PlasTHER

THERAPEUTICAL APPLICATIONS OF COLD PLASMAS

1st Training School 2022
Fundamental Aspects of Plasma Medicine

February 14th-16th, 2022

Hybrid Edition

EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

NOVA School of Science and Technology
Caparica, Portugal
COST Action CA20114

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PREFACE

This book contains the final scientific program and the abstracts of the pitch presentations of the 1st Training School “Fundamental Aspects on Plasma Medicine” that occurred within the scope of COST Action CA20114 “Therapeutical applications of Cold Plasmas” – PlasTHER and was held as a hybrid event from the 14th to 16th of February 2022 at Nova School of Science and Technology, FCT NOVA, Caparica, Lisbon, Portugal.

COST Action CA20114 has the main aim and objective of exploit the possibilities of atmospheric pressure plasmas in medicine to share, develop and consolidate suitable therapies.

The main focus of the training school “Fundamental aspects on Plasma Medicine” was to present both basic level and state-of-the art in cold atmospheric pressure plasmas and plasma treated liquids in the field of medical applications. The idea was to establish a structured link between theoretical and practical interdisciplinary knowledge allowing participants to bridge the gap between the theory and experiments and also to promote interlink between research fields.

Target Audience was PhD students and early career investigators with background knowledge in plasmas, medicine, chemistry, biology, physics, engineering etc. An outstanding line up of invited speakers from different scientific fields have been assembled to present the topics related to the applications of plasma in medicine and for medical therapies. The main idea of the talks was to present the foundations and overview of the related topics to the school participants that can benefit also from the lectures which are not in their field of research. Participants also presented their work in the format of pitch session (online and onsite poster) to share their research topic and practice scientific presentations in public. Special care towards gender equality and students from Inclusiveness Target Countries (ITC) was also considered during the selection process.

This type of events are also meeting places where collaboration projects can emerge from social contacts amongst the participants. Therefore, in order to promote the development of research networks the 1st Training School included in its program Social activities & Sunset in the afternoon of 15th February 2022.

This Training school was sponsored by the COST (European Cooperation in Science and Technology).

We would like to express our thanks to all participants, all members of the Scientific Committee, and to the secretariat. The Organizing Committee also acknowledges to the Dean (Professor Virgílio Machado), Physics Department Director (Professor José Paulo Santos), Events and Special Projects Division, e.learning Office, General Support Division and Division of Communication and External Relations from NOVA School of Science and Technology.

Susana Sério
Chair of the 1st Training School

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SCIENTIFIC PROGRAM

	Monday 14th February	Tuesday 15th February	Wednesday 16th February
8:45	**Opening**		
9:00-10:30	Introduction to Nonthermal Plasmas ***Vasco Guerra***	Basics of cell biology ***Lars Boeckmann***	Plasma for antimicrobial applications ***Paula Bourke***
10:30-11:00	Coffee break	Coffee break	Coffee break
11:00-12:30	Nonthermal Plasma sources ***Joanna Pawlat***	Plasma-cell interactions ***Angela Privat-Maldonado***	Plasmas for Wound healing and tissue regeneration/repair ***Eric Robert***
12:30-13:00	Student short presentations	Student short presentations	Student short presentations
13:00-14:00	Lunch break	Lunch break	Lunch break
14:00-15:30	Plasma-driven liquid chemistry ***Kristian Wende***	Social activities	Plasmas for Cancer ***Nofel Merbahi***
15:30-16:00	Coffee break		Coffee break
16:00-17:00	Good practices in performing and reporting NTP experiments: how to write a paper ***Yury Gorbanev***		Regulatory and Clinical translation of plasmas ***Sarah Cousty***
17:00-17:30	Patents – ***IRIS office NOVA.id.FCT | FCT NOVA***		Student short presentations
17:30-18:00	Student short presentations		Closing session
18:00-20:00	Free time		Free time
20:00	Dinner		Dinner

TRAINERS - SHORT BIOGRAPHIES

Vasco Guerra

Vasco Guerra is Associate Professor at the Department of Physics of Instituto Superior Técnico (IST) and Head of the modelling and simulation activities of the N-PRiME group of Instituto de Plasmas e Fusão Nuclear (IPFN). He is graduated in Engineering Physics at IST in 1991 and received the PhD degree in Physics from Universidade Técnica de Lisboa (IST) in 1998. He held a visiting professor grant from the Royal Netherlands Academy of Arts and Science in 2020 and was invited professor at Sorbonne Université in 2016.
His research focuses on the modelling of non-equilibrium kinetics of low-temperature molecular plasmas. He is currently involved in the study of the fundamentals and mechanisms of CO_2 plasmas interacting with surfaces, for applications on Earth and on Mars. In 2016, he was awarded the William Crookes Plasma Prize, co-sponsored by the European Physical Society and the Institute of Physics Publishing, “for the outstanding contribution to the modeling of molecular low-temperature plasmas.

Joanna Pawłat

D. Sci. Eng. Joanna Pawłat is professor at Lublin University of Technology, Faculty of Electrical Engineering and Computer Science (Poland). She received doctoral degree in Energy and Material Science from Saga University in 2001. During 12 years of her stay in Japan she was awarded with Japanese Government scholarship and Japan Society for the Promotion of Science fellowship. She conducted research in Hi-Tech Centre of Chuo University in Tokyo, Toyohashi Institute of Technology, Saga University and Waseda University. She received Maria Curie Reintegration Grant in 2010 for research on plasma sterilization potential.
J. Pawłat is a member of Committee on Metrology and Research Equipment of Polish Academy of Sciences, Committee of Plasma Division (PAS Lublin) and senior member of IEEE. Her professional interests are focused on plasma application for food, agriculture, medicine, energy and environment; plasma devices innovations and development of plasma generators; techniques of oxidants’ generation; surface engineering and material science; interdisciplinary research for the crops intensification and food treatment.
Her plasma team was awarded with medals at 47th and 49th International Exhibitions of Inventions in Geneva and at International Warsaw Invention Shows in 2016 and 2019, respectively. She is an author of more than 230 scientific publications and 31 patents.

Kristian Wende

Kristian Wende joined the Leibniz Institute for Plasma Science and Technology (INP) in 2010, at the Centre for Innovation Competence (ZIK) *plasmatis* as a postdoctoral researcher. Currently, he is a group leader heading the junior research group Plasma-Liquid Interactions at ZIK *plasmatis*.
Kristian Wende was born in Berlin, Germany, in 1973. He received the pharmacist diploma from the University of Greifswald, Germany, in 1998 and the Doctorate Degree of Pharmaceutical Biology in 2003 from the same university.
His research is located in the redox biochemistry field and focuses the biochemical impact of reactive species on biomolecules. A special emphasis is given to the plasma-derived reactive species and their impact on small metabolites, proteins, and lipids to achieve knowledge on transport processes, life times, and reactivity. A further interest extent to plasma-aided synthesis. Chromatographic systems and mass spectrometry are the central analytical tools in his work.

Yury Gorbanev

Yury Gorbanev graduated from the Rostov State University (now – Southern Federal University) in Rostov-on-Don, Russia in 2006, with a specialisation in green chemistry and chemistry of natural products. In 2012, he obtained a PhD degree in catalysis and sustainable chemistry from the Technical University of Denmark (Copenhagen, Denmark). Since 2013, he has been working as a post- doctoral research fellow in the non-equilibrium plasma field. In 2013-2016, while employed at the University of York, United Kingdom, he performed research on biomedical plasma jets in contact with liquids, which yielded several publications becoming state-of-the-art and receiving various prizes. In 2017, he joined Research Group PLASMANT at the University of Antwerp, Belgium, where he was awarded the Marie Curie research fellowship. In addition to biomedical plasma-liquid systems, his present-day work also encompasses plasma in polymer industry, and plasma for nitrogen fixation – both as a stand-alone green technology, and in combination with catalysis. His current activities involve doing research in plasma-enabled gas conversion, plasma catalysis, and plasma-liquid interactions, and supervision of PhD and undergraduate students.

Pedro Almeida

Pedro Almeida is a team member of IRIS (Innovation, Research & Impact Strategy Office) at FCT NOVA. IRIS mission is to offer strategical and integrated support in all key areas of innovation management to make a reality the valorisation and commercialization of Academia-based technologies.
IRIS provides strategical support to the entire research & innovation cycle of Academia ecosystem, from the idea to its market application, fostering sustainable knowledge generation to increase the societal and economic impact of our customer's R&D.
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Since its inception, IRIS has acquired a strong and large expertise in knowledge valorization of technologies developed in R&D projects. Particularly, the Department of Intellectual Property and Technology Transfer performs state-of-the-art search in patents databases, definition of Intellectual property protection strategy; technology assessment; market search and definition of best market fit; Product fit and customer development; definition of Go-to-Market strategy; definition of business model plan based on the tool Business Model Canvas and partner search and negotiation of contracts.
IRIS supports the valorisation activities of 44 technologies and manages a patent portfolio of 149 active national and international patents and patent applications. It has mediated the execution of 579 partnerships with Industry from which 128 are R&D collaborations with Industry and 11 of Technology Licensing. Additionally, IRIS has been involved in the incorporation 5 spin-offs.
IRIS highly trained team currently counts with seven members with high expertise in different but complementary fields. The Department of Intellectual Property and Technology Transfer counts with two members trained at international and national public and private institutions, including MIT-PT program and The SearchLite Innovators-In-Residence program, USA, complemented with training in Intellectual Property and Technology Transfer from INPI, EPO and IPR Helpdesk.

Lars Boeckmann

Dr. Lars Boeckmann studied biology in Göttingen and received his Ph.D. degree as a fellow of a DFG Graduate School at the University Medical Center Göttingen. Subsequently, he worked as a postdoctoral fellow at the renowned National Cancer Institute in the USA before becoming a group leader at the University of Lübeck. Since 2018 he is head of the research laboratory of the Clinic and Policlinic for Dermatology and Venereology in Rostock. His group conducts basic and translational research in the fields of plasma medicine, dermato-oncology, and rare genetic skin diseases. Dr. Boeckmann is a board member of the German National Center for Plasma Medicine and a member of the Management Committee of the COST Action CA20114 "Therapeutical Applications of Cold Plasmas" (PlasTHER).

Angela Privat Maldonado

Angela Privat Maldonado studied biology at Cayetano Heredia Peruvian University in Lima, Peru. She studied the tropical infectious disease Leishmaniasis in Peru for 5 years, focusing on protein modifications and developing a patented serological diagnostic kit. Her work motivated her to search for novel therapies that could be moved from the researchers' bench to the patient’s bedside. In 2011, she won a Wellcome Trust scholarship to do an interdisciplinary PhD at the University of York, UK. Her research focused on the application of low temperature plasmas for bacteria inactivation. In 2017, she joined the PLASMANT group at the University of Antwerp as a post-doc, investigating the response of tumour cells to plasma. Working in collaboration with the CORE group at University of Antwerp, her research involves the use of biomedical plasmas, 3-dimensional models for cancer research, and determining the role of the tumour microenvironment in the response to treatment.

Paula Bourke

Paula Bourke is a Professor in the UCD School of Biosystems and Food Engineering, a Conway Institute Research Fellow and a member of the UCD Institute of Food and Health. She graduated from the University of limerick in 2001 with a PhD in microbiological safety of novel non thermal processing technologies. During her time as a post-doctoral researcher at University of Limerick and as an Arnold Graves Research Fellow at DIT, she researched both thermal and non-thermal processing and their interactions with food borne pathogens and resistance mechanisms.

Her research is primarily in the areas of sustainable processing innovations and novel antimicrobial technologies. She has a strong interest and research cross over to bio-medically relevant issues including infection prevention and control and Biofilms. She collaborates widely and has been successful in gaining funding awards at a national and international level. Her research group works on a range of cold plasma bioscience and sustainable food processing projects. Current research is supported through SFI Investigator, SFI-UKRI/BBSRC and SFI US-Ireland Tripartite awards, Department of Agriculture Food and Marine FIRM, Irish Research Council, the Royal Irish Academy as well as Enterprise Ireland awards. She was designated as a Highly Cited Researcher by Clarivate Web of Science based on her rankings within the top 1% highly cited researchers. She is a member of the recently initiated EU COST network in Plasma Applications for smart and sustainable agriculture, and the COST network in device associated infection iPROMEDAI.

Eric Robert

Eric ROBERT is CNRS senior Scientist at GREMI laboratory, Orléans, France. He has been involved in the development, diagnostics and applications of gas discharge plasmas for light source, microelectronics, X-rays diagnostics, and from ten years in the biomedical technologies.
His recent publications concern the physics of plasma jets, the antitumor action of atmospheric pressure cold plasma jets, the combination of plasma treatment with electrochimiotherapy, the use of plasmas for skin treatment in anti-aging strategies.
He is deputy director of GREMI laboratory, in charge of the "plasma for biomedical applications" team, and director of the French network "HAPPYBIO" merging forty teams connected with the researches on the use of plasmas, pulsed electric field and dynamic phototherapy for biology. He is board member of the International Society for Plasma Medicine and of the International Plasma Chemistry Society.

Nofel Merbahi

Nofel Merbahi was born in Marrakech, Morocco, in 1978. He received the Ph.D. degree in plasma electrical engineering at University Paul Sabatier (UPS), Toulouse, France, in 2004.,He is Teacher at UPS and Researcher at LAPLACE, Toulouse, in “Non equilibrium reactive plasmas” team where his is strongly involved in the design and the experimental diagnostics of atmospheric discharges (corona and dielectric barrier discharges) applied to gas pollution control, UV emission, surface treatment, and biological decontamination.

Sarah Cousty

Sarah Cousty, DDS, PhD, is an oral surgeon, Head of the Department of Oral Medicine and Oral Surgery in Paul Sabatier University and Toulouse Hospital. She completed a PhD in Electrical Engineering, Electrotechnical studies Telecommunications and Plasmas in 2007. Since then, her research has focused on the biomedical applications of gaseous plasmas, and she is a researcher at LAPLACE (Laboratory on Plasma and Conversion of Energy). She started her research in « plasma medicine » in the theme of sterilization. Now, her interests are focused on therapeutical applications of cold plasmas, for instance in oral mucosa pathology.

ONLINE PITCH PRESENTATIONS

Monday 14th February

Session 1 - 12:30-13:00

Order	Name	Country
1	Giulia Laghi	Italy
2	Thomas Bonnfont	France
3	Asaftei Mihaela	Romania
4	Kristína Trebulová	Czech republic
5	Jovana Petković	Netherlands
6	Gerber Ioana Cristina	Romania
7	Nishtha Gaur	United Kingdom

Session 2 - 17:30-18:00

Order	Name	Country
1	David Sheehan	Ireland
2	Leonardo Zampieri	Italy
3	Beatrice Olayiwola	Ireland
4	Mariachiara Grande	Italy
5	silvia giuditta scaltriti	Italy
6	Mestre Eloïse	France

Tuesday 15th February

Session 3 - 12:30-13:00

Order	Name	Country
1	Slavomír Pásztor	Slovakia
2	Nurzahira Zabidi	Malaysia
3	Giacomo Pierotti	Italy
4	Augusto Stancampiano	France
5	Andreea Mariana Negrescu	Romania
6	Adelina Elena Popovici	Romania
7	SEVDE NURK UTLU	Turkey

Wednesday 16th February

Session 4 - 12:30-13:00

Order	Name	Country
1	Darina Truchlá	Slovakia
2	Zulaika Abdullah	Malaysia
3	LUC TRAN VAN	Romania
4	Alina Bisag	Italy
6	Andrei Vasile NASTUTA	Romania
7	Albert Espona Noguera	Spain

Session 5 - 17:00-17:30

Order	Name	Country
1	Vincenza Armenise	Italy
2	Katayoon Hadian Rasnani	Germany
3	Parisa Shali	Belgium
4	Beatriz Lopes	Ireland
5	Gebremedhin Gebremariam	Italy
6	Apoorva Kashyap	United Kingdom
7	Amaury Rouillard	France

ONLINE PITCH PRESENTATIONS ABSTRACTS

Atmospheric pressure plasma-assisted polymerization of fluorinated silane coatings for antimicrobial applications

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Trabecular titanium scaffolds whose structure imitates trabecular bone morphology are widely used in orthopedic implant applications, as they can greatly match osseointegration and mechanical properties [1]. Despite a generally positive response by the body towards these scaffolds, bacteria may adhere to their surface and produce biofilm, leading to the onset of the so-called nosocomial infections, which are nowadays considered a major health challenge in healthcare units [2]. In the described context, atmospheric pressure plasma-assisted polymerization is particularly appealing since it allows to obtain coatings with surface chemistry and wettability suitable to avoid bacteria adhesion and to reduce biofilm formation, without requiring expensive vacuum systems [3]. With these premises, together with my research group and the Italian Company IBMTech s.r.l., I worked on the polymerization of antimicrobial coatings starting from an atmospheric pressure plasma jet and an aerosolized fluorinated silane precursor. To properly study and control the plasma-assisted polymerization process, coatings were deposited on flat polyethylene substrates, which allow an easier surface characterization than the trabecular titanium ones, and the effects of varying different process parameters (i.e. discharge power and precursor flow rate) on coating properties were investigated. The chemical composition and the wettability of the coatings were assessed by means of Attenuated Total Reflectance – Fourier Transform Infrared spectroscopy, X-ray Photoelectron spectroscopy, and Water Contact Angle analysis. It was demonstrated that a tailored fluorine content in the coatings can be obtained by properly setting the process parameters: the lower is the degree of fragmentation of the precursor in the plasma discharge, the higher is the preservation in the coating of the functional groups of the starting precursor. Biological results showed that antiadhesive properties against *Staphylococcus aureus* and *Pseudomonas aeruginosa* can be achieved thanks to the presence of the fluorinated silane coatings. Considering these promising outcomes, we are currently working on the adaptability of the process on trabecular titanium scaffolds and on the optimization of the coating properties in terms of stability in biological environment and biocompatibility, which are fundamental aspects for the applicability in the biomedical field.

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Modeling of the electrical behavior of a biological cell exposed to an electric field pulse : a Discrete Dual Finite Volume (DDFV) method application

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Electroporation consists in applying a sufficient electrical field in order to modify (reversibly or irreversibly) the cell membrane permeability. This allows the transfer of non-permeable molecules in the cell, e.g. electro-chemotherapy [1]. In this context Nanosecond Pulse Electrical Fields – nsPEF is a promising technique in cancer treatment. New trends consist in ultra-short duration and intense electric field strength. A contactless technique consists in exposing a biological solution containing target cells into a guided Transverse ElectroMagnetic (TEM) structure [2].

We are actually interested in the trans-membrane voltage induced by nsPEF. Some analytical results, for spherical cells [3], and numerical results, through finite-element schemes [4], have already been obtained in 2D. Interpolations and refinements due to the thickness of the membrane are then required.

In our work, we propose a cell electrical model based on the metal-dielectric equivalence. This latter introduces a generalized Poisson equation with jump conditions for each time step. We introduce an adapt finite volumes method, the discrete dual finite volumes method (DDFV) [5], to solve this problem. This numerical scheme allows a rigorous model of discontinuity conditions which can be straightforwardly be extended in 3D. The thickness of the membrane is then solved by a limited number of mesh cells. To the author's knowledge, the metal-dielectric equivalence and the computation scheme have not been yet introduced in this context.

The presented results have been obtained with the model in axisymmetric 2D. An adaptation in order to take ions movement into account is in progress. Finally, some preliminary work on the 3D generalization of the scheme to solve the Poisson equation has recently been done. Future work will include this modelling of the electrical cell behaviour in a plasma exposition device.

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ZnO nanoparticles to modulate growth and biofilms in opportunistic bacteria

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Zinc oxide (ZnO) nanoparticles (NPs) have been studied for the development of next-generation nanoantibiotics against pathogenic microorganisms to combat multi-drug resistance [1,2]. These nanoparticles show unique physicochemical properties including morphology, particle size, crystallinity, and porosity [3]. Based on these characteristics, ZnO NPs have a wide spectrum of antimicrobial activity against microorganisms including *Escherichia coli, Staphylococcus aureus*, *Pseudomonas aeruginosa, Bacillus subtilis*, and the M13 bacteriophage.

The purpose of this study was to evaluate the antimicrobial activity of some newly designed ZnO NPs and reveal their potential synergisms with antibiotics.
We have investigated 2 model microbial species: one Gram positive (*Staphylococcus aureus*) and one Gram negative (*Pseudomonas aeruginosa*).
The results revealed that the analysed ZnO NPs show low minimum inhibitory concentrations (MICs) and possess synergic activity with gentamicin and ciprofloxacin.

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Impact of Cold Plasma Treatment on the yeast Candida Glabrata

K. Trebulová, F. Krčma, Z. Kozáková, P. Matoušková

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Recently, various cold plasma sources have been tested for their bactericidal and fungicidal effects with respect to their application in medicine and agriculture. In recent decades, a number of discovered pathogenic microorganisms has rapidly grown, and their resistance to antibiotics or antifungals has increased as a result of the excessive use of antibiotics, antifungals and immunosuppressants [1, 2]. Therefore, the attention of scientists has focused on finding new ways to inactivate unwanted microorganisms. One of these new ways is also the plasma medicine, which offers new possibilities for food sterilization, sterilization of medical equipment and tools or direct therapeutic treatments of various diseases. Thanks to a high number of active particles generated in a low-temperature plasma operating at atmospheric pressure, this type of plasma has become highly popular, especially thanks to its bactericidal effects. However, its antimycotic effects and mechanisms of fungal inactivation are still not fully understood. Therefore, this study focuses on the antifungal effects of cold atmospheric pressure plasma treatment on Candida glabrata. Candida glabrata is usually well controlled or harmless in healthy people. However, it may cause problems to people with a suppressed immune system. Particularly at risk are hospitalized patients, people with HIV, people being treated for cancer and people who have had organ transplants [3]. The danger of the infections caused by Candida glabrata lies in its innate resistance to the azole antifungal therapy, which is very effective in treating infections caused by other Candida species. Candida glabrata retains many other virulence factors, such as the ability to adapt quickly to stressful conditions and the ability to secrete phospholipases, lipases and hemolysins, expression of the gene adhesin and biofilm formation. Infections caused by the yeasts of the genus Candida are called candidiasis. Candida glabrata is currently ranked the second or third in the world as a cause of superficial (oral, esophageal, vaginal, urinary) or systemic candidiasis [4]. Based on these facts, there is an emergent necessity to find new non-traditional ways to inactivate this yeast.

The use of a cold atmospheric plasma discharge in a form of plasma jet or pen seems like a great option to investigate. So far the microwave plasma jet, corona plasma pen and transitional spark have been tested. For each plasma system several parameters are observed: the effect of the initial cell concentration, the magnitude of the applied power and the exposure time. The assay consists of applying the plasma beam to a freshly inoculated culture of Candida glabrata on agar plates and observing the inhibitory effects in the form of inhibition zones created after the subsequent cultivation.

Acknowledgments: The research was financially supported by the EU cohesion funds, program INTEREG SK-CZ, project No. 304011P709.

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Characterization of a surface dielectric barrier discharge source with segmented electrode for hemp seeds treatment

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Plasma sources of a dielectric barrier discharge (DBD) type have proved their applicability for many different treatments [1,2]. In this work electrical characterization and emission spectroscopy of two configurations of surface dielectric barrier discharge with segmented electrodes were performed. The devices were studied aiming at future application in hemp seeds treatments. A schematic of a side-on view of the configuration used in seeds treatment is shown in Fig.1.

The voltage is brought from the distribution network to the transformer and from it to the powered electrode of DBD. The top dielectric of the source had an upper grounded electrode and a powered electrode on lower side. Both segmented electrodes were made of copper tape. Bottom DBD electrode consisted of a glass with a conductive layer on lower side that was also grounded. The top and bottom electrodes were separated by a spacer of thickness d. Plasma forms in between the electrodes where the seed samples were placed when treated.

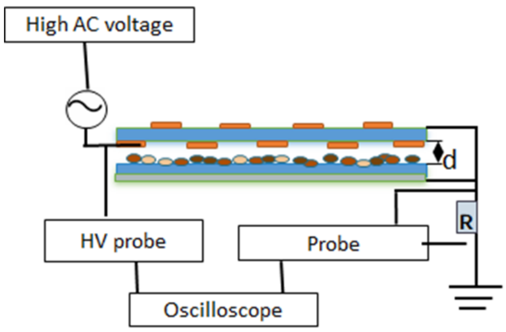

Figure 1.Schematic representation of the first configuration

Electrical characterization was conducted for the range of input voltages between 6 kV and 24 V.Results showed that with greater distance from plasma source less power is delivered to the plasma. Maximum power was measured for d=2 mm electrode gap, for the first configuration it is 1.7W and for the second 2.2W. For d=8 mm dissipated powers in 1st and 2nd configuration were 0.9 W and 1.6 W, respectively. For all conditions discharge current signal had noise structures on signal extremums as a result of filamentary character of discharge [1,2]. From the imaging, done for the first configuration, beside individual filaments, diffuse area of discharge was spotted pointing to formation of a surface discharge [3] The optical emission spectra obtained showed only N_2 molecule bands and N_2+ ion bands as reactive species.

Both electrical characterization and emission spectroscopy were done with the seed samples. For the seeds with higher humidity percentage (≈15%) stabilization effect of additional resistance due to water layer on seed sample [4] was noticed in current signals. Further investigations related to the effects of plasma treatments on hemp seeds sanitation are currently performed.

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Helium Plasma Jet: Evolution of Electrical Parameters with Treatment Time

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Atmospheric pressure plasma sources are an ever-developing field of plasma physics. The need for concrete information regarding its behaviour with various targets coupled with the inherent instabilities these discharges are subject to, a thorough and comprehensive analysis of its behaviour is needed. As treatment times, especially in plasma medicine, are relatively long and many changes can be induced by the target or the environment, understanding its behaviour independently or with stable, controlled targets is vital.

To that extent, a significant amount of data is required to identify any inconsistencies in plasma functioning. The electronic monitoring and control of these discharges are only the first steps in achieving a thoroughly constant and stable discharge and, eventually, treatment method. In order to understand the way plasma interacts with tissues, cells, and biomedical surfaces it is required to understand first the production and behavior of the above-mentioned agents.

These experiments focus on the electrical characterization of a helium-based atmospheric pressure plasma jet with great emphasis on the current behavior. The discharge was generated in helium flow at 3L/min, using high voltage rectangular pulses, with both positive and negative polarity, amplitude between 5 to 8 kV, frequency ranging from 0.5 to 8 kHz, and pulse width from 50 to 500 ms. While investigating the current we focused on the statistical analysis of ignition and extinction time, amplitude, charge, peak duration. The plasma jet evolution was investigated both operating in open air and in contact with a liquid target [1,2].

In order to achieve full parameter stability, these data need to be used in machine learning and the development of feedback loops aimed at adjusting and maintaining the discharge in its desired operational state. This feedback loop, if completely developed could also intervene in plasma operation by taking into account not only the electrical parameters, but also reactive species concentration, environment humidity, target conductivity, target distance from the discharge, and so on, by means of strategically employed sensors monitoring the entirety of controllable and uncontrollable plasma parameters.

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Improving the anti-bacterial efficacy of a plasma jet by modulating the production of hydrogen peroxide using a two-ground electrodes configuration

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Application of cold plasma jets in wound disinfection and healing is getting significant attention in plasma medicine field. The anti-bacterial properties of plasma have been credited to various types of reactive oxygen and nitrogen species (RONS) produced by the plasma jet. Of these RONS, hydrogen peroxide (H_2O_2) is of significant interest due to its high stability in plasma-treated solutions and major roles as a broad-spectrum antibacterial agent [1] and cellular-signalling molecule [2]. Therefore, a major strategy to achieve improved anti-bacterial activity using plasma involves enhancing the production of H_2O_2 in biological target which had been a major challenge for plasma community.

In this study, we developed an argon plasma jet device using two-ground electrodes operated at a peak-to-peak voltage of 7 kV at 23.5 kHz. A 5-minute treatment of water with double ground electrode (DGE) plasma jet resulted in production of 3.10 mM of H_2O_2, which was significantly higher than single electrode plasma jet (1.39 mM). We, then, assessed the potential of DGE plasma jet in killing common wound pathogens (*P. aeruginosa* and *S. aureus*) in planktonic state and within a biofilm. Our results show that in case of planktonic bacteria, DGE plasma jet completely eradicated *P. aeruginosa* and *S. aureus* after a treatment of 40 s and 180 s, respectively. In biofilm model, 99.8& and 99.998% reduction was achieved for *P. aeruginosa* and *S. aureus*, respectively, after 5 minutes of plasma treatment, making it an effective antimicrobial. Finally, we also conducted an *in vitro* toxicity assessment and demonstrated that under certain treatment conditions, DGE plasma jet induced only a low-level of cytotoxicity in HaCaT keratinocyte cells.

In my talk, I will discuss these results in detail to explain the electrical and optical properties of the DGE plasma jet and the varied sensitivity of the two bacterial species to the plasma jet treatment. The talk will provide an insight in the complex physiochemical process which determine production of H_2O_2 in plasma jet treatment and methods to develop next generation plasma jets for wound treatment which are both safe and effective.

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The potential of cold plasma as a treatment for Orthopaedic infection

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The use of cold plasma as a means of infection control is becoming an increasingly studied area. My work will look to further develop this research by studying the effects of cold plasma on the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in the bone. Although my lab work is yet to commence I would be eager to share interesting findings from my lit review and discuss the benefits of using cold plasma as a treatment to date and possibilities for the future. Along with this I would be keen to share ideas for work going forward and our plans for the project in terms of biofilm growth and plasma systems utilised.

In-situ FTIR spectroscopy of a cold atmospheric plasma source for RONS detection

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The large growth of plasma medicine in the last years results in the development of multiple dedicated sources. As of today, various plasma generation technologies are available for different biomedical purposes, resulting in a wide and complex landscape. At the same time, a large variety of diagnostics has been used to characterize the sources, testing them from several points of views and following different experimental protocols. Being each source unique and the data available measured in dissimilar ways, it is difficult to compare the obtained results.

Beyond the electrical properties, which involves the power consumption and the energy deposition on the threated surfaces, the other important parameter to characterize a source is the efficiency in the production of reactive oxygen and nitrogen species (RONS), believed to be the main actors in the triggering of biological chains. It should be studied not only for different sources and configurations, but also in relation to the environmental parameters, such as atmosphere humidity and temperature, and to the substrate the source is interacting with.

It would be useful to define a common protocol, flexible enough to be applied to different sources in different environmental condition but at the same time rigorous enough to allow a meaningful comparison of the results. As a first step, some preliminary studies have been carried out involving a helium plasma source developed at Consorzio RFX in Padova (Italy). In collaboration with EPFL in Lausanne (Switzerland), a custom setup has been built to allow *in-situ* Fourier Transform Infrared Spectroscopy (FTIR) measurements, with the infrared beam going directly through the plasma plume.

The production of RONS has been studied not only varying the source parameters, but also as function of the atmosphere humidity [3]. The first acquisitions already show significant results, managing to detect the presence of ozone, nitrous oxide, and nitric acid; moreover, the diagnostic allowed to detect a strong dependence of the ozone production on the relative humidity. The promising setup can be improved to study more species and combined with other techniques to obtain a more complete diagnostic.

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Deposition of Antibiotic Layers onto Implant Surfaces and bacterial biofilm using Low Temperature Plasma.

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Cold Atmospheric Plasma (CAP) has many uses but the main use that is being focused on in this research is the combination of CAP with an antibiotic to create an antimicrobial environment which will potentially inhibit the growth of life-threatening bacteria such as S. Aureus.

Many Staphylococcal strains are known to cause a large range of community and hospital acquired infections which can lead to fatal prognosis such as septicaemia. The combined use of CAP and the antibiotic Penicillin to treat infected wounds could evolve how bacterial infections are prevented and treated.

The method being used for testing this hypothesis was carried out by creating a design of experiment (D.O.E). This included important parameters such as penicillin load, bacterial load and plasma power. The method for determining the effectiveness of the coating applied was to measure the zone of inhibition created by the penicillin in the bacteria of choice.

The initial stages of the experiment showed that the Penicillin load regulated by flow rate highly impacted the size of the inhibition zones. It was noted that as the plasma power increased the zones of inhibition decreased slightly. Lastly, bacterial load had a major influence on the zones of inhibition, and it was evident that the higher the bacterial load the smaller the zone of inhibition was and the lower the bacterial load the bigger the zone of inhibition was. Further trials will have to be carried out to deduce if the parameters work synergistically to aid in the zone of inhibition or if there is a deciding factor.

Co-deposition of plasma polymerized organosilicon thin films and clay microparticles

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Atmospheric pressure plasma enhanced chemical vapour deposition (AP-PECVD) is a widely used technique for the surface modification of materials [1]. Starting from the use of one or more monomers it is possible to realize thin films (coatings) which possess a large variety of chemical and morphological features suitable for numerous applications such as food packaging, electronics, optical and biomedical applications [2][3]. In the context of biomedical applications, AP-PECVD appears particularly interesting since it allows to obtain coatings which can improve the antimicrobial properties of biomaterials according to two different strategies:

1) **active**, according to which an antimicrobial agent is trapped into the coating and gradually released in time
2) **passive,** which involves preventing the bacterial adhesion thanks to the chemical and morphological characteristics of the coatings.

The process examined in my thesis project was about the creation of a matrix loaded with microparticles of inorganic and clayey nature using AP-PECVD technology. Specifically, for the creation of the nanometric coating tetraethyl orthosilicate (TEOS) was used as precursor with reticulant agent function. TEOS was injected in a Corona Jet source in conjuction with an aerosol solution containing microparticles (4 µm diameter). Polyethylene was used as substrate. The effect of different parameters (applied voltage, feed rates of organosilicon precursors and microparticles, thickness of the aerosolised solution containing microparticles) on matrix chemical composition and microparticles density and integrity was evaluated by means of ATR-FTIR spectroscopy, WCA, and SEM-EDS analysis. According to the results obtained, the integrity and density of the microparticles trapped within the coating strongly affected by applied voltage and by aerosolised solution.

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Cold plasmas for closed environment sanitization

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The Covid-19 pandemic, caused by the SARS-CoV-2 virus, has put into evidence the importance of indoor air quality control and its depuration. The development of devices based on non-thermal plasmas (NTPs) can be exploited to control and reduce aerosol transport and aerosol infectivity of airborne pathogens in air.

At the Laboratory of Magnetofluidodynamic and Plasmas Engineering, LIMP, University of Bologna, various NTP sources, such as atmospheric pressure dielectric barrier discharge (Figure 1), are studied and utilized for different applications. Among them there are also applications in the biomedical field [1], such as for the inactivation of pathogens (Figure 2).

Recently, LIMP's research group, in which I work, has focused attention on the study of a NTP system as an air sanitizer for closed environments. The actual project's goals will include the construction of a small-scale test facility, where several plasma modules will be mounted; the plasma modules will feature different electrode arrangements and applied voltages. Investigations will be carried out to optimize the electric supply system [2] in order to minimize power consumption and noxious species production (ozone and NOX). The effectiveness of the proposed technique against viruses and bacteria will be assessed. The device is integrated with diagnostic systems for the measurement of active species (such as reactive oxygen species (ROS), reactive nitrogen species (RNS) and free radicals) which can be exploited in the abatement processes. Emission spectroscopy will be carried out on the plasma produced by the discharge, to evaluate electron, rotational and vibrational temperature. The impact of applied electric field and the possibility to exploit electrostatic forces to precipitate electrically charged droplets will be investigated as well.

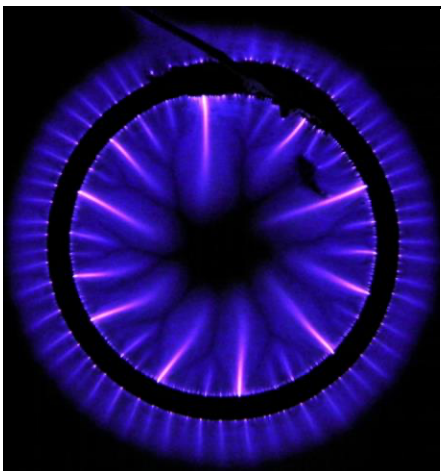

Figure 1 – Image of an annular surface DBD

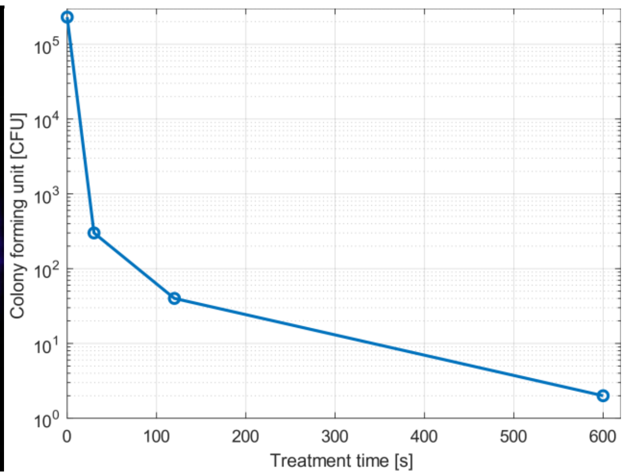

Figure 2 - Candida Albicans cfu logarithmic redaction

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Electrical characterization of an argon/CO_2 and helium/CO_2 plasma jet for wound healing

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Since more than ten years, cold plasma at atmospheric pressure is investigated for medical purposes. Indeed, it’s a close room temperature medium producing UV radiations, electrical field and a large population of reactive species which together has anticancer, sterilizing and wound healing effect.

In large quantities, carbon monoxide (CO) is a lethal poison. In our lungs it binds to hemoglobin and takes the place of dioxygen (O_2) by forming carboxyhemoglobin (COHb) and participates in tissue asphyxiation.

However, medical studies have shown that at lose doses, meaning less than 10% COHb in the blood, CO has anti-inflammatory, vasodilatory, anti-apoptotic and anti-proliferative properties and could thus be used for wound healing. [1]

Plasma is able to produce CO from the dissociation of CO_2. The idea is to use a plasma jet to produce a small amount of CO to combine the beneficial effects of plasma and CO.

This poster will be focused on the electrical characterization of helium and argon plasma jets as a CO sources for biomedical use. The influence of the amount of CO_2, the frequency, and the gas flow rate on the discharge’s properties will be investigated.

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Chemical Analysis of Four Types of Plasma Activated Liquid Stored at Different Temperatures

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Plasma activated liquids (PAL) and their antibacterial effects represent an important field of research especially in medicine. *Escherichia Coli* causes severe urinary tract infections every year. Our research was focused on the bactericidal effects of different types of PALs stored at different temperatures, which determine the changes in concentrations of reactive oxygen and nitrogen species (RONS). The aim of this study is to understand the influence of freezing and refrigerator storage of PALs at their chemistry and RONS concentrations changes.

We tested four types of PALs prepared by different atmospheric air discharge settings, Streamer Corona (SC), Transient Spark with Electrospray (TS-ES), Transient Spark Batch (TS-B) and Glow Discharge (GD). Besides different types of discharges, we tested different types of liquids – saline, PBS (Phosphate Buffered Saline) and deionised water. Each of these PALs, except SC, was tested for the concentration of H_2O_2, NO_2^-, NO_3^- at different storage temperatures (-18 °C, 4 °C, 23 °C). SC PAL was measured only at -18 °C because of its weak bactericidal effect. The chemical analysis was carried out immediately after plasma activation and after 24-hour storage at each tested temperature.

The most important result is the influence of storage of the PALs prepared from PBS at -18 °C. The freezing of PAPBS resulted in the strong decrease of the NO_2^-concentration, which resulted in a decreased bactericidal effect. This discovery changed our approach in microbial experiments which were done after 24 hours PAL freezing in our previous research. The effect can be caused by the crystals of salt which can be formed in the frozen PAL. It is important to mention the same result of losing NO_2^- in plasma activated saline, where the decay of NO_2^- is caused by its reaction with *hydrogen peroxide* in acidic environment (peroxynitrite formation) [1]. The acidic environment is not presented in PAPBS, the peroxynitrite formation is substantially slowed down, and so the real reason of the decay of NO_2^- will be the point of our further research.

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

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A Brief Review on Atmospheric Air Plasma

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Atmospheric pressure air plasma is an attractive technology because it is easy to set up and handle, cheap, and safe. In this paper, a brief review on air plasma configuration and the results of air plasma is presented. One of the important properties of any plasma treatment is the generation of reactive atom species such as oxygen (O), nitrogen (N) also known as RONS, and other ions molecules particles. Air plasma has been found to have the same effect when treating surfaces. Surface modification, hydrophilicity, and decontamination effect were observed when materials are exposed to air plasma. The advancement of air plasma technology will improve the surface processing technology by reducing its operating cost.

A 0D Kinetic Model for Air Non Thermal Plasmas for Sanitation

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An innovative and promising approach to air flow sanitation is that based on the action of non-thermal plasmas (NTPs). The germicidal properties of NTPs are mainly related to the presence of electrons and reactive species of oxygen and nitrogen. This work is focused on the kinetic simulation of some heavy species such as ozone, NO, NO_2, etc.… According to many published papers [1, 2], their number density appears to be linked to the bactericidal and virucidal effect of the cold plasmas. Various diagnostic techniques are available to measure the concentration of some of the species mentioned above. Among these, diagnostic systems based on UV absorption spectroscopy allows a cost-effective, non-invasive and viable option to simultaneously track the dynamic of the species of interest. A computational model is a useful tool for interpretating the experimental results and better understanding the behaviour of the plasmas used in applications under investigation. A 0D simplified kinetic model of a discharge in air has been implemented. The model includes 4 reaction paths, to track ozone and NO concentration and uses 3 fitting parameters as input parameter, that are the transient time constant and the regime value of molecular excited nitrogen vibrational temperature, and the number density of the atomic oxygen. The ozone's number density given by the model has been compared with experimental data obtained investigating a surface dielectric barrier discharge reactor by the research group of Milano Bicocca University. The comparison shows good agreement, as reported in the plot below.

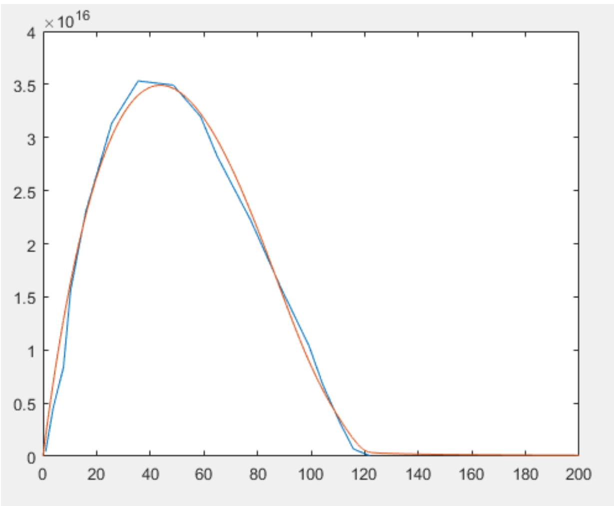

Fig.1 Ozone number density [cm^{-3}] over time [s]. In red the model result, in blue experimental data

The ozone's concentration is observed to increase in the first phase, but the subsequent NO formation causes the ozone to decrease to negligible values. This first model computes only the ozone and NO concentration and can't explain some phenomena. A more detailed model including 35 reaction paths has been also implemented. The comparison with experimental data is currently underway.

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In-vivo safety assessment for fractioned and continuous direct plasma treatments

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Cold plasma have already proven their potential in many biomedical applications. New plasma devices and targets are being developed to close the gap between laboratory investigations and real clinical conditions and requirements [1,2]. To consolidate their use and impact in clinical practice it is essential to assess plasma devices safety and optimize treatment strategies [3-5]. In this work, we assessed the potential effect on skin of a novel multi-jet plasma device designed for medical use. The device was applied on male nude mice and female C57Bl/6J mice (7-week-old, 5 mice/group). Two different operating modes were compared: diffuse mode (uniform plasma volume, device-mouse distance 2 mm) and multijet mode (five separate jets, 5 mm distance, Fig.1). The nude mice were continuously treated for 3 min in the two modes and the skin condition was assessed using an IR camera (Flir i7) and standard photographs to record the skin condition right after the treatment and up to 4 days after. The skin temperature (initially 32°C) rise during the treatment up to 47°C (diffuse mode) and 44°C (multi-jet mode). None or small redness was registered on the treated skin areas right after the treatment. Nevertheless, follow-up 1 day after showed the appearance of skin marks that evolved to scabs 4 days after treatment (Fig.1). A fractioned treatment was investigated dividing the 3 min exposure into three session of 1 min separated by pauses (no plasma) of 80-100s. With the fractioned approach the skin temperature was maintained below 39°C. No scab formed for these cases in the follow-up and only a limited redness was recorded in some cases.

Overall, the results show that continuous exposure to plasma for several minutes may induce skin damages in the form of redness and scabs, and that these effects may take days to express. This highlights the importance to follow-up in plasma in vivo experiments. The monitoring of skin temperature through IR camera appears to be a viable approach to monitor treatments and therefore optimize protocols. In this sense, the results confirm the possibility to avoid adverse damages to the skin by fractioning the treatment with the introduction of short pauses without plasma applications so to maintain the skin temperature below 40°C.

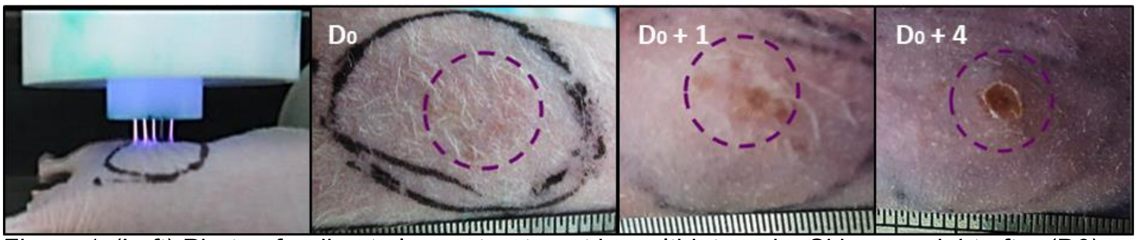

Figure 1: (Left) Photo of a direct plasma treatment in multi-jet mode. Skin area right after (D0) 3min of continuous treatment and follow-up one day (D0+1) and four day (D0+4) after.

Acknowledgments: PEPS ACCUMULTIPLAS of CNRS

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Modulation of macrophage polarization state via cold plasma treatment

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Even though in the last few decades much progress has been made in the field of bone tissue regeneration, the clinical innovation and application of various bone implants can sometimes be hindered, mainly due to the lack of a deeper understanding of the biological processes involved in bone tissue remodelling. In this context, the most recent studies in the bone tissue regeneration and engineering fields focused on the understanding of the bone formation process via the intimately cross-talk of multiple systems [1], with results revealing the importance of the immune response in the biomaterial mediated osteogenesis and inflammatory fibrous tissue encapsulation [2]. It is well known that the elicited immune reaction involves a wide range of activated cells, including one of the key players of the innate immunity, macrophages [3]. Data found in the specialised literature revealed that, both the imediate and the long-term immune reactions to various biomaterials are controlled by macrophages, therefore turning them into primary targets for immune system modulation. Taking this into account, the modulation of macrophage polarization has gained special attention in the field of regenerative medicine, with various strategies being employed. Amongst them, cold atmospheric plasma treatment, applied directly on biomaterials and/or cells could represent a fesible strategy to control and drive the immune cells activity from a pro-inflammatory phenotype M1 to an anti-inflammatory M2 state.

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Determination of antimicrobial activity of packaging materials functionalized with silver, zinc oxide nanoparticles and *Cymbopogon nardus essential oil*

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Nowadays, most of the materials used as food packaging consist of petrochemical polymers. But these polymers are non-biodegradable, which leads to a growth of the chronic environmental pollution issue. Therefore, there is a pressure on the food industry to develop new packaging materials. As they must come from natural sources or nature-safe biopolymers, they also ought to have the ability to inhibit or slow the microbial growth, thus, extending the stability of the packaged product and improving food quality. The aim of this study was to test the antimicrobial activity of some biopolymers functionalized with Ag, ZnO nanoparticles and *Cymbopogon nardus* essential oil against Gram positive and Gram negative bacteria.

The antimicrobial activity of the materials was conducted on four bacterial strains, namely, *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 14023 (Gram positive bacteria), *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* (Gram negative bacteria). The qualitative analysis was performed by disk-diffusion method and determination of inhibition zone, while the quantitative analysis was evaluated by spectrophotometric measurement of the bacterial culture absorbance and colony-forming unit (C.F.U/mL).

The screening of the tested materials by the qualitative methods led to the conclusion that all tested biopolymers have antimicrobial activity. Out of all the materials, one could perceive that the most efficient were those represented by biopolymers funcionalized with silver nanoparticles. The quantitative evaluation proved to be effective for the tested compunds and the results of the statistical test suggested that the antimicrobial activity is influenced by the type of the compound, by its concentration and by the type of microorganism.

The conclusion shown, based on the above, is that the tested nanoparticles and the *Cymbopogon nardus* essential oil could be successfully used as antimicrobial additives incorporated in food packaging systems. Such additives could be viable, environmentally friendly alternatives to conventional polymers, with high potential for development and use in the food industry.

Antibacterial polymeric coating of titanium by atmospheric cold plasma

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Implant-associated infection is one of the factors that determine the success of implantation. Moreover, it causes some problems such as destruction of bone tissue, implant failure, and amputation. Thus, it is important to make in reducing bacterial adhesion and inhibiting biofilm formation through antibacterial modification of the surface of the implant materials. For this reason, cold plasma (CP) has been used as capable of ameliorating surgical implants using various strategies of interface biotechnology, such as surface modification, coating deposition and drug delivery [1].

The aim of this work is to modifiy surface of Ti material with antibacterial N-Halamine polymer through advantage of CP technology. Ti (Grade 2) was used in the study, with a diameter of 5 mm and a thickness of 3 mm. Argon is used as the plasma gas and used a 15 cm long 3 mm inner diameter (1 mm wall thickness) borosilicate glass material. 6 kV 50 kHz power supply was used for discharge item. Ti surface was exposed to plasma for 20 minutes. Afterwards; Poly (2-(acrylamido)-2-methyl-1-propanesulfonic acid) solution (PAA) was sprayed into the plasma medium for 20 minutes and deposited on the Ti surface. After the final Ti-PAA products were obtained, firstly amination (Ti-PAA-NH) and secondly chlorination (Ti-PAA-NCl) were performed [2]. Surface characterization was determined by SEM-EDX analysis. The process steps are shown in Figure 1.

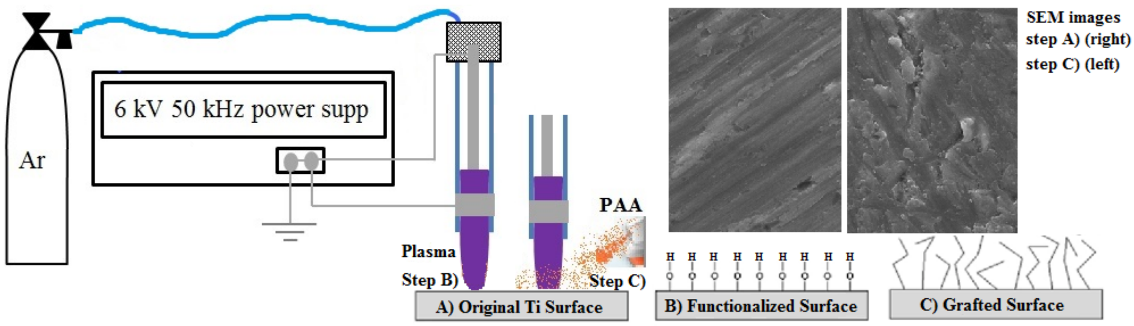

Figure 1. Steps of process and SEM image of Ti surfaces

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Atmospheric Plasma Jet (APJ) for Chronic Wound Disinfection

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Irradiation with Atmospheric Plasma Jet or APJ provides a promising method for chronic wound disinfection especially for diabetic foot ulcers. To be efficient for this purpose, APJ should meet the following criteria: it should significantly reduce bacterial density in the wounded area weather for single microbe or polymicrobes (D-value) and increases the confidence level. In order to design APJ that would satisfy these requirements, we demonstrate two different methods which are direct and indirect APJ irradiation with respect to microbial decontamination. Indirect method or Plasma Activated Water (PAW) has several advantages over direct method

The objectives of this study were to investigate the content of PAW after APJ irradiation, to elucidate the effect of PAW for deactivation of microorganisms and to compare the effectiveness between direct and indirect (PAW) on microbial decontamination. Plasma parameters used for both methods were same which are helium as a working gas, gas flow rate varied from 0ml/min to 2000 mL/min and exposure times varied for 0s,30s,60s, 90s and 120s. both methods were compared based on the D-value and confidence level.

The results of this study suggest that indirect method is more effective compared to direct method because of the least D-value and the highest confidence level recorded for single and polymicrobes.

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Antimicrobial efficiency of essential oils in antibiotic resistant bacteria

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Pseudomonas aeruginosa is a non-fermentative Gram negative opportunistic pathogen frequently encountered in difficult-to-treat hospitals-acquired infections and also wastewaters. The natural resistance of this pathogen, together with the frequent occurrence of multidrug-resistant strains make current antibiotic therapy inefficient in treating *P. aeruginosa* infections. Antibiotic therapy creates a huge pressure to select resistant strains in clinical settings but also in the environment, since high amounts of antibiotics are released in waters and soil. Essential oils (EOs) and plant derived are efficient, ecologic and sustainable alternatives in the management of various diseases, including infections. In this study, we have evaluated the antibacterial effect of four essential oils is Tea tree, Thyme, Sage and Eucalyptus on 36 *P. aeruginosa* strains isolated from hospital infections and wastewaters. Bacterial strains were phenotypically and genotypically characterized in terms of virulence and antimicrobial susceptibility. The results show that most strains express soluble pore toxin virulence factors such as lecithinase and lipase and antibiotic resistance rates is high, most of them being resistant to Cabapenems.

Statistical comparison of the EOs antimicrobial results showed that Thyme gives the lowest the minimum inhibitory concentrations (MIC) and minimum biofilm eradication concentrations (MBEC).

Cold atmospheric pressure plasma-assisted inactivation of *Staphyloccoccus aureus* and *Pseudomonas aeruginosa* biofilms

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In nature, many of the micro-organisms coexist in complex structures commonly known as biofilms; their development depends on biological, chemical, and physical factors. More specifically, biofilms are defined as an assemblage of microbial cells embedded in an extracellular polysaccharide matrix which could be found on a wide variety of surfaces [1]. The development of this complex "community" confers several advantages to the cells that make it up physical and mechanical resistance and chemical protection.

Some of the methods used for the biofilm inactivation consist in the use of chemical solutions (e.g. chlorhexidine (CHX), sodium hypochlorite, hydrogen peroxide, ozonated liquids) or ultrasound where it is possible; however, in many cases, the effectiveness of these methods is very low. Nowadays it is evident that the biofilm formation is the major problem in more than one industrial sectors (e.g. pharmaceutical and food industries, hospitals, etc…) [1]. In this context, the use of cold atmospheric pressure plasma (CAP), thanks to its blend of free radicals, electrons, ions, reactive species, UV radiation, and electric field could represent an innovative and efficient solution for the inactivation of biofilm. Until now, several authors have investigated the efficacy of different CAP sources for biofilm inactivation achieving good results using biofilms made of different type of bacteria [2,3].

In this work, the efficacy of a scalable surface dielectric barrier discharge (sDBD) source was assessed for the eradication of one-, three- and eighteen-days aged biofilms of *S. aureus* and *P. aeruginosa* (a Gram-positive and a Gram-negative, respectively) using four different operating conditions which differ in terms of power density and treatment time. All the investigated conditions have been chosen to work in O_3- and NO_x- enriched atmospheres. A high anti-bacterial effect (LogR > 3.5), which induced cell and biofilm damage, was assessed in all plasma treatments. Moreover, CHX was used as positive control, resulting to be less efficient in the biofilm eradication compared to the plasma treatments. In addition to the biological experiments, electrical characterization and time resolved UV/VIS optical absorption spectroscopy (OAS) were performed for the measurement of power density and the kinetics of the concentration of O_3 and NO_2 produced by the sDBD plasma source.

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He plasma jet optimization towards enhancing polymeric surface biocompatibility

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Polymeric surfaces are of great importance nowadays in many fields, going from industry to bio-medicine, and even food related fields. A convenient and environmentally friendly method for customize polymer surface properties is the usage of non-thermal, atmospheric pressure plasma discharges. Nowadays studies rely on the direct usage of an atmospheric pressure, low temperature, sinusoidal He plasma source for enhancement the biocompatibility properties of polyamide 6 (PA6) foils. The plasma jet source, based on a cylindrical dielectric barrier discharge, was diagnosed by electrical and optical methods. We used a flow rate of 2 SLM of He through the quartz discharge tube and applied a 12 kVpp sinusoidal voltage on the discharge electrodes, at 48 kHz, keeping a gap of 5 mm between the discharge tube and the sample.

Commercial PA6 foils, carefully cleaned with ethyl alcohol and distilled water prior to all experiments, were plasma treated for 60 s on both sides. Atomic force microscopy, static contact angle method and ATR-FTIR spectroscopy were used for polymer characterization before and after plasma treatment. The biocompatibility of untreated and plasma treated polymer samples was tested using Albino rabbit primary fibroblast cells. The cells were cultured for 24 / 48 / 72 h on the PA6 foils, the cell viability being assessed using standard MTT assay protocol. The cellular adhesion onto the polymeric samples were also studied. For these experiments, cell staining was done using Calcein-AM and Giemsa dyes, further on an inverted laboratory microscope being used for imaging the cells onto the samples under investigation.

The electro-optical characterization of jet plasma source revealed a discharge with a mean power of up to 50 W, which has in the emission spectrum along working gas lines (He) also bands and lines of reactive species such as: OH radicals, N_2, N_2^+, O. The polymer characterization revealed good surface properties after plasma exposure: increased roughness, water work of adhesion, as well as surface oxidation. No cytotoxic effect of the untreated/treated PA6 was observed. Increased cell adhesion was found on the treated samples in respect to the untreated and control ones.

These experimental findings, sustained by the good correlation of the obtained results from plasma diagnosis, surface characterization methods, coupled with cell viability and adhesion tests, justify the usage of plasma sources in polymer processing for biocompatibility purposes.

Dual action plasma-treated composite hydrogels: stimulating osteogenesis while selectively killing cancer cells in osteosarcoma treatment

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Cold atmospheric plasma (CAP) is a source of a complex cocktail of chemically reactive species, which can interact with living cells and tissues, thus exerting many biological effects on cells. Interestingly, these reactive species have been shown to selectively kill cancer cells without damaging the surrounding tissues. Thereby, it would enable the development of alternative anticancer therapies, which would reduce undesired side effects found in radio- and chemotherapy. Among all plasma-derived reactive species, it has been shown that the oxygen and nitrogen reactive species (RONS) have a major contribution to this selective cytotoxic effect [1]. Currently, the way to apply a plasma treatment on a patient is still challenging because most of the direct plasma treatments of the tumor area would involve surgery. Interestingly, many studies demonstrated that RONS can be transferred to liquids, which can exert very similar anticancer effects compared to direct plasma treatment [2]. However, the delivery of RONS through non-viscous liquids is fast and poorly controlled, thus reducing the efficacy of the treatment [3]. To ameliorate the delivery of RONS, it has been shown that CAP-treated biopolymer solutions with the ability to form hydrogels can enhance the generation, storage, and release of RONS, resulting in an ideal approach for indirect CAP treatment of tumors [4].

Based on these findings, in our group, we are exploring the capacity of alginate-based hydrogels for generating, storing, and releasing RONS to efficiently deliver RONS in osteosarcoma cancer treatment. In addition, we are also incorporating bioactive molecules like gelatin and hyaluronic acid to the alginate formulation with two objectives; on the one hand, to maximize the amount of RONS in the hydrogel after plasma treatment thus increasing the anticancer potential, and on the other hand, to favor the survival of healthy bone cells while promoting osteogenesis to stimulate the patient' osteo-regeneration processes.

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Cold atmospheric plasma to synthesize activated liquids and hydrogels for biomedical applications

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Cold atmospheric plasmas (CAP) attract remarkable interest in material processing due to the absence of complex and very expensive vacuum systems, the employment of easy-to-handle apparatuses and their easy integration into continuous production lines [1]. In the last decades, these plasmas have promoted the development of the Plasma Medicine, an emerging field of research based on the use of plasma sources or plasma modified liquids in contact with cells, tissues, or organs. Indeed, CAP have been successfully used to promote wound healing, sterilization, blood coagulation and for cancer treatment [2,3].
CAP are particularly emerging in medicine as a tool to generate reactive oxygen and nitrogen species (RONS) to be transferred to liquids of clinical interest [4]. The obtained plasma treated water solutions (PTWS) can be applied in contact with biological targets (i.e., indirect treatment) being useful for potentially therapeutic purposes [3,4-7]. The variety and concentration of RONS and oxidated products of organic molecules in PTWS is related to the experimental conditions of the applied plasma as well as to the chemical composition of the exposed liquid [3,4,8]. Experimental results obtained by the treatment of Dulbecco's modified Eagle medium (DMEM) with a dielectric barrier discharge (DBD) PetriPlas+ source fed by synthetic air, oxygen, or nitrogen, for example, showed that the hydrogen peroxide and nitrite generated in the DMEM, as function of the plasma processes, can modulate astrocyte or cancer cells behaviour in a selective way [3,5].

The direct injection of PTWS to the biological site, as well as the oral administration of such liquids, could be however affected by washing away by body fluids. Thus, the design of biomaterials acting as vehicles of PTWS represents a very new fronter of the research [4,10].
The upcoming work focuses on the utilization of CAP to synthesize PTWS and biocompatible hydrogels for biomedical applications. For this aim very preliminary experiments were performed by using distilled water as liquid sample and a plasma jet with a coaxial cylindrical DBD configuration (Figure 1) fed by helium. The jet consists of a glass tube acting as dielectric barrier, an internal the high voltage (HV) electrode and an external ground one with a 2 mm annular discharge gap.

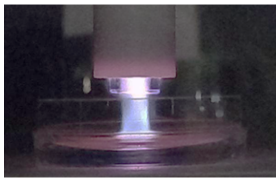

Figure 1. Photograph of the plasma plume on water

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Electrical and optical investigation of the long term operation of an endoscopic plasma device

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In the current situation, facing a worldwide pandemic with SARS-CoV-2, methods to interrupt chains of infection are required. The pandemic fight is based on two main areas: The direct clinical treatment of infected patients and the reduction of viruses released into the environment. However, there are hardly any treatment options to locally reduce the viral load in the respiratory tract of a patient to reduce their infectivity and thus the spread of the virus. Using atmospheric pressure plasma to treat the respiratory tract could be a solution to this challenge [1]. An existing flexible plasma jet [2] is aimed to be studied for direct treatment of the respiratory tract, and in this study, this plasma jet has been investigated in long term operation regarding its electrical and optical behavior to ensure stability and reproducibility of the device for biology investigations. The plasma jet is ignited with a voltage generator that features an oscillating circuit with a frequency of around 400 kHz and peak to peak voltage of around 5 kV. The plasma source is operated with Helium as working gas and supplied with a shielding gas, here CO_2 or N_2. Voltage, frequency, transferred charge (Figure 1), and optical emission spectra were measured and the consumed power was derived for two plasma jets over 25 hours and 17 hours. From the optical emission spectra, the temporal development of the intensity of excited species like O, N_2, N_2^+, and He were recorded. The results show that electrical properties, i.e., voltage, frequency, and power, were steady during the operation of both jets. Production of gaseous species was also reproducible and the same trend recorded for gaseous species after turning on the plasma.

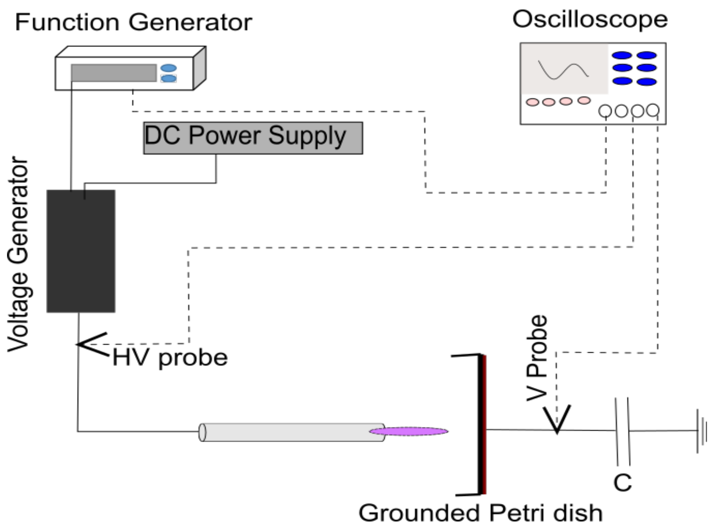

Figure *1*. 'The electrical setup used for measurements of Voltage, Transferred charge and Frequency.

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Plasma directly generated in liquids as an innovative method to treat cancer

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In the recent years, atmospheric pressure plasma technology has been increasingly explored as a possible cancer treatment strategy after being integrated into the medical field as sterilization and wound healing method. In fact, plasma leads to the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as H_2O_2 $^-$, OH, O and NO_2 that trigger cancer killing mechanisms and cannot be produced by any other treatment approach. In contrast to the traditional cancer treatments such as radiotherapy and chemotherapy, one of the major advantages of plasma is its selective anti-cancer ability, which has been shown over many cancer cell lines.

In a more general perspective, the use of plasma in oncology can be applied following 2 different approaches:

1) The direct treatment in which living cancerous cells, tissue or organs are directly exposed to plasma and are considered as one of the electrodes thus actively participating in the discharge plasma processes.

2) The indirect treatment in which a physiological fluid is first subjected to a plasma treatment then the obtained plasma-activated liquid (PAL) is brought into contact with cancer cells. This approach is currently attracting the interest of researchers since most cells and tissues are surrounded by liquids and PAL in contact with such tissues can lead to several desirable physiological responses.

Therefore, within this study, an atmospheric pressure plasma jet will be used to first activate water in its bulk unlike other current studies. The effect of different operational parameters such as plasma exposure time, applied voltage, feed gas and gas flow rate will be studied on different liquid characteristics believed to play a key role in cancer cell death such as: H_2O_2 concentration, NO_2^- concentration, pH, temperature and conductivity.

After excessive fine-tunings of the plasma parameters, a treatment time of 10 min, and a gas flow rate of 2 liters per minute (lpm), an applied voltage of 3 kV and 4 kV for Ar and He feed gas, respectively, gave the optimal results. In fact, a solution having anti-cancer properties was obtained since it was characterized by an NO_2^- concentration of 16.7 µM, an H_2O_2 concentration of 2284.85 µM, a pH of 4.29 and a conductivity of 33.73 µS/cm. In order to confirm the beneficial cancer-responsive properties of the obtained PAL, in vitro cell tests will be performed on different healthy and cancer cell types. One step ahead, several control groups are applied to enhance understanding of abovementioned species on cancer and healthy cells. Results show that hydrogen peroxide plays a key role in decreasing the cell metabolic activity while the role of nitrite is almost negligible.

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Plasma Activated Water and Topotecan: A possible combinatory approach for glioblastoma cells?

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Cancer is the second leading cause of death globally, causing nearly 10 million deaths in 2018, and cancer incidence will continue to rise with an aging population. Resistance to traditional therapies such as chemotherapy or radiation as well as some severe side effects of the current treatment methods are also a matter of concern, generating a need for alternative therapies[1]. Glioblastoma multiforme (GBM) is the most common, malignant and aggressive brain cancer[2].

Highly energized, ionized gas – or plasma is being studied as a new potential anticancer approach. Liquids treated with plasma, which are referred to as "plasma-activated liquids" (PAL) also possess the ability to kill cancerous cells[3].The anti-cancer activity of "plasma-activated water" (PAW) is based on reactive chemical compounds present in solution, which are able to affect multiple cellular targets[4-5]. By analysing the chemistry of diverse PAW, the responsible active ingredients may be identified and used to generate a liquid with optimal anti-cancer effects[6-7] and minimal toxicity on other cells[3].Possible combinatory effects with conventional therapies, such as chemotherapeutics may expand the potential of PAW for cancer treatment.

The research aim is to investigate the therapeutic properties of a combination of different types of PAW and Topotecan (TPT), an antineoplastic agent with major cytotoxic effects during S-phase of the cell cycle (inhibiting DNA topoisomerases I)[8]. Two types of plasma discharges are used to produce PAW with different chemical compositions[9], and evaluated on a human brain cancer cell line (U-251mg). Next, the IC50 values are determined for PAW and TPT alone. Then, the anti-cancer effect of different combinations between PAW and TPT are analysed to establish possible additive, synergistic or antagonistic effects. Finally, the effect obtained is characterized, evaluating short and long term survival, cell proliferation, cell cycle and cell death.

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Applications of cold atmospheric plasma as green technology on Detoxification of Aflatoxins from food to prevent cancer

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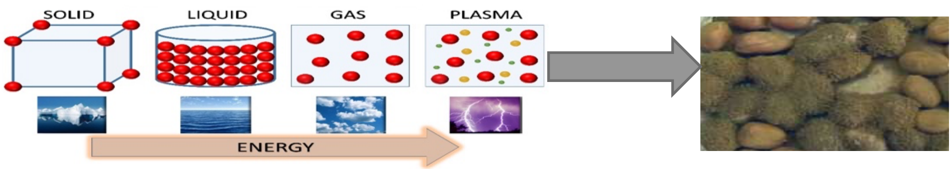
Aflatoxins are secondary metabolites produced by *Aspergillus* species. These aflatoxins have chronic and acute toxic effect on human and animals, and are mutagenic, carcinogenic, negatively affecting the immune system, and even causing death [1].. According to FAO, 25% of the world's crop is contaminated with mycotoxins during growth or storage [2,3] causing significant health problematic and economic damages at all levels of the food production supply chains. The detoxification strategies have been applied such as thermal, physical, chemical and biological [4]. Though some of these processes showed a reduction in aflatoxin level, they tended to be costly, inefficient and often showed undesirable negative effect on quality parameters. These reasons encouraged to develop a novel method that will efficiently degrade aflatoxins without altering the characteristics of the food during decontamination. Cold (non-thermal) plasma is a novel and robust detoxification technology with a potential to fulfills the aforementioned issues in the food industry because of its advantages like high efficiency, short treatment time, no waste residue in the environments, and it has a minimal impact on the quality of treated food products [5]. Plasma is formed by strong electric field accelerating free electrons, which dissociate, excite, or ionize gaseous molecules [6].



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The impact of OH and H_2O_2 fluxes on DNA fragmentation and denaturing

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In comparison to radiolysis studies, plasma jets provide a significant flux of radicals including OH. We are investigating interactions between radicals formed from H2O dissociation (OH, H, HO_2,) and biomolecules (DNA) using a He-H_2O RF plasma isolated from the environment to supply solely H, OH, HO_2, and H_2O_2 flux (estimated >1019 m^{-2} s^{-1}) to DNA solution.

Gel-electrophoresis is used to investigate the fragmentation of DNA with exposure time and radical flux, Figure 1, displaying a linear relationship with increased treatment time. From this we estimate an effective double strand breakage rate constant of 0.516 min^{-1} for the reaction of OH with DNA via the OH reaction with H atoms in the DNA sugar phosphate backbone.

Further investigations involve determining the effective OH flux and rate constant as the distance between the sample and plasma are increased and the use of water microdroplets to enhance OH flux, for exploring possibility of remote non-contact plasma treatments of tissue and in-vivo.

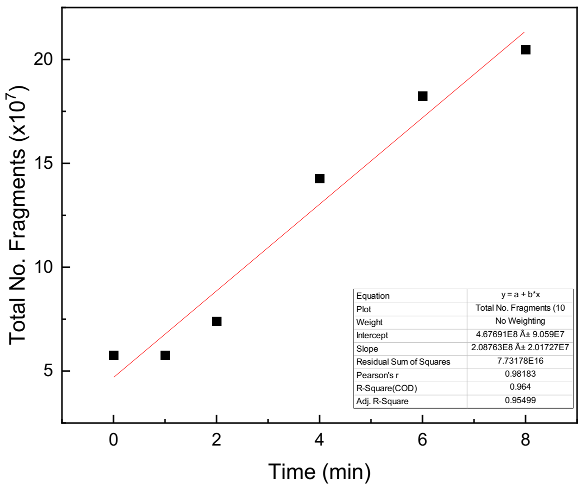

Figure 1. Number of fragments of DNA treated at 50mm from plasma source between 0-8 minutes

Continuously Plasma Treated Water Spray for Medical and Cosmetic Applications

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Cold atmospheric pressure plasma (CAP) bactericidal and anticancer effect is nowadays established. Water and other liquids treated by CAP can also possess antibacterial and antitumor properties and show some advantages compared to direct CAP treatment in terms of handling, storage and ease of use. Moreover, plasma-treated aerosol can cover large surface area thus overcoming the usual dimensional limitations associated to atmospheric pressure plasmas [1]. Recent evidence have demonstrated the potential of CAP and CAP treated liquids in modulating the permeability of cells membranes and tissues [2, 3]. Since active cosmetic ingredients efficacy is controlled by their diffusion in the skin, new possibilities open for the use of CAP in cosmetic applications. In the present work, we developed a device capable of providing a continuous spray of CAP-treated liquid controlling its RONS production. The final aim of the project is to study the correlation between plasma–treated liquid properties and their skin's penetration. The liquid (distilled water in this) is treated thanks to a He DBD plasma jet (10 kV_{peak}, 20 kHz) and then nebulized through a piezoelectric membrane. This allows to produce a continuous flow of treated water (2 mL/min) with significant concentrations of H_2O_2 (10 mg/L), NO_2^- (5 mg/L) and NO_3^- (25 mg/L). The delivery in aerosol form allows the prompt application on a surface of several cm of diameter (Ø 4 cm at 5 cm distance). Beyond the simple design of the plasma treated aerosol device developed in this work, and the efficient generation of long-lived species, work is in progress to assess the delivery of short lived highly oxidant droplets on targets relevant for biomedical applications.

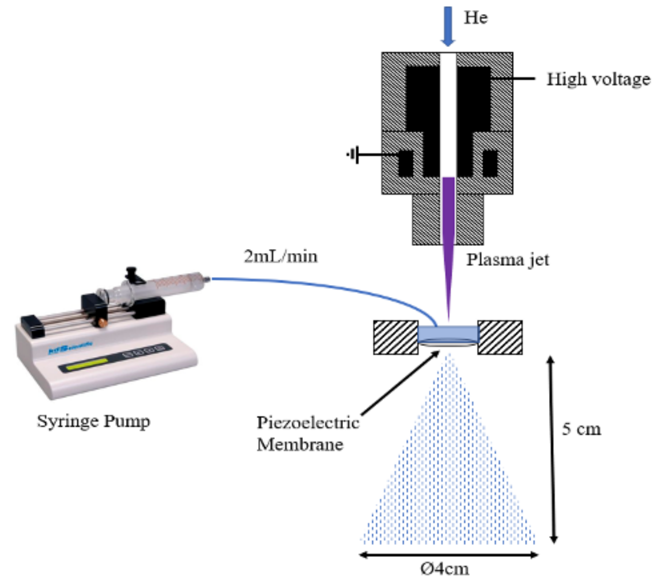

Fig. 1 Plasma treated water spray system

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POSTER PITCH PRESENTATIONS

Order	Name	Country
1	Pasquale Isabelli	Italy
2	Roberto Montalbetti	Italy
3	Caterina Maccaferri	Italy
4	Milica Zivanic	Spain
5	Miguel Mateu Sanz	Spain
6	Matic Resnik	Slovenia
7	Michal Wojcik	Poland
8	Vladyslav Vivcharenko	Poland
9	Oleksandr Boiko	Poland
10	Ruben Verloy	Belgium
11	Benjamin Harris	United Kingdom
12	Ana Sainz García	Spain
13	Nourhan Hendawy	United Kingdom
14	Valeria Veronico	Italy
15	Aleksandra Lavrikova	Slovakia
16	Anna Michalicha	Poland
17	Andjelija Petrovic	Serbia
18	Tijana Lainović	Serbia
19	Xavi Solé-Martí	Spain
20	Berrak Kurt	Turkey
21	Zuzana Okruhlicová	Slovakia
22	Francesco Tampieri	Spain
23	Thais Priscilla Pivetta	Portugal
24	Mostafa Elsayed Hassan	Slovakia
25	Paulo Morgado Zagalo	Portugal
26	Pankaj Pareek	Slovakia
27	Nadja Ivašková	Slovakia
28	Barbora Gromová	Slovakia

POSTER PITCH PRESENTATIONS ABSTRACTS

Cold Plasma Systems to reduce airborne transmission of Hospital Acquired Infections & COVID-19

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The airborne transmission is considered one of the most likely mechanisms explaining the diffusion of Health Acquired Infections (HAIs) and more recently the spread of the SARS-CoV-2 virus, especially in indoor environments [1][2]. In this field, the possibility to use plasma-assisted solutions to deactivate bioaerosol could improve air quality and limit infections diffusion. Indeed, cold atmospheric plasmas producing a blend of bio-active species, e.g. reactive oxygen and nitrogen species, can inactivate airborne pathogens [3]. Recently, our research team evaluated the efficacy of a lab-scale dielectric barrier discharge plasma device in the inactivation of aerosolized *Staphylococcus epidermidis*, purified RNA of SARS-CoV-2, and SARS-CoV-2 virus. Results (through bacterial log reduction and RT-PCR assay) show that the interaction between plasma and bioaerosol flow rate can induce a log R around 3.76 on bacteria and fully degrade viral RNA and SARS-CoV-2 [4][5]. Actually, we are developing a portable plasma device to contrast airborne transmission and we are working on a plasma device to be inserted in Heating, Ventilation and Air Conditioning systems (HVACs) which could improve air quality in healthcare environments (Fig.1).

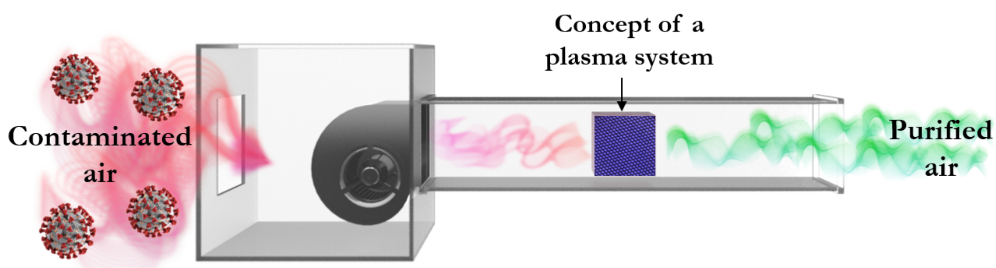

Figure 1 – Concept of plasma system in a lab-scale HVAC system.

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Design, development, implementation, and functional characterization of atmospheric pressure cold plasma sources for thin film deposition

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Atmospheric pressure plasma is widely used in many application fields such as surface treatment of materials, air purification, biomedicine, and film deposition[1]. In the medical field, cold plasma is used for the coating of scaffolds and prostheses. The coating allows the deposition of antimicrobial surfaces on the treated sample, preventing the onset of infections. Atmospheric plasma sources have recently received increased attention due to their many advantages, such as having no need for expensive vacuum equipment, being low-cost, simple systems, and being easy to operate. My research project is focused on Atmospheric Pressure Glow Discharge (APGD) source. The plasma from an APGD source is most suitable for industrial applications among various plasma sources. My research project aims to develop a new APGD source suitable for thin film deposition (coating) [2]. Among the various existing coating deposition techniques, assisted plasma deposition treatments to stand out since they allow to obtain coatings with characteristics (chemical, morphological and structural) suitable for a wide range of applications, such treatments also carry on peculiar advantages such as low process temperatures, low treatment times and eco-sustainability. The process of assisted plasma deposition involves the introduction of a precursor, usually dragged from a gas (carrier gas), inside the plasma discharge. Into the discharge zone, the precursor is fragmented through in-flight reactions and is recombined on the surface of the material, leading to the formation of the desired solid coating. In the literature, there are several examples of plasma sources used at atmospheric pressure for coating deposition. Among these, the most use ones are the planar dielectric barrier discharge (DBD) type with horizontally oriented electrodes and gas mixture (plasma gas and precursor) flushed into the interelectrode gap. In these configurations, the substrate to be treated is positioned into the interelectrode gap and constitutes an obstacle to the motion of the gas mixture, determining fluid dynamic variations that affect negatively the quality of the deposit and affect the applicability of the process at an industrial level. The goal of my research project is to overcome this problem by designing, realize and developing an APGD plasma source with the electrodes oriented perpendicular to the substrate to be treated. This configuration involves the placement of the substrate to be treated below the plasma source, outside the discharge zone. My work is divided into two phases: a first phase in which I have to choose the design and the materials suitable for the production of the APGD source and test the geometries chosen by rapid prototyping. Once the plasma source has been realized, the second phase starts; mapping the possible regions of use of the source itself. The source is electrically characterized using voltage and current probes. The coating is analyzed using Fourier Transform Infrared Spectroscopy (FTIR), which allows a chemical characterization.

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Cold Plasma Systems to decontaminate surfaces from SARS-CoV-2 RNA

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SARS-CoV-2 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 technology [1]. The focus of the PASS project is to develop plasma assisted sanitation systems for food packaging materials, equipment and tools, used for processing and handling food products. The main goal of the research is the identification of specific plasma parameters able to induce the SARS-CoV-2 inactivation, to be used as input for the realization of a preindustrial prototype.

A plasma sanitation system, consisting in a climatic chamber equipped by a specific plasma source connected to a high voltage generator, has been developed and validated at research level in terms of inactivation of bacteria and viruses contaminating foods in University of Bologna pilot plant area. However, the verification of its antiviral efficiency on material surfaces and the optimization of the process parameters has to be performed. In particular, the antiviral activity is being performed, investigating different parameters, such as voltage, frequency, treatment time, etc.., on different target materials. The final aim is to identify the optimized set of process parameters inducing the highest microbial inactivation, minimizing the decrease of functional and technological performances of the material, such as “inertness”, barrier properties and/or mechanical ones.

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Cold atmospheric plasma-treated hydrogels in immuno-oncology

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Given the heterogeneity of the cells within and across tumors, the key to robust and successful cancer therapy lies in the reversal of the immunosuppressive microenvironment of the tumor and activation of the patients' own immune system. This can be achieved by eliciting immunogenic cell death (ICD) in the tumor, a type of regulated cell death that leads to activation of adaptive immune responses for systemic and specific anti-tumor immunity. In ICD, dying cell releases variety of endogenous molecules called damage associated molecular patterns (DAMPs) which act as adjuvants. DAMPs can serve as surrogate markers for detecting ICD-inducing therapies, but the golden standard for determining *bona fide* ICD inducers is a vaccination assay [1]. Recently, cold atmospheric plasma (CAP) has emerged as a promising ICD inducer for cancer therapy [2].

CAP represents an adjustable source of reactive oxygen and nitrogen species (RONS) which can be used for selective cancer therapy. The RONS are delivered to cancer cells either directly via CAP treatment, or indirectly through applications of plasma treated liquids (PTL) [3]. However, PTL can be poorly controlled and are quickly diluted by the body liquids. Thus, our group has proposed plasma treated hydrogels (PTH) as an alternative to PTL, as they could enable better controlled and more localized delivery of RONS. Furthermore, PTH can be designed to be injectable for minimally invasive delivery [4] and to mimic the extracellular matrix of the tissue to favour the survival of non-malignant cells [5]. We have designed an injectable hydrogel for treatment of osteosarcoma, a particularly metastatic and drug-resistant cancer. Our PTH is composed of alginate, for *in situ* crosslinking in Ca^{2+}-rich bone environment, and other biomolecules, to favour the post-treatment survival of healthy osteoblast cells and regeneration of the bone. Since osteosarcoma has not been studied in the context of CAP- of PTH-induced ICD, the aim of my PhD Thesis is to determine tumoricidal and immunogenic potential of PTH for osteosarcoma and investigate strategic combination with existing immunotherapies.

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Impact of Gas Plasma-Treated Ringer's Saline on Stem-Like Properties in Osteosarcoma Tissue-Engineered Models

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Cold Atmospheric Plasma (CAP) has been proved as an anticancer agent against Osteosarcoma (OS), although most research has been conducted in traditional monolayer cultures [1], lacking the Tumor Micro-Environment (TME) which plays a key role in challenging the effectiveness of novel therapies [2]. In a recent research, an OS tissue-engineered model was developed to study CAP effects, based on an osteo-mimetic scaffold containing MG-63 cells [3]. This OS-3D model showed that Plasma Treated Ringer's (PTR) saline increased Cancer Stem-Like Cell (CSLC) subpopulation. CSLCs can evade cell death routes and may be responsible of tumor recurrence.

In this work, we aimed to explore the molecular mechanisms employed by CSLC to survive in response to PTR. For this purpose, we have employed three phenotypically different human OS cell lines (G-292, SaOS-2 and U-2 OS) cultured in the previously described scaffolds to allow them to proliferate in a 3D spatial distribution and increase osteomimicry, where cell viability and gene/protein expression were evaluated after PTR exposure.

Our data presented significantly contrasting results between 2D and 3D cultures. We found that 3D cultures protect cells from PTR-induced cytotoxicity in all cancer cell lines used, related with the increase of antioxidant expression. PTR reduced cell viability while greatly improving the expression of stem-like genes SOX-2, OCT3 / 4, or NANOG and decreasing the osteogenic markers OPN and ALP. In contrast, these harmful effects were abrogated in combination with a specific inhibitor that down-regulated antioxidant expression, sensitizing in this way CSLCs against PTR cytotoxicity.

Taken together, this work confirms the great importance of studying the potential effects of CAP-based therapies in a relevant disease context given the TME and the presence of subpopulations with CSLC properties. We have been able to propose a new mechanism used by OS cells to escape PTR-induced lethality in 3D cultures.

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Cold plasma treatment of polypropylene medical tools

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Disposable medical tools such as containers, tubes, pipette tips, etc, are most commonly made out of polypropylene due to its heat and chemical resistance combined with recyclability. It is both convenient and practical, but the surface interactions between these medical tools and bodily fluids are rarely considered. The morphology, surface charge, top-surface-layer chemistry, degree of crystallinity, wettability all play an important part when delicate nanoparticles come in contact with such surface.

In present work, the interactions between extracellular vesicles and inner surfaces of 1.5 mL tubes were studied. Extracellular vesicles are promising biomarkers that can be noninvasively obtained from body liquids especially blood [1]. Precise diagnosis of early stage diseases is possible when enough extracellular vesicles are successfully isolated. Beforementioned tubes are used for centrifugation of samples and therefore it is expected for particles to come into close contact with the tube walls. Due to the strong centrifugal forces and different surface properties, extracellular vesicles can decompose or permanently attach to the surface. This is where the cold atmospheric pressure plasma jet can be utilized to achieve a more desired surface finish and improve extracellular vesicle isolation yields.

A cold atmospheric pressure plasma jet was constructed, consisting of an electric power source and a copper wire electrode inside a quartz tube. Argon was used as the shielding gas. To ensure equal treatment of all surfaces and a proper repeatability, an optimisation of plasma treatment was required. The optimisation process led to a vertical configuration with plasma jet coaxially placed inside the polypropylene tube moving in and out multiple times. The tube was additionally enclosed to provide predominantly argon atmosphere. Afterwards, the plasma jet treatment time was optimised according to wettability, where the highest hydrophilicity was considered superior (polypropylene in untreated state is hydrophobic). Tubes made by different manufacturers were treated and analysed as notable differences were observed in material properties amongst them.

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New hydrocolloid type biomaterials based on natural polymers for potential applications in regenerative medicine

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The problem of treating difficult-to-heal wounds is a widespread topic in regenerative medicine. Repeatedly the complexity of disturbances in the wound healing process requires the use of specialized bioactive biomaterials, which is a severe challenge for engineering of biomaterials [1]. The use of dressings based on natural polymers is becoming a modern trend due to their non-toxicity, biocompatibility, biodegradability, support of cell proliferation, easy production process, and low costs [2]. An example of such a natural polymer, which is more often used as a matrix of the dressing, is curdlan. It is one of the β-1,3-glucans that exhibits anti-inflammatory properties and accelerates the wound healing process [3].

My research topic is an attempt to develop superabsorbent biomaterials with typical hydrocolloids properties for the potential management of exudative wounds. In order to obtain biomaterials with the desired properties, polymers like curdlan, agarose or chitosan were mixed together in an aqueous medium and then subjected to a high-temperature gelling process. The obtained gels-like materials were frozen at -80°C and subjected to a freeze-drying process. Foam-like biomaterials were obtained as the final product, which after being soaked in a physiological fluid, take the form of a gel acting as hydrocolloid materials. The materials were tested to assess their potential for clinical use as a dressing for highly exuding wounds.

In my studies produced biomaterials were subject to comprehensive biological analysis using cellular model - normal human skin fibroblasts obtained from ATCC. Cytotoxicity in indirect test was conducted in accordance with ISO 10993-5. To assess cell viability of fibroblasts grown next to and on the biomaterials, Live\Dead staining was performed and cells were visualized by CLSM. To assess the biocompatibility of the biomaterials, WST-8 cell proliferation test was performed. Collagen deposition was evaluated by ELISA and immunofluorescence staining followed by observation using CLSM.

The microstructural properties of biomaterials were characterized by SEM and micro-CT techniques. To verify the gas exchange through biomaterials, a water vapor permeability test was performed. Additionally, exudate absorption ability test was conducted to assess absorption of simulated exudate by biomaterials after their immersion in human blood plasma and serum.

Based on the results collected so far, it can be concluded that the produced biomaterials show high biocompatibility. Additionally, they hinder the adhesion of fibroblasts to the surface, which might contribute to the reduction of the feeling of pain when changing the dressing. Tested biomaterials were characterized by high total porosity, which was about 70%. The foam-like biomaterials ensure the optimal gas exchange and have the ability to transform into typical hydrocolloid dressings with superabsorbent properties after contact with physiological fluids. The obtained results indicate the promising potential of the biomaterials as a dressing for highly exuding wound management. Additionally, the developed production method of wound dressings enables the introduction of bioactive compounds into their structure at the production stage.

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Bioengineered materials enriched with bioactive compounds dedicated for tissue regeneration

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I perform interdisciplinary studies in the field of biomedicine, regenerative medicine and tissue engineering, with main focus on the development of novel biomaterials for biomedical applications. The new approach to wound repair combines modern strategies consisting of the application of progressive bioactive wound dressings. The aim of my PhD research was to develop a novel production method of hydrogel and foam-like biomaterials for skin regeneration. As the consequence, two completely different biomaterials have been developed and comprehensively evaluated with respect to their structural, mechanical, physicochemical, and biological properties:

- a thin chitosan/agarose film, supportive to adhesion of skin fibroblasts and keratinocytes, that could serve as a potential artificial skin substitute for regenerative medicine applications (patent applications no. P.430458) [1,2],
- highly porous and superabsorbent chitosan/agarose foam-like biomaterial for an external wound dressing applications (patent applications no. P.430457) [3].

Recently, I have started my scientific cooperation with prof. Waldemar Turski from the Medical University of Lublin aimed at determining the influence of kynurenic acid and its derivatives on the proliferation and mineralization of mouse osteoblasts. The planned research will allow a selection the most promising chemical compounds that may positively affect the bone formation process and can be used, for example, in the prevention of osteoporosis. In addition, I perform research aiming to evaluate the effects of chestnut honey (rich in kynurenic acid) on skin regeneration process. For this purpose, the dressings developed as part of my doctoral dissertation were enriched with chestnut honey to improve their biological properties.

Also in cooperation with prof. Tomasz Mroczek (Medical University of Lublin) I do research on the determination of the antitumor activity of plant extracts (endemic propolis from various regions of the world). The research involves assessing the anti-cancer properties of various plant compounds on selected cancer cell lines. The aim of these studies is also the immobilization of bioactive compounds within the polymer matrix in order to create an optimal drug carrier that releases active substances in a controlled manner.

I am also involved as a principal investigator in the implementation of PRELUDIUM 20 grant (financed by National Science Centre in Poland) aiming to develop bioabsorbable nanocomposite granules (hydroxyapatite/biopolymers) with a high specific surface area, which would show increased osteoconductivity (the ability to support the adhesion, proliferation, and differentiation of osteoblasts), antioxidant, anti-inflammatory and antibacterial properties. During this project it is planned to produce hydroxyapatite-based granules, which will be produced as a nanocomposite material made of nanohydroxyapatite (nanoHA) powder sintered at high temperature (1100 °C) and a matrix based on chitosan (agarose/chitosan or curdlan/chitosan) that will serve as the binder for nanoHA. The produced granules will be then subjected to a comprehensive evaluation of their mechanical, physicochemical, microstructural and biological properties.

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Selected supply systems for non-thermal plasma reactors: construction peculiarities, power electronics used and application potential

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This work presents the brief overview of supply systems for non-thermal plasma reactors with dielectric barrier discharge (DBD), atmospheric pressure plasma jets (APPJ) and gliding arc discharge (GAD). The main advantages of these types of reactors are: they produce non-thermal plasma at atmospheric pressure, their design is well developed and relatively simple, the potential area of application is large, they can be powered from similar sources using non-linear transformer magnetic circuits and power electronics [1].

The most important construction element of presented plasma reactor power supply systems is the transformer, which in appropriate implementation can meet most of the requirements for plasma power supplies. Because of the nonlinearity of transformers cores, the simple, dependable, low cost and efficient power systems, especially suitable for industrial applications, can be constructed. Furthermore, transformer-based systems demonstrate good resistivity to radiated and conducted interference generated by electrical discharges. As for disadvantages, the transformer power supply have the relatively narrow range of the discharge power control [2].

Static semiconductor voltage and frequency converters are commonly used high energy electronic devices for the most of reactors power supply [3]. Plasma reactors with DBDs, surface and co-planar discharges, atmospheric pressure plasma jets (APPJ) are often energized from frequency resonant converters, RF and pulse power systems, flyback and forward converters. In case of DBDs, the use of electrical resonance phenomenon allows to obtain higher power density factor of the power electronic converter [4]. Plasma reactors with arc discharges can be supplied with both direct and alternating voltage and pulse voltage.

Highly efficient AC/DC/AC converters are more and more commonly applied in numerous applications of the plasma-chemical process [5]. They enable adjustment of the voltage waveform, as well as the current, voltage, power and frequency regulation, for the purpose of a given plasma process. In the voltage regime inverter is suitable to supply DBD reactor while the current regime is most suitable to supply GAD reactor.

Research on the properties and applications of non-thermal and non-equilibrium plasma at atmospheric pressure requires interdisciplinary cooperation of scientists representing both basic and applied sciences, such as: plasma chemistry and physics, medicine, chemical technology, environmental engineering, material engineering, agricultural engineering, bioengineering, metrology and electrical engineering.

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Triple co-culture spheroid model of pancreatic cancer for plasma research

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Pancreatic ductal adenocarcinoma (PDAC) has low five-year survival rates of 2-9% and is characterized by high resistance towards chemo- and radiotherapy due to extreme desmoplasia [1, 2]. PDAC is predicted to become the third global leading cause of cancer death due to its significant rise in incidence [2]. Therefore, new alternative or combinational treatment approaches are necessary to target this disease and improve survival outcome. Cold atmospheric plasma (CAP) has received increasing attention for oncological research in the last decade. Previous reports have shown that CAP also influences cells in the tumour microenvironment (TME), such as pancreatic stellate cells (PSCs) and endothelial cells (ECs) [3, 4]. In their activated state, PSCs play a crucial role in the propagation, growth and survival of PDAC tumours [5]. However, the effect of CAP on PSCs is not yet fully understood. In addition, knowledge on the effect of CAP on PDAC using complex models including PSCs and ECs is currently lacking. Three-dimensional *in vitro* models are experiencing a rising importance in cancer research.
The aim of this study is to investigate the effects of CAP on PDAC, considering the effects on other cells in the TME. We established triple co-culture (TCC) spheroids of pancreatic cancer cells, PSCs and ECs to better resemble PDAC tumours *in vitro* and improve our study on the impact of CAP in complex TMEs. To optimize this model, we have studied the seeding densities of each cell type and evaluated spheroid diameter, shape, the distribution of each cell type, etc. using live-imaging. Using such complex 3D *in vitro* models will highly increase the predictive power of *in vitro* work and reduce the abundant need of animal studies, which are time consuming and costly while sacrificing animals. We aim to use this model for our plasma research in the near future and combine it with chemotherapeutic drugs to investigate a potential synergistic effect.

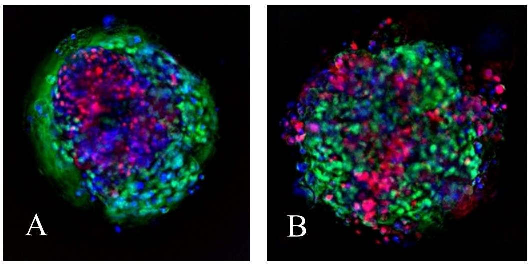

Fig. 1 Two different types of TCC spheroids of PDAC cells (red), PSCs (green) and ECs (blue).

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Bioactive Species Production in He+H_2O Pin-Pin Pulsed Helium Discharges: Dependence on Cycle Frequency

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Nanosecond-pulsed He+H_2O discharges between pin-pin electrodes are an established method for producing biologically relevant species, including H_2O_2, O_3, and OH. In this study the chemistry of such a plasma is analysed using the zero-dimensional chemical kinetics model GlobalKin, with a He+H_2O chemistry set [1][2]. Of particular interest is the relationship between the density of key species and the cycle frequency of the plasma, and by extension the afterglow duration. The duration of the power pulse is kept constant across all cases.

Species densities are averaged during the pulse across ten separate cycles late into the plasma lifetime, when all species have reached equilibrium. It is found that the densities of species with short lifetimes, such as O, H, OH, and e^-, increase consistently with cycle frequency. An example of this is shown in the case of OH, in *Figure 1*. It is also found that the density of longer-lived species, namely H_2O_2 and O_3, exhibit a more complex relationship with the frequency, such as that shown for H_2O_2 in *Figure 2*. Despite the density of OH having a positive relationship with cycle frequency and the dominant reaction producing H_2O_2 being the three-body recombination of two OH radicals, the density of H_2O_2 peaks at 100kHz and decreases steeply at higher frequencies.

Species densities are subsequently analysed with respect to time during a single cycle in equilibrium, for a range of cycle frequencies. It is concluded that despite the increase in OH, the production of H_2O_2 at high frequencies is limited by the reduced afterglow period before it is destroyed during the next pulse, primarily through electron dissociation.

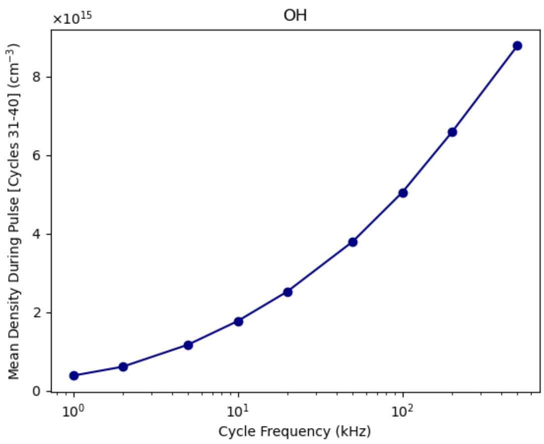

__Figure 1:__ The density of OH during the power pulse when the plasma is at equilibrium, for a range of cycle frequencies from 1kHz to 500kHz. The density is found by averaging the densities during the pulse for cycles 31-40.

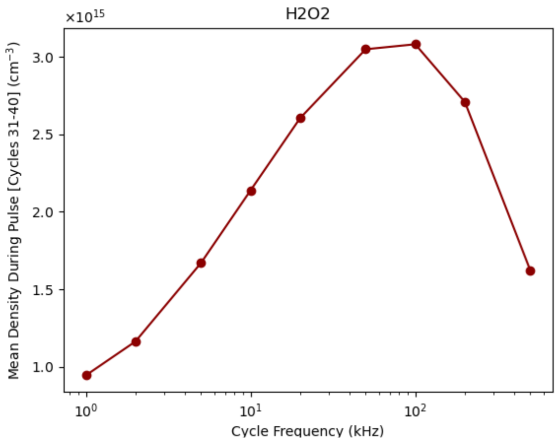

__Figure 2:__ The density of H_2O_2 during the power pulse when the plasma is at equilibrium, for a range of cycle frequencies from 1kHz to 500kHz. The density is found in the same manner as with Figure 1.

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Face Masks Disinfection using Atmospheric Pressure Cold Plasma

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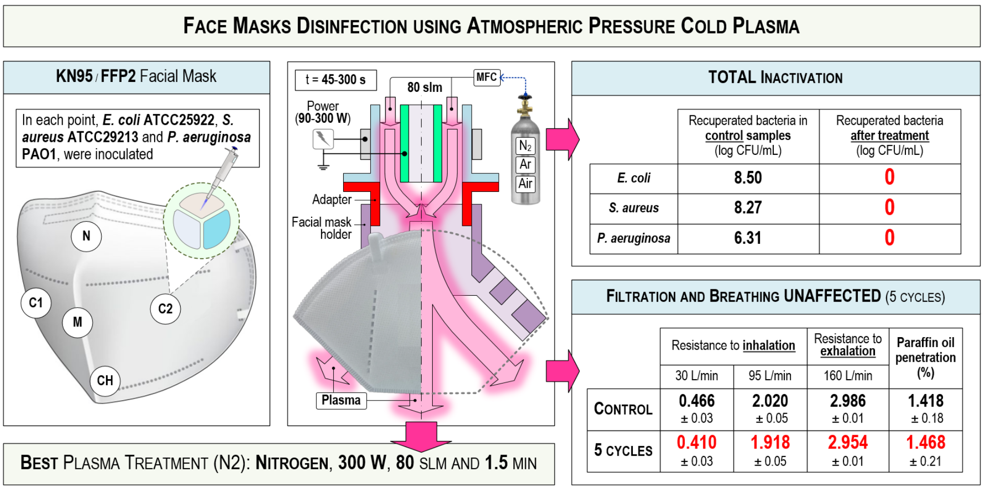
COVID-19 pandemic is worldwide known and has led to a mask shortage around the world. On the other hand, mask diseases could be the result of an incorrect mask usage by either prolonging the time using them or disinfecting through wrongly ways (such as water detergents). For that reason, the present work studied atmospheric pressure cold plasma (APCP) as an alternative technology for correct masks disinfection. Twelve different microorganisms were analyzed (PAO1, *P. aeruginosa* ATCC15692GFP, *E. coli* and *Staphylococcus* strains) which were inoculated in mask disks and full masks. Several parameters were modified in order to study how they affect inactivation. They went from gas used (nitrogen, argon and air), plasma power (90 – 300 W) to treatment time (45 s – 5 min). Then, the best APCP treatment was chosen being the one generated by N_2 gas, 300 W and 1.5 min. Furthermore, filtration capacity (FC) and breathing test were carried out showing no negative effects after 5 cycles plasma treated masks. Besides, neither morphological modifications nor visual deformations on masks were identified after plasma treatments. Finally, there are only two papers where APCP is used with the aim of disinfecting mask disks [1-2]. However, as there are not researches where APCP is used for disinfecting full mask, this work can be considerate as innovative. To sum up, APCP could be a cheap, eco-friendly and sustainable technology for mask disinfection that will let their reusage, ending with mask shortage.



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Delivering plasma-activated microdroplets inside the body in real-time for ultra-low energy cancer treatment

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The possibility of generating low temperature non-thermal equilibrium atmospheric pressure plasmas in contact with liquid has led to a large number of biological and medical applications such as cancer treatment through plasma-induced apoptosis, dermatological applications, and wound healing [1]. The major plasma reactive species mainly rely on the plasma temperature. Gas temperature can vary rapidly in the presence of liquids therefore, gas temperature is a vital factor which impacts the determination of the technological application of each type of plasma [2]. Plasma activated microdroplets has a great importance in developing cancer treatment therapy, however, microdroplets significantly affects the gas temperature so it requires more control especially for biomedical applications that needs heat control such as skin cancer treatment [3,4]. This project aims at studying the chemical kinetics of low-temperature atmospheric pressure plasma. My initial focus is on measuring and controlling gas temperature measurements in the presence of microdroplets using infrared temperature sensor (IR) that provides instant and accurate measurements. Measured gas temperatures, without droplets, varied from 25 ^{o}C – 50 ^{o}C, increasing with absorbed power and decreased gas flow. The introduction into the plasma of a stream of microdroplets (~12 um diameter) led to a reduction in gas temperature of ~10 ^{o}C, for the same absorbed power, Fig. 1. This study also will include monitoring the effect of low energy electrons (LEE) generated by the plasma on the damage of DNA cancer cells and the possibility of delivering activated microdroplets inside the human body for effective cancer treatment.

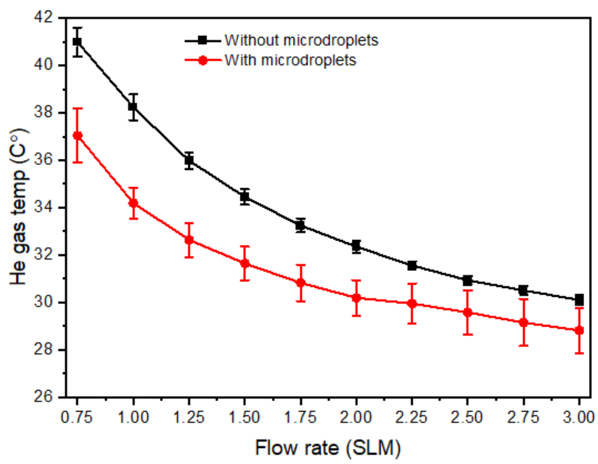

Fig.1. The relation between the He gas total flow rate (Q) and the outer wall temperature with and without microdroplets

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Critical Aspects in the study of Plasma Treated Water Solutions for therapeutic goals

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Non-thermal plasmas (NTP) recently affirmed in the field of life sciences for therapeutic applications due to their ability to generate gaseous blends of Reactive Oxygen and Nitrogen Species (RONS). These molecules can be administered to living matter to target pathological states based on cellular oxidative stress [1–3], including the healing of wounds [4] and cancer treatments. [2,5,6] Beside direct NTP treatments, the delivery of RONS has also been tested by using Plasma Treated Water Solutions (PTWS), namely, biologically inspired liquids (physiological and injectable solutions, cell culture media, etc.) treated with NTP and used as vectors for delivering RONS to cells or tissues.[7]

The immediate advantage of PTWS-based therapies is the possibility to inject the active solution with controllable and detectable chemical composition into various district of the body. However, many critical aspects confine the potentialities of these liquids as novel therapeutic agents. The most relevant aspects include: the control of plasma treatment and the choice of the liquid to be treated [8], its analysis after plasma treatment[9] and the biological protocols used to test PTWS with living matter[10]. Inhomogeneity in these aspects currently limits the understanding of the clinical efficacy of these liquids, and makes it difficult to compare results from different laboratories. The aim of this short presentation is to briefly summarize the possible approaches to produce, to analyse and to test plasma-treated liquids to selectively attack cancer cells, with the aim to provide an overall view on the topic and highlight the major challenges in the use of these liquids for the biomedical field.

Keywords: Plasma Medicine | Reactive Oxygen and Nitrogen Species | Cancer treatment | Plasma-Liquid Interactions

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Antibacterial action of air transient spark discharge: Physical disruption vs. chemical oxidation

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Inactivation of microorganisms in liquids by cold plasmas (CPs) is a promising alternative to conventional decontamination methods as it is efficient, gentle to heat-sensitive materials, and environmentally friendly. CP is a highly reactive gas (air) mixture of reactive oxygen and nitrogen species (RONS), charged particles, and UV radiation [1]. CP-induced cell damages are the result of physical and chemical effects. Depending on plasma processing parameters and microbial physiological characteristics, a certain effect might prevail and lead to different inactivation pathways. The microbial inactivation by plasma species is due to their interaction with membrane lipids, proteins and DNA. Strong oxidative properties of plasma species and their accumulation during plasma treatment can cause cell membrane damages, cytoplasmic deformations, and leakage of intracellular content [2][3].

The present work is focused on the study of bacterial inactivation mechanisms induced by air transient spark (TS) discharge plasma [4]. Planktonic *E. coli* and *S. aureus* in physiological saline solution were treated for 5 - 20 min. Plasma induced changes in the membrane integrity and accumulation of intracellular reactive oxygen species (ROS) were analysed and correlated with the survival data. The morphological changes of bacteria exposed to plasma were analysed by SEM. Physicochemical properties of plasma-activated saline were also evaluated to better understand the complex molecular mechanisms of the plasma–bacteria interaction pathways.

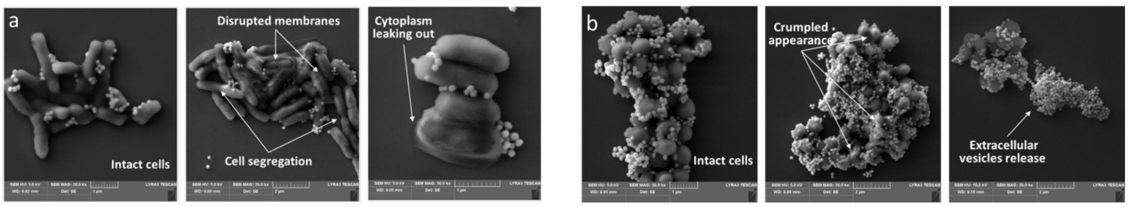

Figure 1. SEM images of intact and plasma (15 min) treated a) *E. coli* and b) *S. aureus*.

It has been shown that plasma treated *E. coli* rapidly lost the membrane integrity (in 5 min) and accumulated intracellular ROS that led to a complete inactivation (in 15 min). Multiple morphological changes were observed (Fig. 1a). On contrary, plasma treated *S. aureus* exhibited only slight cell deformations (Fig. 1b), better preserved its integral membrane, and did not accumulate intracellular ROS. Most likely, *S. aureus* could maintain the redox balance of integral cells, which helped them to survive. The results highlighted the selective role of plasma species on different bacteria in plasma-based inactivation mechanisms.

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Polycatecholamines as biosafe and innovate curdlan hydrogel modifiers

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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 risk of bacterial infection is one of the problems encountered when treating a difficult-to-heal wound or other similar medical procedures.

Currently, various synthetic and natural polymers are used for regenerative medicine. One of natural polymers of high biomedical potential is curdlan which forms hydrogel of high strength and elasticity. Also, it exhibits high water-absorption and retention capacity, therefore may be useful in fabrication of e.g. hydrogel dressing materials or other biomaterial resources [1]. Coupling of therapeutical substances to curdlan matrix could increase its biological potential. However, the disadvantage of curdlan is the lack of active groups for modifications, such as amino and carboxyl groups which could enable binding of antibacterial or anti-inflammatory agents. Strategies used for curdlan functionalization include, among others, sulfonation, amination, oxidation, esterification or phosphorylation [1, 2] but they typically lead to curdlan solubilization and loss of beneficial physicochemical properties.

A possible solution to this challenge is noninvasive (by surface deposition) modification of curdlan fibers with highly adhesive polydopamine (PDA). Dopamine polymerizes (in most cases pH 8.5 and room temperature) to create PDA which spontaneously deposits on various types of organics and inorganics substrates with a layer width of up to 50 nm [3]. However, levodopamine (L-DOPA) being a dopamine precursor in the biochemical synthesis pathway (with free carboxyl group in its structure) also forms a highly adhesive coatings but it is very rarely used for surface functionalization [4].

Curdlan hydrogel functionalization was performed by dopamine monomer or L-DOPA monomer polymerization [5, 6]. It is extremely important to carefully select the conditions for polycatecholamine deposition mode in the polymer matrix because it may exert very high impact on physicochemical and biological properties of such-produced biomaterial.

We have proved that the curdlan matrix modified with these polycatecholamines allows for efficient binding of the aminoglycoside antibiotic - gentamicin, demonstrating antibacterial activity against three reference strains of bacteria. In comparison with polydopamine, little is known about biological safety of poly(L-DOPA) coatings and their efficacy in binding of therapeutically active substances. In connection with the above we decided to carry out a comprehensive biosafety characterization of matrices made with the use of L-DOPA monomer. We confirmed nontoxicity of poly(levodopa)-modified hydrogels for primary fibroblasts culture and in zebrafish model. Moreover poly(L-DOPA)-deposits on curdlan matrix showed stability in organic liquids and safety for blood properties. Our results encourage further research on polycatecholamines as surface modifiers. We believe that it would be worth to verify the effect of plasma treatment on stability and biological properties of manufactured matrices.

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Treatment of liquids by DBD type of plasma jet for application in plasma therapy

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In the last decades, it has been shown that the desired effects on the living matter can be achieved by indirect application of plasma. The scientific effort on atmospheric pressure plasma jets (APPJs) has gradually shifted from their investigation in free flow conditions to the study of their interaction with different liquid targets [1] [2]. In the case of liquid target, the gas phase plasma chemistry and the plasma chemistry in the gas/liquid interface induce chemical reactions in the liquid phase. As a result, reactive species are created in the liquid phase that are, after implementing the treated liquid to the sample, responsible for the effects on the samples (bacteria, plant cells, human cells, cancer cells, stem cells, etc.)[3] [4].The chemistry, both in a gas phase and liquid phase-sample, depends on the plasma parameters (type of gas mixture, gas flow, concentration of electrons, temperature of electrons, deposited power etc.), and on the type of liquid that is being treated by plasma.

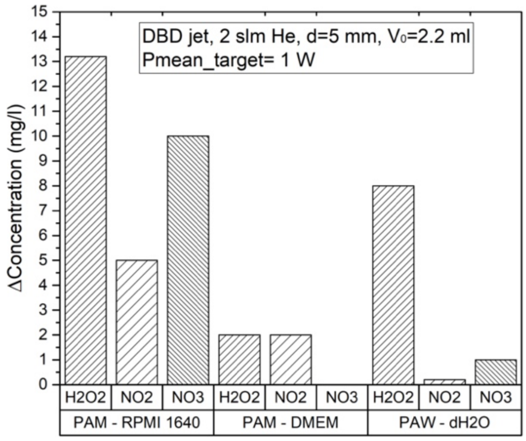

Fig. 1 Concentration increase of reactive oxygen and nitrogen species in plasma activated liquids after 5 min of treatment and 1 W power deposited in the plasma treatment by DBD jet

Fig. 1 shows the increase in concentrations of reactive species in three different liquids – cell culture mediums RPMI 1640, DMEM and distilled water after 5 minutes of plasma treatment. Plasma source was a dielectric barrier discharge (DBD) type of the APPJ system with two electrodes powered by a kHz sine wave high-voltage. For all liquids, the deposited power in plasma treatment was 1 W. Helium was used as working gas with the 2 slm gas flow. In the untreated RPMI 1640 medium the concentration of nitrate was 32 mg/l, while there were no nitrite and hydrogen peroxide. There were no reactive oxygen and nitrogen species measured by colorimetric and spectrophotometry methods in DMEM medium and distilled water before the treatment. Although the treatment conditions are the same, the concentrations of H_2O_2 is the highest in PAM-RPMI 1640. Nitrate concentration in PAM-RPMI 1640 also increased after treatment while in PAM-DMEM the colorimetric method did not show any nitrates after treatment. The concentration of H_2O_2 predominates over nitrates and nitrites in PAW–dH_2O. The differences in concentrations stem from the different chemical compositions of these three liquids that induces different chemical reactions during plasma treatment. Consequently, different concentrations of reactive species in activated samples will give different effects on the living matters.

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The influence of Cold Atmospheric Plasma on the morphology of dentin-adhesive interfaces

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The use of plasma in dental medicine has begun thanks to its antimicrobial and surface modifying properties [1], so it has been tested in various dental professional fields, such as adhesive and prosthetic dentistry, implantology, and periodontology. Due to the surface free energy increase caused by the plasma treatment, many dental tissue-material interfaces have been improved in terms of bond strength [2] and the structural changes affecting the interface durability.

In our study, we used Cold Atmospheric Plasma (CAP) to modify the surface properties of dentin after the conventional procedure of caries removal and etching protocol for dental adhesives. Human molar teeth were cut in half to ensure the use of the same biological sample, for the experimental and the control group. Dentin was treated by orthophosphoric acid as usually done for the "etch-and-rinse" adhesive procedure (ER) and then treated with CAP produced by a RF plasma needle. He was used as a feeding gas, gas flow 1 slm, deposited power in the plasma of 1 W or 2 W, and tip-to-surface distance of 2 mm. After the CAP treatment, Single Bond (*3M* ESPE) was applied. As this adhesive is suitable for both ER and self-etch adhesive techniques (SE), the CAP treatment was also applied on dentin prior to SE bonding placement. The control samples in ER and SE was prepared according to standardized procedures. After the CAP modification of the dentinal surface, contact angles of adhesive were measured, and the surface free energy (SFE) was calculated for distilled water, ethylene-glycol, and diiodomethane, in treated and control samples. The morphology of dentin adhesive interfaces was examined by the Nonlinear Laser Scanning Microscopy (NLSM) [3].

Due to the increase of dentinal surface free energy caused by CAP treatment, the adhesive created lower contact angles on CAP-treated dentin. This caused the drastically changed microstructure of the dentin-adhesive interface, as observed by NLSM. The adhesive flow to dentinal tubules was considerably enhanced, causing the more frequently present, way longer resin tags in dentin, and the thicker hybrid layer, more interdigitally intertwined. CAP treatment demonstrated considerable enhancement of bonding at dentin-adhesive interface, thus making a promising result for application of plasma in dentistry.

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Hydrogels for the delivery of plasma-generated reactive oxygen and nitrogen species

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Plasma-treated liquids (PTL) are gaining increasing attention in the medical field, especially in oncology, as it has been reported that PTLs can selectively kill cancer cells by triggering apoptotic mechanisms [1]. In this context, PTLs represent a very interesting alternative to direct cold atmospheric plasma (CAP) treatment because they may allow treatment of malignant tumors located in inner organs of the body by means of an injection. However, the injection of a liquid in the tumor site may result in fast diffusion throughout the body due to extracellular fluids and blood flow, thus reducing the efficiency and efficacy of the PTL treatments. Besides, multiple PTL injections are needed to induce biological effects in vivo [2]. For these reasons, new strategies with hydrogel-forming polymer solutions have been recently investigated [3,4], with the aim of designing new efficient vehicles that allow a controlled and localized release of reactive oxygen and nitrogen species (RONS) directly to the tumor site. Methylcellulose (MC) is a water-soluble cellulose derivate that can form a thermoreversible hydrogel in water upon heating. Sol-gel transition of pure MC is ~ 60 ºC, too high for in vivo purposes as an injectable hydrogel. However, the gel point can be altered by the addition of salts.

In this work, we developed a thermosensitive hydrogel with the ability to form a gel at physiological temperatures while remaining in liquid phase at room temperature to allow a suitable and homogeneous formation of CAP-generated RONS. Treatment times from 60 up to 300 s were studied using a kINPen® plasma jet as CAP source. Concentrations of 350 μM ,75 μM and 175 μM of H_2O_2, NO_2^- and NO_3^-, respectively, were detected for the longest CAP treatment (Fig 1i). Characterization techniques such as infrared spectroscopy, nuclear magnetic resonance, size exclusion chromatography were performed in order to study physical and modifications of MC chemical structure after plasma treatment. In vitro experiments showed that cytotoxic effects of CAP-treated MC towards MG63 cancer cells are time-dependent, showing a direct relation with the concentration of RONS (Fig 1i,ii)

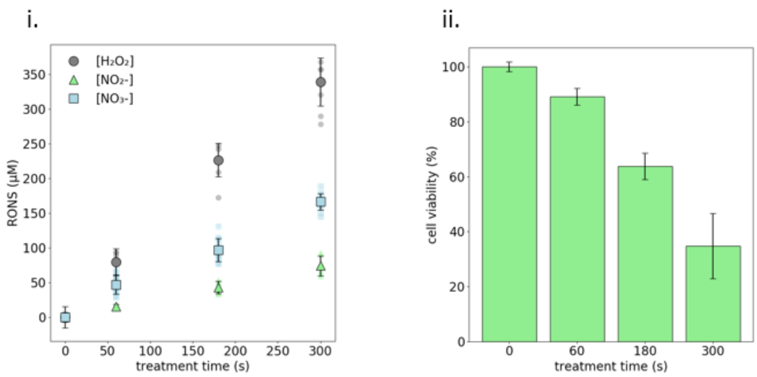

Figure 1. Influence of CAP treatment time on i) the generation of RONS in the MC polymeric solution and on ii) the metabolic activity of MG63 osteosarcoma cell line after 24h exposure of CAP-treated MC.

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Cold Atmospheric Plasma: Its role in Cancer Research

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Cold atmospheric plasma is a form of treatment used in various fields of medicine, especially in cancer treatment. This treatment method, which has in vitro and in vivo effects, triggers apoptosis by producing reactive oxygen species. The selective lethal effect of this treatment method on cancerous tissue without destroying the normal tissue has been tested in various studies. Because cold atmospheric plasma has a temperature below 40 C^0, it does not show thermal effect and can be applied to living tissue [1].

Studies show that CAP application in melanoma, colon, bladder and breast cancer selectively causes reduction in tumor volume. It has been determined that CAP application triggers apoptosis in tumor cells independent of p53 status. It has been shown that 20-40 seconds of CAP administration inhibits tumor growth in breast adenocarcinoma and triggers apoptosis in patients with metastatic head and neck cancer [1].

Since the basis of this application is based on the increase of reactive oxygen and nitrogen species, it has also been evaluated in terms of potential genotoxic effects. Studies show that CAP application does not cause a significant increase in genotoxicity risk. It has been shown that the ROS/RNS produced by CAP application causes an increase in the expression of NOX-1, catalase and SOD in tumor cells. The increase in NOX-1 expression triggers the formation of extracellular superoxide anion. These anions inhibit catalase, trigger apoptosis in HOCl and NO/peroxynitrite pathways. Since this effect is limited to the area of superoxide anion production, the tumor tissue regresses and the healthy tissue is not damaged by CAP application [2].

In summary, CAP application can be considered as an adjunctive treatment method, especially in treatment-resistant and advanced cancer cases.

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Low-temperature plasma and ozone disinfection of FFP2 type respirators

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With prevailing COVID-19 pandemic situation, single-use respirators represent the first personal protection, but also an increasing environmental pollution burden as significant sources of microplastics to the environment [1]. A possibility of safe reuse of respirators would be both environmental and economical friendly. Cleaning of reusable respirators can be done by immersing them in warm cleaning solution, suitable disinfectant or by using UV radiation. Another efficient method represents non-thermal plasmas generated by numerous types of electrical discharges, known for their highly efficient antimicrobial properties and decontamination effects of various materials.

In this study, we used Gram-negative bacteria *Escherichia coli* as a non-pathogenic model microorganism for investigations of antimicrobial properties of two atmospheric air plasma discharges (multi-electrode pulsed corona and DBD) under different conditions. FFP2-type respirators were inoculated by overnight culture of *E. coli* using an airbrush and then dried for one hour. The antimicrobial effects of cold plasmas were examined on dried and humidified respirators. Since ozone is produced by air plasma discharges and acts as an antibacterial agent, we used an ozone generator to also separately evaluated the role of ozone on the decontamination of respirators.

The results showed a promising potential of using air plasma discharges for surface decontamination of disposable respirators. The surfaces of the respirators were successfully disinfected up to 4 log reduction without causing any visible material damage. Methodology for bacterial application on the respirators was optimized. Generally higher efficiencies were achieved for humidified respirators than dry ones. The decontamination rate increased with the plasma processing time and the plasma power, and it also depended on the plasma chamber volume. Separate effect of ozone only was shown to be weaker than the direct effects of plasma discharges.

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Characterization of the non-thermal plasma treatment outcomes on biopolymer solutions

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Non-thermal plasma has been extensively investigated in the last decade, as a potential therapy for tumour treatment due to its selectivity in killing tumour cells without negatively affecting the surrounding healthy tissues. As drawbacks, direct plasma treatments would have a large impact on patients since they would require surgery and indirect treatments, using physiological liquids or cell culture media, would have a very limited effect since the reactive species introduced in the tumour site would be quickly washed away from the blood stream.

A promising strategy to overcome these limitations is the use of hydrogel-forming biopolymer solutions as carriers for plasma-generated reactive species. These liquids have been shown to be able to store these species and to generate stable hydrogels under suitable external conditions in order to deliver these species locally in the site of interest [1, 2]. What still need to be assessed, for a safe use of plasma-treated biopolymer solutions on patients, is the direct effect of the treatment on the polymers structure.

The aim of this contribution is the investigation of the nature and extent of the modifications induced by non-thermal plasma treatment on biopolymer solutions. The approach we use is twofold: i) Direct study of polymers, by conventional techniques used in polymer science (size exclusion chromatography, rheology, vibrational spectroscopy) and ii) Study of molecular models with similar chemical properties, by chromatography with UV-vis or MS detector.

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Study of non-conventional Photosensitizer and its encapsulation in Liposomes for Phototherapy Application

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Photodynamic Therapy (PDT) has been investigated for several diseases including some types of cancer [1]. The efficacy of the PDT action depends on three main factors that are the presence of oxygen in the target tissue, a light source and the photosensitizer. With these conditions occurs the formation of reactive oxygen species that will consequently lead to cell death [2]. However, some photosensitizers present problems such as poor solubility and aggregation which interferes with the proper accumulation of molecules in the target tissue [3]. Therefore, nanocarriers such as the liposomes present many advantages and can be used to overcome these problems, enhance the cellular uptake and the efficacy of PDT [1,4]. In our studies a group of potential photosensitizers were investigated regarding their phototoxicity in skin cancer cell line. The most promising molecules were used for the encapsulation in the nanoparticles. Liposomes were produced by the thin-film hydration method followed by extrusion through a 0.2 μM polycarbonate membrane and different compositions were tested in the optimization process to achieve the most suitable formulations. The lipids tested were the zwitterionic lipid 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), the electrical negative lipids 1,2-dipalmitoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (sodium salt) (DPPG) and 1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt) (DOPG), and cholesterol. The addition of surfactants and their effect on the nanoparticles size was also investigated.

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Solvation of Gaseous H_2O_2, HNO_2, NO_2, NO, and O_3 into Water Aerosol and Electrospray

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Plasma–liquid interactions have become of high-rank interest and importance in many applications because they produce reactive oxygen and nitrogen species (RONS) which are delivered into liquids [1]. Electrospray (ES)/Aerosol of liquids is one of the efficient ways to accelerate the transport of RONS into water by producing droplets of liquid in the sub-micrometer dimensions with a large surface-area-to-volume ratio [2]. The solubility of the gas species in liquids, e.g., water under the equilibrium conditions is given by Henry's law solubility coefficient (K_H) where the achieved concentration for each species dissolved is significantly different [3,4].

This work presents an investigation of the transport mechanism of gaseous RONS: H_2O_2, HNO_2, NO_2, NO, and O_3 generated by several external sources separately into water microdroplets. Deionized water microdroplets are produced in two ways. First: charged microdroplets with different sizes (~20-300 µm) during ES produced by the positive dc high voltage applied on the needle electrode. Second: non-charging microdroplets using the atomizer compressor nebulizer to produce mist/aerosol of water microdroplets with the same sizes (~5 µm).

During the ES process, the solvation of the gaseous H_2O_2, HNO_2, NO_2, NO, and O_3 into water is enhanced by increasing the applied voltage which increases gas-water interface surface area [5]. H_2O_2 was solvated in water with 4 orders of magnitude more efficiently than O_3, despite the 7 orders of magnitude larger Henry's law coefficient. This is because of the insufficient amount of gaseous H_2O_2 which is completely depleted from the gas [6]. HNO_2 is solvated into the water as a dominant source of NO_2^- with 3 orders of magnitude higher than O_3, which corresponds well with Henry's law coefficient [7]. NO_2 is solvated in water also making aqueous NO_2^-, with 2 orders of magnitude higher efficacy than O_3 despite similar Henry's law coefficients due to the quick diffusion of NO_2^- into the volume. NO also seems better soluble than predicted, especially at higher voltages where a weak corona discharge is ignited: OH radicals are generated and interact with NO to produce the highly soluble HNO_2. In the nebulizer results, the solvation of H_2O_2, NO, and O_3 is higher than that found in ES due to the larger surface-area-to-volume ratio obtained during the mist/aerosol. While the solvation of HNO_2 and NO_2 as NO_2^- is limited because they also ionize to NO_3^- once entering water, where NO_3^- was found higher than NO_2^- in the collected water which makes it saturated. Based on the obtained results, the solubility of gaseous species is not determined purely by Henry's law. These results can lead to a better understanding of the transport mechanism of gaseous RONS generated by plasma into water and will enable optimization of plasma–liquid interaction systems.

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Detecting emergent organic contaminants in water matrices

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Detecting traces of emergent pollutants in water matrices throughout the ecosystem remains to this day a pivotal endeavour. These pollutants originate from a myriad of sources like industrial products and by-products, pesticides, human waste, pharmaceuticals and personal care products (also referred to as PPCP), endocrine disrupting chemicals (EDC), amongst many others [1]. Although these compounds are present in the environment at rather low concentrations, which by itself should not pose a large threat, it is their continued growth and build-up from the day-to-day usage that has been leading to significant, and pernicious, consequences both in flora and fauna. These consequences range from elevated risk of cancer (prostate and breast cancer in particular) to atrophy of reproductive organs and their functions on various animals (sheep, cows, fish) [2]. In order to tackle this issue, several policies have been devised and implemented to ensure that these contaminants are monitored, studied and in some more dire cases even banned from use as to prevent further damage to the ecosystem [3]. To this end, the present work was envisioned and put into practice to develop new sensor devices based on molecularly thin-films capable of detecting and possibly monitoring the presence of these emergent compounds such as Triclosan (TCS, 2,4,4'-Trichloro-2'-hydroxydiphenyl ether) and 17α-Ethynylestradiol (EE2) through impedance spectroscopy.

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Optical diagnostic of transient spark with electrospray

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Interaction of non-equilibrium plasma with liquid are of great significance in a wide range of plasma-liquid system because the properties of plasma as well as of liquid charge in time under their interactions [1]. Atmospheric air plasma in contact with liquid water generates a unique functional solution called as plasma activated water (PAW). This solution contains various reactive oxygen and nitrogen species (RONS) for example H_2O_2, NO_2^-, NO_3^- and O_3, as well as other short-lived reactive species [2]. This activity in plasma activated water is the result of transport/dissolution of RONS into the water from gas. The plasma and liquid interface area is an important parameter that determines the obtained plasma activated water solution by maximizing the contact between plasma and water. The fine droplets compared to bulk water have more surfaces to volume ratio, thus accelerating the transport of RONS into the water. This concept has been adapted by our research group in our previous work [3]. The electro hydro dynamic spray/ electro spray is a simple process to produce micro droplets from bulk liquid by a stray electric field. In our previous work, Transient Spark (TS), a DC-driven self-pulsing discharge generating highly reactive atmospheric pressure air plasma, was employed as a rich source of NOx. It was observed that transient in dry air generates high concentration of NO and NO_2 as compared to humid air where the concentration of NO_2 decreased in favor of HNO_2. The advantage of transient spark is its capability of simultaneous generation of the plasma and the formation of microdroplets by electrospray of water directly into the discharge zone. It was shown that gaseous HNO_2, rather than NO or NO_2, plays a major role in the formation of NO_2 (aq.) in PAW. This was studied by comparing TS with ES in a one stage system and TS operated in dry or humid air followed by water ES in two stage system [4]. Currently, the research is going towards the optimization of the HNO_2 generation by TS and of the two stage system with a shorter distance between TS and ES reactors. Apart from the use of humid air and dry air, various input mixtures of $N_2/O_2/NO/H_2O$ will be used so that the treated air spray by microdroplets contains different reactive oxygen and nitrogen species, such as NO, NO_2, HNO_2, H_2O_2 or O_3. Investigation will be performed with different gas and water flow rates and electrospray settings. The phosphate buffer solution will be used as the input liquid for the electrospray to avoid acidification of water solution and to suppress chemical reaction in collected liquid solution that would occur under acidic condition. We will study the transport of reactive species from plasma to water droplets by measuring change of RONS concentration in the gas phase after application of water electro spray and by analyzing RONS in the water by UV/Vis absorption spectroscopy, namely H_2O_2, nitrites, nitrates, and O_3. The gaseous RONS will also be measured using UV/Vis absorption spectroscopic method, in combination with electrochemical sensors spectrometry.

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The effect of Plasma Activated Liquid on Uropathogenic *E. coli*

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Uropathogenic *E. coli* (UPEC) is a prevalent pathogen causing urinary tract infections (UTI), with potentially severe consequences in case of recurrent infection. While antibiotic administration is currently the treatment standard, their overuse can lead to acquired resistance [1], increasing the need for novel treatment approaches. Cold plasma sources produce a variety of agents such as reactive oxygen (ROS) and nitrogen species (RNS), and antimicrobial effect of plasma activated liquid (PAL) have already been showed [2]. Thus, the aim of this study was to investigate whether PAL has antimicrobial effect on UPEC with potential application in animal model of UTI.

Four types of PALs prepared from PBS by different discharge settings (Streamer Corona (SC), Transient Spark with Electrospray (TS-ES) and Transient Spark Batch (TS-B), Glow Discharge (GD)) were tested in inactivation of UPEC. Overnight culture of E. coli was diluted in Luria-Bertani medium to 8×10^8 CFU/ml and 100-fold dilutions were prepared. 10 µl of bacterial culture was mixed with 10 µl of liquid Luria-Bertani medium and 180 µl of PBS/PAL (SC/TS-ES/TS-B/GD) in 96-Well Plates. Optical density of samples was measured every hour up to 32h with seeding of bacteria on agar plates performed at 5 timepoints (0, 4, 8, 12, 24h). Antimicrobial effect of the strongest PAL was further tested in a 1:1 ratio with a mouse urine. 10 µl of bacterial culture was mixed with 10 µl of liquid Luria-Bertani medium and 90 µl of the mouse urine/90 µl of GD PAL/90 µl of the mouse urine + 90 µl of GD PAL. Optical density was measured every hour up to 24h with bacteria seeding on agar plates performed at 5 timepoints (0, 4, 8, 12, 24h).

Based on *in vitro* tests using uropathogenic *E. coli*, GD PAL showed the strongest antimicrobial effect in comparison to other types of PALs and was further tested with the mouse urine. GD PAL mixed with the mouse urine surprisingly had not decreased the bacterial burden at all. Moreover/Furthermore, the mixture of urine and GD PAL allowed faster bacterial growth than the urine itself. This effect could be explained by the ability of urine to scavenge •OH radicals or the change in pH when PAL is mixed with urine, but this was not proved in our experiment.

In conclusion, PAL itself is a promising in inactivation of bacteria. Although GD PAL had the highest antimicrobial effect on the bacterial growth in comparison with SC, TS-ES, TS-B PALs, and PBS, our results showed a loss of antimicrobial effect when GD PAL was mixed with the mouse urine. Therefore, we would suggest testing another type of PAL, which may not be so potent on itself, but carries another type of ROS/NOS and in combination with urine could result in antimicrobial effect. Based on our results, the detailed mechanism of the effects of urine on PAL requires further investigations.

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The effect of plasma activated water in mouse model of colitis

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The usage of plasma activated water (PAW) in biomedicine is progressing thanks to the described beneficial effects [1,2]. Ulcerative colitis, global chronic disease with unknown pathogenesis, is characterized by uncontrolled inflammation and imbalance between reactive oxygen species (ROS) and antioxidant capacity in the intestinal mucosa and even more [3]. The main aim of our study was to investigate how daily application of PAW affects the development of colitis and oxidative status in the mouse inflamed colon.

Colitis was induced by 7-day intake of 3% dextran sulphate sodium in tap water. PAW or control water was administrated to the colon by colonoscopy instrument daily. Colitis mice with PAW treatment started to progressive lost weight the day before colitis mice with control water. However, colon length as another marker of disease activity was unchanged. Furthermore, markers of oxidative stress, were measured in colon homogenates, including thiobarbituric acid reacting substances (TBARS), advanced oxidation protein products (AOPP) and response to oxidative stress - total antioxidant capacity (TAC). All markers were increased in colon of PAW-treated mice, but most in an inflamed colon. The presence of certain bacterial strains in colon contributes in part to the development of inflammation. Therefore, 16S rDNA from stools collected at last day of the experimental colitis were analyzed. The PAW application led to significant shifts in gut microbiota composition in both healthy and colitis mice. Analysis of metabolic pathways associated with oxidative stress revealed that the effect of PAW was only in healthy mice.

Taken together, PAW increased oxidative stress in inflamed colon and therefore may not to be the optimal therapeutic tool for colitis. Regarding to microbiome analysis and metabolic pathways, more analyses are needed to draw conclusions.

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