient of the Global-Women Inventors and Innovators Network platinum prize.

Recently, she was also elected Fellow of the Material Research Society and awarded with the Distinguished Women Award by the International Union of Pure and Applied Chemistry. Gathered research funding for over 26 M€, comprises several European contracts as well as national and regional projects. Torsi is committed to the role-modeling for younger women scientists. She has been giving a number of talks, including a TEDx.

References

- <https://www.nature.com/articles/s41467-018-05235-z>

- <https://www.science.org/doi/10.1126/sciadv.abo0881>

- <https://pubs.acs.org/doi/full/10.1021/acs.chemrev.1c00290>

- <https://link.springer.com/article/10.1007/s00216-020-02540-9>

Daniela Boehm

Daniela recently moved to a lecturing position in the School of Chemical and Bioprocess Engineering at University College Dublin, Ireland after working as a Science Foundation Ireland Starting Investigator and assistant lecturer in the School of Food Science and Environmental Health at Technological University Dublin. Her research focuses on investigating the application of plasma and plasma activated liquids in microbial decontamination, with an emphasis on understanding the translation of liquid chemistry to biological effect. The safety of plasma activated liquids is one of Daniela's topics of interest including studies on cytotoxic and genotoxic effects, the action of reactive oxygen and nitrogen species and development of resistances.

Cristina Canal

Cristina Canal Barnils (Barcelona 1977) is Associate professor at the Department of Materials Science and Engineering, at the Technical University of Catalonia (UPC), head of the PlasmaMedLab [\[link\]](#): Plasmas for BioMedical Applications Laboratory and of the Medical Technologies: Biomaterials & Tissue Engineering research group at the IRSJD [\[link\]](#).

Before joining UPC, she did different research stages at pre and postdoctoral level in different national and international research centres. She has participated and lead a number of research projects, as well as technology transfer projects in the areas of Textile materials, Biomaterials and Cold Plasmas. Her research has led to above 80 publications, and several invited conferences. Her research has been recognized with different awards, including the L'Oreal-Unesco fellowship "For Young Women in Science" (2012), the "2018 Early Career Award in Plasma Medicine" and the ICREA Acadèmia 2020.

Her interests are focused in cold plasmas for biomedical applications, particularly: i. Surface modification of biomaterials to control parameters such as adhesion or biological behaviour; ii. Control of drug release from biomaterials; and iii. Therapeutical applications of cold plasmas, for instance, in bone cancers.

She is currently Chair of COST Action CA20114 PlasTHER and ERC APACHE project [\[link\]](#) Starting Grant leader in a project in the field of atmospheric pressure plasma therapy, her main axis of research being currently focused in the atmospheric pressure plasma therapy of bone cancer treatment in combination with biomaterials.

Zdenko Machala

Prof. Zdenko Machala, D.Sc. has a background in plasma physics. His research is focused on various environmental, biomedical and agriculture applications of non-thermal (cold) atmospheric plasmas. He pioneers new research areas of plasma-liquid interactions, their optical diagnostics, transport of plasma active species into water, and fundamental mechanisms of physico-chemical and biomedical processes of cold plasma interaction with live cells and animal/plant organisms. He has served as a

president of the International Society for Plasma Medicine, is a work group leader in COST Action CA 19110 PIAgri, and is responsible for Environmental Physics study program at the Faculty of Mathematics, Physics and Informatics of Comenius University Bratislava.

Ita Junkar

Assoc. Prof. dr. Ita Junkar is a chemical engineer who obtained her PhD in 2010 and is currently employed as a senior researcher at “Jožef Stefan” Institute in Slovenia. Her main field of work are biomaterials and modification of surfaces by highly reactive gaseous plasma. She developed several methods for surface treatment of materials by highly reactive gaseous plasma, mainly for the use in bio-applications. She has seven granted patents, among them three European. She is actively involved in the interdisciplinary research activities mostly based on surface modification of biomaterials and has many years' experience in working with medical doctors and microbiologist in the field of biomaterials.

Davide Bavaro

Bavaro DF is working as an Infectious Disease specialist and clinical researcher at University Hospital of Bari since 2019. His main clinical and research interests are: infections in immunocompromised or critically ill hosts, infections caused by multidrug resistant organisms, endocarditis and bone and joint infections, antimicrobial stewardship and infection control.

He is author of more than 50 publications in indexed journal of Infectious Diseases and Internal Medicine, total H-Index= 10, total citations=498.

Vandana Miller

Dr. Vandana Miller, MD is an Associate Professor in the departments of Microbiology and Immunology and Surgery at Drexel University College of Medicine. Her research focus is on harnessing the immunomodulatory capability of non-thermal plasma for applications in cancer treatment, wound healing, skin diseases, viral diseases and vaccine delivery. She works closely with tumor immunologists, surgeons, dermatologists, virologists and immunologists, and collaborates with plasma scientists all across the world.

Dragana Vukovic

Dragana Vukovic holds a full professor position at the Faculty of Medicine, University of Belgrade, where she teaches medical and clinical microbiology to medical students as well as to PhD students at various stages of their PhD programmes. She has over 25 years of demonstrable work experience in both fundamental and applied aspects of microbiology. Her main research interests include antimicrobial resistance, genotyping of clinical bacterial isolates, bacterial biofilms and assessment methods for biofilm growth, and antibacterial efficacy of cold atmospheric plasma.

Antonio Moretti

Antonio Moretti is a Research Leader at CNR-ISPA, Member of National Scientific Committee of CNR; Responsible of CNR-ISPA Foggia Unit; President of General Assembly of MIRRI-IT, Microbial Resource Research Infrastructure – Italy Joint Research Unit; Secretary of “International Society of Mycotoxicology” (around 650 members worldwide). He is author of around 160 scientific publications in peer review Journals and 15 book chapters.

Main field of activity: biodiversity of the main toxigenic fungi of agri-food interest, and their pathogenicity, genetics, phylogenetics and mycotoxin biosynthetic gene cluster occurrence and variability. He has coordinated studies on metabolomics and genomics of *Aspergillus*, *Fusarium*, *Alternaria* species. Through such comparative omics approach, a better evaluation of key genetic and mycotoxin differences among the toxigenic main fungal species has been achieved.

Main invitations abroad as invited professor in USA, China, Japan, Spain, and Argentina.

Project Responsibility: Responsible of several National and International Projects on Mycotoxins

He has got around 60 invited and plenary lectures on toxigenic fungi and mycotoxins at National and International conferences.

Editor in Chief of Fungal Secondary Metabolites and Mycotoxins Section of *Frontiers in Fungal Biology*. Editorial Board of *Frontiers in Microbiology*; *International J. of Food Microbiology*; *Toxins*; *Phytopathologia Mediterranea*

H-Index: ISI Web of Knowledge: 44; Scopus: 47; Google Scholar: 55

Romolo Laurita

My main expertise concern the biomedical and industrial application of plasmas, the plasma chemistry in gas and liquid phase, the interaction of cold plasmas with matter, and the plasma assisted decontamination processed. I am currently MC member of COST ACTION CA20114 “Therapeutical applications of Cold Plasmas (PlasTHER)”, WG2 leader of “Antimicrobial effects of Plasmas”. Since October 2020, member of the COST ACTION CA19110 - Plasma applications for smart and sustainable agriculture Since September 2019: member of the Research Group on gynecologic neoplasms (UNIBO) Since September 2017 member of the International Society for Plasma Medicine 2014-2015: member of the American Ceramic society 2013-2016: member of COST ACTION TD1208 Electrical discharges with liquids for future applications 2012-2015: member

COST CA20114 COST CA19110 – Cold Plasmas to fight microorganisms, viruses & toxins for medical and agricultural applications - 2nd Training School 2023

of COST ACTION MP1101 Bio-Plasma - Biomedical applications of atmospheric pressure plasma technology Since 2012 member of the International Plasma Chemistry Society (IPCS).

I am referee of international journals: Plasma Processes and Polymers, Plasma Medicine, Applied Sciences, European Physical Journal, Journal of Advanced Oxidation Technologies, IEEE Transactions on Radiation and Plasma Medical Science, Oxidative Medicine and Cellular Longevity, Molecules.

Judit Buxadera-Palomero

Dr. Judit Buxadera Palomero (Granollers, 1986) is Bachelor in Chemistry (UB), Materials Engineer (UB-UPC) and PhD in Materials Science and Engineering (UPC). She is currently working as a research technician at the Biomaterials, Biomechanics and Tissue Engineering group (BBT), in areas regarding in vitro and in vivo materials characterization, and she is in charge of the microbiology lab of the group. Dr. Buxadera is author of thirteen papers in JCR journals, a book chapter and has done several contributions to scientific meetings. She has taken part in several national projects.

Assunta Bertaccini

Plant Pathology professor at the Alma Mater Studiorum – University of Bologna, Italy. Master in Biology and PhD in Plant Pathology; invited speaker and chairperson at national and international meetings and seminars. Research devoted to detection and management of plant diseases associated with phytoplasmas and bacteria. Editor and reviewer for several scientific international journals. Author of about 360 peer reviewed publications and 65 books and book chapters. Founder and leader of the International Phytoplasma Working Group (IPWG) (<http://www.ipwgnet.org/>). Member of SiPAV, APS, IOM, ISHS, Accademia dei Georgofili, International Committee on Systematics of Prokaryotes (ICSP) Subcommittee on Taxonomy of Mollicutes and of the EASIN board (<https://easin.jrc.ec.europa.eu/easin>).

Nicoletta Contaldo

Dr Nicoletta Contaldo (MSc, PhD) has been a junior assistant professor (fixed-term) in Plant Pathology in the frame of the European project Horizon H2020 “Tropicsafe”, at the Department of Agricultural and Food Sciences, University of Bologna from 2017-2022. She carries out research in the field of plant pathology and epidemiology and molecular diagnosis of plant diseases, actively collaborating with leading international research groups in the field of plant pathology in several countries. She started the academic career working with plant viruses and studying some of the molecular mechanisms underlying plant-virus interactions. Since 2008, after PhD, she has been working on phytoplasmas and phytoplasma-associated diseases, with all the available molecular techniques and eventually succeeded in phytoplasma axenic cultivation, depositing a patent number PCT/IB2012/052965 about this topic. Currently she is involved in national and international projects addressing eco-sustainable control strategies towards plant diseases in open fields and greenhouse conditions. In her career she has published more than 60 papers in indexed journals and five book chapters.

Roberto Gristina

I got the PhD in "Cell and Developmental Biology" in 1992 t the University of Palermo, Sicily, Italy. Examining my PhD thesis no mention of statistics can be found.

At the beginning of this century, when plasma processes suddenly entered in my scientific life, exploring data become more intriguing and I found the exciting power of "Statistics" in helping to understand scientific data and show them in the best charming way.

I spent my everyday scientific time looking at how eukaryotic cells respond, in a cell culture environment, to stimuli from materials and/or liquids modified by plasma processes, assessing whether the data obtained from culture biology in vitro experiments can generalize the effect of plasma in modifying cell behavior in different field of science from Biomaterial Science to the more innovative tools in the fight against cancer.

List of abstracts - LESSONS

Monday 13th February

Plasmas, Plasma Sources and Decontamination/Desinfection

Jean-Michel Pouvesle

GREMI, UMR7344 CNRS/Université d'Orléans, Orléans, France

The recent multiplication of epidemics and problems related to food poisoning, coupled with the increase in the number of bacteria resistant to antibiotics, have reinforced the search for new ways to fight against pathogenic microorganisms and viruses. In this context, plasmas (ionized gases) appear particularly promising whether they are applied directly or whether they are used to produce media (liquids, surfaces) that are themselves effective against the targeted microorganisms. The last two decades have seen an impressive development of the research dedicated to the biological applications of low temperature plasmas, especially with plasma sources working at atmospheric pressure. In this new trend, beside decontamination/ disinfection/ sterilization and surface treatment that have already a quite long story through low-pressure plasma research and developments, medical applications are tacking an increasing place underlined by the actual numerous clinical trials. Biological applications of plasmas are also now extended to agriculture and, more recently, to cosmetic. In all these domains, pathogenic organisms play a deadly role that low temperature can help to fight.

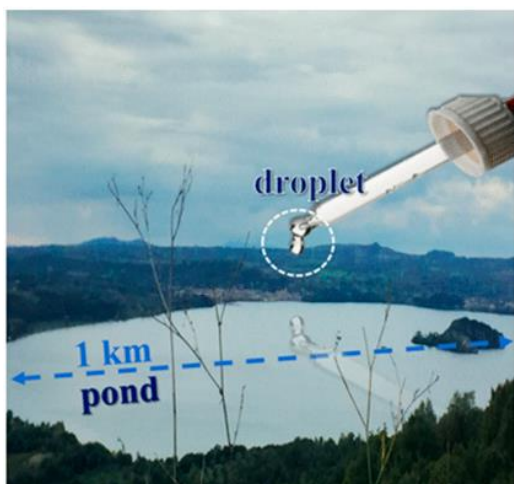
In this lecture, I will first introduced the general concept of plasma for non-plasma specialists and then present a simple description of the physics involved, including species production through basic reaction kinetics, in order to better understand some conventional diagnostic results. Attention will then be drawn to the importance of the plasma/target interaction, of which the failure to take into account can lead to serious problems in the in vitro/in vivo translation. I will then present some plasma sources in use for decontamination/disinfection together with some applications in the concerned domains. I will have a brief part on the new opportunities for plasma parameter or plasma device control. All along the talk, that I hope interactive with the audience, I will emphasize on simple concept that can help everyone to “image” the invisible for an easier understanding of the very complex processes involved.

Single-molecule reliable bioelectronic large area sensors for biomedical and food applications

Luisa Torsi

Dipartimento di Chimica, Università degli Studi di Bari Aldo Moro, Bari (I)

A large millimeter-wide electronic interface can detect at a single-molecule/entity limit-of-detection. The technology is called SiMoT - Single-Molecule with a large Transistor.¹ So far, antigens (Immunoglobulins, C-reactive proteins, spike 1, HIV p-24), antibodies (anti-immunoglobulins, anti-spike1), peptides, viruses (SARS-Cov-2), bacteria (*Xylella fastidiosa*), and even DNA strands (KRAS, miR-182) have been detected. Selectivity is assured by covering the gate electrode with a large number (10¹¹-10¹²/cm²) of recognition elements to affinity binding the target element.



SiMoT detects directly in a droplet (0.1 mL) of a real fluid such as saliva from COVID-19 patients, blood serum, pancreatic cysts juice, and olive saps from infected trees. Relevantly Brownian diffusion enables the entity to statistically hit the millimeter-wide interface in a few minutes.² Considering the footprint of a molecule on a millimeter-wide interface, it is like spotting a droplet of water falling on the surface of a 1 Km wide lake as depicted in the Figure on the left.

The applications span from a handheld intelligent single-molecule binary bioelectronic system for fast and reliable immunometric point-of-care testing of COVID-18 patients³ and *Xylella fastidiosa* single bacterium detected in infected plants sap. The phenomenon enabling such outstanding performance level was discovered in 2018.⁴ While still under investigation, it is supposed to involve an amplification that starts from the single affinity binding that triggers a propagating collaborative response.

Future actions include the deepening of our understanding of the sensing mechanism and the engagement in a campaign of thousands of clinical trials that will bring SiMoT beyond TRL5.

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Tuesday 14th February

Microbiology in Medicine

Daniela Boehm ^{a,b}

^a Environmental Sustainability and Health Institute and School of Food Science and Environmental Health, Technological University Dublin, Dublin 7, Ireland,

^b School of Chemical and Bioprocess Engineering, University College Dublin, Dublin 4, Ireland

Microorganisms are ubiquitous and essential to human life but they can pose major threats to health, well-being and life as causative agents of disease and infection. Close to 14 million infection-related deaths were estimated in 2019 and *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were identified as the five leading causative pathogens¹. The increase in microorganisms resistant to common antimicrobial and antibiotic treatments, in particular, is cause of concern and highlights the need for alternative antimicrobial strategies².

The topics covered in this lecture will include an outline of different types of microorganisms (prokaryotic - gram negative/positive, eukaryotic, viruses), an overview of microorganisms of particular concern in medicine, and an introduction to biofilms (formation, structure, removal, and related challenges). The threat of rising antimicrobial resistance will be discussed as well as commonly used antimicrobials and antibiotics and their mechanism of action. Finally, the role of ROS/RNS both in normal cellular pathways and in the inactivation of microorganisms will be outlined and linked to the role of reactive species generated through cold plasma discharges.

This lecture will provide an introduction into the fundamentals of microbiology in medicine, highlight the current challenges and set the scene for how/where cold plasma can be used (cellular targets) and why it may present a promising alternative for microbial decontamination and the treatment of infections.

Keywords: *antimicrobial resistance, biofilms, pathogen*

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Introduction to microbiology: from Soil to Seeds and Plants

Jure Mravlje a, Marjana Regvar a, Katarina Vogel-Mikuš a,b

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Microbiology is a rapidly developing biological discipline that studies microorganisms (MO). MO comprise prokaryotes and eukaryotes from all three domains: Bacteria, Archaea and Eucarya. They can be found on land and in water, in soils and air (even in clouds), and both on and inside other living organisms. Microbial activities play critical roles in biogeochemical cycles, including nitrogen circulation and fixation and the disintegration of organic matter. In nature, no plant lives alone; thus, the concept of the plant as a “holobiont” has been introduced, meaning the plant and all its associated microorganisms and their interactions. MO can be found either on (epiphytic) or inside plants (endophytic); they can be beneficial or harmful and play an important role in plant growth and development, its physiological state and immunity against biotic and abiotic stressors. Fungi are one of the main concerns in plant production, as they are responsible for more than 70% of all plant diseases worldwide. Fungal infections can occur at all stages of production, from preharvest to postharvest and storage processes, whenever the conditions are favourable. They can affect seed quality by suppressing germination or causing spoilage of the stored seeds. Many of them can produce mycotoxins, which are harmful to humans and animals, significantly impacting global food security. Fungi associated with seeds are traditionally classified into two groups, namely “field fungi” (colonising seeds in the field during growth season or harvest process) and “storage fungi” (colonising seeds during transport and storage). In the last few years, a new interdisciplinary and rapidly developing field of research known as “plasma agriculture” has been formed, including the branch on plasma decontamination of seeds, with some encouraging results. However, much is still unknown and yet to be revealed, especially regarding mechanisms underlying fungal decontamination¹.

In the following lecture, microbial diversity and its importance for planet Earth will be presented. We will focus on MO associated with plants and their interactions, with an emphasis on fungi (e.g. mycorrhizal and endophytic fungi). The concept of the endophytic continuum and its importance in plant production will be explained. In the last part, a brief review of state of the art in the field of plasma decontamination of seeds will be presented, focusing on issues when working with fungi.

Acknowledgements: The researchers acknowledge the Slovenian Research Agency (ARRS) for financing: program group P1-0212 (Plant Biology), young researcher grant (J. Mravlje) and project J1-3014 (Alternative approaches to assuring quality and security of buckwheat grain microbiome).

Keywords: microorganisms, fungi, endophytes, decontamination

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EU Opportunities for early career investigators

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This talk will introduce different possibilities for funding the career of early career investigators, like Marie Skłodowska-Curie Actions or European Research Council Grants. Marie Skłodowska-Curie Postdoctoral Fellowships are European funding available to researchers regardless of their nationality or field, which allows for undertaking research within an institution based in Europe¹. The European Research Council (ERC) grants support to individual researchers of any nationality and age who wish to pursue frontier research², and Starting Grants are particularly interesting for early career researchers.

During the talk, we will introduce these funding structures and provide some tips and strategies that might be useful in the preparation of proposals.

Acknowledgements: The authors acknowledge the support of ISCIII-HEALTH in IHRC22/00003 (Next Generation EU, MRR), AEI for PID2019-103892RB-I00 and COST Action CA20114 PlasTHER.

Keywords: *career progression, funding, project preparation, tips, grant writing*

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<http://erc.europa.eu/funding-and-grants>

Plasma gas and liquid chemistry - emphasis on bioactive compounds and their interaction with microorganisms relevant for agriculture and food processing

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Non-thermal (cold) atmospheric plasmas, especially in or in contact with air generate a cocktail of charged and neutral active particles, such as O, N, H, OH, HO₂, O₂⁻, H₂O₂, IO₂, NO, NO₂, N₂O₅, HNO₂, HNO₃, in addition to radiation and electric field. Many of these particles are called reactive oxygen and nitrogen species (RONS) that have specific roles in antimicrobial action, cell biochemistry, cell-to-cell signaling, immune response to pathogens, etc. When cold plasma interacts with a liquid, typically water solutions, it induces chemical activation of a liquid and generates plasma activated liquid (PAL). Many of the plasma-gas phase RONS are then transported into PAL. Some species transfer through the plasma-liquid interface very well (e.g. highly soluble H₂O₂), while some are very badly soluble (e.g. O₃ or NO). Their concentrations and ratios in specifically plasma and PAL depend on the plasma discharge and dissipated power (e.g. low power corona or DBD vs. high power spark, glow or gliding arc discharges), the gas flow system, the way of interaction with liquid, and the liquid properties (pH, buffering capacity and storage temperature). Various strategies enhance the transport of badly soluble species, e.g. bubbling/stirring or aerosolization to microdroplets. Many of the reactive species are very short-lived, so their effects are limited to a small dissipation volume (e.g. O, N, H, OH, HO₂, O₂⁻, IO₂). The most typical long-lived RONS in PAL are H₂O₂, nitrites and nitrates. Some of the transported species from plasma into liquid get ionic forms upon entering into the solution (e.g. NO₂⁻, NO₃⁻), and many undergo further reactions among themselves (e.g. leading to peroxyxynitrite formation), and also with other organic components present, e.g. in cell cultivation media.

In direct plasma treatments, plasma-generated RONS directly interact with microorganisms, cells, tissues, food matrix, seeds and other targets. The natural environment of microbial or higher cells is typically humid, so in addition to direct effect of primary RONS, secondary and long-lived RONS in PAL take prolonged action even after plasma discharge is turned off. Both primary and secondary RONS may act together with pulsed electric field and (UV) radiation, the plasma components antimicrobial or stimulative by themselves, which often leads to synergic effects.

In the lecture we will look in detail on (air) plasma RONS, their lifetimes and penetration volumes, their transport into liquids depending on the plasma-liquid interface. Then we will analyze the liquid RONS, their reactivity and diffusivity and possible interactions with organic components of the media. Finally, we will briefly describe how these primary and secondary RONS affect microorganisms, as well as cells and tissues, food matrix, seeds and plants. We will mention how their effect can be synergized with the plasma pulsed electric fields and UV radiation.

Wednesday 15th February

Use of plasma technologies for medical and agricultural applications

Ita Junkar and Metka Benčina

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Use of plasma technologies for medical and agricultural applications will be presented, with the focus on innovative approaches.

In the first part of the talk biocompatibility of surfaces will be discussed, with the emphasis on multifunctional surfaces. Desired biological response (improved cell adhesion of one cell type over the other, prevention of bacterial adhesion and biofilm formation, protein adhesion, etc.) presents the crucial factor influencing on the life-span of all implantable medical devices. Surface finishing procedures are one of the important aspects in this field, as biological response of all biomaterials is closely linked with its surface features (micro and nanostructured topography, surface chemistry, wettability). The importance of simple and environment friendly approach based on gaseous plasma for surface modification of custom-made implants, will be discussed.

In the second part we will shortly discuss also the use of plasma technologies for applications in agriculture. In this part interaction of plasma species for decontamination of surfaces will be presented. Case studies of different approaches will be given and discussed.

Acknowledgements: This research was funded by the Slovenian Research Agency (ARRS), grant numbers P2-0232, J3-2533, J3-4502, J3-3074 and CRP project V4-2001.

Keywords: *biocompatibility, antibacterial surfaces, decontamination, gaseous plasma*

Difficult to treat infections in clinical practice

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Despite the advances in many medical and surgical disciplines, modern Medicine is still facing the issue of “difficult-to-treat” infections (DTT-I). Currently, in the field of Infectious Diseases, the term “DTT-I” refers to serious infections caused by bacteria or fungi resistant to first-line antibiotics, or infection affecting deep sites of the body, including bone and joints infections, endocarditis, surgical site, and foreign body infections (like intra-abdominal abscesses).

Interestingly, all these diseases share similar pathogenesis and cornerstones of therapy, although affecting different sites of the human body (or implanted prosthetic devices).

At first, in the context of DTT-I, bacterial biofilm formation should be taken into account: a biofilm comprises any syntrophic consortium of microorganisms in which cells stick to each other and often also to a surface. In this form, bacteria produce an extracellular matrix, composed of extracellular polymeric substances (EPSs), in which they can grow and resist to antibiotics.

Second, these infections are usually caused by hospital-acquired pathogens, often very resistant to common antibiotics due to the heavy selective pressure occurred in the nosocomial setting.

Third, DTT-I need, in all cases, a complex multidisciplinary approach, that should start from a precise diagnostic pathway, up to a pre-established combined surgical and medical therapy, with the aim of eradicate biofilm, kill bacteria and, consequently, reduce the risk of mortality or life-long disability of the patient (according to site and severity of the infection).

The aim of this talk is to guide the audience deep into the DTT-I state of art, current issues, and future perspectives.

Considerations for non-thermal plasma for inclusion as a therapeutic for patients with bacterial and viral infections

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The number of infections associated with drug resistant pathogens is steadily increasing and development efforts from pharmaceutical and biotechnology companies are unable to keep up with the emerging drug resistance. The COVID-19 pandemic underscores the escalated threat posed by emerging superbugs and the urgent need for new strategies to combat these infections. This lecture will explore the potential of non-thermal plasma (NTP) for inclusion in the clinical therapeutic armamentaria against bacterial and viral infections. The antimicrobial effects of plasma in vitro and their application for disinfection and sterilization are well documented. This lecture will discuss how NTP can be useful in treating superficial infections with bacteria and viruses through direct or indirect application of NTP. We will also propose the possibility of engaging the immune system to treat local and systemic infections and to prevent long term sequelae of infections in patients. We will also speculate on the use of NTP to augment vaccination efficacy for prevention of infectious diseases. This information will be used as the basis of an interdisciplinary group discussion which will examine the opportunities and challenges for the translational potential of NTP for treatment of infectious diseases.

Thursday 16th February

Foodborne pathogens and their influence on human health: a microbiology perspective

Dragana Vukovic

University of Belgrade

Foodborne diseases are recognized as a major public health issue, and are associated with high morbidity and mortality worldwide. The true burden of these diseases is difficult to determine as they often remain unrecognized, unreported or uninvestigated. According to the World Health Organization's estimate, around 600 million people fall ill after eating contaminated food and 420,000 die annually. Foodborne pathogens are biological agents that can cause a foodborne illness event. They are phylogenetically diverse, and include eucaryotic parasites and fungi, procaryotic bacteria as well as their toxins, and subcellular infectious agents such as viruses and prions. The major foodborne pathogens are bacteria (e.g. salmonellae, *Campylobacter jejuni*, *Listeria monocytogenes*, *Escherichia coli*), viruses (e.g. noroviruses, hepatitis A virus), and parasites (e.g. *Toxoplasma gondii*, *Trichinella spiralis*). The most obvious interpretation of the term "foodborne" is consumption of food contaminated with microorganisms or their toxins, but it should be noted that foodborne pathogens are also transmitted through contaminated water, through contact with contaminated environment or contact with infected animals and humans. Foodborne pathogens differ in their virulence traits and pathogenesis of the diseases they cause. *E. coli*, for example, displays at least six different mechanisms of pathogenicity leading to diarrhea. The illnesses caused by foodborne pathogens vary considerably in severity and clinical manifestations, from a self-limiting diarrhea to a serious life-threatening disease associated with extra-intestinal complications. The overall threat of foodborne diseases is continually increasing due to the growing number of emerging foodborne pathogens, changes of virulence of known pathogens, and, in particular, acquisition and spread of antibiotic resistance. Foodborne diseases of microbial origin are prevented by safe production and handling of foods. Microbiological investigations in conjunction with epidemiological and clinical data should enable identification of erroneous practices in food production, distribution and preparation that allow foodborne pathogens to contaminate food. To that end, a multitude of microbiological techniques are applied, ranging from basic plate counts to sequencing of microbial genomes.

Toxigenic Fungi and related Mycotoxins in Cereals

Moretti Antonio

CNR- Institute of Sciences of Food Production (CNR-ISPA), via Amendola 122/O 70126 Bari, Italy;

Among the food safety issues, the occurrence of fungal species able to produce toxic metabolites on the agro-food products has acquired a general attention. These compounds, the mycotoxins, generally provided of low molecular weight, are the result of the secondary metabolism of the toxigenic fungi. They may have toxic activity toward the plants, but mostly represent a serious risk for human and animal health worldwide, since they can be accumulated on many final crop products and they have a broad range of toxic biological activities. In particular, mainly cereals are the most sensitive crops to the colonization of toxigenic fungal species which accumulate in the grains the related mycotoxins both in the field, until the harvest stage, and in the storage. According to a Food and Agriculture Organization study, approximately 25 % of the global food and feed output is contaminated by mycotoxins. Therefore, since a large proportion of the world's population consumes, as a staple food, the cereals, the consumption of mycotoxin-contaminated cereals is a main issue for health risk worldwide. Furthermore, mycotoxin contamination can have a huge economic and social impact, especially when mycotoxin occurrence on the food commodities is over the regulation limits established by different national and transnational institutions, implying that contaminated products must be discarded. Finally, the climate change due to the global warming can alter stages and rates of toxigenic fungi development and modify host-resistance and host-pathogen interactions, influencing deeply also the conditions for mycotoxin production that vary for each individual pathogen. New combinations of mycotoxins/host plants/geographical areas are arising to the attention of the scientific community and require new diagnostic tools and deeper knowledge of both biology and genetics of toxigenic fungi. Moreover, to spread awareness and knowledge at international level on both the hazard that mycotoxins represent for consumers and costs for stakeholders is of key importance for developing all possible measures aimed to control such dangerous contaminants worldwide. A wide overview on toxigenic fungi and related mycotoxins in cereals will be provided.

Keywords: *Aspergillus, Fusarium, climate change*

Prospects of plasma decontamination processes in agriculture and food industry- how to implement plasma technology efficiently

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Cold Atmospheric Pressure plasmas (CAP) and Plasma activated liquids (PAL) have recently drawn considerable attention as a novel non-thermal technology for food product and packaging decontamination. Indeed, the emergence of new pathogens contaminating the products and changes of production technologies, consumers' lifestyles and requirements are posing new and peculiar challenges. In this frame, CAP could present several possibilities of high interest. A substantial number of scientific studies appeared in the literature during the last decade, demonstrating the effectiveness of plasma to inactivate degradative enzymes, food pathogens, and spoilage and spore-forming microorganisms. Nevertheless, before a possible application, many aspects still need clarification, mainly related to consumer safety. The main objective of this lecture is to provide deeper knowledge about aspects still scarcely investigated about CAP treatment of food products and examples of scaling up of CAP system in the agri-food sector. Results on the use of CAP or PAL on food matrices demonstrated the feasibility of this process for food decontamination. Nevertheless, results on the nutritional profile after CAP treatment have to be deeply investigated. Finally, an introduction concerning the intellectual protection will be given, focusing on the main tools, such as journal and patents.

The use of plasma activated water (PAW) to improve health and fitness of agricultural crops

Assunta Bertaccini, Nicoletta Contaldo

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Current global food sustenance by intensive agriculture is mainly based on crop monocultures that drastically reduces the biodiversity, increasing the yield losses due to the presence of biotic and abiotic stresses. In the frame of a sustainable agriculture the use of cold atmospheric pressure plasma (CAP) was applied to sterile distilled water, inducing the production of a hydrogen peroxide, nitrite and nitrate, and a pH reduction. In particular, an atmospheric pressure dielectric barrier discharge (DBD) has been initially used to produce plasma activated water (PAW), that was firstly assayed in in vitro experiments and then in planta through application at the root apparatus of tomato plants, against *Xanthomonas vesicatoria*, the etiological agent of tomato leaf spot. PAW did not show direct antimicrobial activity however it was effective in reducing the disease severity by giving relative protections of ca. 61, 51 and 38% when applied 1 h, 24 h and 6 days before the experimental inoculation, respectively. In addition the pal gene transcription levels significantly increased from 1 to 48 h until 192 h after the PAW application. In PAW- treated micropropagated periwinkle shoots, periwinkle and grapevine plants, qRT-PCR and small RNAs high-throughput sequencing were used to analyse the differential expression of genes involved in the major plant defence pathways confirming the increased expression of some defence/fitness-related genes. Different systems to produce PAW were evaluated to control grapevine yellows diseases in open field and greenhouse for qualitative and quantitative yield parameters, pathogen presence, and gene expression. The results show the capability of PAW to enhance plant defense mechanisms and, as demonstrated in the field trials, confirmed its ability to improve the health status of the treated plants enhancing the plant defence responses and providing inputs for its applications in plant disease management programs.

If on a winter's night a scientist encounters data...

Roberto Gristina

CNR - Institute of Nanotechnology (CNR-NANOTEC), via Amendola 122/D 70126 Bari, Italy

Once experimental data have been obtained from our carefully planned experiments, they have to be presented to the scientific world. They can be presented in very different ways ranging from seminar to running text to lists to tables and figures. Statistics, plural since there are many type of these tools, can help us in doing this in the most precise and understandable way, since our results presentation have a marked effect not only on how readers perceive and understand the research results, but also on how authors analyze and interpret those results. In our one hour walk through the statistic way of thinking we will meet the most used statistic tools useful to describe the obtained experimental data in order to better understand and evaluate information in the world. We will look at some example that can help us in organizing and summarizing the data using descriptive statistics .Then, we will look at the use of inferential statistics to formally test hypotheses and make estimates about the population. Finally, we can interpret and generalize our findings.

Poster/Pitches Program

Monday 13th February
Time 17:00 – 18:30

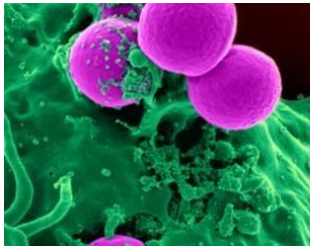
N.	Name	Institution	Country
P1	Neda Babucic	Institute of Physics Belgrade	Serbia
P2	Neusa Silva	Faculdade de Medicina Dentária da Universidade de Lisboa	Portugal
P3	Olivera Jovanović	Institute of Physics Belgrade	Serbia
P4	Darina Kužmová	Faculty of Mathematics, Physics and Informatics, Comenius University	Slovakia
P5	Mostafa Elsayed Hassan	Institut Pprime	France
P6	Valentina Puca	University G. d'Annunzio Chieti-Pescara	Italy
P7	Aleksandra Nurzyńska	Medical University of Lublin	Poland
P8	Anna Michalicha	Medical University of Lublin	Polska
P9	Pankaj Pareek	Comenius University Bratislava, Slovakia	Slovakia
P10	Domenico Aceto	CNR-ISTP; Instituto Superior Tecnico Lisboa	Italy
P11	Silvia Giuditta Scaltriti	University of Bologna	Italy
P12	Rouillard Amaury	GREMI	France
P13	Francesco Tomelleri	Università di Bologna	Italy
P14	Palma Rosa Rotondo	University of Bari	Italy
P15	Vladyslav Vivcharenko	Medical University of Lublin	Poland
P16	Tijana Lainović	Faculty of Medicine, School of Dental Medicine, University of Novi Sad	Serbia
P17	Alina Maria Holban	University of Bucharest	Romania
P18	Lorenzo Ibba	EPFL	Switzerland

Tuesday 14th February
Time 12:00 – 13:30

N.	Name	Institution	Country
P19	Caterina Maccaferri	Università di Bologna	Italy
P20	Leonardo Zampieri	Università degli Studi di Milano Bicocca	Italy
P21	Berrak Kurt	Ankara University Faculty of Veterinary Medicine Department of Biochemistry	Turkey
P22	Dawid Zarzeczny	Lublin University of Technology	Poland
P23	Aleksandra Lavrikova	Comenius University in Bratislava	Slovakia
P24	Rita Agus	EPFL	Switzerland
P25	Marianna Roggio	University of Bari Aldo Moro	Italy
P26	Sara Lotito	University of Bari Aldo Moro	Italy
P27	Regina Del Sole	University of Bari Aldo Moro	Italy
P28	Nikola Milojević	School of Dental Medicine University of Belgrade	Serbia
P29	Kristína Trebulová	Brno University of Technology	Czech Republic
P30	Marwa Balaha	D'Annunzio University of Chieti–Pescara	Italy
P31	Merve Şensöz Turgut	Ankara University Faculty of Veterinary Medicine Department of Biochemistry	Turkey
P32	Sara Covella	Università degli Studi di Perugia	Italy
P33	Zuzana Měšťánková	Brno University of Technology	Czech Republic
P34	Vincenza Armenise	University of Bari Aldo Moro	Italy
P35	Michal Wojcik	Medical University of Lublin	Poland
P36	Marta Trzaskowska	Medical University of Lublin	Poland
P37	Zuzana Okruhlicová	Division of Environmental Physics, Comenius University in Bratislava	Slovakia

Tuesday 14th February
Time 17:30 – 18:30

N.	Name	Institution	Country
P38	Vikas Rathore	Institute for Plasma Research	India
P39	Morena Pinti	Università degli studi "G. d'Annunzio" Chieti-Pescara	Italy
P40	Ewelina Godek	Maria Curie-Skłodowska University in Lublin	Poland
P41	Mariangela Mazzone	University "G. d'Annunzio" Chieti-Pescara	Italy
P42	Ştefana-Marcela Şimon	Alexandru Ioan Cuza University of Iaşi, Doctoral School of Romania	România
P43	Sevde Nur Kutlu	Suleyman Demirel University, Graduate School Of Natural and Applied Sciences, Bioengineering	Turkey
P44	Gebremedhin Gebremariam	Bologna University	Italy
P45	Giulia Tomei	University of Padova	Italy



2nd Training School

Cold plasmas to fight microorganisms, viruses & toxins for medical and agricultural applications

P1

Nonthermal Plasma at atmospheric pressure with aerosols: applications in agriculture

Neda Babucić¹, Nikola Škoro¹, Nevena Puač¹

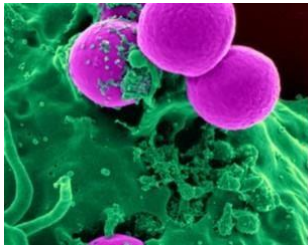
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Applications of low temperature plasma (LTP) are investigated due to its versatile use in water decontamination, wound treatment by using plasma activated water (PAW) and in agriculture. One of the key challenges in plasma-water interaction is to increase flux of reactive species from the plasma. The addition of micrometer-scale droplets of aerosols immersed in plasma provides a high surface-to-volume ratio, increases the contact area for a given amount of water and potentially enhancing the rates for chemical interaction between plasma in gas phase and liquid. Apart from development of plasma-liquid applications, the plasma-aerosol configuration is also enabling greater scientific insights into a complex problem with potentially thousands of transient and non-equilibrium chemical reactions¹. In this regard, we made the first step in assembling an experiment where microwave (MW) plasma source is used for aerosol treatment. The setup will enable characterization of the plasma, interaction between the plasma and droplets and characterization of treated water in order to better understand gas-liquid reactions of high chemical reactivity. At the moment, MW plasma is operated using Ar flow from 1-7 slm without addition of aerosols. Optical emission spectroscopy together with images of plasma provided information about the distance from the source where role of reactive species is important. After introducing aerosol into the reactive volume, in this setup we will be able to assess the influence of droplets to the plasma. The main idea is to better understand the interaction of plasmas with aerosols as there is potential of plasma-aerosol interaction at atmospheric pressure in treatment of biology samples. This research will be linked to the topics of WG 3 (treatment of plants with PAW made through aerosol) and WG4 (determination of PAW properties) of PIAgri COST action.^{2,3}.

Keywords: *Nonthermal plasma, aerosols, MW source, activated water*

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2nd Training School

Cold plasmas to fight microorganisms, viruses & toxins for medical and agricultural applications

P2

Cold Atmospheric Plasma for periodontal and peri-implant disease treatment: antibacterial effects and cellular response

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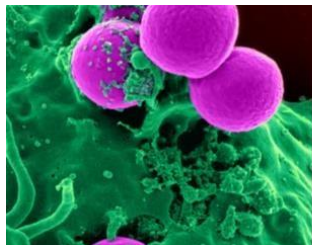
Periodontal and peri-implant diseases are the major cause of tooth and implant loss and remain the most common non-communicable diseases worldwide, affecting approximately 3.6 billion people, of whom 18.45 million are edentulous^{1,2}. An unbalanced oral microbiota favors the entry of many microbial species that promote the onset of periodontitis, leading to tooth loss with a significant impact in quality of life^{1,3}. The most effective treatment approaches involve biofilm removal and are highly dependent on patient compliance. Cold atmospheric plasma (CAP) is a promising tool for disinfection and tissue regeneration. However, its ability to successfully disrupt oral biofilm and prevent posterior formation in implant surfaces is not yet assessed. This PhD project aims to optimize and validate a novel point-of-care CAP device developed by our team for the chairside treatment of tooth root or implant surfaces affected by periodontal diseases. *In vitro* models will be used to assess antimicrobial effects of CAP treatment in root and implant surfaces evaluating oral multispecies biofilm inhibition or eradication. We will also evaluate the effects of CAP treatment parameters on *in vitro* periodontal cell behaviour, specifically on cytotoxicity, differentiation, inflammatory and wound healing responses. At the end of this project we expect to optimize and validate a prototype to be tested *in vivo* for periodontal treatment applications.

Acknowledgements: This project was funded by FCT - PhD Scholarship - Cold Atmospheric Plasma for periodontal and peri-implant disease treatment: antibacterial effects and cellular response, 2022. 13655.BD

Keywords: Cold atmospheric plasma, periodontal cells response, Biofilm eradication

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2nd Training School

Cold plasmas to fight microorganisms, viruses & toxins for medical and agricultural applications

P3

Varying the plasma-treated liquids' characteristics for applications in biomedicine

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The development of new plasma sources operating at atmospheric pressure in contact with liquid samples over the past 20 years has mostly been driven by new biomedical applications such as wound healing, cancer treatment, as well as the deactivation of bacteria and viruses and sterilization¹⁻². Recent research is focused on several aspects of plant biology and wastewater treatment³⁻⁴ because low-temperature plasmas are environmentally acceptable and the active gaseous environment is at room temperature.

Plasma Activated Liquids (PALs) are essential in this application. PALs have been used in a substantial number of successful experiments, but there are still many questions regarding the mechanisms governing the interaction between the plasma and liquid including the production of a broad number of chemical species.

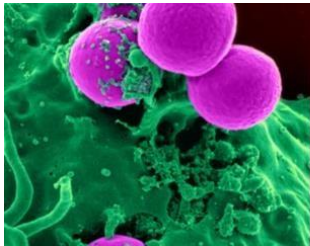
In this work, we utilized atmospheric pressure non-equilibrium plasma jets for the treatment of liquids, and the production of PALs. PALs were employed for the treatment of plant tissue culture, then also for application on wound healing in diabetic mice and finally in a study that tested their toxicity after oral intake by rats. Diagnostics of liquid samples were performed to assess the effectiveness of plasma treatment. The analysis of physicochemical parameters was conducted by measuring conductivity, pH, temperature, and the amount of deposited RONS (H_2O_2 , NO_3^- , NO_2^-). The mechanisms of interaction between plasma and liquid samples were determined depending on the conditions in the system. Through this research, we wanted to show that the used plasma systems provide the possibility to adjust the properties of treated liquids for a particular application in biomedicine by changing the treatment conditions.

Acknowledgements: This work was supported by MSTDI Republic of Serbia grant number 451-03-68/2022-14/200024, and The Science Fund of the Republic of Serbia, grant No. 3114/2021 - Project APPerTAin-BIOM.

Keywords: *plasma jet, plasma biomedicine, plasma activated liquids*

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P4

Investigations of the effects of plasma-activated phosphate buffered saline to monolayers and 3D tumour models of breast adenocarcinoma cell lines

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High toxicity level of conventional cancer therapies often burdens patients with severe side effect. In many cases the tumour becomes resistant to chemotherapeutic agents, therefore it is necessary to search for new strategies to cure cancer. One of the promising strategies is found in plasma medicine. Cold plasma has the potential to prolong patient's life and make it more valuable if the previous conventional cancer therapies fail. The main role in plasma therapy is played by reactive oxygen and nitrogen species (RONS) generated in the plasma and plasma-activated liquids (PAL) ¹.

This study is focused on the effects of indirect application of cold plasma, via plasma-activated phosphate buffered saline (PBS), to drug sensitive human breast adenocarcinoma cell lines MCF-7 and the Paclitaxel-resistant sub-lines MCF-7/PAX ^{2,3}.

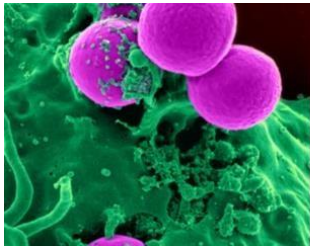
We tested the effect of plasma-activated PBS (PAPBS) treated for 5 and 10 minutes by cold atmospheric plasma of streamer corona discharge added to 2D cell monolayer and 3D tumour models – spheroids. The concentration of RONS in PAPBS linearly increased with the plasma activation time⁴. Incubation of cells in PAPBS significantly reduced the viability of both cell lines. Metabolic WST-1 assay has shown a decrease of the number of viable cells depending on the plasma treatment time. The following microscopic analysis proved that PAPBS induced processes leading to cell death in both sensitive cancer cell line and the resistant sub-line of human breast tumor cells. PAPBS also seems to inhibit the growth and induce disintegration of the spheroids.

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

Keywords: *plasma, plasma-activated liquid, cancer, spheroids*

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P5

Production of Gaseous Species in Water Bulk and Microdroplets Depending on their Surface Area and Charge

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Atmospheric air plasmas in contact with water produce “plasma-activated water” (PAW), which contains various solvated reactive oxygen and nitrogen species (RONS). PAW solution has many potential applications in agriculture e.g., plant growth promotion and in biomedicine in which PAW can be used in killing microbes and some PAW solutions have shown anticancer effects ¹.

This work presents experimental investigations of the transport mechanisms of the typical long-lived air plasma species: H₂O₂, O₃, NO, NO₂, and HNO₂, having different Henry’s law solubility coefficients (k_H), into the water under nonequilibrium conditions. Two experimental setups were used. First, the transport of gaseous RONS from external sources to bulk water with fixed surface area, and to aerosol of charged or non-charged water microdroplets with a larger surface-area-to-volume ratio is investigated. Second, the transport of RONS produced by streamer corona discharge in direct contact with water aerosol is investigated.

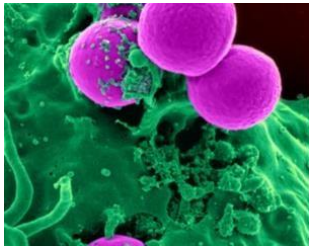
The solvation of RONS to water is increased by the enhanced surface area of the water microdroplets compared to the bulk water. Compared to O₃, relatively less H₂O₂ is dissolved than expected according to k_H ². HNO₂, NO₂, and NO are dissolved in the water as NO₂⁻ anion, with higher concentration as predicted by their k_H . HNO₂ has been proven to be the dominant source of NO₂⁻ in the treated water/PAW ³. The charge of the electrosprayed water microdroplets positively influences the solvation of the gaseous species HNO₂ and NO₂, while the gas/plasma–water interface area is the key factor influencing the solvation of O₃, and NO. The solvation of H₂O₂ is dependent on the amount of OH radicals created near plasma–water interface. This fundamental research contributes to a better understanding of the transport mechanism of the gaseous plasma RONS into water. Obtained results enable us to optimize the design of plasma–liquid interaction systems to efficiently produce PAW and to tune its composition.

Acknowledgements: This work was supported by the Slovak Research and Development Agency under the Contract No. APVV-17-0382, by the Slovak Grant Agency VEGA, project no. 1/0596/22.

Keywords: plasma–liquid interactions, plasma-activated water, Henry’s law solubility, aerosol microdroplets

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P6

Cold Atmospheric Plasma (CAP) for the Treatment of Oral Bacterial Biofilms

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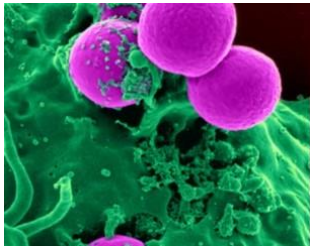
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In recent years, plasma-based technologies have emerged as a promising approach for non-chemical and low-temperature decontamination. Plasma is the fourth state of matter, an electrically neutral ionized gas which can be divided in “thermal”, “non-thermal” such as Cold Atmospheric Plasma (CAP). The CAP sources have the possibility of expanding the plasma treatment to living tissues, in relation to temperatures less than 40°C at the point of application¹. An interesting CAP application is within the oral medicine field, especially in periodontology. Based on these observations, the aim of this study was to evaluate the potential use of CAP in removing oral biofilms.

S. mutans UA 159 biofilm was developed in Brain Heart Infusion (BHI) plus 1% of sucrose (BHIS) for 24 hours of incubation at 37°C in static conditions with 5% of CO₂. A saliva sample, from a patient with periodontitis was used to develop a second *ex vivo* biofilm. The saliva biofilm was developed in BHI in anaerobic conditions for four days of incubation at 37°C. After incubation, *S. mutans* biofilm was treated with the CAP source at a distance of 6 and 12 mm at different times, corresponding to 30s, 60s, 120s, 180s and 240s. The biofilm developed from a saliva sample was treated with the same CAP source at a distance of 6 mm and for 60s and 120s. Both biofilms were immediately stained with XTT (sodium 3'-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis-(4-methoxy-6-nitro) benzene sulfonic acid hydrate) for 2h at 37°C. The CAP Minimum Biofilm Eradication Concentration (MBEC) was determined with the evaluation of viability by using both the Colony Forming Units (CFU) count and XTT metabolic assay. The CAP treatment was effective in eradicating the biofilms developed by both *S. mutans* and saliva sample. A statistically significant reduction of both metabolic activity and CFU count was already detected after 60s of CAP treatment while the eradication of both biofilms with a -4Log₁₀CFU/mL reduction was reached after 120s of treatment. The data obtained in this study showed that CAP treatment is able to eradicate a preformed biofilm developed by both *S. mutans* and the complex mixture of saliva microorganisms, representing a potential innovative strategy against oral pathogens responsible of peri-implantitis and periodontal diseases.

Keywords: CAP, biofilm, new biofilm eradication strategies.



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P7

Multifunctional curdlan-based hydrogels: Can they be also used in agriculture?

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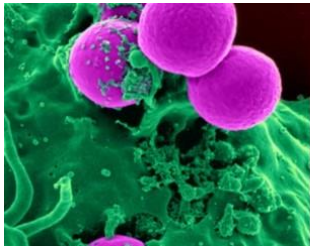
Polysaccharides are polymers of natural origin, synthesized by plants, animals, and microorganisms. These polymers exhibit a number of beneficial characteristics, such as biodegradability, non-toxicity, biocompatibility, bioactivity, and hydrophilic character. These features make them an excellent raw material in many industries. For instance, hydrogels based on natural polymers have been widely applied as components of bioactive materials for regenerative medicine applications. Furthermore, biopolymers have also found application in agriculture as soil conditioners and fertilizer carriers. In this case, agricultural polysaccharide hydrogels (APH)s have gained particular attention of scientist because they support the water-holding ability, porosity of soil, and promote viability of plant as well as total vegetable yield¹.

Curdlan is an exopolysaccharide, consisting exclusively of glucose monomers that are linked by β -1,3-glycosidic bonds. Importantly, curdlan possesses the ability to form tasteless, colourless, and odourless hydrogels, which are characterized by biocompatibility towards human body as well as environmental safety. Curdlan has been used as a thickening and gelling agent as well as stabilizer in foodstuffs. In the pharmaceutical industry, it has been used to encapsulate certain drugs, while in the biomedical sector it has been widely applied for fabrication of bioactive biomaterials [2]. Nevertheless to date, the possibility of using curdlan-based hydrogels in agriculture has not been recognized. Our research team produced two types of absorbent curdlan-based hydrogels using ion-exchanging dialysis. One of them contained copper ions and possessed antimicrobial properties, but at the same time exhibited cytotoxicity towards human cells [2]. In turn, the second one was improved with calcium ions. This biomaterial was not only non-toxic, but also supported viability and proliferation of human cells. However, it did not show antibacterial properties [3]. Therefore, we believe that plasma treatment can improve desired antimicrobial properties of curdlan-based hydrogels, without adverse cytotoxic effect. The biomaterials produced in this way can be especially used in the biomedical and food industry. However, we believe that they will be also applicable in agricultural sectors. For example they can promote viability of plant and total vegetable yield as well as protect plants against bacterial infections.

Keywords: natural polymers, curdlan, agricultural polysaccharide hydrogel, antimicrobial activity

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P8

Curdlan Modified With Functional Nanoparticles Via Poly(L-Dopa) Deposits As Biocompatible, Antibacterial Wound Dressing Candidate

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The need to produce new functional materials is one of the challenges faced by modern regenerative medicine. These materials include among others dressing materials for the treatment of difficult wounds, pressure ulcers or burn wounds, and for the regeneration of tissue defects, such as bone tissue. The significant problem in the area of wound dressings design and improvement is the increasing problem of bacterial resistance to conventional drugs used in the treatment of infections. The risk of bacterial infection is one of the problems encountered when treating difficult-to-heal wounds or other similar medical procedures. The significant problem in the area of wound dressings design and improvement is the increasing problem of bacterial resistance to conventional drugs used in the treatment of infections. Therefore, it seems to be crucial to develop wound dressings which will exhibit strong antibacterial properties without use of antibiotic substances.

One of natural polymers of high biomedical potential is curdlan which forms hydrogel of high strength and elasticity. Coupling of therapeutical substances to curdlan matrix could increase its biological potential. However, the disadvantage of curdlan is the lack of active groups susceptible to modifications. Strategies used for curdlan functionalization lead to curdlan solubilization and loss of beneficial physicochemical properties.

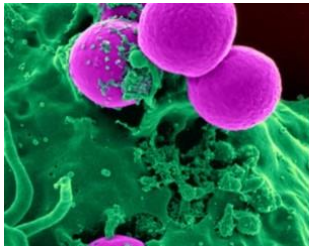
It was shown that catecholamines (dopamine and L-DOPA) are useful for creating polycatecholamine deposits within curdlan polymer matrix for the immobilization of active molecules to its structure. I demonstrated the possibility of curdlan functionalization using the above-mentioned polycatecholamine deposits with the use of an antibiotic [1,2] and enzymatic proteins [3]. Matrices obtained using this functionalization method showed high potential as dressing materials, exhibited antibacterial properties and inhibited the immune response from the immune system.

Therefore, it was interesting to verify how the other types of molecules will affect the matrices properties. Bearing in mind the growing problem of antibiotic resistance, we decided to use nanoparticles with antibacterial potential as the alternative to antibiotic molecules. Our aim was the verification, whether the addition of functional nanoparticles to the curdlan-polycatecholamine network could positively affect the properties of manufactured matrices and increase its suitability for biomedical purposes. We believe that the development of modification mode of the matrices obtained in this way using the cold atmospheric plasma technique may be a valuable clue for further research on curdlan-based dressing materials. Exploring the knowledge about the double mechanism of antibacterial action presented by hydrogels modified simultaneously with nanoparticles and poly(L-DOPA) deposits as well as by cold atmospheric plasma will give new research perspectives.

Keywords: natural polymers, antibacterial, polycatecholamines,

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P9

Transport mechanism of various reactive oxygen and nitrogen species in direct and indirect plasma-liquid interaction in transient spark and electro spray system

Pankaj Pareek ^a, Saeed Kooshki ^b, Mário Janda ^b

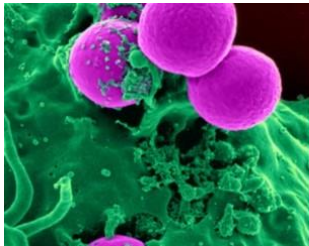
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The generation of plasma-activated water (PAW) has become an important research topic in recent years due to its many potential applications in medicine, agriculture, and food industries [1]. PAW may contain various reactive oxygen and nitrogen species (RONS), generated either by the transfer of reactive species from plasma or by secondary chemical reactions in water. Hydrogen peroxide (H_2O_2), ozone (O_3), nitrites (NO_2^-), and nitrates (NO_3^-) belong to the important long-lived species in PAW. In previous research in our group, it was found that the increase of gas/water interface by electro spray (ES) of water to microdroplets enables a higher transport rate of RONS from gas to the liquid phase in comparison to bulk water [2]. In this study, we employed transient spark (TS) discharge with water ES in a one-stage system (1SS) in dry oxygen (O_2) gas and TS operated in dry or humid O_2 followed by water ES in a two-stage system (2SS). The aim was to show the significance of direct plasma-liquid interaction in 1SS for the generation of high concentrations of reactive species in PAW. TS discharge operated in dry and humid synthetic air can produce NO_2 , HNO_2 , and NO in the gas phase which can then be transported to ES microdroplets to produce PAW in 1SS and 2SS. To limit the interactions of nitrites (NO_2^-) with H_2O_2 , pure O_2 was used as input gas. We observed that, unlike NO_2 and HNO_2 , no significant amount of H_2O_2 is produced in the gas phase. It is probable that OH radicals which are transported from plasma to water combine to generate H_2O_2 in the liquid phase. For this reason, the concentration of H_2O_2 was much higher in 1SS (TS+ES) than in 2SS (TS and ES separated) because direct contact between plasma and liquid water is possible only in 1SS. These results are beneficial for understanding the mass transport from the gas phase to water and for the tuning of plasma-activated water.

Keywords: *Transient Spark, Electro spray*

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P10

Electric Field Measurements by E-Fish in nanosecond humid air discharges

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The application of low-temperature plasma to several biological systems has in the last decades attracted the attention of researchers working in the field of low-temperature plasmas. Several physical agents are interacting with biological tissues when plasma treatment is applied. Nonetheless, very few papers report applications of advanced laser diagnostic in such discharges. In this contribution, we report on the direct measurements of the electric field by the E-Fish¹ technique in discharges in the typical condition of a low-temperature plasma used in decontamination processes. The local electric field produced by plasma may have major importance in such treatment. Usually, in the literature, such field is indirectly measured by applying optical emission spectroscopy and following the emission of the first negative and second positive systems of the nitrogen molecules². The fact that such measurements can only be performed during the plasma-on phase of the treatment, is a strong limitation in processes where the plasma is amplitude modulated to keep the gas temperature as close as possible to ground one.

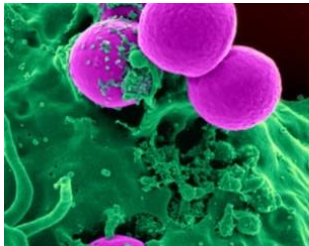
Besides the E-fish measurements spatially resolved optical emission spectroscopy and plasma imaging by ICCD were also performed.

Acknowledgements: I.V.A. kindly acknowledge the CNR STM 2019 mobility programme for financial support of his stay at ISTP-Bari.

Keywords: E-Fish, Nanosecond, Pulsed Low-Temperature
Plasma, Plasma, Diagnostics, Plasma Decontamination

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P11

Cold plasmas for closed environment sanitization

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The Covid-19 pandemic has put into evidence the importance of indoor air quality control and its depuration. The development of non-thermal plasmas (NTPs) based device can be exploited to control and reduce aerosol transport and aerosol infectivity of airborne pathogens in air. At the Plasma Technology Laboratory, PLT, University of Bologna, various NTP sources are studied and utilized for different applications. Recently, PLT's research group, in which I work, has focused attention on the study of a NTP system as an air sanitizer for closed environments¹. The actual project's goal is to design and construct a small-scale test facility, which will be used to test different electrode arrangements, applied voltages and electric power supplies. Investigations on those different configurations will be carried out to optimize the electric power supply system in order to minimize power consumption and noxious species production (ozone and NO_x). Currently, we are testing a configuration made of a grid-like coated electrodes powered by a 1 μ s square bidirectional pulse voltage waveform² with ± 1 kV peak (Figure 1.a). The above-mentioned device is integrated with diagnostic system for the measurement of the ozone concentration, which can be exploited in the abatement processes. Different time intervals between pulses (50,100,200 μ s) have been chosen to investigate the ozone concentration over treatment time. The preliminary results are shown in Figure 1.b. Once the optimal working conditions have been obtained, the effectiveness of the device against viruses and bacteria will be assessed.

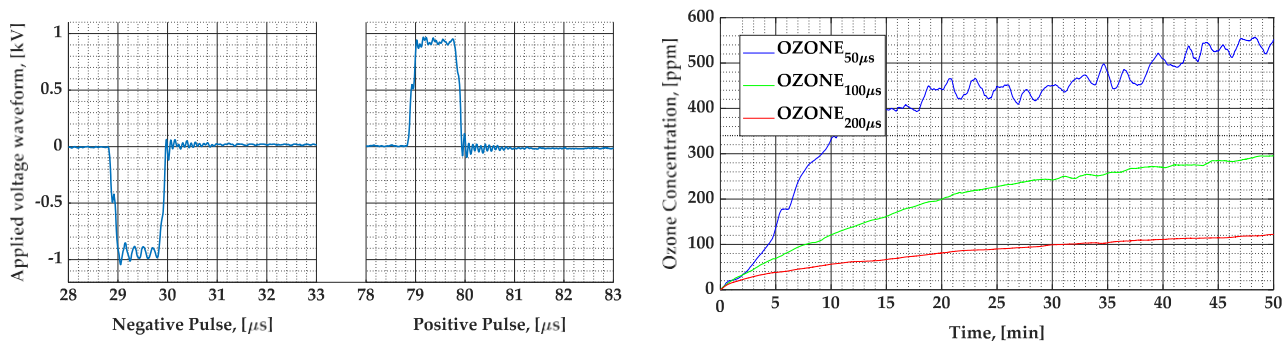
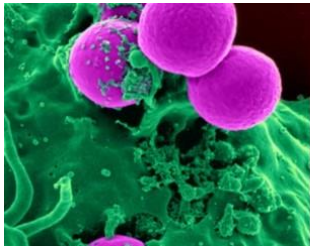


Figure 1.a - Applied voltage waveform; Figure 1.b - Ozone concentration comparison

Keywords: electric power supply, airborne pathogens, non-thermal plasma device.

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P12

Cold Atmospheric Pressure Plasma for Bacteria Decontamination in Space Conditions

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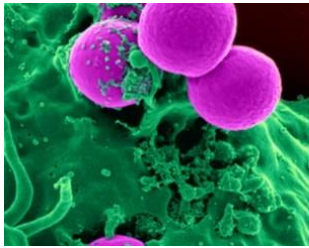
The future space expeditions will have a long duration and many challenges to overcome. Spacecrafts have special environments; they are under low gravity, confined with very limited resources and virtually isolated. Thus, they need a versatile and compact medical supply capable to face drug-resistant bacteria, virus and potentially alien pathogens. Cold atmospheric pressure plasmas (CAPs) antimicrobial effect and their multiple applications in medicine are nowadays established¹. We developed an experimental setup allowing us to test a CAP in the microgravity conditions achievable during parabolic flights². The CAP used is a “Plasma-Gun” (PG) developed in the GREMI laboratory and well-characterised³. It is a microsecond pulsed DBD jet plasma reactor (7 kV and 20 kHz) operating in a mixture of He and O₂ (1 SLM). The first obtained results were oriented at demonstrating the compatibility of the PG with microgravity conditions. Then, inactivation potential of the PG in microgravity is studied under varying conditions (electrical parameters, O₂ concentration, treatment time, reactor-target gap). The bacteria used are non-pathogens E. Coli. The operational range of the PG for various O₂ concentration is enlarged in microgravity conditions and its inactivation potential is enhanced compared to terrestrial conditions. The final aims of this project is to optimize the PG inactivation and to correlate the microgravity impact on the plasma jet structure and the reactive species generation.

- *Acknowledgements: This project is in the scope of Amaury Rouillard PHD: Study on the potential of cold plasma jets for the treatment of (multi)resistant bacterial strains in terrestrial and space conditions, supported by MITI (80 PRIME 2022 – PLANES) from the CNRS, and is supported by the CNES (7900-SdV - SAFE)*

Keywords: Microgravity, Cold atmospheric pressure plasma, Bacteria, Decontamination, Space.

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P13

Assessment of plasma systems' antimicrobial efficacy in food industry

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In recent years food safety is assuming a role of relevant importance due to the new risks associated with changes in pathogens' behavior and changes and production systems¹. In food industry, traditional food and packaging decontamination technologies are based on thermal or chemical treatments. These kind of treatment have some limitations associated with their interactions with food matrix, waste residues and energy cost²⁻³: it is therefore essential to develop new strategies in microbial inactivation those lacks these limitations, to be implemented in food production systems. Cold atmospheric plasmas (CAP) are one of the major candidates to fulfill this purpose, thanks to their efficacy, lack of waste residues and low energetic cost¹.

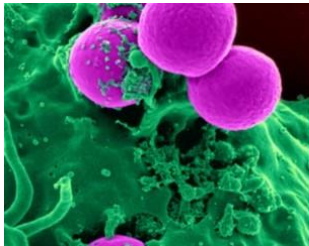
Our research project intents are to assess cold plasmas efficacy in food and packaging microbial inactivation; to optimize treatment sets and conditions in a product and pathogen specific manner; to implement plasma systems in food production industry.

In order to accomplish these goals, we are going, firstly, to determine an experimental procedure through which assay efficacy of plasma treatment compared to traditional decontamination system employ. Analysis like colony count, colorimetric assays, death and vitality assays in florescence microscopy, morphological analysis by scansion electron microscopy on treated foods and packages could give us answers to this first question. Subsequently, through the same set of experiments we are going to test different working conditions of plasma systems, to determine which are the best to use on different products and in the presence of different pathogens. In this phase of our research we are going to determine, in addition to efficacy, the use of energy and the time taken for every working condition, in order to determine advantages and disadvantages in industrial employment of plasma systems. In the end we are going to implement optimized decontamination system with traditional decontamination technology, in order to obtain synergic effects and to concretely include plasma systems in food production industry.

Keywords: *Cold Atmospheric Plasma, Food Industry, Food safety, Microbial inactivation*

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P14

Evaluating the inhibitory effect of low-temperature volume barrier discharge against phytopathogenic fungi

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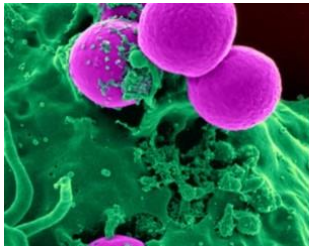
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The inhibitory effect of a square wave modulated plasma (duty cycle 20%) Volume Dielectric Barrier Discharge (VDBD) at atmospheric pressure in ambient air was evaluated on conidial germination of different fungal species (*Botrytis cinerea*, *Monilinia fructicola*, *Aspergillus carbonarius*, *Fusarium graminearum* and *Alternaria alternata*). Several factors potentially influencing the efficacy of plasma treatment were considered, including total treatment time, applied voltage, and agarized medium composition. As previously observed¹, the inhibitory effect of the treatments progressively increased with the total time of exposure to VDBD and decreased with the complexity of cellular structures of the analyzed fungal species. Another main factor influencing efficacy is the uniformity of the distribution of the plasma microdischarges. In in vitro experiments, one crucial point is the response of agarized media (Water Agar (WA), Glucose Agar (AG), Malt Extract Agar (MEA), and Potato Dextrose Agar (PDA)) to the electrical applied field that could strongly influence plasma generation. To better understand this point, agarized media structure was analyzed through cryo-scanning electron microscopy (cryo-SEM) images and correlated to the different plasma electrical responses in terms of electrical impedance and charge transfer characteristics in the plasma phase. In the tested experimental conditions, complete inhibition of conidial germination was achieved with different treatment times. For instance, 5 s total treatment time, inhibited completely the conidial germination of *B. cinerea*, while 20 s were required for both *M. fructicola* and *F. graminearum* instead, the total inhibition of conidial germination was shown after 60 and 180 s of treatment for *A. alternata* and *A. carbonarius*, respectively.

Keywords: Plasma Agriculture, Decontamination, Volume Dielectric Barrier Discharges, Fungi

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P15

Promotion of MC3T3-E1 preosteoblast proliferation and differentiation by low temperature atmospheric nitrogen plasma

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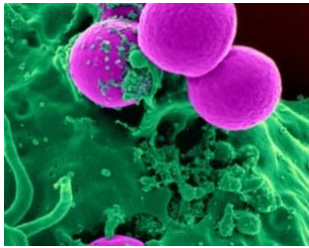
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There is a wide range of atmospheric pressure plasma applications which start from the food industry, biomaterial engineering, agriculture and end with water, exhaust gas and wastewater treatment ^{1,2}. The main goal of the present research was to estimate the influence of atmospheric pressure nitrogen plasma on viability, proliferation, and osteogenic differentiation of MC3T3-E1 Subclone 4 cell line (normal mouse calvarial preosteoblasts). During the plasma treatment, the above-mentioned cells were maintained in Hanks' balanced salt solution (HBSS). MC3T3-E1 were exposed to plasma for 4 s, 8 s, and 16 s. Obtained results revealed that short-time nitrogen plasma application possesses a non-toxic effect on preosteoblasts viability. Moreover, plasma treatment promotes cell proliferation, as well as enhances osteogenic differentiation by increasing osteocalcin and bone alkaline phosphatase concentration. Nevertheless, inhibition of an extracellular matrix mineralization process was also noted. According to the conducted experiments, the best results were obtained after 16 seconds of the plasma treatment when the preosteoblasts were left in HBSS for 3 hours after plasma treatment. The presented work demonstrates the great clinical potential of cold atmospheric nitrogen plasma, especially in the case of regenerative medicine applications.

Keywords: *plasma activated cells, cell viability, ECM mineralization*

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The influence of Cold Atmospheric Plasma dentin pretreatment on endogenous MMPs activity and bond strength – a pilot study

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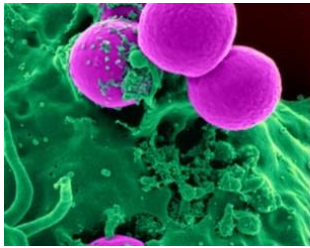
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The present research aimed to investigate the influence of Cold Atmospheric Plasma (CAP) pretreatment on dentinal endogenous enzymatic activity and dentin-adhesive bond strength, by means of in situ zymography and microtensile bond strength (μ TBS) test, respectively. Materials and methods: Sound human extracted molars (N=30) were cut to expose middle/deep dentin. Teeth were randomly assigned to the treatment groups: a) no pretreatment (control – CTR); 15s plasma pretreatment (15s); c) 30s plasma pretreatment (30s). Further, a universal adhesive was applied on dentin surfaces either in the self-etch (SE) or the etch-and-rinse (ER) mode, with or without dentin surface rewetting (RW) after plasma treatment. Hence, 10 different treatment groups were formed: 1) self-etch control – SE-CTR; 2) SE 15s DRY; 3) SE 15s RW; 4) SE 30s DRY; 5) SE 30s RW; 6) etch-and-rinse control – ER-CTR; 7) ER 15s DRY; 8) ER 15s RW; 9) ER 30s DRY; 10) ER 30s RW. Composite resin build-ups (4 mm) were made on all teeth. Bonded specimens were further cut into 1-mm²-thick sticks and subjected to μ TBS. Dentin slices were obtained from additional 3 teeth (8 slices per tooth) and were treated in the same way as for μ TBS to investigate the dentinal endogenous enzymatic activity. Bonded slices were ground down and covered overnight with fluorescein-conjugated gelatin and observed using a confocal microscope. Data were statistically analyzed ($p < 0.05$). Results: Direct dentin treatment with CAP influenced μ TBS significantly ($p < 0.05$). The influence was more notable in the ER groups, where 15s RW increased the bond strength results significantly, while 30s DRY led to the complete debonding of all the specimens. The differences in the SE groups did not reach statistical significance. Enzymatic activity was higher in the ER groups compared to SE. Plasma treatment influenced more the ER groups, demonstrating the lowest enzymatic activity in the 15s RW group, which is in accordance with μ TBS results. Conclusions: Direct plasma pretreatment could influence bond strength, particularly of ER adhesive systems, possibly also due to the reduction in endogenous enzymatic activity. Adequate CAP treatment duration and dentin moisture level should be further analyzed in detail and applied.

Acknowledgements: The work is supported by the PlasTHER COST Action and is in accordance with the topics related to WG1 and WG3. The authors would like to thank the DIBIDEM department from the University of Bologna, for their great hospitality.

Keywords: Cold Atmospheric Plasma (CAP), microtensile bond strength, MMPs, dentin



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Atmospheric plasma treatment of enamel specimens for increased fluoridation and modulation of biofilm development

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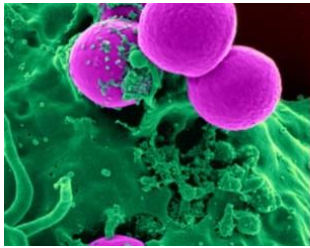
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Despite the technological progress of the last decades, dental caries is still the most frequent oral health threat in children and adults alike. Such condition has multiple triggers and is caused mainly by enamel degradation under acidic attack of microbial cells, which compose the biofilm of the dental plaque. Fluoride is the most used remineralizing agent. The cariostatic effect of fluoride is primarily due to its ability to decrease the rate of demineralization by forming fluorapatite and enhancing the remineralization of incipient carious lesions, while showing antibacterial effects.

The aim of this study was to obtain a cold-plasma based technology with dual effect exhibited through microbial biofilm modulation and enamel strengthen through fluoridation, intended for dental applications. In this study we have developed two cold (atmospheric) plasma models: 1) DBD (Dielectric Barrier Discharge) plasma optimized to improve fluoridation after the application of a commercial fluoride gel and 2) DBE (Discharge with Bare Electrodes), optimized to ensure enamel fluoridation by the incorporation of a fluoride gas (SF₆ - sulfur hexafluoride).

The results revealed a two fold increase in the fluoride retention in enamel samples treated with DBD source after the application of the commercial gel, and a eight fold increase in fluoridation when DBD+SF₆ plasma treatment was used, as compared to classical fluoride gel application of the enamel samples. Increased fluoride retention rate correlated with a diminished bacterial biofilm development on the plasma treated enamel specimens. Here again, monospecific biofilms developed by the Gram-positive *Staphylococcus aureus* and the Gram-negative *Pseudomonas aeruginosa* opportunist bacteria were significantly inhibited. Biofilm formation was reduced by un to 3 folds under DBD+fluoride gel treatment and up to 5 folds under DBE+SF₆ treatment, suggesting the ability of the two plasma sources to inhibit biofilm development on natural enamel by increasing local Fluoride content. Moreover, DBE plasma source was very efficient to eliminate mature biofilms developed after 24h on enamel specimens, when applied to scan the enamel surface containing monospecific biofilm. After 10 plasma scans of the surface, more than 50% of the biofilm cells have lost their integrity and viability, as revealed by scanning electron microscopy and viable counts.

Keywords: enamel plasma treatment, biofilm modulation, dental caries, antibacterial plasma



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Laser diagnostics for Low-Temperature Plasma Characterization

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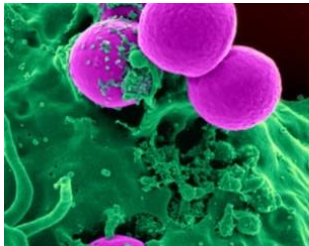
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Low-Temperature Plasma (LTP) applications for medicine, sterilization and agriculture are rapidly increasing. A comprehensive characterization, electrical and chemical, of LTP is necessary. Electrical characterization of the plasma is needed to understand the dynamics of the ionization wave and the electric field evolution during a plasma discharge. Optical Emission Spectroscopy (OES) can be used to measure indirectly the electric field only in the plasma phase. However, advanced laser diagnostic such as E-Fish can measure the electric field inside the plasma discharge locally, with better space resolution and in the plasma off phase. We report on the direct measurements of e-Fish¹ inside a Volume Dielectric Barrier Discharge (VDBD) plasma at atmospheric pressure, showing the electric field is of major importance in the understanding of LTP. As already mentioned, chemical characterization is fundamental, especially since RONS, at atmospheric pressure, are considered the main factor in biological treatments. Laser Induced Fluorescence (LIF) is used to measure the concentration of different molecules like NO, O, OH². These molecules are of major importance in the understanding of interaction mechanisms with biological substrates. The advantages of LIF with respect to other diagnostics, like FTIR, is the space and time resolution, which ultimately depends on the pulse duration of the laser. Absolute measurements of picosecond LIF of NO were performed in a nanosecond pulsed Surface Dielectric Barrier Discharge plasma at atmospheric pressure, showing NO concentration to be relevant only in the close proximity of the plasma. Since the locality is crucial in LTP, laser diagnostics are needed to complement the data from other diagnostics such as FTIR or OES.

Keywords: Laser Diagnostics, E-Fish, E-Field, LIF, Plasma Diagnostics, Plasma Decontamination

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Plasma-assisted systems for packaging decontamination

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Pathogens contamination of external surfaces of food packaging, processing, and handling materials represents a novel and crucial issue to consider. Food industries are interested in the use of novel systems able to inactivate viruses and microorganisms on both packaging and food processing tools, avoiding the use of chemicals. On the other hand, plasma-assisted treatments exploit the antimicrobial action of several active components, such as reactive species, thus representing a cheap, green and safe technology¹. The pre-industrial PASS prototype having shown good inactivation capabilities on SARS-CoV-2 in 10 minutes, without causing a significant increase in the temperature of the treated substrate and without reducing the shelf-life of the packaged food, constitutes a proof of concept and can therefore be considered a promising starting point for the future research. However, the main limitation of this type of source lies in the limited volume of the treatment chamber. In fact, the results were obtained with a chamber of the order of tens of cubic centimeters, certainly not usable for large-scale industrial processes.

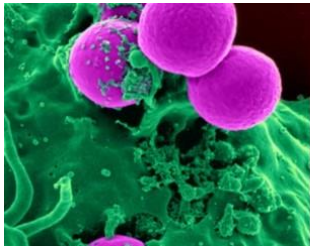
First of all, a systematic review of the state of the art has been carried out to analyze several characteristics of plasma-assisted surface decontamination studies, such as treated material, type of plasma source, operating conditions, pathogen strain, and maximum inactivation achieved.

The analysis is essential to redesign of the prototype, taking into account the requirements of an in-line industrial process. For this purpose, the design and construction of different sources of cold plasma and different mechanisms of action will be evaluated, such as the presence of aerosols to convey the reactive components of the plasma or the possible synergistic action with traditional disinfection systems, such as hydrogen peroxide vapors.

Keywords: *packaging, foodborne, sanitization, in-line process*

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Combining diagnostics for characterizing antibacterial effect of a cold atmospheric plasma source

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Following the large growth in plasma medicine of the last years, a wide landscape of different plasma sources is now available in the various research groups. Different layouts, power levels, application methods, gases and peculiar characteristics make each source unique. At the same time, a large variety of different diagnostics has been proposed to characterize the apparatus. The absence, however, of a standardized analysis protocol leads to important difficulties in comparing the various plasma sources, and does not allow to properly reconstruct the activated biochemical chain.

In the process of optimizing the plasma sources, it is of fundamental importance to recognize which agents have the key role in triggering the aimed effects. It is therefore of interest to correlate physical parameters of the source with the production of reactive oxygen and nitrogen species and the biological consequences. A contribute to this project is proposed using a helium pulsed plasma jet, originally developed in Consorzio RFX in Padova (Italy) [1], and in collaboration with EPFL in Lausanne (Switzerland).

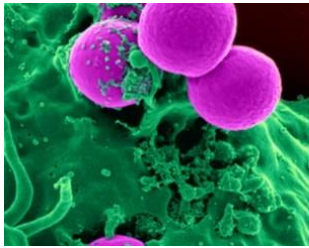
A variety of diagnostics has been applied on the same setup, which involves the treatment for 10 minutes of 6 ml of deionized water. The jet has been electrically characterized, describing the voltage and current waveforms and the power as a function of the different tunable parameters. *In-situ* Fourier Transform Infrared spectroscopy allowed to observe the variations in the production of ozone and nitrous species in the gas phase between the nozzle and the water surface. Using photochemical surveys, the presence of peroxides, nitrates, nitrites and ozone in the water after the treatment has been described. Finally, the killing of *E. Coli* suspended in water has been described using CFU counts.

The correlations between the measurements from the different diagnostics, combined on the same source in the same experimental setup, shows promising results in contributing to outline the pathway which leads from plasma to disinfection. Following them, the tunable parameters of the source can be connected to the final biological effects, allowing for an effective optimization of the jet.

Keywords: jet, RONS, combined diagnostics

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Cold Plasma Applications: Antiviral and Antineoplastic Effects in Cervical Cancers

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The antiviral and antineoplastic effects of cold atmospheric plasma applications (CAP) have been shown in various studies. CAP application draws attention as an alternative method in oncoviral diseases such as cervical cancer¹.

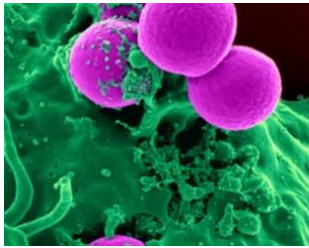
Persistent infections caused by the *human papilloma virus (HPV)*, which is an oncogenic virus, is the most common cause of cervical cancer. Despite successful vaccination policies around the world, cervical cancer with an incidence of 6.6% remains important among cancer deaths in women. In addition to chronic wound management, promising antineoplastic effects of CAP application have been observed in precancerous and cancerous lesions of the cervix². Reactive oxygen and nitrogen species regulates cell proliferation and migration at low doses, while at high doses it controls cell senescence and apoptosis. In studies to investigate the antitumorogenic effects of CAP sources, it has been shown that ROS formation pauses the cell cycle in G1 phase and induces apoptosis by activating p53 and apoptotic factors Puma and Bax³. In addition, ozone, a reactive oxygen species, has been observed to have a maximum virucidal effect at concentrations of 20-25 ppm. It has been determined that non-thermal plasma applications cause regression in warts in a period of 3-4 months in relation to the formation of ROS/RNS in HPV infections¹. Cytotoxic and cytostatic effects of the Martin Argon Plasma Beamer System (MABS), an argon plasma source, were observed in cervical tissue (NCCT) and cervical cancer cell lines (CC). In addition, the application of MABS triggered a greater decrease in the metabolic activity of cancerous tissues than non-cancerous tissues².

In summary, argon plasma applications, which is a CAP application, is an area open to development as an alternative treatment method in both regression of warts and prevention of cancer progression in cases such as cervical cancer caused by oncoviruses.

Keywords: Cold atmospheric plasma, Martin Plasma Beamer System, cervical cancer, human papilloma virus

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Plasma treatment for conditioning of juice

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The aim of the research was to investigate the effect of Cold Atmospheric Plasmas (CAP) treatment on various parameters of freshly pressed NFC (Not From Concentrate) apple juice, including on microbiological quality, physicochemical and structural properties. In addition, the conducted research will be used to assess the usefulness of CAP in extending the shelf life of fresh apple juice with minimized loss of valuable nutrients.

Currently conducted research, described by many authors, confirms the rightness of using CAP in order to eliminate unwanted bacteria and fungi from food products. However, there is little evidence of the effectiveness of the disinfection of fruit and vegetable juices. The most frequently performed experiments are based on the analysis of the effect of CAP on specific microorganisms in pasteurized juices. An interesting area, due to the prevailing trends related to healthy eating, is the possibility of extending the shelf life of unpasteurized juices ^[1-6].

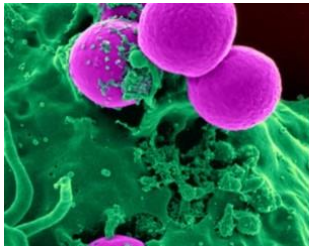
Using a modified GildArc (GAD) reactor connected to a flow system, the potential use of low-temperature plasma technology to condition unpasteurized apple juice was investigated. The conducted experiments were aimed at determining the optimal conditions of food processing with the use of CAP.

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Keywords: *Cold Atmospheric Plasma, GAD reactor, Plasma reactor, Conditioning of food*

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P23

Antibacterial action of cold plasma for biofilm infection control

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Most microbial and chronic infections are caused by biofilms, i.e. bacterial populations that have a distinct phenotype from free-living planktonic cells. They are ubiquitous, resilient, and extremely difficult to eradicate due to high resistance to microbicides, changes in physiology, and toxic compounds accumulated in the biofilm matrix¹. One of the promising techniques for biofilm infection control is a cold plasma which is believed to combine both physical (ion bombardment, electroporation, electrostatic disruption) and chemical (oxidative and nitrosative stress) antibiofilm mechanisms^{2,3}. Yet, knowledge of complex network of interactions between bacteria in mixed-species biofilms as well as plasma-biofilm inactivation pathways is limited. With respect to occurrence of more adaptive biofilms, persistent cells, or resistance to reactive oxygen and nitrogen (RONS) generated by plasma, it is essential to find adequate eventual optimal parameters of cold plasma treatment and improve its overall efficiency.

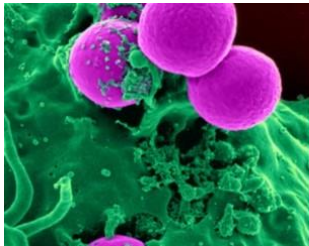
A pulsed streamer corona discharge generated in ambient air was used to combat single- and mixed-species biofilms of Gram-positive *S. aureus* and Gram-negative *P. aeruginosa* of different growth stages. These bacterial species are most commonly isolated from co-infected wounds⁴. Both types of biofilms underwent significant loss of biofilm biomass, suppression of metabolic activity, and removal from polystyrene or glass substrates. Despite the strong immediate effect on bacterial viability, the regrowth of biofilms on the next day showed an inadequacy of the used plasma treatment that requires further improvement. Also, hydrated form of biofilms was found more resistant than dry-surface one. The gaseous species including O₃ and NO_x combined with the etching of biofilms are probably the main factors responsible for overall antibiofilm eradication induced by the pulsed streamer corona discharge plasma.

Acknowledgements: This work was supported by Comenius University grant UK/206/2022 and Slovak Research and Development Agency grants APVV-17-0382 and APVV-20-0566.

Keywords: cold plasma, bio-decontamination, *Staphylococcus aureus*, *Pseudomonas aeruginosa*

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Investigation of plasma activated water inactivation mechanisms of *E. coli* through single cell microfluidic experiments, flow cytometry and scanning electron microscopy

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Despite bactericidal properties of plasma treated liquids have been widely reported in the literature, the bacteria inactivation mechanisms are still subject of debate^{1,2}. We have developed and fully characterized an in-house Plasma Activated Water (PAW) reactor, optimized for sterilization purposes. In our reactor, PAW is produced by exposing a continuous flow of sterile deionized water to cold atmospheric plasma, generated by a surface dielectric barrier discharge (SDBD). The design of the reactor allows to modulate the water flow and produce rather different samples.

Chemical characterization of PAW samples is performed for different treatment times and reactor configurations by measuring RONS concentration, pH, electrical conductivity (EC) and oxidation-reduction potential (ORP).

The effect of different PAW samples is then tested on non-pathogenic *E. coli* (K-12 strain MG1655) and the corresponding inactivation curves are measured, revealing a clear correlation between the nitrites and nitrate ratio and the inactivation efficacy.

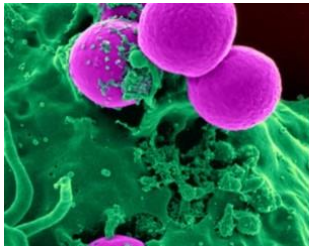
To shed light on the inactivation mechanism, three different diagnostics are coupled together for the first time: single-cell phase contrast and fluorescence time-lapse microscopy³, for the investigation of the real time effect of PAW on bacterial size and duplication rate, scanning electron microscopy for the identification of microscopic bacteria membrane damage and morphology modification, and flow cytometry for the discrimination of specific cell death pathways through specific fluorescent dyes. In light of the above-mentioned measurements, we propose a mechanism for the bacteria inactivation by PAW, which will be a key factor for the design and optimization of future PAW sterilization applications.

Acknowledgements: EPFL Flow Cytometry Core Facility (FCCF) and Biological Electron Microscopy Facility (BioEM) staff.

Keywords: *Plasma Activated Water, Inactivation Mechanisms, Sterilization, Single-cell microscopy*

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P25

Cold Plasma Processes and Technologies for Sustainable Agriculture and Food Processing

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Seeds and plants can be contaminated by pathogens. Contaminations by mycotoxins, secondary metabolites of fungi, can cause acute and chronic toxicity in humans and animals. Toxines of the *Fusarium* fungi, in particular, are common in cereals and their by-products; different *Fusarium* species can colonize such crops from the first growth stage and, in certain conditions, they can produce extensive amount of toxins. Pesticides, with all their undesired side effects, are to date the most effective tool available in Agro-Food technology for controlling *Fusarium* in cereals, to be used during all the critical steps of the vegetative cycle ^[1]. The “from farm to fork” strategy aims to turn the whole Agro-Food system safer and more eco-compatible by reducing the use of pesticides and fertilizers. In this direction, Low Temperature Plasma (LTP) processes recently developed for applications in Agriculture and Food technologies are very promising ^[2]. A home-made AP-DBD plasma reactor powered by an electric generator in the KHz range, equipped with a back-and-forth movable stage, will be used to homogeneously process the seeds and plant propagative materials under the discharges. Two different electrode configurations will be tested as shown in **Figure 1**, to optimize the AA-AP plasma deposition processes with an aerosol generator (atomizer) of the different active coatings, namely: 1) a volume DBD, where the seeds are placed between the electrodes, with the movable stage acting as ground electrode; 2) a laminar jet configuration, where the discharge is ignited between two vertical electrodes and it is expanded towards the substrates to be treated. In this case the movable stage acts just as substrate holder. The volume DBD configuration is known to be very effective in increasing the growth rate of the coatings, but it could reveal more sensitive to inhomogeneities due to the different shape and size of the seeds. In the jet configuration, instead, the plasma is scarcely influenced by inhomogeneities of the substrates but the growth rate of the coating should be lower than in the previous case.

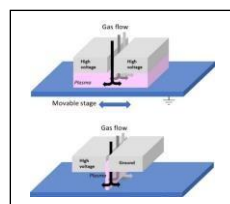


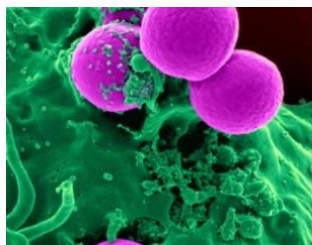
Figure 1. Electrodes configuration of the AP-DBD reactor for plasma coating of seeds.

Acknowledgements: Mr Danilo Benedetti is grateful acknowledged for his technical support

Keywords: Agriculture, Plasma, Seeds, coating

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Low-pressure plasma surface modification of Spanish Broom fibers for water remediation

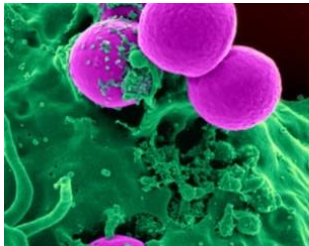
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Due to rapid global development over the last century, anthropogenic activities have left elevated amount of heavy metals and hydrocarbons into surface and ground water through discharges of wastewater produced from metallurgical, mining, chemical and battery manufacturing industries. Considerable efforts are being addressed to develop cost-effective methodologies, among which the use of low-cost adsorbing materials. In this contribution we present different plasma processes for the surface modification of Spanish Broom cellulose fibers, as low-cost, eco-sustainable adsorbents with great affinity towards petroleum hydrocarbons or Hg(II). To this aim, Spanish Broom fibers were treated in low-pressure plasmas fed with either tetrafluoromethane or tiophene. Raw and plasma functionalized fibers were analysed by fast Fourier Transform-InfraRed (FT-IR) and X-ray Photoelectron Spectroscopy (XPS) for the chemical characterization confirming, the fluorination or sulfurization of the treated fibers. Scanning electron microscopy (SEM) was used to assess potential degradative effects induced by the plasma treatments. Both fluorine or sulfur functionalization turned the fibers into hydrophobic. Batch experiments were performed with the aim of studying kinetic and thermodynamic aspects of the adsorption process, as a function of the initial total pollutant load (hydrocarbon or Hg²⁺). Polluted aqueous solutions were specifically prepared in order to simulate typical polluted water conditions. The kinetics data showed that the hydrocarbon removal efficiency of fluorinated SB cellulose fibers ranged between 80-90% after one minute of contact time, in dependence of the initial hydrocarbon/fiber weight ratio (20-240 mg/g), with a maximum adsorption capacity larger than 270 mg/g. Such results demonstrate a significant hydrocarbons removal action if compared to other cellulosic materials reported in the literature. On the other hand, the sulfurized fibers performed a mercury removal efficiency of 82% which slightly decreased to 76% as the pollutant concentration increased up to 10ppm. The experimental results are very encouraging, indicating that plasma treated Spanish Broom fibers could be promising materials for water remediation, implemented for example, inside filtering devices.

Keywords: water remediation, low-pressure plasma processing, surface modification, Spanish Broom, lignocellulosic sorbent.

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Aerosol-Assisted Atmospheric Pressure Plasma Deposition of TiO₂/rGO and TiO₂/C₆₀ polymeric nanocomposite coatings for photocatalytic applications

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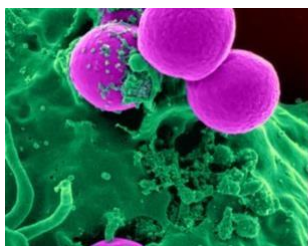
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Nanocomposites have been a topic of increasing interest in the last decades because of their superior properties when compared to their single components. Nanomaterials can be used as fillers for polymeric matrices, thus leading to an enhancement in the pristine mechanical, electrical, thermal and chemical properties of the material components. Among the others, nanostructured semiconductors may be relevant candidate in photocatalytic applications, including wastewater treatment, photodegradation of organic pollutants or antimicrobial activity. In particular, NPs made of TiO₂, an n-type semiconductor, are often used in photocatalysis. However, its wide band gap of 3.2 eV leads to a strong absorption in the UV region, thus requiring a high energy irradiation. In addition, TiO₂ NPs are characterized by a fast electron-hole recombination rate that limits their photocatalytic performance. In order to overcome these limitations, nanocomposites combining TiO₂ and CNMs, and in particular with rGO (reduced Graphene Oxide) and C₆₀, can be manufactured, in order to profit of the charge sink properties of the carbon derivatives that contribute in enhancing the electron-hole recombination time. Furthermore, the immobilization onto a solid support can make photocatalysts suitable and technologically viable for environmental applications, as it could be easily recovered and recycled. In this work, we addressed the Aerosol Assisted Atmospheric Pressure Plasma Deposition (AA – APPD) of nanocomposites formed by TiO₂/rGO and TiO₂/C₆₀ thin films in a polysiloxane matrix. This technique has been already used for the deposition of TiO₂ nanocomposites in siloxane matrix¹ as it allows to embed thermodegradable or scarcely volatile species, to deposit also on 3D structures and thermodegradable materials and to reduce chemicals and materials waste with respect to traditional methods of thin films deposition. The chemical composition and the morphology of the obtained films were characterized by means of FT-IR, EDX, profilometry, SEM and TEM analyses. The nanocomposite coatings deposited onto glass were tested for the photodegradation of a model organic pollutant, methylene blue (MB), and their photocatalytic activity under UV light irradiation was demonstrated and rationalized.

Acknowledgements: Prof. Gianni Barucca (UNIVPM) for TEM analysis; Savino Cosmai is gratefully acknowledged for his technical skill assistance

Keywords: *Plasma processing of materials, Nanocomposites, Photocatalysis*

References¹ C. Lo Porto, M. Dell'Edera, I. De Pasquale et al. *Nanomaterials*, 12, 21 (2022), 3758



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Title: Examination of the antimicrobial potential of different sources of low-temperature atmospheric plasma *in vitro* against methicillin-resistant *Staphylococcus aureus*

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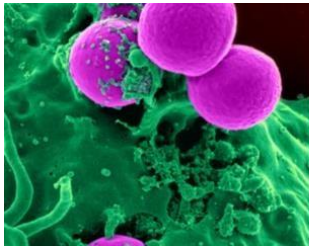
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The growing prevalence of infections caused by multiresistant bacteria represents a global medical problem, given that there is no adequate therapeutic solution for these infections. Infections caused by resistant, multi-resistant, and even pan-resistant bacteria occur primarily in the hospital environment, but their frequency is also increasing in the general population. The emergence and spread of resistance to antibiotics and chemotherapeutics is observed in most medically important bacteria. However, the problem of resistance is particularly significant with Gram-positive bacteria of the genera *Staphylococcus* and *Enterococcus*. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the leading nosocomial pathogens, but it is increasingly occurring as a cause of infections in the general population. These strains are resistant to all beta-lactam antibiotics and represent a very big problem today. Abscess is a common form of odontogenic infections and is a reflection of a favorable defensive reaction of the organism that tries to localize the infectious focus. The microbiological flora of odontogenic infections consists of an association of anaerobic and aerobic species with a dominant role of anaerobic species. One of the facultative anaerobes in odontogenic abscesses is MRSA, whose prevalence, according to some studies, ranges from 3.3% and even up to 12%¹. Previous research shows that low-temperature atmospheric plasma exhibits a strong antimicrobial effect without toxic effects on human stem cells, which is why it could be applied in the field of biomedicine for *in vivo* decontamination of surfaces colonized by bacteria and antimicrobial therapy^{2,3,4}. The aim of this research is to examine the antimicrobial effect of different sources of NTAP on the reference strain and clinical isolate of MRSA from an abscess of odontogenic origin and to determine whether resistance to plasma treatment develops, as well as whether plasma treatment increases the sensitivity of MRSA to different antibiotics. **Keywords:** MRSA, low temperature atmospheric plasma, antimicrobial effects

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Antimycotic effects of different CAPP sources tested on different surfaces

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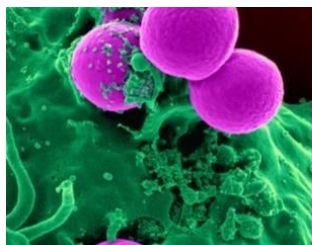
Cold atmospheric-pressure plasma (CAPP) technology has become a very discussed topic in the past few decades, thanks to its wide range of applications in various fields of science. This work is aiming to broaden the knowledge about antimicrobial effects of CAPP, mainly focusing on the antimycotic effects of different CAPP sources as a potential alternative method for yeast inactivation. *C. glabrata* was chosen as a representative of non-albicans species, in order to establish basic plasma set-up of chosen CAPP sources for further studies and experiments hopefully leading to clinical studies *in-vivo*. So far, 5 different cold plasma sources, namely: microwave discharges (unipolar¹ and surface-wave²) in the form of plasma torches, transient spark³ and corona discharge⁴ in the form of corona pen and plasma jet⁵ were tested. From the preliminary experiments microwave plasma jet was chosen to be the most effective in yeast inactivation, thus subsequent measurements with the usage of more complex inoculation substrate (pork skin) have been done with this discharge. With regard to a specific application, the dog ear treatment, the plasma jet with its capillary design was tested. The preliminary tests have been done on the Petri dishes to establish the basic plasma parameters. To render this research more appropriate to real application more complex inoculation substrates, pork skin and 3D printed models, have been used. The results of this work confirm the high efficiency of CAPP in the inhibition of yeasts on different surfaces and will lead to further experiments and international cooperations.

Acknowledgements: This work was conducted in cooperation with the GREMI laboratory at the University of Orleans, and was financed by the COST action grant CA20114, part of the work was done at the Comenius University in Bratislava and results were evaluated in cooperation with the University of Chemistry and Technology Prague

Keywords: *Candida glabrata*, antimycotic effects, CAPP, inoculation substrate

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CAPE synthetic derivatives as adjuvant anticancer therapy with cold atmospheric plasma

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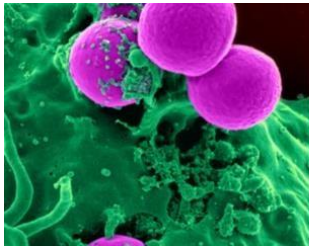
Caffeic acid phenethyl ester (CAPE), one of the main components of honeybee propolis, is reported to have broad-spectrum biological activities, including antibacterial and anticancer effects¹. Anyway, since it is reported that CAPE can be easily hydrolysed or metabolized either in vitro or in vivo², two different novel series of CAPE derivatives were designed and synthesized in our lab. The first series has an ester linkage, whereas the second series would contain an amide linkage, with different heterocyclic rings such as quinoline and isoquinoline, which are known to have both antimicrobial and anticancer effects³. The twelve synthesized compounds were administered in different concentrations (0-100 μ M) to breast (MCF-7), prostate (PC3) and oral cancer cell lines (HSC-2, CAL-27 and HOC-621) and two of them, namely MB10 and MB14, showed very promising IC₅₀, especially toward the HSC-2 cells.

Cold Atmospheric Plasmas (CAP) is a new promising therapeutic option for tumour treatment, being able to enhance the efficacy of traditional drugs. Anti-proliferative effects of the two selected compounds were investigated in combination with CAP in non-malignant oral cells (HGFs) and in the oral squamous cell carcinoma cells. Combined treatment with CAP for 30 and 60 sec at a distance of 12 mm enhanced the anti-proliferative effects with a slightly different sensitivity among the cell lines. In particular, 5 μ M MB14 combined with 30 sec CAP was the mildest treatment being effective in reducing CAL-27 and HOC-621 proliferation by 40% and 50%, respectively. More importantly, HGFs were unaffected by the combination of 30 sec CAP and 5 and 10 μ M MB14 showing selectivity for this approach.

Keywords: cold atmospheric plasma, CAPE, anticancer

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Effect of Cold Plasma and Liquids exposed to Cold plasma against Covid-19

Merve ŞENSÖZ TURGUT^a, Görkem KISMALI^b

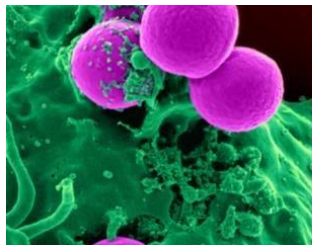
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With the outbreak of the Covid-19 pandemic, the importance of air decontamination has increased against respiratory viruses. The use of plasma has come to the fore in the evaluation of protection methods, with many studies showing that plasma neutralizes viruses in the air. However, Cold Atmospheric Plasma (CAP) applied to viruses doesn't cause a serious change in the DNA of viruses, but it inactivates their entry into the human body and provides decontamination in the environment¹. In a study conducted for this purpose, virus samples obtained from a patient with Covid-19 were inoculated into Vero E6 cells and after it was determined that it had a cytopathic effect on the cells, contamination was ensured on 6 different surfaces. Inactivation of Covid-19 with argon fed CAP was done in 180 seconds. On metal surfaces, this time is reduced to 30 seconds. On the surface of plastic, cardboard, football and basketball balls, this time was between 30-60 seconds. However, it was observed that the inactivation rate decreased as the surface roughness and absorbency of the surface increased². On the other hand, it is possible to use liquids such as water or saline solution exposed to CAP for a long time for virus inactivation. These liquids are known as Plasma Active Media (PAM). In addition, these liquids have the advantage of reaching body cavities that CAPs cannot reach. A study was conducted with PAM containing H₂O₂, which was produced to develop a preventive drug against Covid-19, and it was revealed that its effect is stronger at low pH and high concentration. In line with the studies, it has been suggested that supporting ventilation systems with CAP or PAM or solutions such as nasal and throat spray containing PAM can be an important decontamination method to protect humans and animals against diseases³.

Keywords: Cold Plasma, Covid-19, Corona virus, Plasma Activated Media

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P32

Plasma Processes on Metal Halide Perovskite Interfaces for Photovoltaic Applications

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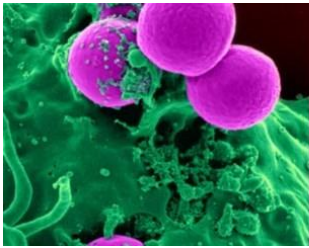
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Metal halide perovskite (MHPs) solar cells represent a promising newcomer in the front of emerging photovoltaic technologies to address the dramatic energy crisis and climate change that we are facing. The exceptional properties of MHPs derive by their hybrid organic-inorganic nature, which also allows a low-cost and straightforward fabrication process. Their structure can be described by the formula ABX_3 , where A is usually an organic cation, B is a metal cation and X is a halogen anion, typically I or Br . Solar cells containing MHPs as absorbing layer have already achieved a power conversion efficiency of about 25,7 %, close to the efficiency of silicon-based devices. Nevertheless, a major limitation is related to the controversial stability of the material when exposed to operative conditions, namely temperature, light and moisture, which still prevents the uptake of this technology. Therefore, to further improve the performances of these devices, many surface processes have been applied to solar cells interfaces, most of which include a solution-based methodology¹. The aim of these treatments is not only to improve the efficiency of solar cells in terms of carrier concentration and transport properties, but also to improve device stability under working conditions. Among the different surface treatments exploitable, the use of plasma represents a solvent-free and non-invasive promising strategy to boost MHP solar cells performances. Plasma-deposited coatings on perovskite, as fluorocarbon polymers, have shown to improve material resistance to humidity and photoluminescence properties². We have explored an innovative low-pressure plasma treatment applied to Methylammonium Lead Iodide surface. Different gases were tested and an interesting improvement in device performance (with a record efficiency of 17.08 %), was obtained for the 2 second Ar plasma-treated perovskite film. A similar improvement was obtained for H_2 plasma, object of current research. These preliminary results open interesting new opportunities in the use of plasmas for the optimization of perovskite-based devices and photocatalytic technologies.

Keywords: Metal Halide Perovskite, Plasma Treatment, Interface Engineering, Solar Cells

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P33

Use of direct application of plasma for therapeutic purposes

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In recent decades, there has been a significant shift in the field of cold plasma systems, which have a wide range of applications across disciplines, most notably in materials processing, surface treatment and biomedical applications^{1,2}.

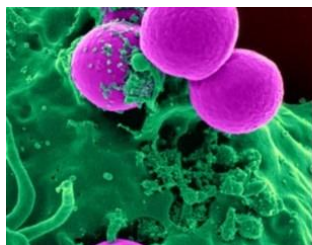
In this work, the antimicrobial effect on nosoclonal pathogens was investigated using microwave torch discharge with direct gas supply. *Staphylococcus epidermidis* and *Escherichia coli* were selected as representatives of nosoclonal pathogens. *Staphylococcus epidermidis*, one of the most widespread species found on human skin and mucous membranes, is able to easily colonise implanted medical devices and thus be dangerous to patients with compromised defence mechanisms³. *Escherichia coli* is found in the gut flora of humans and warm-blooded animals, but the evolution of the microorganism has led to the emergence of pathogenic strains that cause serious extraintestinal infections in humans and animals⁴.

At the beginning of the work, tests were performed on Petri dish in order to determine the parameters for the experiment. Then, we switched to a more complex inoculation substrate, namely pig skin, to simulate a real application on human skin. Two types of tests were performed namely application of plasma to porcine skin in a closed or open area. The results of this work confirm the efficacy of cold atmospheric-pressure plasma in inhibiting both bacteria on different surfaces and the higher efficacy of plasma in a closed space. Further experiments will be conducted in this area.

Keywords: *Escherichia coli*, *Staphylococcus epidermidis*, antimicrobial effects, inoculation substrate

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Cold atmospheric plasma processes to generate reactive oxygen and nitrogen species in water

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In the last years, cold atmospheric plasmas (CAPs) have exhibited a huge potential for biological and clinical applications ¹. The efficacy of CAP in these fields primarily derives from their ability to deliver gaseous blends of reactive oxygen and nitrogen species (RONS), well-known as redox modulators and oxidative stress inducers in redox biology ^{1,2}. Particularly, CAPs are emerging as a tool to enrich liquids with stable RONS (e.g., H₂O₂, NO₂⁻, NO₃⁻), to be incubated with cells in vitro or injected into tissues or organs in vivo ². The plasma treated water solutions (PTWS) obtained in this way revealed often as efficacious as the direct CAP applications for therapeutic purposes ^{3,4}. Atmospheric pressure dielectric barrier discharges (DBDs) can be applied to generate PTWS and the investigation of plasma sources' configuration as well as experimental conditions and the chemical composition of the liquid are of paramount importance to vary the dose of so produced RONS ²⁻⁵.

This research work is a case study in which the RONS generation (i.e., H₂O₂ and NO₂⁻) in water exposed to two different DBD plasma sources (Figure 1) is compared ⁶. Specifically, a plasma jet-DBD and a planar-DBD have been used. The obtained results demonstrate that the reactor configuration, the treatment modality, and the experimental conditions strongly impact the generation of reactive species.

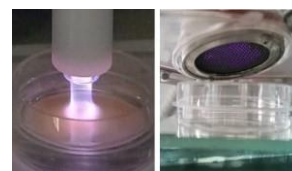


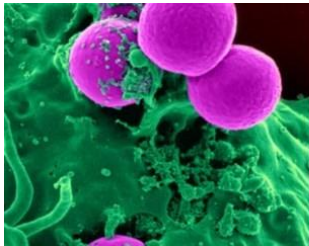
Figure 1. Photographs of ignited discharges.

Acknowledgements: The POR Puglia 2014/2020 –FSE/2019 “Research for Innovation (REFIN) D9C5DF93, and the Horizon Europe Seeds “INTERGLIO” projects of the University of Bari are acknowledged for partially funding this research.

Keywords: atmospheric pressure plasma, plasma medicine, plasma–liquid interactions.

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P35

Plasma activation of Fe_xO_y/NPs-loaded biomaterials for potential use in regenerative medicine applications

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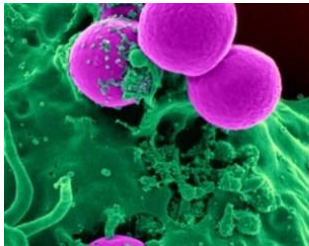
Low-temperature atmospheric pressure plasma, due to its rich properties, has found wide application in numerous fields [1]. Due to its ability to generate different reactive oxygen species (ROS) and reactive nitrogen species (RNS), cold plasma has also found purpose in medicine and healthcare [2, 3]. The primary idea of this study was to evaluate the effect of the cooperative action of cold plasma and fabricated bone scaffolds on the proliferation and osteogenic differentiation of human mesenchymal stem cells isolated from adipose tissue (ADSCs). Cells were seeded onto biomaterials modified with mesoporous silica nanoparticles (NPs) or Fe_xO_y/NPs catalysts. Study hypothesized that incorporation of catalysts into the biomaterial's structure would enhance plasma-induced reactive oxygen and nitrogen species (RONS) formation, which will have biological effects on living cells. Results showed that a short 16-second nitrogen plasma treatment of stem cells cultured on Fe_xO_y/NPs decorated biomaterial accelerates their proliferation. Additionally, plasma had stimulatory effect on osteocalcin production by ADSCs cultured on the material containing only NPs. The results obtained indicate that plasma-activated Fe_xO_y/NPs-enriched biomaterial show increased biocompatibility, signaling its high potential for use in regenerative medicine to accelerate bone regeneration.

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Keywords: biomaterials, osteogenic differentiation, bone regeneration, proliferation, regenerative medicine

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The effect of biomaterial treatment with different cold plasma reactors on its surface chemistry and biological properties

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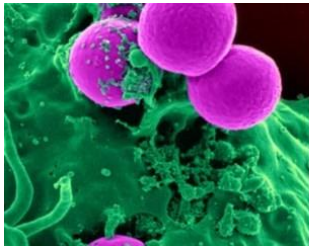
Methods of biomaterials surface modification with the use of plasma treatment are currently becoming more and more popular in regenerative medicine^{1,2}. Due to its effectiveness and economy, this technique has been used to improve the mechanical properties, morphology and surface energy as well as the biocompatibility of materials, especially those with complicated shapes³. The aim of the research was to enhance cell adhesion on the biomaterial by surface modification using cold plasma treatments. The biopolymer-based biomaterial, made of agarose and curdlan, was treated with plasma generated by three reactors: dielectric barrier discharge (DBD) plasma jet, gliding arc discharge (GAD) reactor, DBD surface reactor. The effect of plasma treatment on the chemical properties of the surface of the samples was investigated using ATR-FTIR analysis. Next, cell adhesion to the surface of biomaterials was examined using the BJ cell line (normal human skin fibroblasts). The results of the study showed that the plasma treatment could possibly have created new functional groups on the surface of the samples. Nevertheless, no increase in cell adhesion to the surface of the biomaterial was observed. Based on the obtained results, it can be assumed that the application of plasma treatment generated by the tested reactors: DBD plasma jet, GAD reactor and DBD surface reactor did not result in the expected modification of the surface of biomaterials and did not affect its biocompatibility⁴.

Acknowledgements: This research was funded by National Science Centre (NCN) in Poland within OPUS 16 grant no. UMO-2018/31/B/ST8/00945.

Keywords: biomaterial, plasma, cell adhesion, biocompatibility,

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Elimination of Green Moulds Using Cold Atmospheric Plasma and PAW

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A green spoilage usually found on lemon zest is caused by a filamentous fungi *Penicillium digitatum*. This progressively spreading mould is one of the biggest post-harvest pathogens of citrus fruits, resulting in high economic losses. Commercial chemical fungicides used for sterilization lead to environmental and health issues and to antifungal resistance as well¹. Thus, searching for suitable and sustainable alternatives to provide food safety and to extend the shelf life of citrus fruits is of a great importance.

Cold atmospheric plasma (CAP) with its well-known antimicrobial properties is considered as a novel potential “greener” technology for fungi inactivation. Although the eukaryotic fungi are more resistant to antimicrobial agents than prokaryotic bacteria, it was shown, that CAPs act on multiple levels in fungal inactivation by causing permeabilization of the cell wall leading to efflux of cytoplasm and leakage of organelles, DNA damage of spores or disruption of mycotoxins². It was confirmed that ozone and UV radiation are not dominant plasma components responsible for the inactivation of *P. digitatum*, contrary to reactive oxygen species (O, OH, O₂)³. Oxygen radicals decreased number of *P. digitatum* spores by oxidative degradation of cell wall and organelles⁴. However, the mechanisms of CAP in fungal inactivation have not been described in detail yet.

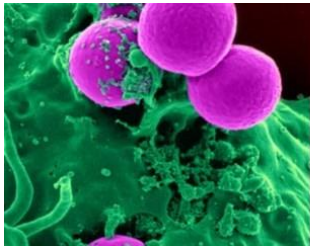
Transient spark (TS) discharge was used for inactivation of spores of *P. digitatum* in this work. TS discharge was applied in two ways: 1) for a direct treatment of spore suspension and 2) for water treatment, PAW was subsequently incubated with spores or applied on infected lemon zest. PAW was analysed for its physical-chemical properties (pH, conductivity, red-ox potential and reactive oxygen and nitrogen species content, i.e., concentration of H₂O₂, NO₂⁻, and NO₃⁻). Overall, we analysed direct plasma treatment, post-treatment delay effect and indirect effect of plasma by PAW on fungal spores.

This work was supported by Slovak Research and Development Agency grant APVV-17-0382 and APVV-20-0566.

Keywords: cold atmospheric plasma, plasma activated water, *P. digitatum*, inactivation

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Study of Plasma Activated Water and its Applications (Pathogen Inactivation, Food Preservation, Agriculture, Aquaculture)

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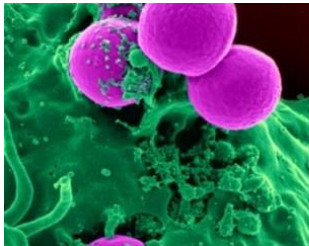
The present work discusses design and development of multiple plasma device setup to produce plasma-activated water (PAW) on a large scale with low plasma discharge power^{1,2,3}. The optimization of plasma device and process parameters of PAW generation is the main highlight of present work^{4,5}. PAW is characterized by measuring the physicochemical properties (pH, oxidizing potential, electrical conductivity, etc.) and reactive species concentration (nitrate and nitrite ions, hydrogen peroxide, dissolved ozone). PAW is used as a disinfection solution for inactive pathogenic microbes (bacteria, fungi, etc.)^{6,7}. The results clearly indicate the long-term effectiveness (~ 4-weeks) of PAW and it has been observed that stored PAW is able to inactivate pathogenic microbes ($> 6 \log_{10}$ CFU ml⁻¹) with significantly low treatment time (~10 s)⁶. The damaged morphology and compromise in the membrane integrity of pathogens showed the PAW microbial inactivation mechanism^{6,7}. The current work also shows PAW treatment enhances shelf life of citrus limon L. (lemon) ($6+ \log_{10}$ CFU ml⁻¹ reduction in lemon spoilage fungi)⁷. Moreover, the lemons washed with PAW showed lower weight loss, lesser spoilage, better texture, better sensory (smell, appearance, taste), and color (L, a, b) evaluation compared to the control. At last, agriculture and aquaculture applications of PAW have been reported^{8,9}. The PAW-treated seeds have higher germination and plant growth and better agronomic traits compared to control⁸. This is due to PAW treatment with seeds removes the hydrophobic wax and enhances the rate of water absorption. In addition, it is also a rich source of nitrogen, hence, can be used as a nitrogen replacement for various agriculture and aquaculture applications as shown by the results of pea plants and freshwater green chlorella algae^{8,9}. In conclusion, PAW has the affinity to be used in numerous applications such as healthcare, food preservation, agriculture, aquaculture, etc.

Acknowledgements: Department of atomic energy doctorate fellowship for pursuing Ph.D.

Keywords: Plasma Activated Water, Pathogen Inactivation, Agriculture and Aquaculture, Food

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COLD ATMOSPHERIC PLASMA INDIRECT TREATMENT OF ORAL CAVITY SQUAMOUS CARCINOMA CELL LINE HSC-2

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Plasma medicine investigates cold atmospheric plasma (CAP) as an innovative non-invasive anti-cancer solution due to the possibility of activating liquids – plasma-activated media (PAM) - through the generation of a mixture of reactive oxygen and nitrogen species (RONS), that can be applied to a biological target¹. HNSCC have a low response to conventional therapies, presenting a 5-year survival rate of about 60%^{2,3}. Therefore, this work aims at studying the anti-tumoral effects of different PAMs on an oral cavity squamous carcinoma cell line, namely HSC-2.

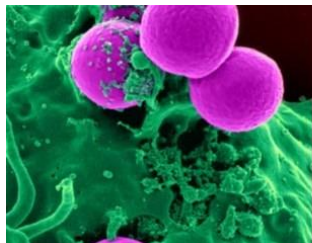
PAM was obtained using a plasma jet with air gas, provided by PBRC. Two volumes of culture media - 2 mL and 5 mL - were treated by CAP for 5, 10, and 20 min at a 6 mm working distance. HSC-2 viability was assessed by MTS assay at 24, 48, and 72h, and apoptosis induction was analyzed by flow cytometry at 24 h. HSC-2, incubated with 2 mL PAM treated for both 5 and 10 showed a vitality reduction of 75%, and 96%, respectively. While the treatment with 5 mL PAM showed less marked effects in all the examined conditions. Flow cytometry analysis revealed that all the generated PAMs induced apoptosis (early and late) and that the treatment with 2 mL PAM for 5 min appeared the most effective, while after the treatment with 5 mL PAM the cellular response, although milder, would seem related to PAM times of production. These preliminary results may open a new opportunity for an innovative approach to the management of HNSCC in the future.

Acknowledgments: This work was funded by The European Union NextGenerationEU and National Recovery and Resilience Plan (NRRP) and by the National Research Foundation (NRF) of Korea.

Keywords: cold atmospheric plasma (CAP), head and neck squamous cell carcinoma (HNSCC), plasma-activated medium (PAM), anti-cancer therapy.

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The influence of cold plasma on the stabilizing and flocculating properties of a colloidal suspensions containing sodium salt of carboxymethyl cellulose and glauconite

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The influence of cold plasma (CPT) on the stabilizing, adsorptive and electrokinetic properties of the sodium salt of carboxymethyl cellulose/glauconite system was investigated.

As for the influence of CPT on the properties of polymers, it has been shown that CPT can be used as an effective method to improve the dissolving capacity of polymers by changing its structure and increasing the oxygen containing functional groups on the polymer surface¹. Plasma is known to charge organic surfaces negatively². However, the influence of CPT on the stabilization and adsorption properties of colloidal suspensions has not been studied so far, and this factor can ensure significant colloidal stability of polymer/solid suspensions, therefore research in this direction is very promising.

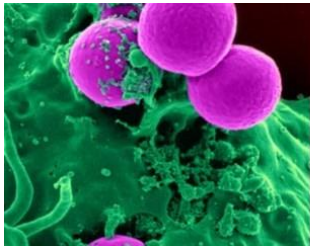
In the experimental part, the following methods were used: UV-Vis spectrophotometry, FT-IR, XPS, SEM HRTEM and STEM-EDS spectrophotometry. The data obtained show that cold plasma causes changes in the composition of the surface and chemical bonds in both NaCMC macromolecules and GT particles - both methods detected the incorporation of functional groups related to oxygen³. CPT protonates the sodium carboxyl group and cross-links the polymer, while the GT sample has a smaller surface area and pore volume, but its surface is more oxidized⁴. It was also noted that CPT significantly improved the stability of the GT/NaCMC system and the adsorption of NaCMC on the GT surface.

The obtained data have application potential, especially in the cosmetics industry, and can be used to determine the colloidal stability of polymer/solid colloidal systems, which opens up new opportunities for the chemical industry; especially in the preparation of new functionalized materials.

Keywords: sodium salt of carboxymethyl cellulose; glauconite; stability; cold plasma

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The anti-tumoral effects of plasma activated media (PAM) on human tongue squamous carcinoma cell line HSC-3

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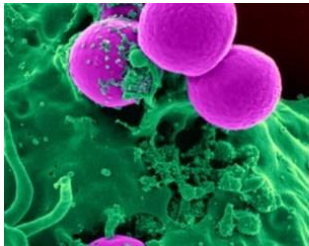
The effectiveness of cold atmospheric plasma (CAP) as an anti-cancer therapy has been reported in human cancers¹; however its use is limited by the accessibility of the tumor. Thus, its nature renders it suitable to the treatment of primary tumors arising from skin or mucosal surfaces, such as head and neck squamous cell cancer (HNSCC). EGFR, a receptor of the ErbB family, plays a key role in tumorigenesis and is overexpressed in 80–90% of HNSCCs. Cetuximab, a monoclonal antibody against EGFR, is the only approved targeted therapy for HNSCC, but for which resistance has been demonstrated. Thus, the present work aimed at investigating the anti-tumoral effects of different plasma activated media (PAM) on HSC-3 human tongue carcinoma cell line. PAM was obtained using a Jet plasma with air gas, provided by Kaushik NK. The working distance between the capillary of the device and the liquid medium surface was fixed at 60mm and the volumes of liquid activated with CAP (5, 10, 20 min) were 2ml and 5ml. Cell viability was analysed by MTS assay at 24, 48 and 72h and apoptosis induction by flow cytometry (FCM) at 24h. EGFR expression and activation was evaluated by Western Blotting assay (WB). MTS assay revealed that all the PAM treatments remarkably reduced cell viability in HSC-3 cells in a range from about 90 to 10%. FCM analysis showed that PAM treatments induced apoptosis, and specifically, the late apoptotic response was enhanced in a PAM production time-dependent fashion and was higher with 5ml PAM at 20 min of treatment. EGFR was poorly expressed by HSC-3 cells. Cetuximab alone was not able to reduce cell viability compared to untreated cells. However, in the cells treated with the combination of 100µg/ml Cetuximab and 5ml PAM obtained after 5 min of irradiation, the cell viability was inhibited by ~30% compared to untreated cells. These preliminary results highlight that CAP is a promising treatment for HNSCC and that its combination with Cetuximab should be further explored on other cancer and normal cell lines in order to evaluate a possible selective effect.

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Keywords: cold atmospheric plasma (CAP), head and neck squamous cell carcinoma (HNSCC), epidermal growth factor receptor (EGFR), anti-cancer therapy.

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Direct and indirect effects of atmospheric pressure plasma on complex structures

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Research in plasma physics has established various applications of plasma sources in the medical field in terms of surface sterilisation, induced changes in protein structure, wound healing, blood coagulation.

The first objective of the thesis is of potential interest in the field of radiotherapy, as in the future the use of amino acids subjected to atmospheric pressure plasma treatment as targeted therapy is envisaged. The aim is to alter the metabolism of cancer cells.

The effects of plasma on protein structure and function can be compared for different parameters such as treatment time, temperature and degree of plasma ionisation.¹ The results obtained in this test phase will be compared with those already existing in the literature.

The use of plasma sources for indirect applications in life sciences forms a reactive environment at the interface with the biological medium.² Exposure of biological samples to the plasma source, e.g. He plasma jet, driven in high voltage pulses (of the order of KV) will result in the formation of free radicals (OH, O⁻, N₂⁺).³

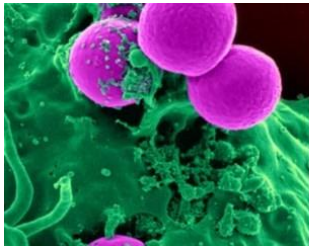
In this stage of the thesis I test a typical experimental setup to determine some quantities for plasma samples. The experimental device consists of a pump, a syringe, a cuvette and a light source. The whole device is coupled to a computer. With the help of a program called PUMPTERM, droplets of certain volumes are generated for which the surface tension coefficient for the falling liquid droplets is currently determined. This data in the form of images is processed in another program which calculates the parameters according to the droplet contour.

In this research program, I propose to follow the behaviour of liquids by exposing them to the action of plasma sources at atmospheric pressure and determining macroscopic parameters such as the surface tension coefficient. I also aim to gain insight into the effects of plasma action on proteins.

Keywords: *plasma, protein, treatment time, temperature*

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Plasma-activated water as an oral sterilization product

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In recent years, plasma applications have attracted attention as an innovative technology in the field of dentistry. Especially, the usability of plasma activated liquids (PALs) in oral sterilization is promising. PALs efficiently inactivate bacteria and bacteriophages by damaging biological macromolecules. Guo et al reported that in the experimental model using SARS-CoV-2 S protein pseudoviruses, plasma activated water (PAW) effectively inhibited pseudovirus infection through S protein inactivation¹. Shen et al. reported that PAW preserved its bactericidal activity on Saureus and its physicochemical properties for up to 30 days².

In this work, PAW was obtained by using argon plasma at different times. As the control group, Listerine, which is used as mouthwash in the market, was preferred. Oral culture was propagated in glass tubes in an incubator at 37 °C in Luria Bertani (LB) growth medium. At the end of the incubation, microorganisms were cultivated at a concentration of 10⁶ cell/mL for each group. Each group was treated with PAW or Listerine for 1 min. The process steps are shown in the Figure 1.

Depending on the time, variable amounts of RONS such as H₂O₂, NO₂ and NO₃ were detected in the PAW. The oral culture was reduced to less than 20 colonies at the end of the PAL treatment period. Anti-bacterial effectiveness rate was determined as 67%.

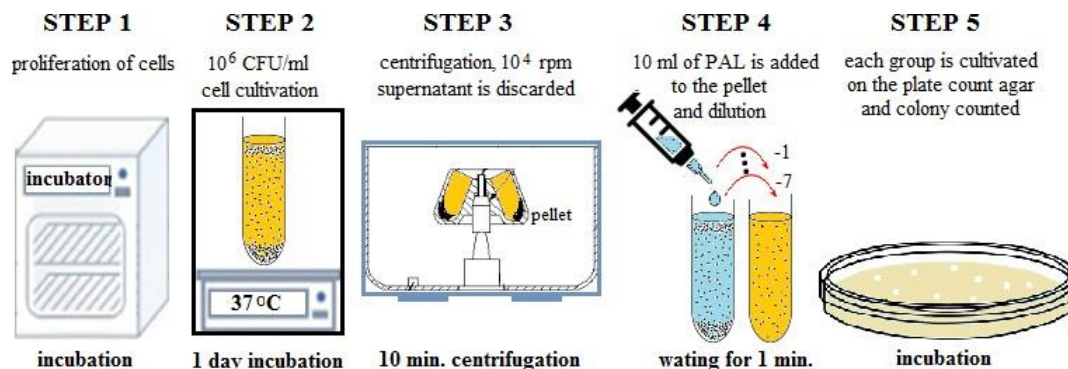
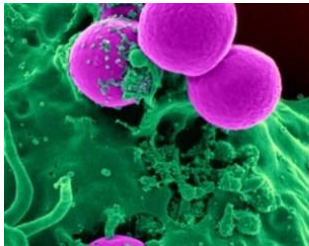


Figure 1. Steps of process

Keywords: plasma activated water, anti-bacterial effect, mouthwash,

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Inactivation effects on *Aspergillus flavus* and *Aspergillus parasiticus* on Peanut seeds by Cold Plasma

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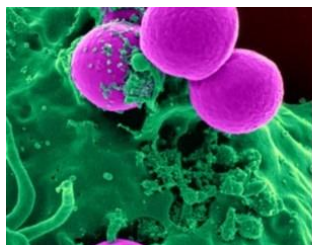
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Peanut (*Arachis hypogaea* L.) is an economically crucial oilseed crop in the world due to its high production ¹ and is a major source of income ². However, it is susceptible to pre and post-harvest aflatoxin infection caused by *Aspergillus flavus* and *Aspergillus parasiticus* ³ and subsequently severe food safety issues in the globe ⁴. *Aspergillus* species are the most common toxigenic species in various grains, legumes, oilseeds, foods and feed which are found throughout the world, being present in both the soil and the air. These species produce secondary metabolites which are aflatoxins infecting numerous food and feed crops, affectation serious health threats for both humans and animals ⁵. Therefore, eradication of fungus before the toxins are formed must be the real objective rather than the elimination of aflatoxins once produced. It is, therefore, of pronounced attention to develop novel, practical, and cost-effective post-harvest approaches to reduce or if likely eliminate fungus before aflatoxins are formed ⁶. Deactivation of fungi by cold plasma leftovers a topic of high attention to the food industry. Therefore, inactivation of aflatoxins producing *Aspergillus parasiticus* and *Aspergillus flavus* in peanut was studied using cold plasma.

Keywords: *Aspergillus* Species, aflatoxin, atmospheric cold plasma

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Atmospheric plasma for water reuse – PlasWARE

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According to the WHO, nearly one billion people in the world do not have access to drinking water and more than 500 million people die every year due to the intake of water contaminated by pollutants and pathogenic microorganisms.¹ Therefore, both a sustainable use of natural resources and the development of methods for treating contaminated water for its purification and possible reuse are necessary. The class of contaminants that raises the greatest concern for possible adverse effects on the environment and on humans is that of Persistent Organic Pollutants (POPs), which includes Per- and Polyfluoroalkyl substances (PFAS) since 2001.²

Due to their high chemical stability and to their solubility in water, PFAS are resistant to natural degradation mechanisms and have widely spread throughout aquatic environments and soil; moreover, they are able to bioaccumulate in animal and human organisms, causing great concern for human health. In addition to being persistent in the environment, these compounds are resistant to most conventional water treatment systems.³ It is therefore necessary to develop alternative methods of degradation, based on advanced oxidation and reduction processes that are eco-sustainable and effective against this class of contaminants. Among the technologies being studied and developed, atmospheric plasma is particularly promising.⁴⁻⁶

This PhD project aims to contribute to the development of an atmospheric plasma system for the removal of PFAS from water to decontaminate water resources, with the objective of reusing them for irrigation. The aim is to study the mechanism of degradation of PFAS induced by non-thermal plasma as a function of the characteristics of the plasma and the aqueous matrix, to optimize the degradation process in terms of kinetics and yield of mineralization and promote its application. A Radial Discharge Plasma (RAP) reactor is used to treat solutions prepared in tap water at different initial concentrations and real samples of water contaminated by PFAS; moreover, the characterization of atmospheric plasma is conducted through the analysis of reactive species with spectroscopic techniques and with appropriate molecular probes.

Keywords: *Non-thermal plasma, radial-discharge plasma, PFAS, plasma treatment.*

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