https://www.plasther.eu/events-archive/2nd-annual-meeting-plasther-bologna-2023/

1.1 2nd Annual Meeting – 4th – 7th September 2023, Bologna, Italy

The PlasTHER COST Action's primary objective is to exploit the unprecedented potential of atmospheric pressure plasmas in medicine. This endeavor aims to foster the sharing, development, and consolidation of ongoing research into plasma-assisted viable therapies. The ultimate goal is to establish Europe as a leading force in the scientific and healthcare arenas concerning plasma therapies.

The 2nd annual meeting, held in person in Bologna (Italy), September 4th – 7th, 2023, assumes significant importance as it provides a pivotal opportunity for participants to convene, thus contributing to creating a cohesive and solid community. This gathering aims to collectively address community-shared objectives, primarily enhancing plasma therapies' performance to maximize patient benefits.

The essential purpose of the meeting is to facilitate the exchange of the most recent advancements in comprehending the fundamental mechanisms underpinning plasma actions. Additionally, it seeks to generate harmonized protocols that can be adopted throughout the community, extending to various therapeutic domains. A critical outcome of the meeting will be the formulation of individual roadmaps for each area of interest.

Throughout the meeting, we will focus on the COST tasks undertaken by all our working groups, fostering collaboration to produce specific deliverables. From this perspective, the meeting will also involve presenting outcomes from Short-Term Scientific Missions (STSMs) conducted in recent months by the researchers of the cost community.

1.2 Working Groups and Memorandum of Understanding (MOU)

WG1: Fundamental plasma-biological interaction mechanisms

Dr Angela PRIVAT-MALDONADO University of Antwerp, Belgium

angela.privatmaldonado@uantwerpen.be

Dr Ana SOBOTA Technische Universiteit Eindhoven, Netherlands a.sobota@tue.nl

WG2: Antimicrobial effects of plasma

Dr Romolo LAURITA Alma Mater Studiorum- Bologna University, Italy romolo.laurita@unibo.it

Dr Daniela BOEHM Technological University Dublin, Ireland daniela.boehm@tudublin.ie

WG3: Tissue regeneration

Dr Eloisa SARDELLA Institute of nanotechnology – CNR- Nanotec, Italy eloisa.sardella@cnr.it

Dr Marwa BALAHA Università degli Studi G. d'Annunzio Chieti e Pescara, Italy

marwa.balaha@unich.it

WG4: Plasma cancer therapy

Dr Sander BEKESCHUS Leibniz Institute for Plasma Science and Technology (INP), Germany

sander.bekeschus@gmail.com

Dr Lars BOECKMANN University Medical Center Rostock, Germany lars.boeckmann@med.uni-rostock.de

WG5: Combination therapies

Dr Joanna SADOWSKA Royal College of Surgeons in Ireland, Ireland joannasadowska@rcsi.ie

Dr Cédric LABAY Universitat Politécnica de Catalunya (UPC-BarcelonaTECH), Spain

cedric.labay@upc.edu

WG6: Regulatory, ethics, dissemination & technology transfer

Dr Sara LAURENCIN-DALICIEUX Université Paul Sabatier, France laurencin.s@chu-toulouse.fr

Dr Eric ROBERT CNRS/University of Orléans, France eric.robert@univ-orleans.fr

PDF of the TECHNICAL ANNEX OF THE MOU

1.3 Local Organizing Committee

Romolo Laurita - *Alma Mater Studiorum-Università di Bologna*

Pasquale Isabelli - *Alma Mater Studiorum-Università di Bologna*

Matteo Gherardi - *Alma Mater Studiorum-Università di Bologna*

Filippo Capelli - *Alma Mater Studiorum-Università di Bologna*

Giulia Laghi - *Alma Mater Studiorum-Università di Bologna*

Roberto Montalbetti - *Alma Mater Studiorum-Università di Bologna*

Maria Chiara Grande - *Alma Mater Studiorum-Università di Bologna*

Caterina Maccaferri - *Alma Mater Studiorum-Università di Bologna*

Andrea Marchetti - *Alma Mater Studiorum-Università di Bologna*

Francesco Tomelleri - *Alma Mater Studiorum-Università di Bologna*

Vittorio Colombo - *Alma Mater Studiorum-Università di Bologna*

1.4 International Scientific Committee

Cristina Canal - *Universitat Politècnica de Catalunya, Spain, Action Chair*

Sarah Cousty - *CHU Toulouse, France*, *Action Vice-Chair*

Angela Privat Maldonado - *University of Antwerp, Belgium*

Romolo Laurita - *Alma Mater Studiorum – Università di Bologna, Italy*

Eloisa Sardella - *CNR-NANOTEC, Italy*

Sander Bekeschus - *Leibniz Institute for Plasma Science and Technology, Germany*

Joanna Sadowska - *Royal College of Surgeons in Ireland, Ireland*

Sara Laurencin-Dalicieux - *Paul Sabatier University, France*

Susana Serio - *FCT/UNL, Portugal*

Nikola Skoro - *Institute of Physics Belgrade, Serbia*

Ana Sobota - *Technische Universiteit Eindhoven, Netherlands*

Daniela Boehm - *Technological University Dublin, Ireland*

Lars Boeckmann - *University Medical Center Rostock, Germany*

Cédric Labay - *Universitat Politécnica De Catalunya (Upc-Barcelonatech), Spain*

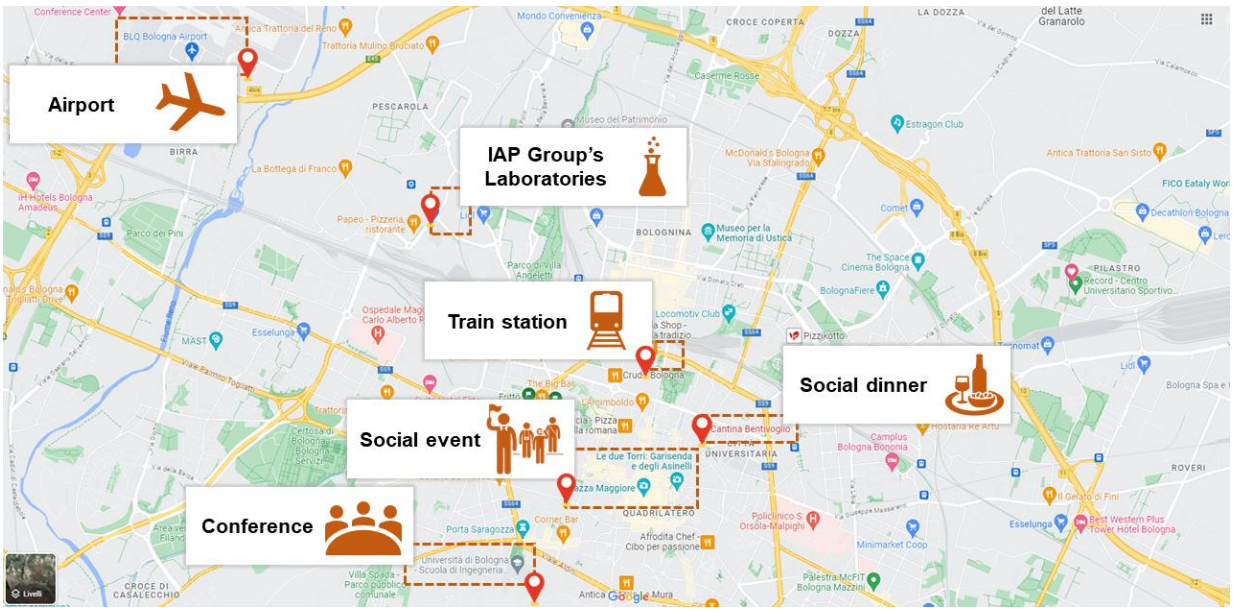
Eric Robert - *Cnrs/University Of Orléans, France*

1.5 Venue

The 2nd Annual Meeting of COST Action PlasTHER "Therapeutical applications of cold plasmas" is an in-person event held in Bologna at the Alma Mater Studiorum-Università di Bologna, Faculty of Engineering, II floor, Viale del Risorgimento 2.

Bologna is a city in northern Italy known for its rich history, architecture, and cuisine. It is the capital of the Emilia-Romagna region and has a population of about 390,000 people. The city is home to the oldest university in the Western world, the University of Bologna, founded in 1088 (The history of the University of Bologna). Bologna is also famous for its beautiful medieval architecture, including the Two Towers and the Basilica di San Petronio. The city's cuisine is renowned for its delicious pasta dishes, such as tagliatelle al ragù and tortellini in brodo.

Bologna is strategically well located in the heart of northern Italy, a long-standing transit, exchange, and destination hub for many major travel infrastructures. Located on the Via Emilia, the city lies a short distance from the sea of the Romagna Riviera and towns such as Florence, Venice, and Verona. It can therefore be easily reached by car, train, bus, or plane.



BY TRAIN

Bologna's Central Station serves as the final destination for a large number of local, intercity, and high-speed trains connecting neighboring locations and the country's main cities. Visit Trenitalia or Italo websites to find out about trains inbound to Bologna. The station is also the terminus of the Marconi Express, a monorail link service to Bologna airport.

BY PLANE

Guglielmo Marconi Airport is located about 10 km from the city center. Dozens of flights from Italian and international destinations land here daily.

The Marconi Express service connects the airport to the central railway station, which takes only 7 minutes to cover the route. The monorail costs € 9.20 one way and € 17.00 round trip.

Line 944 also connects the airport to the city for € 4.00, reaching as far as the nearby Ospedale Maggiore.

Visitors wishing to travel more cost-effectively can instead catch bus 81/91 at Birra, a 20-minute walk from the airport, which both travel to the center of Bologna and the Central Station at the standard city fare.

From the airport, you can also reach the center by taxi. A ride to/from the center takes about 20 minutes, with the price ranging from € 20.00-25.00.

BY CAR

If you travel by car, you may want to know that Bologna is one of Italy's major motorway junctions, connecting the north and south of the country.

Bologna is located along the A1, only 1 hour from Florence (southbound) and just over 2 hours from Milan (northbound).

Bologna is also the gateway to the A14, running along the Adriatic side of the peninsula from Romagna all the way to Taranto.

Another important thoroughfare is the A13 towards Veneto, connecting Bologna to cities such as Ferrara and Padua, the gateway to Venice.

BY BUS

Buses from outside the city travel primarily from the main coach station – located a short distance from the train station – with connections throughout Italy and even to foreign countries.

Visit the coach station website for information on bus routes arriving from outside the city.

1.6 Visit to the IAP Group's Laboratories (Tuesday, September 5th)

The Research Group for Industrial Applications of Plasmas (IAP Group) studies and develops **numerous processes related to the use of atmospheric pressure plasmas for therapeutical purposes**, including:

- plasma-assisted bioaerosol inactivation;
- plasma-assisted surface decontamination;
- plasma-assisted deposition of antimicrobial coatings;
- plasma-activated liquids production.



To support these processes, over the years, the IAP Group has set up several Laboratories equipped with dedicated instrumentation:

- **Tesla Research Laboratory** (3D printer, oscilloscope, and electronic probes, Fourier transform infrared spectrometer, drop shape analyzer);
- **Raizer Plasma Diagnostic Laboratory** (optical emission spectrometer, optical absorption spectrometer, high-speed camera with or without Schlieren setup, ICCD camera);
- **Von Ardenne SEM Laboratory** (scanning electron microscope);
- **Langmuir BioPlasma Laboratory** (biohazard class II microbiology lab);
- **Golgi BioPlasma-Cell Laboratory** (cell culture lab).



The visit to the IAP Group's Laboratories will take place on Tuesday, September 5th, in Via Terracini 28 (40131, Bologna, BO) starting from 17.00. To reach the Laboratories from the venue of the Annual Meeting, the following options are available:

- **By bus**. Bus 32 (*Departure bus stop: Aldini. Arrival bus stop: Don Minzoni – Mambo*) + Bus 35 direction Facoltà di Ingegneria – Osp. Maggiore (*Departure bus stop: Don Minzoni – Mambo. Arrival bus stop: Terracini*). Total time: around 45 minutes. Ticket price: 1.50 € (2.00 € if purchased on bus).
- **By car**. Total time: around 15 minutes.
- **On foot**. Total time: around 50 minutes.

1.7 Social event (Wednesday, September 6th)

The social event is programmed for Wednesday, September 6th, from 2:30 PM to 5:00 PM. You will be guided on a suggestive field trip through some of the many places of great historical and cultural interest in the city center. The meeting point is **Piazza Scaravilli**, where two different groups will separately start their journey. The first place you will visit is **Palazzo Poggi**, located in front of the meeting point. As well as being the actual seat of the university, its majestically painted rooms house a museum with astonishing collections of geography and nautical science, military architecture, physics, natural history, chemistry, human anatomy, and obstetrics. Then, you will be led through **Via Zamboni**. Since the first day of the world oldest university history, this street has been the designed place of its main faculties' headquarters. Millions of students from every side of the world, since 1088 A.D., have crossed this path, encountering themselves and discovering together for the sake of human knowledge. Then, you will visit **Archiginnasio**, which was the university seat until 1803. Today, it is one of the most important libraries of Bologna and the largest library in the region, Emilia Romagna, at present it boasts 850000 volumes and pamphlets. It also houses the anatomical theater, where historically anatomy lessons were held for medicine students. Ultimately, you will be led to the more significant (as the name says) and most important square in the city: **Piazza Maggiore**. Located almost in the perfect geographical center of the medieval city, it has been for many centuries the focal political and religious point of Bologna, as it lays in front of San Petronio Basil (the 14th largest church in the world) and Palazzo d'Accursio, the historical municipal seat of the city. Piazza Maggiore is also famous for harboring two of the Seven secrets of Bologna, particularly, the Neptune shadow and the "Wireless phone".

1.8 Social dinner (Wednesday, September 6th)

The social dinner will take place on Wednesday, September, at a typical Bolognese osteria (Cantina Bentivoglio). The Osteria is in the heart of Bologna, just a few minutes walk from Piazza Maggiore (Via Mascarella, 4/b,40126 Bologna BO). Dinner will be served in the Music Room of the Osteria, where traditional jazz-type musical entertainment is planned from 9:30 p.m. The menu is designed. to taste excellent traditional Bolognese food.

Menu

First Courses

- Traditional Bolognese lasagne
- Gnocchi in parmesan cheese

Main Course

- Bolognese Cotoletta with potato pie

Dessert

- Mascarpone with chocolate

Mineral water and wine are included.

Program

TIME	04/09/2023
15.00	Registration
16.00	Opening Ceremony
16.20	WGs intro
16.35	WG6 intro
16.40	WG6-L1 Stancampiano
16.55	WG6-S1 Muro-Fraguas
16.58	WG4-S1 Pavlakis
17.01	WG 6 discussion
17.30	WELCOME RECEPTION

TIME	05/09/2023
9.00	WG1 intro
9.05	WG1-L1 Gerling
9.20	WG1-S1 Constantin
9.23	WG1-S2 Verloy
9.26	WG1-L2 Lukeš
9.41	WG1-S3 Zampieri
9.44	WG2-L1 Krčma
9.59	WG2-S1 Mentheour
10.02	WG1-S4 Tampieri
10.05	WG5 intro
10.10	WG5-S1 Gaur
10.13	WG5-S2 Navascués
10.16	WG1-S5 Junkar
10.19	WG1-S6 Ozdemir Gizem Dilara
10.22	WG1 - WG5 discussion
10.52	COFFEE BREAK
11.20	WG2 intro
11.25	WG2-L2 Myers
11.40	WG2-S2 Rouillard
11.43	WG2-S3 Petrová
11.46	WG2-S4 Měšťánková
11.49	WG2-S5 Okruhlicová
11.52	WG2-L3 Bostanaru-Iliescu
12.07	WG2-S6 Ozdemir Mehmet Akif
12.10	WG2-S7 Nic Shiurdain
12.13	WG2-S8 Alba-Elías
12.16	WG2-S9 Holban
12.19	WG2-S10 Vazquez
12.22	WG2-S11 Scaltriti
12.25	WG2-S12 Popoli
12.28	WG5-S3 Ditu
12.31	WG2-S13 Furno
12.34	WG2 - WG5 discussion
13.00	LUNCH
14.30	WG3 intro
14.35	WG3-L1 Masur
14.50	WG3-L2 Weiss
15.05	WG3-L3 Cortazar
15.20	WG3 discussion
15.50	End of the discussion
17.00	Laboratory of Industrial Applications of Plasmas Group visit (facultative)
19.30	

TIME	06/09/2023
9.00	WG4 intro
9.05	WG1-L3 Malousi
9.20	WG4-S2 Baroen
9.23	WG4-L1 Heirman
9.38	WG4-S3 Oliveira
9.41	WG4-S4 Kužmová
9.44	WG4-S5 Kurt
9.47	WG4-S6 Gomez-Casado
9.50	WG4-S7 Gristina
9.53	WG4-S8 Sensoz Turgut
9.56	WG5-L1 Lopes
10.11	WG5-S4 Cevik
10.14	WG4-S9 Kutasi
10.17	WG4-S10 Raud
10.20	WG4-S11 Erdağ
10.23	WG5-L2 Verswyvel
10.38	COFFEE BREAK
11.05	WG4-L2 Živanić
11.20	WG4-L3 Bauwens
11.35	WG4-S12 Miletić
11.38	WG4-L4 Boeckmann
11.53	WG4-L5 Pavy
12.08	WG4-S13 Cremer
12.11	WG4-S14 Coşkuntan
12.14	WG4 - WG5 discussion
12.45	End of the discussion
14.45	Social event
20.30	Social Dinner

TIME	07/09/2023
9.00	STSM intro
9.05	WG2-STSM1 Laghi
9.20	WG2-STSM2 Lavrikova
9.35	WG2-STSM3 Sainz-García
9.50	WG2-STSM4 Trebulová
10.05	WG3-STSM1 Lainović
10.20	COFFEE BREAK
10.50	WG4-STSM1 Jasna-Tinea Jelinek
11.05	WG4-STSM2 Espona-Noguera
11.20	WG5-STSM1 Balaha
11.35	Closing Ceremony
12.45	LUNCH
14.00	MC meeting
15.30	TEA BREAK
16.00	MC meeting

Detailed Program

Mon, September 4

TIME	04/09/2023
15,00	Registration
16,00	Opening Ceremony
16,20	WGs intro
16,35	WG6 intro
16,40	WG6-L1 Stancampiano
16,55	WG6-S1 Muro-Fraguas
16,58	WG4-S1 Pavlakis
17,01	WG 6 discussion
17,30	WELCOME RECEPTION

Mon, September 4

15:00 - 16:00 Registration

16:00 - 16:20 Opening Ceremony - Canal, Cristina; Laurita, Romolo

16:20 - 16:35 WGs intro

16:35 - 16:40 WG6 intro - Laurencin-Dalicieux, Sara; Robert, Eric

16:40 - 16:55 WG6-L1 Stancampiano, Augusto

Easily accessible ex-vivo animal and electrical model for the technology transfer in plasma medicine

16:55 - 16:58 WG6-S1 Muro-Fraguas, Ignacio

Low-friction and safe coatings on suture needles via atmospheric pressure plasma polymerization

16:58 - 17:01 WG4-S1 Pavlakis, Christoforos

Public and Patient Involvement (PPI) in knowledge production of plasma cancer therapy

17:01 - 17:30 WG 6 discussion

17:30 - Welcome reception

Tue, September 5

TIME	05/09/2023
9,00	**WG1 intro**
9,05	WG1-L1 Gerling
9,20	WG1-S1 Constantin
9,23	WG1-S2 Verloy
9,26	WG1-L2 Lukeš
9,41	WG1-S3 Zampieri
9,44	WG2-L1 Kučma
9,59	WG2-S1 Mentheour
10,02	WG1-S4 Tampieri
10,05	**WG5 intro**
10,10	WG5-S1 Gaur
10,13	WG5-S2 Navascués
10,16	WG1-S5 Junkar
10,19	WG1-S6 Ozdemir Gizem Dilara
10,22	**WG1 - WG5 discussion**
10,52	**COFFEE BREAK**
11,20	**WG2 intro**
11,25	WG2-L2 Myers
11,40	WG2-S2 Rouillard
11,43	WG2-S3 Petrová
11,46	WG2-S4 Měšťánková
11,49	WG2-S5 Okruhlicová
11,52	WG2-L3 Bostanāru-Iliescu
12,07	WG2-S6 Ozdemir Mehmet Akif
12,10	WG2-S7 Nic Shiurdain
12,13	WG2-S8 Alba-Elías
12,16	WG2-S9 Holban
12,19	WG2-S10 Vazquez
12,22	WG2-S11 Scaltriti
12,25	WG2-S12 Popoli
12,28	WG5-S3 Ditu
12,31	WG2-S13 Furno
12,34	**WG2 - WG5 discussion**
13,00	**LUNCH**
14,30	**WG3 intro**
14,35	WG3-L1 Masur
14,50	WG3-L2 Weiss
15,05	WG3-L3 Cortazar
15,20	**WG3 discussion**
15,50	**End of the discussion**
17,00	**Laboratory of Industrial Applications of Plasmas Group visit (facultative)**
19,30	

Tue, September 5

09:00 - 09:05 WG1 intro - Privat-Maldonado, Angela; Sabota, Ana

09:05 - 09:20 WG1-L1 Gerling, Torsten

Absolute ion density measurement (MAID) by evaluating ion acoustic waves in the plasma – an approach via external excitation

09:20 - 09:23 WG1-S1 Constantin, Catalin

Maximization of RONS generation by selection of gas nature and injection type in one-electrode tubular RF cold plasma source

09:23 - 09:26 WG1-S2 Verloy, Ruben

Eliminating the effects of ambient conditions during a plasma jet treatment of a liquid sample with a gas shield

09:26 - 09:41 WG1-L2 Lukeš, Petr

Chemical and bactericidal properties of cell culture media DMEM and RPMI modified by He/O2 plasma treatment

09:41 - 09:44 WG1-S3 Zampieri, Leonardo

Correlating the production of reactive oxygen and nitrogen species with biological effects using combined diagnostics

09:44 - 09:59 WG2-L1 Krčma, František

Surface distribution of RONS generated by cold microwave plasma torches

09:59 - 10:02 WG2-S1 Mentheour, Robin

Characterization of RONS delivery following helium jets treatments of infected wound tissue models

10:02 - 10:05 WG1-S4 Tampieri, Francesco

Hydrogels as models to evaluate the effect of non-thermal plasma treatment on living tissues

10:05 - 10:10 WG5 intro - Sadowska, Joanna; Labay, Cédric

10:10 - 10:13 WG5-S1 Gaur, Nishtha

Plasma-Activated Hydrogel Therapy: An On-Demand Drug Delivery Platform for Multiple Clinical Indications

10:13 - 10:16 WG5-S2 Navascués, Paula

Nanoporous plasma polymer films as functional layers for the delivery of reactive oxygen species

10:16 - 10:19 WG1-S5 Junkar, Ita

Combining hydrothermal and gaseous plasma treatment for fabrication of nanostructured surface for vascular

10:19 - 10:22 WG1-S6 Ozdemir Gizem Dilara, Gizem Dilara

Cold Atmospheric Plasma for Dental Restoration Materials: An Investigation on Bleaching and Surface Properties

10:22 - 10:52 WG1 - WG5 discussion

10:52 - 11:20 COFFEE BREAK

11:20 - 11:25 WG2 intro - Laurita, Romolo; Boehm, Daniela

11:25 - 11:40 WG2-L2 Myers, Brayden

Elucidating the mechanisms of plasma inactivation of E. Coli using singlecell impedance flow cytometry

11:40 - 11:43 WG2-S2 Rouillard, Amaury

Continuous vs fractioned treatment modes: achieving effective bacterial inactivation with minimal thermal stress

11:43 - 11:46 WG2-S3 Petrová, Veronika

Study of low temperature plasma direct application on yeasts Candida Glabrata and bacteria

11:46 - 11:49 WG2-S4 Měšťánková, Zuzana

Influence of plasma and plasma treated water on fungus Aspergillus niger

11:49 - 11:52 WG2-S5 Okruhlicová, Zuzana

Cold atmospheric plasma against fungal pathogens

11:52 - 12:07 WG2-L3 Bostanāru-Iliescu, Andra-Cristina

Effect of Plasma-Activated Water on Young and Mature Aspergillus Conidia

12:07 - 12:10 WG2-S6 Ozdemir Mehmet Akif, Mehmet Akif

Machine Learning-Based Regression Analysis for Predicting Antimicrobial Activity of Cold Atmospheric Plasma-Treated Liquids

12:10 - 12:13 WG2-S7 Nic Shiurdain, Orla

Optimisation of plasma functionalised liquids for the treatment of orthopaedic implant infections

12:13 - 12:16 WG2-S8 Alba-Elías, Fernando

Bubbles-PAW for L. monocytogenes inactivation

12:16 - 12:19 WG2-S9 Holban, Alina Maria

Biofilm inhibition and decontamination of medical surfaces by cold plasma treatment

12:19 - 12:22 WG2-S10 Vazquez, Thomas

Dielectric barrier discharge combined with photocatalysis for the treatment of indoor air

12:22 - 12:25 WG2-S11 Scaltriti, Silvia Giuditta

Electrical Characterization and Efficacy Assessment of a Non-Thermal Plasma System for Closed Environment Sanitization

12:25 - 12:28 WG2-S12 Popoli, Arturo

A oD kinetic model of a humid-air DBD reactor

12:28 - 12:31 WG5-S3 Ditu, Lia-Mara

Light-Activated Antimicrobial Agents as Photocatalytic Method for Protection of Surfaces with Increased Risk of Infections

12:31 - 12:34 WG2-S13 Furno, Ivo

Low-temperature plasmas for biological applications at the Swiss Plasma Center

12:34 - 13:00 WG2 - WG5 discussion

13:00 - 14:30 LUNCH

14:30 - 14:35 WG3 intro - Sardella, Eloisa; Balaha, Marwa

14:35 - 14:50 WG3-L1 Masur, Kai

Cold Plasma for accelerated healing of chronic wounds in diabetic patients

14:50 - 15:05 WG3-L2 Weiss, Martin

Non-invasive physical plasma in gynaecology and regenerative surgery – focus areas of the 'Division of Plasmamedicine and Medical Technology'

15:05 - 15:20 WG3-L3 Cortazar, Osvaldo Daniel

Electric Field in Cold Air Plasma Jet as a Key Factor for Wound Healing

15:20 - 15:50 WG3 - WG5 discussion

17:00 - 19:30 Industrial Applications of Plasmas Group Laboratory Visit (facultative), Via Umberto Terracini, 24, 40131 Bologna BO

Wed, September 6

TIME	06/09/2023
9,00	**WG4 intro**
9,05	WG1-L3 Malousi
9,20	WG4-S2 Baroen
9,23	WG4-L1 Heiman
9,38	WG4-S3 Oliveira
9,41	WG4-S4 Kužmová
9,44	WG4-S5 Kut
9,47	WG4-S6 Gomez-Casado
9,50	WG4-S7 Gristina
9,53	WG4-S8 Sensoz Turgut
9,56	WG5-L1 Lopes
10,11	WG5-S4 Cevik
10,14	WG4-S9 Kutasi
10,17	WG4-S10 Raud
10,20	WG4-S11 Erdağ
10,23	WG5-L2 Verswyvel
10,38	**COFFEE BREAK**
11,05	WG4-L2 Živanić
11,20	WG4-L3 Bauwens
11,35	WG4-S12 Miletić
11,38	WG4-L4 Boeckmann
11,53	WG4-L5 Pavy
12,08	WG4-S13 Cremer
12,11	WG4-S14 Coşkuntan
12,14	**WG4 - WG5 discussion**
12,45	**End of the discussion**
14,45	**Social event**
20,30	**Social Dinner**

Wed, September 6

09:00 - 09:05 WG4 intro - Bekeschus, Sander; Boeckmann, Lars

09:05 - 09:20 WG1-L3 Malousi, Andigoni

Shared transcriptomic patterns of cold atmospheric plasma treated cancer cells elucidate the role of immunogenic cell death

09:20 - 09:23 WG4-S2 Baroen, Jana

Comparing the induced cancer cell death pathways by direct and indirect non-thermal plasma treatment

09:23 - 09:38 WG4-L1 Heirman, Pepijn

The effect of plasma-induced oxidation on the cancer – natural killer cell inhibitory axis: a computational-experimental approach

09:38 - 09:41 WG4-S3 Oliveira, Maria Cecilia

Effect of Plasma-induced Lipid Oxidation on the Permeability of Hemichannels for Treating Cancer Cells

09:41 - 09:44 WG4-S4 Kužmová, Darina

Impact of plasma-activated PBS on human prostate cancer cell line and noncancer prostatic cell line

09:44 - 09:47 WG4-S5 Kurt, Berrak

Cold Plasma Applications: Antineoplastic Effects in Chronic Lymphocytic Leukemia

09:47 - 09:50 WG4-S6 Gomez-Casado, Eduardo

Osteosarcoma cell lines susceptibility to plasma treated water

09:50 - 09:53 WG4-S7 Gristina, Roberto

Anticancer effects of plasma treated L-tyrosine enriched water solutions

09:53 - 09:56 WG4-S8 Sensoz Turgut, Merve

Plasma Therapy for Pancreatic Cancer

09:56 - 10:11 WG5-L1 Lopes, Beatriz

Cold Plasma Deposition as a novel technology for targeted cancer drug delivery

10:11 - 10:14 WG5-S4 Cevik, Ozge

Cold Plasma application may have the potential to be used in cancer treatment as it stimulates exosome release

10:14 - 10:17 WG4-S9 Kutasi, Kinga

RONS and Zn nanoparticles enriched alginate hydrosols

10:17 - 10:20 WG4-S10 Raud, Jüri

The influence of cancer cells FeCl2 pretreatment on the cytotoxicity of reactive chlorine species

10:20 - 10:23 WG4-S11 Erdağ, Demet

The effect of silica coated superparamagnetic iron oxide nanoparticles on cold plasma treated skin cancer cell lines

10:23 - 10:38 WG5-L2 Verswyvel, Hanne

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

10:38 - 11:05 COFFEE BREAK

11:05 - 11:20 WG4-L2 Živanić, Milica

Exploring alginate hydrogels for minimally-invasive treatment of internal tumors with plasma

11:20 - 11:35 WG4-L3 Bauwens, Mauranne

Patient-derived Organoids as a state-of-the-art 3D tumor model for HNSCC

11:35 - 11:38 WG4-S12 Miletić, Maja

Modulating chemosensitivity of oral carcinoma to Cisplatin by combination with plasma activated medium on 3D cell models

11:38 - 11:53 WG4-L4 Boeckmann, Lars

The effect of silica coated superparamagnetic iron oxide nanoparticles on cold plasma treated skin cancer cell lines

11:53 - 12:08 WG4-L5 Pavy, Allan

Unraveling the multifaceted antitumor effects of cold atmospheric plasma on cholangiocarcinoma

12:08 - 12:11 WG4-S13 Cremer, Isabelle

Cold plasma as a new promising therapeutic approach in lung cancer

12:11 - 12:14 WG4-S14 Coşkuntan, İrem

The Fourth State Matter: Potential of Cold Atmospheric Plasma Treatment in Malignant Mesothelioma

12:14 - 12:45 WG4 - WG5 discussion

12:45 - 14:45 FREE LUNCH

14:45 - 17:30 Social event

20:30 - Social dinner, Cantina Bentivoglio, Via Mascarella, 4/b, 40126 Bologna BO

Thu, September 7

TIME	07/09/2023
9,00	**STSM intro**
9,05	WG2-STSM1 Laghi
9,20	WG2-STSM2 Lavrikova
9,35	WG2-STSM3 Sainz-García
9,50	WG2-STSM4 Trebulová
10,05	WG3-STSM1 Lainović
10,20	**COFFEE BREAK**
10,50	WG4-STSM1 Jasna-Tinea Jelinek
11,05	WG4-STSM2 Espona-Noguera
11,20	WG5-STSM1 Balaha
11,35	**Closing Ceremony**
12,45	**LUNCH**
14,00	MC meeting
15,30	**TEA BREAK**
16,00	MC meeting

Thu, September 7

09:00 - 09:05 STSM intro - Serio, Susana

09:05 - 09:20 WG2-STSM1 Laghi, Giulia

Control strategies for atmospheric pressure plasma polymerization processes to produce antimicrobial coatings

09:20 - 09:35 WG2-STSM2 Lavrikova, Aleksandra

Effects of Plasma Functionalised Water on MRSA Biofilms

09:35 - 09:50 WG2-STSM3 Sainz-García, Ana

Evaluation of the Antimicrobial efficacy of Cold Atmospheric Pressure Plasma on Surfaces

09:50 - 10:05 WG2-STSM4 Trebulová, Kristína

Antimycotic effects of plasma gun tested on C. glabrata on various surfaces

10:05 - 10:20 WG3-STSM1 Lainović, Tijana

The influence of Cold Atmospheric Plasma treated hydrogels on dentinal MMPs activity

10:20 - 10:50 COFFEE BREAK

10:50 - 11:05 WG4-STSM1 Jasna-Tinea Jelinek, Jasna-Tinea

Atmospheric Pressure Plasma-assisted synthesis of two types of Polypyrrole/Carbon Quantum Dot Nanoparticles for Photothermal Therapy

11:05 - 11:20 WG4-STSM2 Espona-Noguera, Albert

Exploring the anticancer potential of multicomponent plasma-treated hydrogels in an in ovo pancreatic cancer model

11:20 - 11:35 WG5-STSM1 Balaha, Marwa

Antioxidant effects of cape derivatives in treating cancer cells in combination with direct cold atmospheric plasma

11:35 - 12:45 Closing

12:45 - 14:00 LUNCH

14:00 - 15:30 MC meeting

15:30 - 16:00 TEA BREAK

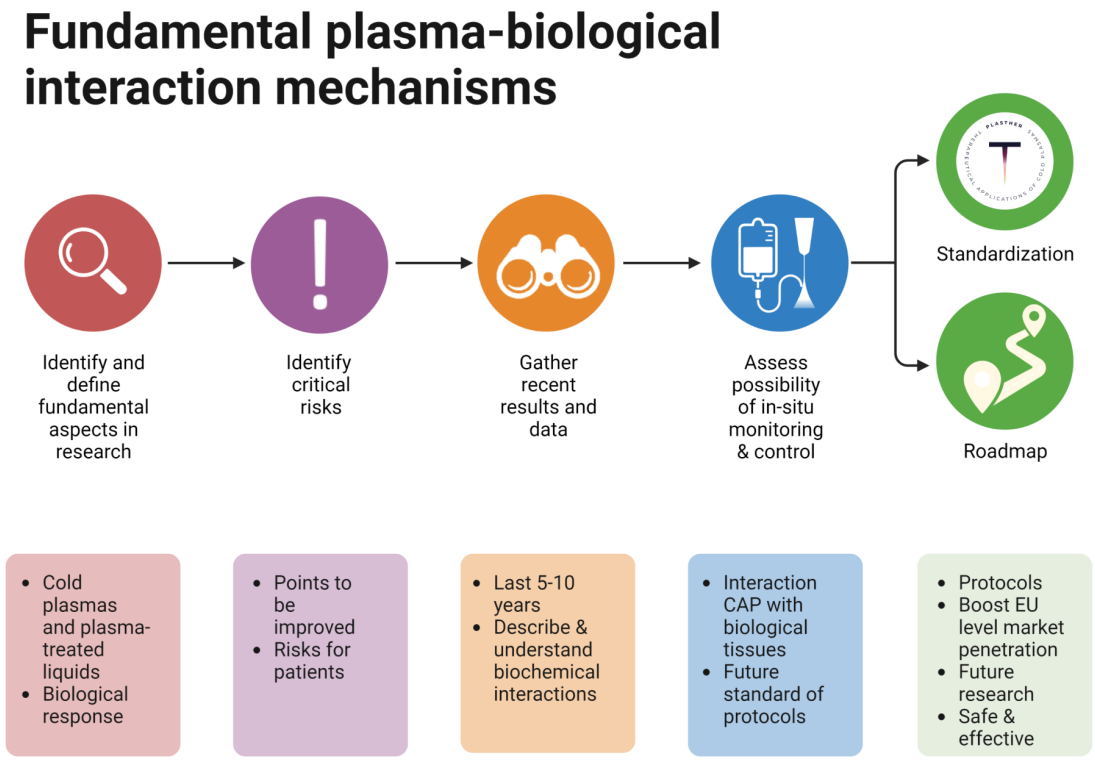
16:00 - 19:00 MC meeting

Abstracts

WG1 - Fundamental plasma-biological interaction mechanisms

The interaction mechanisms of plasma with biological systems are not trivial, due to the complexity of both plasmas and the biological target. Therefore, unification of all the known physical and biochemical mechanisms will allow for the advancement towards a general vision and a better understanding of plasma technology in biomedical applications. Besides, different plasma sources have been licensed as medical devices and are already in use in hospitals and healthcare centers for treating patients affected by different dermatological diseases (diabetic wounds, infected wounds, burns, etc.).

There's a need for a deep and systematic recollection of data emerging from CAP treatment of biological substrates in-vivo, with a special interest in the standardization of physical/chemical measurements related to such interaction.



This task will address this issue by surveying different CAP sources available on the market worldwide, with a deep characterization of their physical principle of operation and related effect in the specific field of application.

Working Group Deliverables

D1	Review of the existing bioactive physical and chemical components from CAP/PTL.
D2	Description of the currently known molecular interactions between CAP/PTL and eukaryotic cells or procariotes, prions, virus
D3	Definition of standard protocols measuring physical and chemical characteristics of CAP and PTL and its induced effect in-vivo treatment of biological samples
D4	Definition of standard protocols measuring physical and chemical characteristics of CAP and PTL and its induced effect in-vivo treatment of biological samples

Absolute ion density measurement (MAID) by evaluating ion acoustic waves in the plasma – an approach via external excitation

Hans Höft[1], Tom Huiskamp[2], Aleksander P. Jovanović[1], Detlef Loffhagen[1], Markus M. Becker[1], Philipp Mattern[1,3,4], Torsten Gerling[1,3,4]

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For a fundamental understanding of the biological effect of physical plasma treatment, a thorough knowledge of the plasma properties is an essential prerequisite. Previously, the appearance of weak and erratically occurring oscillations of the current signal was observed in an argon-based transient spark discharge [1]. These oscillations were attributed to ion acoustic waves and their frequency was used to estimate ion densities in argon [1,2]. Identifying the excitation mechanism and dominant ions responsible for the excitation of ion acoustic waves could allow using the same procedure for ion density estimation in other discharges as well. A related project aims to combine the experimental results with modelling data for an improved understanding of the underlying the physical processes.

Furthermore, within a STSM in WG1 in July 2023 of the COST Action CA20114 PlasTHER we try to improve the diagnostic possibilities by bringing together electrical engineering and plasma diagnostics with a focus on highly resolved, erratic electrical measurements. It will be tested, if it is possible to excite ion acoustic waves within the conductive discharge channel by externally applying an electrical high frequency signal into the electrical circuit. The concept is depicted in Fig 1, where a calculated 100 MHz signal is artificially combined with a measured current. If this approach can be successfully proven during the visit of the electrical engineering department at the TU/e, this MAID method could be transferred to multiple different plasma sources and hence become a powerful tool for the community.

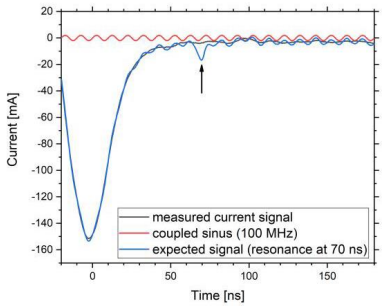

Fig. 1 Visualisation of the overlap of a measured electrical signal (black) with an external high frequency excitation signal (red). The result is expected to generate a local resonance allowing a scoping of ion densities over time

Acknowledgement
This work was funded by the DFG (German Research Foundation) project number 466331904 and by a STSM through CA20114 PlasTHER.

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[2] T. Gerling, C. Wilke and M. M. Becker, Journal of Physics D: Applied Physics, 54, 85201 (2020)

WG1: Fundamental plasma-biological interaction mechanisms

Chemical and bactericidal properties of cell culture media DMEM and RPMI modified by He/O_2 plasma treatment

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The application of cold atmospheric plasma (CAP) in medicine is a perspective and rapidly increasing research topic. Besides direct treatment of living tissue or cells with CAP source, properties of plasma-treated biologically relevant liquids such as cell culture media gain significant interest and have been applied for various medical and biological trials. Various transient reactive oxygen and nitrogen species such as OH•, $O_2^{\bullet-}$, NO• and NO_2• radicals, peroxynitrite may be produced in plasma-treated liquids. These species have highly cytotoxic properties and cause biochemical and antibacterial activity of plasma-treated solutions through post-discharge processes. In addition, culture media contain a complex mixture of inorganic salts and organic compounds such as amino acids, vitamins, glucose, antibiotics, etc., significantly influencing the properties and activity of these plasma-treated liquids. Nevertheless, diagnostics of reactive species and reaction pathways responsible for biological responses caused by these multicomponent liquids are affected by many factors which significantly influence the selectivity, sensitivity, and precision of the analytical methods applicable to detect and quantify the chemical species produced by plasma in these liquids and evaluation of reaction mechanisms.

In this work, we studied He/O_2 plasma chemical modifications of cell culture media DMEM and RPMI. The COST-Reference plasma jet operated at a helium flow rate of 1.4 slm with a 0.6% oxygen admixture was used as a plasma source to treat the culture media [1-5]. We performed a chemical analysis of the plasma-treated media and correlated the chemical properties with their antibacterial properties on *E. coli*. We compared the direct treatment (plasma treatment of bacterial suspensions) and indirect treatment (incubation of bacteria in post-discharge plasma-treated media) of bacteria. Chemical changes in the treated media were analyzed by various diagnostics regarding the possible interferences taking place in these complex mixtures. The contributions of the specific components in plasma-treated media to their antibacterial effects were evaluated. Special attention was paid to the role of amino acids in the plasma-induced biocidal activity of DMEM and RPMI.

Plasma-treated cell culture media have shown long-term post-discharge chemical and bactericidal activity. Formation of organic mono- and dichloramines of amino acids in DMEM/RPMI by hypochlorite produced by the reaction of plasma-generated O atoms with Cl^- ions present in culture media were the major chemical contributors to the observed biocidal activity of plasma-treated cell culture media. This behavior was attributed to the action of tertiary chemical products formed by the decay of organic dichloramines. The thiobarbituric acid assay revealed malondialdehyde formation from glucose oxidation as the major component of the media.

References

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[3] V. Jirasek, P. Lukes P, *J. Phys. D: Appl. Phys.*, 53, 505206 (2020).
[4] V. Jirasek, Z. Kovalova, B. Tarabova, P. Lukes, *J. Phys. D: Appl. Phys.*, 54, 505206 (2021).
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WG1: Fundamental plasma-biological interaction mechanisms

Shared transcriptomic patterns of cold atmospheric plasma treated cancer cells elucidate the role of immunogenic cell death

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[3]Veterinary Research Institute of Thessaloniki, Greece
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To explore the therapeutic potential of cold atmospheric plasmas (CAP) in oncology, we first need to understand the molecular interactions between cold plasmas with living systems. Systems biology provides efficient multi-omics methods to find shared deregulated mechanisms in CAP-treated cancer cells yet, so far, there is limited evidence about the differences among cancer types, the utilization of heterogeneous CAP-generating devices and carrier gases. In this study, we analyzed data from whole transcriptome studies aiming to provide a holistic view of the biological pathways and transcriptional networks that are invariably activated in response to CAP treatment. Following a PRISMA-based strategy, we identified publicly available raw microarray transcriptomic data obtained from CAP treatment experiments in different human cancer cell lines and analyzed them separately, following the same approach for normalization, differential expression, and pathway analysis. *Limma* package and *pathfindR* were used to identify differentially expressed genes (adj. *p-value*< 0.05, $|\log_2 FC)| \geq 1$) and to perform enrichment analysis, respectively.

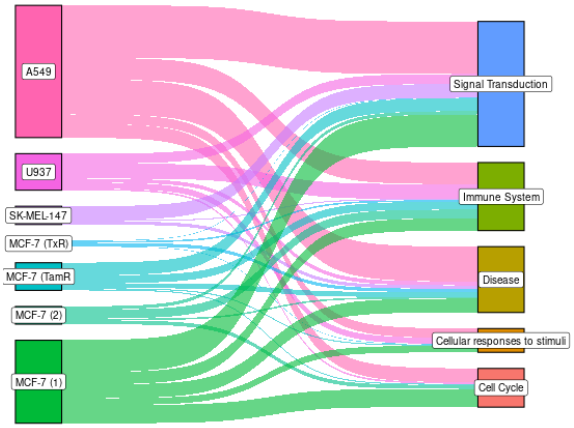

Fig. 1 Shared deregulated pathways in response to CAP treatment across seven cancer cell lines.

Seven microarray data series were eligible, corresponding to CAP-treated A549 (Lung Ca), U937 (Lymphoma), SK-Mel-147 (Melanoma), and four MCF-7 (Breast Ca), including paclitaxel and tamoxifen-resistant cell lines. Most studies employed direct CAP treatment using a dielectric barrier discharge device to produce CAP (4/7), whereas argon was mainly used as carrier gas (5/7). Among the top 82 common deregulated pathways, 34 associated with signal transduction, including ERBB2, MAPK, NOTCH, Insulin/IGF1R, Rho-GTPases, PI3K/AKT, NGF/NTRK signaling (Figure 1). The second most represented category comprised immune-related pathways, including cytokine signaling pathways (IL-4, IL-13, IL-17, IFN) and Toll-like receptor (TLR) cascades, mainly TLR3 and TLR4. The HMGB1/TLR4/MYD88 axis as well as the TLR3/TRIF/Caspase-8 signaling pathway have been implicated in cancer cell apoptosis [1]. Despite the marked heterogeneity of the microarray datasets this study suggests that the molecular mechanisms of CAP treatment are not cancer type-specific [2] and provides further insights into the induction of immunogenic cell death in human tumors [3], underpinning the potential as an emerging immunotherapeutic approach in oncology.

References

[1] L. Galluzzi et al., Nat. Rev. Immunol. 17(2): 97–111 (2017)
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[3] A. Azzariti et al., *Sci Reports*, 11;9(1),1–13 (2019).

WG1: Fundamental plasma-biological interaction mechanisms

Maximization of RONS generation by selection of gas nature and injection type in one-electrode tubular RF cold plasma source

Catalin Constantin[1], Ciprian Dumitrache[3], Marian Bazavan[2], Bogdana Mitu[1],Valentina Marascu[1], Cristian Stancu[1], Sorin Vizireanu[1] and Gheorghe Dinescu[1]
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The reactive oxygen and nitrogen species (RONS) that are generated using cold plasmas at atmospheric pressure have a crucial role in bio-medical applications such as: wound treatment, oncological therapies, inactivation of microorganisms, surface or water decontamination, as well as producing structural changes in proteins. Therefore, considering the utility of RONS, measuring their amount and understanding their evolution in such plasmas is important.
This study presents a comparative analysis of two DBD plasma sources with different gas injection geometries. Radio frequency plasma jet is generated with one annular electrode in a glass tube using Argon as the main gas [1] and injecting various secondary gases in the discharge such as nitrogen, oxygen and compressed air. The main difference between the geometries consists in the way that the secondary gases are introduced in the discharge: for first geometry, all gases are injected through the same tube, while for the second one, the secondary gases are injected in the discharge through a lateral tube placed downstream the powered electrode.
The spectral analysis consists of identifying and comparing the emissions of reactive species (RONS) such as NO, OH, O, etc.[2] and obtaining quantitative information on several plasma parameters such as the electron number density, vibrational and rotational temperatures. The electron number densities were calculated in the expanding jet, near the opening of the tube, using the Stark broadening method, similar values, around 10^{14} cm^{-3} were obtained for both geometries. Rotational and vibrational temperature were obtained by fitting the N_2(C-B) emission bands using a spectral simulation code. Analyzing the rotational temperature values as compared to those previously determined by OH band simulation[3], we conclude that the N_2(C) state is not populated primarily through electron impact from N_2(X), while the energy transfer from the metastable Ar* impact is another significant mechanisms for N_2 (C) state population. Thus, by correlating emission spectra evolution and the quantitative data, the gas injection geometry that favor the production of reactive species can be identified. It was observed that the plasma generated by the DBD source in which the secondary gases are injected laterally in the discharge is more stable, has a larger operating domain and produce higher amounts of RONS.

References

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WG1: Fundamental plasma-biological interaction mechanisms

Eliminating the effects of ambient conditions during a plasma jet treatment of a liquid sample with a gas shield

Heirman Pepijn[1], Verloy Ruben[1,2], Baroen Jana[1,2], Privat-Maldonado Angela[1,2], Smits Evelien[2], and Bogaerts Annemie[1]

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Atmospheric pressure plasma jets (APPJ), such as the kINPen®, are a typical source of cold atmospheric plasma (CAP) used in plasma medicine research [1]. The plasma-produced reactive oxygen & nitrogen species (RONS) can originate from two possible sources: 1) admixtures or impurities in the feed gas, or 2) mixing of the effluent with the atmosphere. The latter makes the plasma-treatment susceptible to the atmospheric conditions, such as the relative humidity. This affects the RONS-cocktail produced and the reproducibility of the treatment. To prevent this, a gas shield can be employed [2]. A second, concentric gas flow, of which the composition can be controlled, will surround the jet and separate it from the environment. Though research was conducted regarding the effect of the shielding gas composition on the effluent chemistry and biological treatment effect, less attention was given to the effectiveness of the gas shield to prevent mixing of the APPJ effluent with the atmosphere, especially for different setups.

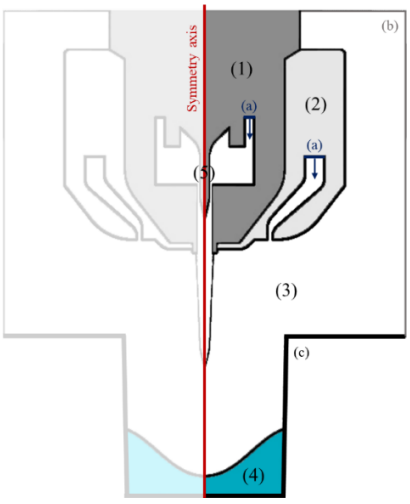

Fig. 1 General model geometry with (1) the plasma jet, (2) the gas shield, (3) gas phase, (4) liquid phase and (5) the pin-electrode. Through boundary conditions the edges of the model are treated as (a) inlets, (b) open boundaries and (c) no-slip

We adapted a computational 2D-axisymmetric model for the kINPen® above a liquid water surface [3], and expanded it to include a gas shield. Experiments regarding the concentrations of RONS and cell viability were also conducted to support our computational results. We investigated how varying atmospheric conditions, such as temperature and relative humidity, affect the effluent and the chemical treatment. This allows us to assess the effectiveness of the gas shield. The treatment of a well, as opposed to a flat surface, inherently creates a backflow towards the jet outlet, which may influence the gas shield flow pattern. Our results indicate that even though the effects of the atmospheric conditions are reduced, they are not completely eliminated. The degree of shielding also depends on the geometry of the substrate. Our results also show that mixing of the jet effluent with the shielding gas is significantly higher than with the air when no gas shield is used. Our findings must be taken into account in experiments, as it means that this gas shield cannot be used to mimic the surrounding atmosphere in a controlled way.

We acknowledge financial support from the Fund for Scientific Research (FWO) Flanders (Grant ID 1100421N, G033020N and 1SD6522N).

References

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Correlating the production of reactive oxygen and nitrogen species with biological effects using combined diagnostics

Leonardo Zampieri[1], Rita Agus[2], Lorenzo Ibba[2], Claudia Riccardi[1], Ivo Furno[2], Matteo Zuin[3], Gianluca De Masi[3], Luigi Cordaro[3], Roberto Cavazzana[3], Emilio Martines[1]
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In recent years, a variety of cold atmospheric plasma sources have been designed by research groups around the world, exploring various configurations in terms of mechanical and electrical characteristics and achieving different performances in biological treatments. Although some layouts prove to be more efficient than others, a clear pathway for the optimization still has to be traced.

Reactive oxygen and nitrogen species are thought to have a major role in triggering the biological chains. Correlating their presence to the effects on live cells can advance the understanding of plasma-biological interaction mechanisms on a fundamental level, helping in converging towards optimized apparatus.

At Università di Milano Bicocca, in collaboration with EPFL in Lausanne, we are contributing to this milestone. Using a helium pulsed plasma jet [1], originally developed in Consorzio RFX in Padova, different diagnostics are combined on the same source. We are exploring the different operating points of the source from different points of view, keeping the same experimental setup and avoiding external parameters variations.

In particular, the chosen substrate consists of 6 ml of deionized water, with 10^8 CFU of *E. Coli* suspended. The excitation voltage of the source and the distance from the nozzle to the substrate can be tuned, allowing to explore different plasma regimes.

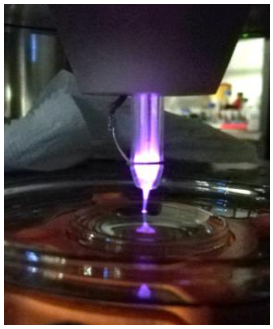

Fig. 1: the chosen setup

From the physical point of view, the electrical characterization of the source is carried out. The gaseous phase is studied via *in-situ* Fourier-transform infrared spectroscopy (FTIR), characterizing reactive oxygen and nitrogen species; on the other hand, the liquid phase is investigated using spectrophotometric techniques, and measuring the concentration of residual nitrites, nitrates and peroxides dissolved in water after the treatment. Finally, biological effects are measured via CFU counting, implanting the treated samples after plasma exposure.

The results are promising: physical, chemical and biological parameters are correlated observing non-trivial relation between them, contributing both to a better understanding at the fundamental level of the plasma-cells interaction and suggesting directions for the source optimization.

References

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Hydrogels as models to evaluate the effect of non-thermal plasma treatment on living tissues

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Hydrogels are employed in a wide range of medical and pharmaceutical applications due to their high biocompatibility, non-toxicity, tuneable mechanical properties and ability to encapsulate and deliver biological agents and water-soluble drugs. Up to now, hydrogels have been explored for two specific objectives in the plasma medicine field: i) to store reactive oxygen and nitrogen species (RONS) for local delivery; and ii) as model systems for living tissues [1].
In particular, agarose and gelatin-based hydrogels have been used for this last purpose: to study the diffusion, penetration and transport of plasma-generated RONS in tissue models. Most of the publications in this field do not compare different types of hydrogels. The purpose of this research is double: 1) to set good protocols to study qualitatively and quantitatively the interaction of plasmas with hydrogels, in terms of distribution and transport of RONS and 2) to compare different types of hydrogels, starting with agarose and gelatin, under the same experimental conditions.
The distribution of RONS on the surface and inside the hydrogels has been asessed using colorimetric and fluorescent chemical probes that were introduced in the hydrogels during their preparation. The transport of RONS through the hydrogels into a liquid was also studied using chemical probes, added to the liquid after the treatment. The effect was studied as function of the treatment time and the thickness of the hydrogel layer. The preliminary results point out a clear time dependence of the amount of RONS that is able to diffuse and penetrate in and through the hydrogel (Fig. 1) and suggest some important differences between agarose and gelatin-based hydrogels. Therefore, for future studies eventually more complex hydrogels that are able to better mimic real tissues should be explored.

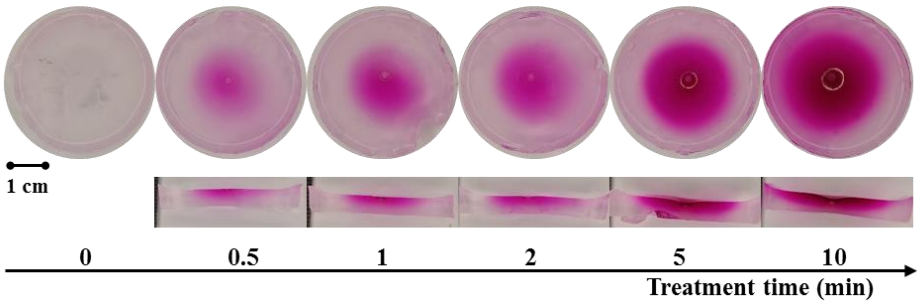

Figure 1. Alginate disks containing Griess reagent (NO_2^- selective colorimetric probe) as function of the plasma treatment time. Surface (up) and cross-section (down).

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WG1: Fundamental plasma-biological interaction mechanisms

Combining hydrothermal and gaseous plasma treatment for fabrication of nanostructured surface for vascular

Niharika Rawat[1], Metka Benčina[2], Domen Paul[2], Janez Kovač[2], Katja Lakota[3], Polona Žigon[3], Veronika Kralj-Iglič[4], Aleš Iglič[5], Ita Junkar[2]

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Cardiovascular diseases (CVDs) present one of the leading causes of death in the modern world. Usually, surgical procedure is used to treat the diseased blood vessels, and an implantable medical device such as cardiovascular stent is inserted to restore the flow through the vessel. The number of cardiovascular surgeries is increasing every year, however the vascular stent material is still far from optimal, as they with high probability induce thrombosis and restenosis. Therefore, there is an increased demand to develop biomaterials with superior surface properties, which would on one side prevent platelet adhesion (stent induced thrombosis), prevent uncontrolled proliferation of smooth muscle cells (restenosis) and improve proliferation of endothelial cells. To optimize surface properties of medical implants two surface treatment procedures were studied; gaseous plasma treatment and hydrothermal treatment. The modified surfaces were analyzed by scanning electron microscopy (SEM), water contact angle analysis (WCA) and X-ray photoelectron Spectroscopy (XPS). *In vitro* biological response evaluated platelet adhesion and proliferation of endothelial and smooth muscle cells on the surface. Our results indicate that combining both treatment procedures significantly improve biological response of surfaces used for vascular implants. In Figure 1 improved proliferation of Human Coronary Artery Endothelial Cells (HCAEC) can be clearly seen.

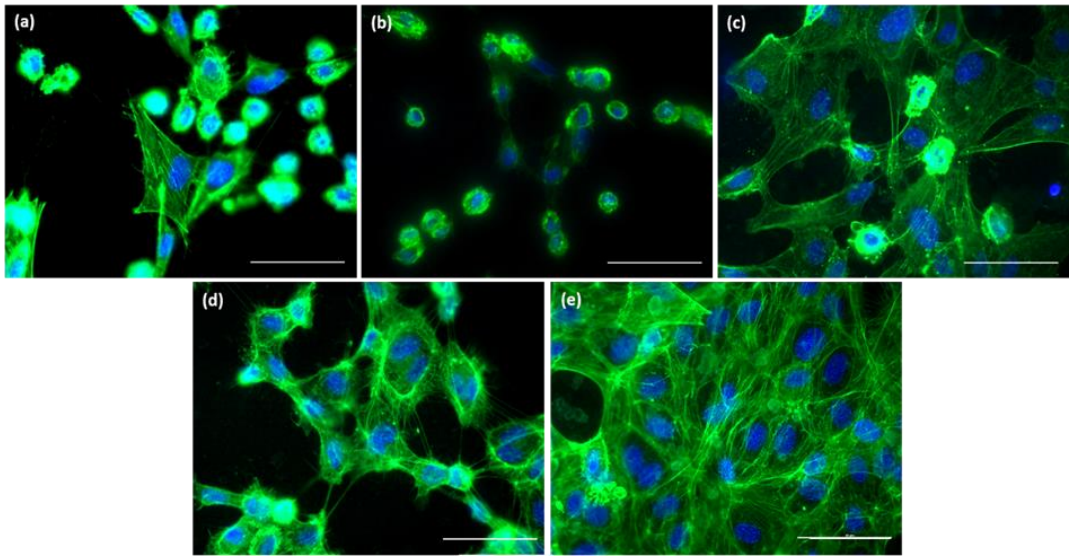

Fig. 1 HCAEC on Ti6Al4V surface (a) untreated (b) treated with plasma, (c) treated with hydrothermal treatment, (d) hydrothermally/plasma-treated, (e) plasma/ hydrothermally treated. Determined by immunofluorescent microscopy. Scale bar 25 µm.

Cold Atmospheric Plasma for Dental Restoration Materials: An Investigation on Bleaching and Surface Properties

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Cold atmospheric plasma (CAP), an emerging discipline in medicine and biomedical fields, offers unique features for various applications. In dentistry, CAP has gained rapid recognition due to its ability to treat living tissues without causing harmful effects at physical, chemical, and biological levels [1]. This study investigates the advantages of CAP application on dental restoration materials, specifically focusing on tooth bleaching and surface properties. CAP has been extensively utilized in dentistry for diverse applications, including biofilm removal, enhancing osseointegration, treating intraoral diseases, adhesive procedures, root canal disinfection, and natural tooth bleaching [2]. Previous studies have demonstrated that CAP not only enhances the mechanical properties of dental restoration materials but also improves their connectivity to living tissues [3]. Furthermore, it has been shown that CAP offers distinct advantages over traditional methods for tooth bleaching. Traditional tooth bleaching agents can lead to surface roughness and compromise the integrity of dental restoration materials due to prolonged contact required for effective bleaching. In contrast, CAP offers a high degree of bleaching within a shorter time frame, while minimizing harm to the surrounding tissues [4]. To explore these benefits, this study focuses on determining the bleaching effect of plasma, as well as its impact on translucency, discoloration, and surface properties of dental restoration materials.

The experimental procedure involved preparing dental restoration material samples, optimizing plasma parameters to achieve desirable coloration, constructing an imaging system with relevant image processing code, and recording the results. Evaluation of the obtained results revealed that CAP application as a pretreatment exhibits an anti-coloration effect alongside its bleaching efficacy on dental restorations. As a result of the study, the treatment of CAP demonstrated effective whitening properties and effectively prevented discoloration in the context of CAD/CAM materials.

In conclusion, this study highlights the potential of CAP as a promising approach for dental restoration materials, specifically for tooth bleaching procedures. By mitigating the side effects associated with traditional bleaching agents, such as surface roughness and compromised integrity, CAP offers an innovative solution for achieving efficient and safe tooth bleaching while preserving the desired surface properties of dental restoration materials. Further research and clinical studies are warranted to fully explore the potential of CAP in dental applications.

Acknowledgement
This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) under the grant number of 120S278.

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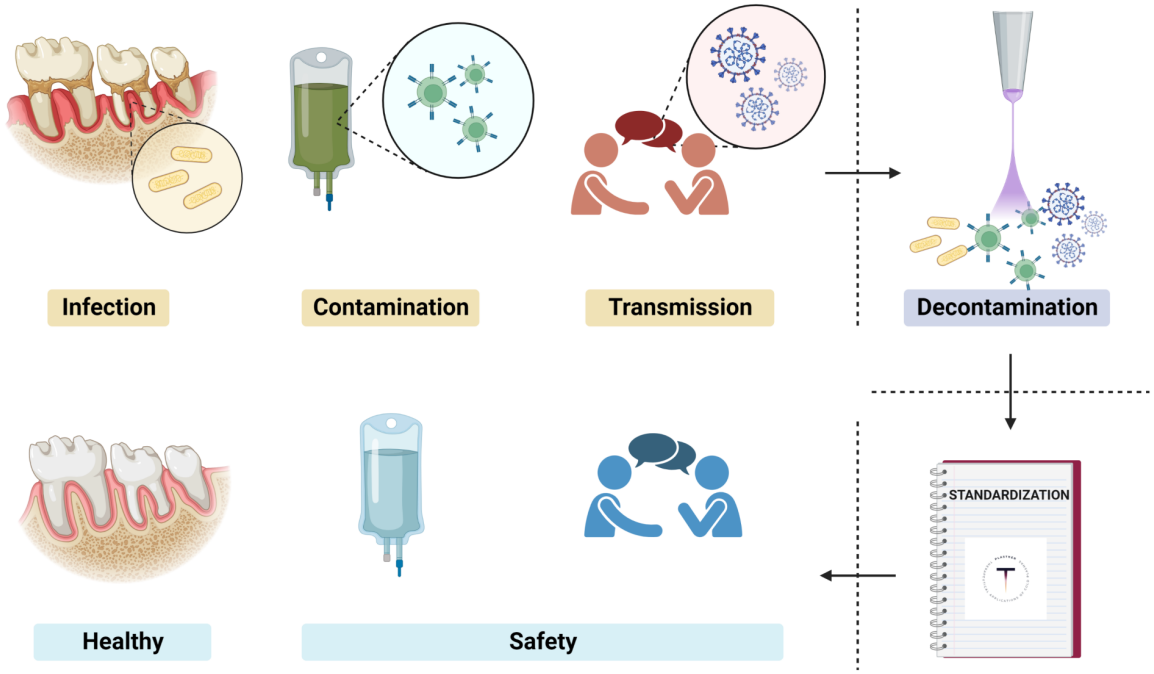
WG2 - Antimicrobial effects of plasma

Plasma decontamination is a promising technique for the treatment of medical devices and implants, as well as for the healing of chronic wounds associating infection, or in decontamination of bucodental cavity. While many mechanisms involved in plasma-driven decontamination have been uncovered, there is still room for deeper investigations, especially when dealing with plasma-treated liquids. Bacteria and related biofilms or spores have been the object of most plasma-decontamination-related studies. In the current COVID-19 pandemic new CAP assisted preventive solutions are envisaged, to mitigate the effects of airborne transmission of SARS-CoV-2 in poorly ventilated spaces through aerosol microdroplets, which can remain in the air for long periods and can be transmitted to others over distances >1 m. Tasks in this WG will be directed towards a better understanding of the role of plasma in decontamination processes and developing standard plasma treatment protocols for antimicrobial applications.



Working Group Deliverables

D5	Development of standard protocols and guidelines to characterize and test CAP and PTL for antimicrobial and antiviral purposes.
D6	Report on the evaluation of standard decontamination protocols on relevant biomaterials, & potential translation of results to industrial scale.
D7	Report on the evaluation of standard decontamination protocols onto pathogenic bioaerosols, and the potential translation of experimental results to industrial scale

WG2: Antimicrobial effects of plasma

Surface distribution of RONS generated by cold microwave plasma torches

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One of the most important points during different cold plasma systems interaction with the surfaces is presence of the active species. Couple of studies were focused on the diagnostics of selected reactive oxygen and nitrogen species (RONS) like OH, O, NO, NO_2, H_2O_2 in the gaseous phase or in the bulk liquid after interaction with plasma. The presented contribution focusses on RONS distribution on the biopolymer surfaces. The results are related to the same plasma systems application on the selected microorganisms, namely *C. glabrata*, *E. coli* and *S. epidermidis*.
The selected biopolymers with embedded specific dyes for RONS detection were prepared and spot treated under different conditions leading to color changes in all biopolymers. Two different microwave-based plasma systems were used for the current experiment. The first one was surface wave driven torch generated in 3 mm i.d. capillary, the second system was corona like MW torch (surfayok) affecting larger surface area. Both systems were operating in argon flow of 5 SLm at powers between 6 and 15 W. The active particles distribution over the entire surfaces were found. Additionally, the UV light influence was verified using UV transparent window just covering dye containing biopolymer. No color change was noticed in this case that means the color changes are due to interaction with RONS, only.
The optimized parameters were used for treatment of agar plates with monocultures of yeasts *C. glabrata*, and bacteria *E. coli*, and *S. epidermidis*. Additionally, the mixed culture *C. glabrata* and *E. Coli*. was treated, too. Plasma treated microbial cultures were photographed after incubation. The surface area which was covered by the microbial culture was calculated using a specially designed SW Aurora. The surfayok microwave torch achieved higher efficiency than the surface wave sustained microwave torch for all tested microorganisms. Correlation with the active species distribution shows that the key role in inhibition plays probably NO species.

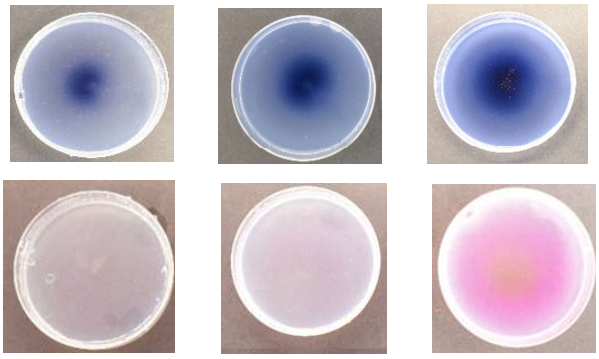

Fig. 1 Point treatment by surface wave sustained discharge of ozone sensitive biopolymer (top) and treatment by surfayok of nitrite sensitive biopolymer (bottom). Applied power of 9 W, treatment times 30, 60, and 120 s.

Acknowledgement: This research is a part of COST CA20114 Action. Both microwave plasma systems were developed under collaboration of both institutions.

WG2: Antimicrobial effects of plasma

Elucidating the mechanisms of plasma inactivation of *E. Coli* using single-cell impedance flow cytometry

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Low-temperature plasmas (LTPs) have been known to inactivate bacteria since the 1990s [1], yet a clear understanding of specific modes of action remains elusive [2]. Deciphering the intricate relationships between the active ingredients delivered by LTPs and subsequent biological effects has proven to be a formidable challenge, inhibiting progress and limiting the scope of potential applications. To overcome this, we look to employ an extension of conventional dielectric spectroscopy new to the field of LTPs: single-cell impedance flow cytometry (IFC). As the name suggests, single-cell IFC allows us to probe changes in the dielectric properties of individual cells and to analyze their discrete components by examining the dielectric response at various frequencies. This powerful technique can provide information on a cell's viability, volume, membrane capacitance, and cytoplasm conductivity. Here, initial experimental results will be shared showing the effects of plasma treatment on the dielectric properties of *E. Coli* for two well-characterized sources, an in-house plasma-activated water (PAW) reactor and the kINPen. The use of the kINPen and PAW reactor are deliberate to highlight the distinction between the different pathways expected to inactive bacteria for direct and indirect treatment methods. Planned work with the single-cell IFC, including the implementation of a three-shell model to examine the dielectric response of the cell to plasma treatment will also be outlined. In this contribution, the application of a novel diagnostic to the field of biological applications of LTPs will be discussed, initial results shared, and a potential path forward presented.

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WG2: Antimicrobial effects of plasma

Effect of Plasma-Activated Water on Young and Mature Aspergillus Conidia

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Aspergillus is a common airborne mold representing up to 40% of food industry, hospital and home fungal contamination. More specifically, the genus Aspergillus, which consists of a few hundred opportunistic mold species found in various climatic conditions, can cause diseases including localized infections, invasive diseases, allergic responses. Also, the conidia can contaminate preharvest and postharvest food contributing to observable or undetectable defects, such as unfavorable flavor and spoilage. In addition, there are species, such as *Aspergillus brasiliensis*, able to colonize and biodegrade a wide range of surfaces. Surface contamination is based on two key-features of the fungal colony: the fungal spores, and the vegetative mycelium.

As a solution to this situation, several studies have previously shown that exposure to plasma-activated water (PAW) has a sterilizing effect on fungal pathogens [1]. The inactivation efficiency of PAW and its mechanism were discussed in this study. Particular attention has been paid to the young and mature conidia that have different melanin concentrations as one of the cell wall components.

In this abstract, we present the preliminary results of an extended study dealing with the effect of PAW against young and mature conidia. *Aspergillus brasiliensis* ATCC 16404, *Aspergillus fumigatus* RTCC 2113, *Aspergillus terreus* RTCC 2214, *Aspergillus flavus* RTCC, *Aspergillus versicolor* RTCC RTCC 2197, *Aspergillus glaucus* RTCC 2200 were used in this experiment. PAW was prepared using distilled water and a GlidArc reactor as previously described [2]. The final parameters of PAW were as follows: conductivity 446 ± 25 µS/cm, pH 2.78 ± 0.12, oxidation reduction potential (ORP) + 1.06 V, NO_2 192 ± 10 mg/L, NO_3 1550 ± 95 mg/L, H_2O_2 2.6 ± 0.12 mg/L, and O_3 1.08 ± 0.07 mg/L. Suspensions of *Aspergillus species* (10^7 CFU/mL) were treated with PAW for different periods of time (5, 15, 30, 45 and 60 minutes) and specific volumes of 1:10 mixtures were further inoculated on Czapek-Dox Agar plates in order to evaluate the reduction of fungal burden after each contact period. In addition, some methods were used in order to assess the impact of PAW treatment on young and mature conidia structure: water quality parameters, melanin concentration, flow cytometric measurements, microelectrophoresis, Atomic Force Microscopy and Scanning Electron Microscopy.

A reduction higher than 4 $\log_{10}$ was observed after various exposure periods ranging between 15-30 minutes depending on the species. Ultrastructural studies detected impairments of the conidial wall with the absence of the outer cell-wall rodlet layer which is composed of hydrophobins.

A marked decrease in the electronegative charge of the conidia and cell surface hydrophobicity was also seen.

This study provides valuable information for describing the mechanism of action of PAW against fungal pollution.

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WG2: Antimicrobial effects of plasma

Characterization of RONS delivery following helium jets treatments of infected wound tissue models

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An infection can increase the healing time, which presents complications for the patient and additional medical fees. When healing time exceeds 6 weeks, it is called chronic wounds. Patients suffering of chronic wounds develop pain, necrosis, which can lead to limb amputation. One of the origins of the chronicity of wounds is attributed to colonization by bacteria of the tissues, blocking the mechanisms of tissue regeneration. Cold plasmas have already shown their effectiveness for acute wound healing [1] and for bacterial decontamination. Then cold plasmas represent hope in the treatment of some patients. There are still many steps before being able to treat chronically infected wounds for clinical treatment.
GREMI has developed multijet based on the Plasma Gun technology for large surface chronic wound treatment.[2] In this work, in the context of PLASFECT project, for in vivo mouse studies a two-jet device is studied. The measure of different reactive oxygen and nitrogen species (RONS) species produced by our jet, the estimation of their area of effect, as well as their penetration into the treated tissues, will be discussed with the objective to design the optimal protocols to promote the antibacterial and healing plasma-based effects. The potentialities of the plasma jets for subcutaneous tissues treatment is also under investigation, considering such relevant situation in a clinical context.

The plasma jets were produced for two different peak voltage amplitudes (3.5 kV and 4.9 kV), for different frequencies (5,8,12 kHz), and with a 2 slm helium flowrate with various oxygen admixtures. We used KI starch which reacts with the oxidizing species of the plasma which we incorporated into lated agar samples as a basic tissue model, and which allows both to visualize the surface exposed as well as to image their penetrations. We added RONS quencher in the KI starch agar, D-mannitol and/or catalase to quench respectively the OH radical and the H_2O_2. The agar plates were also covered with a bacterial lawn and then treated with plasma under different conditions. After an overnight incubation at 37°C the bacterial lawn becomes denser except in the treated areas where the bacteria have been inhibited.

Increasing the oxygen admixture in helium feed gas increase the concentration of ozone O_3 produced by the plasma, as well as the radius of action of the produced RONS, increase the diffusion across the agar sample, decreases the length of the plasma plume, reduce the power coupled to the plasma, and produces different chemical species than in the case of pure helium. The addition of quencher did not change the pattern nor the coloration of the treated KI starch agar petri dish which Indicating the key role of ozone as an active species.

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This work was supported by the PLASFECT project

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Continuous vs fractioned treatment modes: achieving effective bacterial inactivation with minimal thermal stress

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In the coming years, duration of space expeditions will increase. Thus, difficulties related to their special environments are the new challenges to overcome, including decontamination. Cold atmospheric pressure plasmas (CAPs) are nowadays well known and studied for their antimicrobial effect and their medical applications [1]. With the objective to develop a CAP device for space missions, we investigated the behavior of a DBD plasma jet ("Plasma Gun") in microgravity conditions. These conditions are achievable during parabolic flights as those organized every year by Novespace and the CNES [3].
However, parabolic flights impose strict timing requirements since a parabola lasts a maximum of 22 seconds. In the perspective of longer treatments characterization with these time constraints, we decided to compared continuous and fractionated treatment (ie. shorter treatments repeated multiple times so to reach the total desired treatment duration). The effect of these different treatment methods was investigated in terms of inactivation, RONS production and thermal stress.
Three mains results stand out in this study. First, areas of bacterial inactivation on *E. Coli* petri dish are similar for both treatments methods, as well as reactive oxygen and nitrogen species concentration in a plasma treated water.
Second, the thermal stress caused by both plasma treatments was assessed by temperature measurement on the agar surface and within the agar. Surprisingly, the temperature recorded at the surface of the agar is lower than the one recorded few millimetre under the surface, and could be superior to 40°C. Thus, inner temperature of the target during a plasma treatment should be incorporated in the development of therapeutic plasma treatment protocols.
Finally, it is demonstrated that fractional treatment allows to reduce thermal stress driven by a plasma treatment.
These findings combined could be of use to the plasma medicine community both for the comparison of different treatment methods and for the planning of treatments with reduced thermal stress.

Acknowledgments
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WG2: Antimicrobial effects of plasma

Study of low temperature plasma direct application on yeasts *Candida Glabrata* and bacteria

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The aim of this work was to study the interactions of the low-temperature microwave plasma with *Candida glabrata*, *Staphylococcus epidermidis* and *Escherichia coli* and their co-cultivations. The microorganisms were treated on selected solid media and in suspension (medium and PBS). In both cultivation variants the inhibitory effects of plasma were monitored.

The surface wave microwave discharge was used, with argon as a working gas. For the experiment using the solid nutrient media, a constant gas flow rate of 5.0 Slm and a power fluctuating between 8-10 W were maintained throughout the experiment. For the suspension experiment, a constant gas flow rate was 2.0 Slm and the power was 13 W.

The microorganisms treated on solid media were inhibited and uncultivable after the treatment with plasma, however their cytoplasmic membrane remained intact. When the microorganisms were treated in suspension, individual cells were killed, however the efficiency of the inhibition was low. This was caused probably by the experimental setup, specifically by the plasma only being formed in argon (no intermixing with air), as the capillary was submerged in the treated suspension.

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WG2: Antimicrobial effects of plasma

Influence of plasma and plasma treated water on fungus *Aspergillus niger*

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Based on the proven decontamination effects of low-temperature plasma on bacteria and yeasts, my work began to focus on the application of plasma and PTW to fungi. The fungus selected was *Aspergillus niger*, which is a filamentous fungus of the class Ascomycetes. The fungus is found worldwide and grows on a large variety of substrates, mainly cereals, nuts. Exceptionally, it can be pathogenic to humans as it causes the respiratory diseases Aspergillosis and Onychomycosis. *A. niger* is also ingested to produce its secondary metabolite, for example citric acid, oxalic acid [1-3].

At the beginning of the experiment, the number of spores was determined for optimal counting of the colonies growth and then the time for treatment of the samples. Different plasma systems (microwave source, AC, DC) were used. To decontaminate the fungus direct plasma, plasma-treated saline and their combination was used. In the direct treatment, the fungus was first inoculated into 25 ml of saline solution. The prepared sample was pipetted into the wells of the microtiter plate, which were then irradiated with plasma. Afterwards the samples were diluted 10 and 100 times using phosphate buffer. The samples were then spiked onto Petri dishes. When plasma treated saline was used, the fungus was inoculated directly into the treated liquid and sampling was carried out at 10 min; 3; 6; 24 h and then diluted and spiked onto Petri dishes. For the combination of both methods, two types of process were performed, where the first involved inoculation of the fungus into plasma treated saline, the sample was pipetted into a microtiter plate after one hour and then directly treated with plasma for 1, 2.5 and 5 min and the second involved reversal of the first process where irradiation was done first followed by addition of treated saline.

From the measured results it is evident that the highest efficiency was obtained when the sample was first irradiated and then plasma-treated saline was added to it using AC or DC. 2.5 minutes was the time at which there was a significant decrease in viable spores.

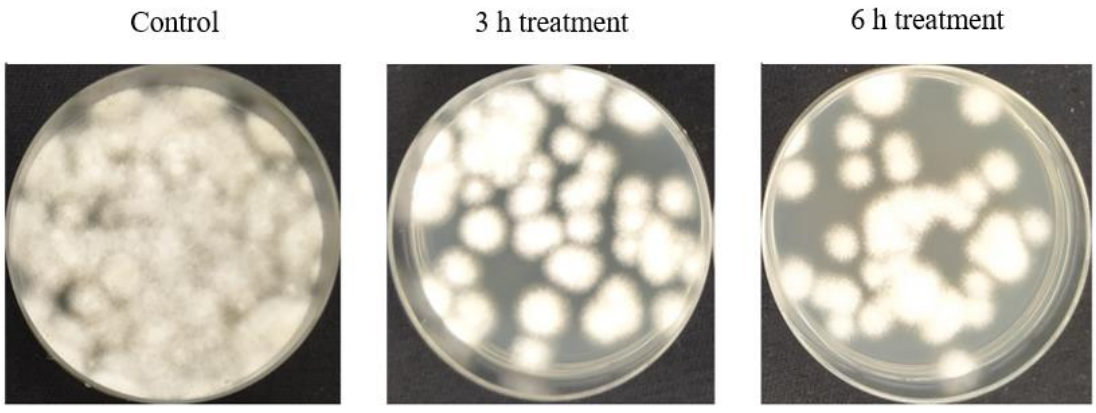

Fig. 1 Demonstration effect of PTW on *Aspergillus niger*

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WG2: Antimicrobial effects of plasma

Cold atmospheric plasma against fungal pathogens

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The kingdom of fungi is rich in various species with beneficial properties used in both nature and industry. On the other hand, a large number of fungi represent a serious threat to plants, animals, and humans, causing significant financial and resource losses by limiting the shelf life of fresh produce and infecting crops. Moreover, infectious diseases caused by yeasts and filamentous fungi lead to more than 1.5 million deaths annually [1]. Fungal pathogens are usually inactivated by various fungicides, which are considered environmental and health risks. Thus, the importance of finding new solutions for the inactivation of pathogenic fungi is high. Furthermore, various species produce harmful and usually carcinogenic mycotoxins.

With continuous deepening of our knowledge of the antimicrobial effects and inactivation mechanisms of cold atmospheric plasma (CAP) and plasma activated water (PAW) [2], this technology can be considered a potential key player against fungal pathogens in medicine, agriculture, and the food industry, replacing fungicides or other undesirable chemicals. Despite plasma's efficiency against the growth of filamentous fungi, little is known about the inactivation mechanisms on the cellular and molecular level. Although eukaryotic fungi are more resistant to antimicrobial agents than prokaryotic bacteria, it was shown, that CAPs act on multiple levels in fungal inactivation by causing permeabilization of the cell wall, resulting in the efflux of cytoplasm and leakage of organelles and macromolecules, DNA damage, or disruption of mycotoxins [3]. Of all the components of plasma, long-lived and short-lived reactive oxygen and nitrogen species (RONS) were shown to be dominant agents responsible for fungal inactivation. Oxygen radicals decreased the number of *Penicillium digitatum* spores by oxidative degradation of the cell wall and organelles [4]. However, the mechanisms of CAP on fungal inactivation need to be studied and characterized more thoroughly.

A transient spark discharge and a pulsed streamer corona discharge produced in atmospheric air were used for spore suspension treatment of two species of filamentous fungi, namely *P. digitatum* and *Botrytis cinerea*. We analyzed the direct effect of plasma discharge on spores, the post-treatment delayed effect of plasma discharge, and the effect of PAW. PAW was analyzed for its physical-chemical properties (pH, conductivity, oxidation-reduction potential and RONS content, i.e., concentrations of H_2O_2 and NO_2^-). The separate contributions of low pH and RONS content on the viability of fungal spores were described. The biological effects of CAP and PAW were evaluated as inactivation of spores on agar plates and metabolic activity of spores.

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

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WG2: Antimicrobial effects of plasma

Machine Learning-Based Regression Analysis for Predicting Antimicrobial Activity of Cold Atmospheric Plasma-Treated Liquids

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Cold atmospheric plasma (CAP) has emerged as a promising technology with diverse applications, including antimicrobial treatments [1]. However, outcomes of CAP treatments may vary due to factors such as variations in plasma sources, treatment parameters, and the diverse nature of biological systems being treated. This study focuses on harnessing machine learning (ML) techniques to develop predictive models for evaluating the antimicrobial activity of plasma-treated liquids (PTLs).

A comprehensive dataset comprising experimental measurements of antimicrobial activity, encompassing a wide range of PTLs, was utilized in this study. Several ML algorithms, including linear regression, support vector regression, random forest regression, and gradient boosting regression [2], were employed to construct accurate predictive models after the required pre-processing and parameter optimization. The regression analysis demonstrated the efficacy of the ML models in promisingly predicting the antimicrobial activity of PTLs. Among all algorithms utilized, the parameter-optimized random forest regressor model outperformed, yielded an R-squared value of 0.745, indicating predictive solid capabilities. Furthermore, permutation importance-based feature contribution analysis [3] was conducted to identify the key factors influencing the antimicrobial efficacy of PTLs. This analysis revealed the significant influence of specific input parameters, such as plasma treatment time, initial microbial load, and liquid composition on antimicrobial activity. The findings highlight the potential of ML algorithms not only in predicting the antimicrobial activity of PTLs but also in the possibility of valuable insights for optimizing the formulation of PTLs with enhanced antimicrobial properties. Thus, the integration of ML techniques in the field of CAP paves the way for the development of targeted and efficient antimicrobial treatments.

In conclusion, this work demonstrates the effectiveness of ML-based regression models in predicting the antimicrobial activity of PTLs and the potential applications of ML algorithms in the field of plasma medicine. The results contribute to a better understanding of the underlying mechanisms governing the antimicrobial properties of PTLs, thereby facilitating the advancement of innovative and tailored antimicrobial treatment strategies. Future studies may build upon these findings, exploring additional ML algorithms and expanding the applications of PTLs in various biomedical and industrial domains.

Acknowledgement

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WG2: Antimicrobial effects of plasma

Optimisation of plasma functionalised liquids for the treatment of orthopaedic implant infections

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Orthopaedic implant infections cause a significant burden on healthcare systems. Orthopaedic implants are commonly used to help heal broken bones or to replace joints. If the implantation site becomes infected the infection can be difficult to treat. This is because of the formation of bacterial biofilms on the surface of the implant and antimicrobial resistant bacteria. Current treatments for these infections include antibiotic treatment and wound debridement, however in some cases this is not adequate, and the implant must be removed via revision surgery. Cold atmospheric plasma (CAP) and plasma functionalised liquids (PFLs) have antimicrobial properties which could be used as a treatment for orthopaedic implant infections. The aim of this research is to optimise a PFL treatment to treat methicillin resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilm.

PFLs generated from several plasma systems including an in-house reactive species specificity (RSS) system in Spark and Glow discharges and a microwave discharge – Midiplex were investigated. The chemistry of the liquids was compared using colorimetric methods. The efficacy of the liquids was assessed against *S. aureus* (planktonic and biofilm forms) and *P. aeruginosa* (planktonic forms). This study has found that PFLs generated from different plasma systems had distinct chemistry as well as different efficacies. This study aims to assess the efficacy and safety of the optimised PFLs against mature biofilms, used both alone and in combination with direct CAP treatments.

WG2: Antimicrobial effects of plasma

Bubbles-PAW for *L. monocytogenes* inactivation

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According to the Food and Agriculture Organization of the United Nations (FAO) the concept "One Health" has been implemented in medical and agri-food fields during the past years. In this regard, there are bacteria such as *L. monocytogenes* that could inhabit in ecosystems, animals or humans and cause several problems; for instance, food-borne illnesses.

This work studies Plasma Activated Water (PAW) generated by an atmospheric pressure cold plasma jet system with dielectric barrier discharge. Each PAW was generated by bubbles method during 20 minutes. For their generation, three gas flows (40, 60 and 80 slm) were used. *L. monocytogenes* was chosen as the target bacterium and antibacterial activity of each PAW was studied after six contact times PAW/bacterium (30 min, 60 min, 2 h, 4 h, 6 h and 24 h). Optical Analysis Spectra, chromatographic, UV-vis and colorimetric methods were used for reactive species characterization (NO_2*, NO*, OH*, NO_2^-, NO_3^-). Moreover, other parameters such as temperature, oxidation-reduction potential (ORP), electrical conductivity (EC) and pH were also analyzed. Finally, in order to understand inactivation results, bubbles simulations were performed using a fluid-dynamic software (Ansys-Fluent) and the water-air interface area was calculated with CFD models.

In terms of bacteria inactivation, it was shown that the higher the air plasma flow, the higher the inactivation rate. OES showed no differences among the reactive species in gas phase regardless the PAW. Biocidal activity of PAW-40 and PAW-60 could be explained by the activity of the identified reactive species from the chromatography (OH*, NO* and NO_2*).Then, taking into account that the RONS identified in PAW-80 chromatography were really low, it could be possible that its biocidal activity was caused by a synergial activity of peroxinitrites and reactive oxygen species not identified (such as singlet oxygen). Those peroxynitrites could originate from H_2O_2 + NO_2^- and O_2*$^-$ + NO* generated by cyclic reactions.

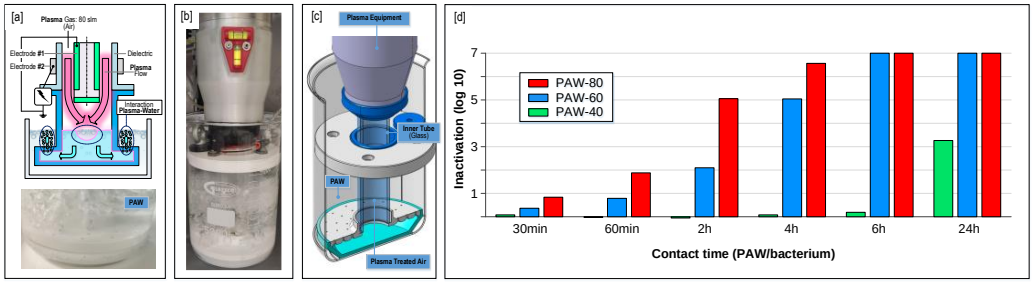

Fig. 1 [a-c] Scheme of PAW generation; [d] Bactericidal activity of PAW against L. monocytogenes.

Acknowledgements

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WG2: Antimicrobial effects of plasma

Biofilm inhibition and decontamination of medical surfaces by cold plasma treatment

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In the context of increasing rates of biofilm-associated infections, physical antimicrobial approaches, such as cold plasmas are receiving a great attention on the field of novel antimicrobial approaches. Studies developed in the last 10 years regarding the antibacterial efficiency and mechanisms of action of cold plasmas proved they could kill planktonic and attached bacteria with limited or no side effects against the host. However, careful optimization of plasma functioning parameters must be performed depending on the treated material and type of infection.

The purpose of this study was to obtain a cold-plasma based technology using a planar DBD (Dielectric Barrier Discharge) source, able to inhibit biofilm development and ensure complete removal of mature biofilms developed in different ceramic substrata.

Energy Dispersive X-Ray Spectroscopy was utilized to determine the atomic content of the plasma treated surfaces, while scanning electron microscopy (SEM) showed the morphological changes in the treated substrata. SEM analysis and viable counts-based methods were used to evaluate biofilm development and attached-bacteria removal ability of the developed plasma.

Bacterial viability studies revealed that viable bacteria could be recovered from the plasma treated surfaces for up to 6 hours, while no viability could be observed after 24h of contact with plasma treated materials. Viability of monospecific mature biofilms developed by relevant opportunistic pathogens (i.e., Gram positive strains of *Staphylococcus aureus* and *Enterococcus faecalis*, and Gram negative strains of *Pseudomonas aeruginosa* and *Escherichia coli*) was differently impaired depending on the number of plasma surface scans. Therefore, biofilms were significantly disrupted after one up to five plasma scans, while being completely destroyed after ten plasma scans of the surfaces. The SEM evaluation of the mature biofilm architectures post-plasma treatment revealed detached biofilm clusters and altered morphology bacteria cells. On the other hand, bacterial attachment and biofilm formation inhibition are mainly related to the hydrophobicity modifications of the plasma treated surfaces.

The developed DBD atmospheric plasma proved very efficient in bacterial attachment inhibition and biofilm removal from various substrata, and they can be further investigated for biomedical applications, such as dental and other medical surfaces.

WG2: Antimicrobial effects of plasma

Dielectric barrier discharge combined with photocatalysis for the treatment of indoor air

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Bioaerosols and chemical pollutants are present in most indoor air environments and can cause respiratory, cardiovascular, and oncological diseases. It is an important health issue as it might be responsible for an excess of millions of deaths each year [1]. Increasing indoor air quality would be therefore a major advance for public health. However, most of the conventional technologies for air purification are using non-destructive methods: the pollutants are trapped by filters that can later become a secondary source of pollution. Innovative technologies such as cold atmospheric plasma and photocatalysis can be used as destructive methods to decompose chemical pollutants and inactivate microbes and viruses without filters. Moreover, combining these two techniques may offer a very effective hybrid air decontamination device, as studies suggest a synergetic effect [2].

In this study, we used cold atmospheric plasma and photocatalysis for the treatment of indoor air at large gas flow rates (>300 L/min). We designed an air decontamination device that combines a surface Dielectric Barrier Discharge (DBD) for the plasma generation (Fig. 1), and a TiO_2 coating which is activated by UV-A LEDs. Experiments aimed at decomposing chemical pollutants to form CO_2 and H_2O and assessing bioaerosol inactivation containing bacteria E. *Coli*.

Despite a short gas residence time, results showed that the device is efficient to decompose chemical pollutants (tests were performed with acetaldehyde, acetone and formaldehyde). However, several passes into the device are needed to completely remove the pollutants. Further investigations are required to assess the effect on bacteria E. *Coli*. We also monitored the concentration of ozone generated by the DBD, which is not desired for human exposure but its excess is well decomposed by the photocatalytic process.

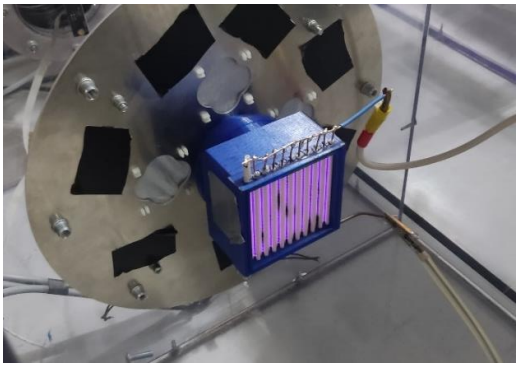

Fig. 1 Plasma DBD module

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

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WG2: Antimicrobial effects of plasma

Electrical Characterization and Efficacy Assessment of a Non-Thermal Plasma System for Closed Environment Sanitization

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The Covid-19 pandemic has highlighted the critical role of indoor air quality control and purification. To address this challenge, non-thermal plasmas (NTPs) based device have emerged as a potential solution for mitigating aerosol transport and reducing the infectivity of airborne pathogens [1]. At the Plasma Technology Laboratory (PLT) of the University of Bologna, our research group has been actively investigating and utilizing various NTP sources for different applications. In particular, we have recently focused on studying an NTP system, based on a Dielectric Barrier Discharge (DBD) as an air sanitizer for enclosed environments.

The ongoing project aims to develop a small-scale test NTP facility and optimize the electric power supply system. The desired optimal working conditions are focused on minimizing power consumption and the production of undesirable byproducts such as ozone and NOx. In the current phase, we are testing a configuration that utilizes grid-like coated electrodes (Rilsan®), powered by a 4 µs square bidirectional pulse voltage waveform with a peak voltage of 1.2 kV [2]. We have electrically characterized the discharge and estimate that the power involved is in the order of a few Watts. Additionally, we have integrated a diagnostic system to measure the ozone concentration, which can be leveraged for effective abatement processes.

To assess the efficacy of these devices in reducing the microbial load, a selected panel of bacterial and viral microorganisms were taken into account. Currently, our focus lies on testing the system's effectiveness against *Herpes Simplex Virus (HSV)* within an enclosed volume. The initial evaluation has yielded promising results. Additionally, we plan to contaminate an air stream with an aerosol containing *SARS-CoV-2* and assess the system's sanitation efficiency under these conditions. The preliminary findings motivate further investigations to unravel the underlying mechanisms and establish a comprehensive understanding of the relationship between electrical parameters and the sterilization process.

Furthermore, in September I will conduct a chemical characterization of the proposed DBD discharge through FTIR analysis at the SPC - Swiss Plasma Center - in Lausanne, supported by the COST STSM grant. The proposed chemical analysis will provide deeper insights into the system's operational mechanisms, facilitating an understanding of fundamental processes underlying sterilization efficacy. By investigating chemical composition and reactions during the discharge, we aim to optimize the NTP system for efficient and targeted sterilization applications.

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A 0D kinetic model of a humid-air DBD reactor

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Dielectric Barrier Discharge (DBD) reactors operating in humid air are widely used in many applications, including plasma medicine [1] and plasma agriculture [2]. Reactive species generated in a humid-air plasma play a central role in these fields and, consequently, plasma-kinetic models of are an essential tool to better understand and improve the operation of these devices. In this work, a 0D model is developed to simulate the chemical kinetics of a DBD reactor powered by a sinusoidal voltage waveform. The reaction set for humid-air (considered as a tri-molecular gas composed by nitrogen, oxygen and water) is based on the one proposed by Sakiyama et al. [3]. The average power delivered to the DBD during one time-period of the voltage source is assumed constant.

The occurrence of microdischarges in a time-period is evaluated assuming that a discharge initiates whenever the electric field E_{gap} in the air gap reaches a given threshold value E_{in}, considered as a simulation parameter. E_{gap} is calculated as the difference between the external electric field E_{ext} (due to the imposed voltage at the electrodes) and the field E_c generated by the charge deposited on the dielectric barrier. When the discharge takes place, E_c is updated to make E_{gap} equal to another parameter of the simulation, i.e. the extinction field E_{end}. The behavior of E_{ext}, E_c and E_{gap} is reported in Fig. 1. ZDPlaskin [4] was utilized to model the DBD kinetics. A microdischarge is simulated introducing a triangular power peak as a source term.

This approach allows one to account both for the input power and the frequency of the power supply.

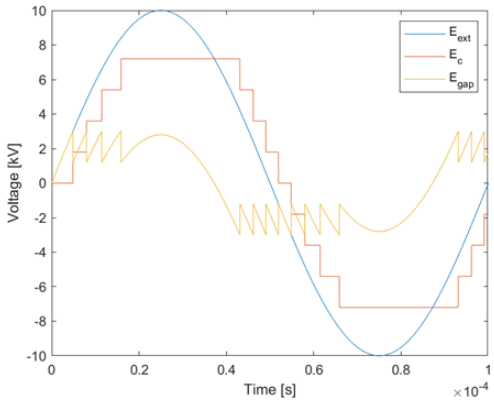

Fig. 1 Electric field due to the voltage at the electrodes (E_{ext}), field generated by the charge deposited on the dielectric barrier (E_c) and field in the air gap (E_{gap}).

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WG2: Antimicrobial effects of plasma

Low-temperature plasmas for biological applications at the Swiss Plasma Center

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The most recent frontier in low temperature plasmas is their use for biological applications. A variety of fields, ranging from plasma sterilization to plasma medicine, are presently being explored with a focus on both the development of industrial applications and the fundamental understanding of the interaction processes between plasmas and biological organisms. At the Swiss Plasma Center (SPC) at EPFL, we recently created a laboratory, dubbed "bio-plasmas laboratory", to investigate biological applications of plasmas. A virtual tour of the bio-plasmas laboratory is available in Ref. [1]. The activities of the group at the SPC are centered on the investigation of Dielectric Barrier Discharges (DBDs) and the effect of DBD plasmas upon biological organisms. The bio-plasmas laboratory is equipped with a variety of DBDs in various geometries and powered by different voltage waveforms, (AC, nanopulsed, etc…) and by state-of-the-art diagnostics to characterize the plasma phase as well as plasma-treated liquids. The DBD performances are investigated from the electrical and material point of view and optimized in a dedicated experimental setup to obtain stable plasma operation under high humidity conditions [2], which are needed when working with biological samples. Characterization of the reactive oxygen and nitrogen species in the plasma phase is obtained by using in-situ FTIR and a tunable diode-pumped high energy picosecond Nd:YAG laser for TALIF and LIF of the relevant species as well as the measurement of the electric field by E-FISH [2]. The liquid phase is characterized by using spectrophotometric techniques as well as Electron Spin Resonance spectroscopy (ESR). These diagnostics are complemented by tools to characterize the biological counterpart of the experiments. Besides standard CFU counting and basic biological protocols, in the bio-plasmas lab we can also perform single-cell time lapse microscopy and single-cell electrical impedance spectroscopy [4], which are used to investigate in real time the effect of plasmas and plasma-activated water on *E. Coli.* In this contribution, I will present a few highlights of our activities to study the inactivation mechanisms of *E. Coli* by plasmas and plasma-activated water, which are relevant to WG1 and WG2.

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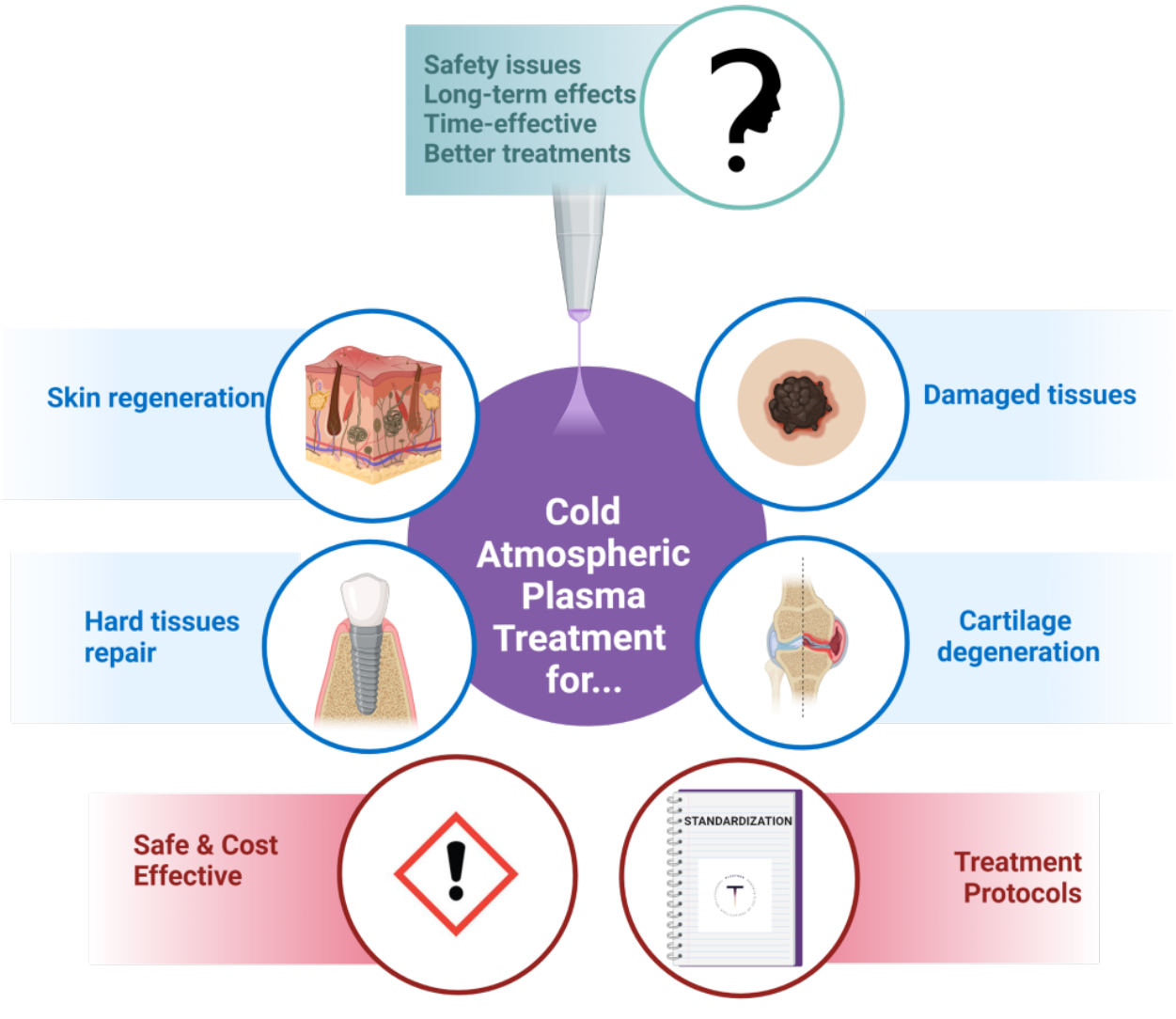
WG3 - Tissue regeneration

This WG will study the role of plasma on the regeneration of different tissues, namely skin in views on wound disinfection, stimulation of wound healing, but also on regenerative processes which are of great interest for the cosmetics industry. In both cases, there is evidence that plasma treatment can be efficacious, but the biological mechanisms involved in these processes are uncertain. In this context, there are concerns about safety, so it is necessary to have a better understanding of the molecular and cellular effects such as the implications of plasma stimulation of cell regeneration. Besides, other tissues are of great interest and research is starting to focus, for instance: mucosae, and cartilaginous and bone tissues.



This knowledge may be critical to the clinical success of this type of therapy, to find suitable treatments providing beneficial effects and avoiding damage.

Working Group Deliverables

D8	Development of standard techniques and protocols to characterize and test CAP and PTL for skin treatment applications.
D9	Report on the mechanism of action, therapeutic effect & safety of CAP and PTL therapies for tissue regeneration & decontamination in wound healing.

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WG3: Tissue regeneration

Cold Plasma for accelerated healing of chronic wounds in diabetic patients

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Diabetic foot syndrome is a common complication of diabetes mellitus and requires specialized treatment. Type 2 diabetic patients are often compromised by wounds with persistent infection and chronic inflammatory processes accompanied by a retarted wound healing.
Cold atmospheric pressure plasma (CAP) is a promising tool for biomedical and clinical application. Applying electric energy to molecular or noble gases generates partially ionized gases that mediate biological response mediated by reactive oxygen and nitrogen species (ROS and RNS) in combination with electric fields and mild UV radiation.
The goal of this placebo-controlled patient-blinded study was to show that application of cold plasma in addition to standard care treatment compared to placebo could accelerate wound healing with a significant regression of wound size. Wound closure progression and microbiological analysis were monitored time dependently to prove the effects. It is known, that wound oxygenation is an important factor of wound healing and scavenging reactive species could impair wound healing. Therefore, some patients were monitored by hyperspectral imaging in order to investigate the underlying processes, such as tissue oxygenation and microcirculation.
Here we show results of a randomized clinical trial with a clear focus on improvement of wound healing. Cold plasma treated wounds significantly earlier turned into healing process, irrespectively from bacterial load reduction and infection status. These results support the hypothesis that cold plasma efficacy do not primarily rely on antimicrobial effects, but directly turn chronic wounds into acute wounds, and therefore stimulate wound healing processes.

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WG3: Tissue regeneration

Non-invasive physical plasma in gynaecology and regenerative surgery – focus areas of the 'Division of Plasmamedicine and Medical Technology'

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Introduction:
Non-invasive physical plasma (NIPP) exhibits remarkable biological reactivity and can be administered non-invasively to human tissues through direct application via non-thermally driven corona discharge (NIPP) or indirectly via plasma activated liquids (PAL). At the Division for Plasma Medicine and Medical Technology, located within the Department of Women's Health in Tübingen, NIPP and PAL have undergone extensive physicochemical and biological characterization, along with rigorous evaluation in clinical trials and developments for clinical use spanning several years.

Results:
Our research group conducted preclinical and clinical studies that unveiled the remarkable therapeutic potential of NIPP in the regenerative treatment of low-grade (LSIL) and high-grade (HSIL) intraepithelial neoplasia of the cervix and vulva [1, 2]. Importantly, this therapy effectively combats these precancerous conditions while preserving tissue integrity and minimizing patient discomfort. This is enabled by transmembranous penetration of the biologically active factors [3]. Furthermore, in vitro investigations have demonstrated the impressive cytotoxic efficacy of NIPP against various gynecological tumor types in patient-specific 2D and 3D tissue models, showcasing its selective targeting properties. Detailed cellular mechanistic studies have illuminated the tumor-selective cytotoxicity mechanism, involving the influx of reactive NIPP species, transient DNA interactions, cell cycle arrest, and apoptosis [1,4].
In vitro studies involving PAL have also revealed selective antiproliferative effects in different tumor and benign tissues, specifically demonstrating promising applications in preventing postoperative adhesions by selectively inhibiting activated fibroblasts, modulating the expression of pro-adhesive factors, cytokines, extracellular matrix components and modulation of immune response [5]. Recent developments seek for the innovative and save generation and intracorporal use of PAL and Plasma activated Hydrogels (PlaG) and will enter preclinical testing soon.
By integrating NIPP with cutting-edge AI-driven molecular image recognition and image integration techniques, personalized and disease-specific in vivo application and dose monitoring has been made possible, significantly advancing the field.

Summary:
The therapeutic concepts revolving around NIPP and PAL / PlaG encompass tissue-sparing procedures for both prevention and treatment in the field of gynecology and regenerative surgery. The Division of Plasmamedicine and Medical Technology seeks for providing consultation and active scientific collaboration to facilitate clinical translation of plasma-based medical technologies.

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WG3: Tissue regeneration

Electric Field in Cold Air Plasma Jet as a Key Factor for Wound Healing

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The first measurements of the electric field within a cold air plasma jet at atmospheric pressure for medical purposes are presented. The values show excellent agreement with those used by medical devices called Wound Healing Electro-Stimulators that have been used for years with recognized success to accelerate wound healing by speeding up the migration of key cells [1], enhancing migration of lymphocytes, fibroblasts, macrophages and keratinocytes [2-7]. These results revitalize the discussion about the competition between the Electric Field as a physical effect and the Reactive Oxygen and Nitrogen Species (RONS) as a pharmacological effect in the healing of chronic wounds such as ulcers [8]. We propose the Electric Field delivered by the air plasma jet on the wound surface and its induced currents as the main therapeutic mechanism for reactivating microcirculation and breaking of wound stagnation until reaching its closure. The synergy between the Electric Field and the selective decontaminating action of RONS is proposed as a determining fact in the effectiveness of cold atmospheric plasmas as a unique tool in medicine for healing torpid wounds.

This work is based upon work from COST Action CA20114 PlasTHER “Therapeutical Applications of Cold Plasmas”, supported by COST (European Cooperation in Science and Technology).

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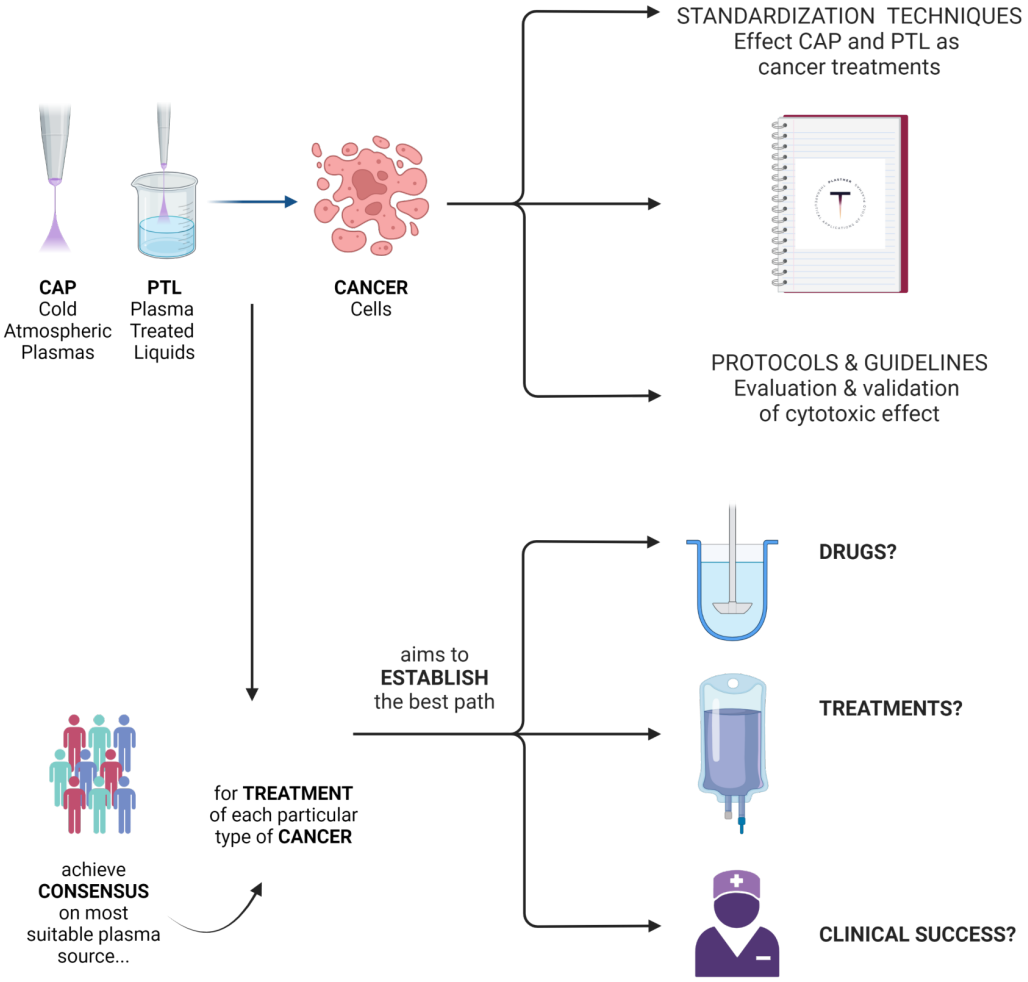
WG4 - Plasma cancer therapy

This WG will focus on CAP and PTL treatments for cancer therapy. This novel therapeutic approach is based on the ability of plasma treatment to kill tumor cells without affecting the healthy cells within the surrounding tissues. It has been demonstrated that indirect treatment using plasma-treated liquids (mainly containing long-lived RONS) exerts very similar effects compared to direct plasma treatment (that contains also short-lived RONS, radiations and electromagnetic fields).



In this sense, plasma generated long-lived RONS are thought to be among the major contributors in selectively killing cancer cells. Therefore, based on this fundamental insight, this Action will take particular attention to studying the field of redox biology to explain and understand the molecular basis behind the biological effects of plasma in cancer treatment.

Working Group Deliverables

D10	Development of standard techniques and protocols to characterize and test CAP and PTL as cytotoxic agent in anticancer therapies.
D11	Report on the mechanism of action and cytotoxic effect of CAP and PTL on cancer cells.
D12	Identify and describe the common therapeutic and side effects to determine the safety of CAP and PTL-based anticancer therapies.

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WG4: Plasma cancer therapy

The effect of plasma-induced oxidation on the cancer – natural killer cell inhibitory axis: a computational-experimental approach

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Plasma-based immunotherapy, a recent addition to the field of plasma-oncology, aims to harness and improve the natural immune response against cancer through treatment with cold atmospheric plasma (CAP) [1]. Natural killer (NK) cells, lymphocytes of the innate immune system that have the ability to directly kill malignant cells, play a major role in the body's cancer immunosurveillance and form an attractive target in immunotherapy. Previous research indicated that treatment of skin cancer cells with CAP augmented NK-cell mediated toxicity [2]. This effect was attributed to the changed amounts of surface ligands observed on the treated cancer cells, induced by the plasma-produced RONS. Indeed, whether or not an NK-cell will attack a certain cell is determined by the balance between activating and inhibiting signals received through binding of surface receptors to relevant ligands on the target cell [3]. However, RONS can also interact with the ligands already expressed on the cells. It is known that plasma treatment can cause oxidation and conformational changes in proteins expressed on treated cells, which in turn can influence their ability to bind their receptors [4].

Here, we computationally investigate the effect of oxidation, as would be induced by plasma treatment, of the cancer cell surface ligands HLA-Cw4 and HLA-E, on their ability to bind their NK-cell expressed receptors, respectively KIR2DL1 and the heterodimer NKG2A/CD94. These two complexes are part of the cancer – NK-cell inhibitory axis, and prevent NK-cell activation and toxicity upon binding to its target cell [5]. By employing molecular dynamics simulations, we determine the free binding energy of the two investigated complexes in both their native and oxidized state. Our simulation results show that the oxidation has a negligible effect on the binding affinity of both complexes. Combined with our previous experiments, the computational results indicate that the improved interaction must be attributed to the changed ligand expression after treatment, and not to oxidative changes in the already expressed ligands.

We acknowledge financial support from the Fund for Scientific Research (FWO) Flanders (Grant ID 1100421N and 1S67621N). The computational resources and services used in this work were provided by the HPC core facility CalcUA of the Universiteit Antwerpen, and VSC (Flemish Supercomputer Center), funded by the Research Foundation - Flanders (FWO) and the Flemish Government.

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WG4: Plasma cancer therapy

Exploring alginate hydrogels for minimally-invasive treatment of internal tumors with plasma

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Cold atmospheric plasma, as a source of reactive oxygen and nitrogen species (RONS), emerged as a promising cancer therapy with good selectivity for cancer cells. Furthermore, it has been shown that plasma could act through different mechanisms to promote anti-tumor immune responses [1]. Such responses are important for long-lasting and systemic therapeutic effects. Despite the promise, plasma faces different challenges that limit its clinical application. To this end, we proposed a novel plasma treatment modality, plasma-treated hydrogels (PTHs), which may help broaden the clinical utility of plasma, e.g., by (i) enabling non-invasive, controlled, and high local delivery of plasma-generated RONS and (ii) facilitating the development of combinatorial therapeutic approaches. We used our previous experience to describe a workflow for the development of PTHs [1]. This study represents the first example following the proposed workflow. We developed an injectable and shear-thinning alginate PTH for minimally invasive applications. We were able to generate, store, and release plasma-derived RONS from the alginate PTH to kill osteosarcoma cells. Moreover, for the first time, we investigated the ability of a PTH to induce immunogenic cell death in cancer to promote immune cell functions. For this, we co-cultured alginate PTH-treated cancer cells with human monocyte-derived immature dendritic cells (iDCs) isolated from healthy blood donors. Despite inherent differences between different immune cell donors, PTH treatment resulted in enhanced phagocytic uptake of osteosarcoma cells by iDCs. This is the first report on plasma-induced immunogenicity in osteosarcoma. Taken together, our study provides an encompassing *in vitro* characterization of an alginate-based PTH, highlighting the promise of this novel plasma-treatment modality and providing a rationale for further development and research.

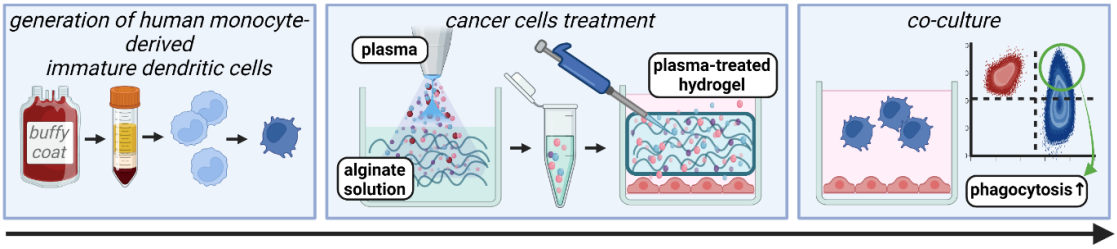

Fig. 1 Plasma-treated alginate hydrogel promotes phagocytic uptake of osteosarcoma cells.

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WG4: Plasma cancer therapy

Patient-derived Organoids as a state-of-the-art 3D tumor model for HNSCC

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Patients with advanced stages of head and neck squamous cell carcinoma often face relapse, metastasis (R/M HNSCC), and detrimental outcomes [1]. The current first-line treatment with immunotherapy alone or in combination with platinum-based chemotherapeutics (CIS) has limited benefits due to low response rates and severe side effects in already weakened patients [2]. Therefore, well-tolerated therapeutic strategies to improve currently established therapies are very much required. However, common *in vitro* tumor models (e.g. 2D monolayers) lack the ability to reflect essential components of the complex tumoral context, impairing *in vivo* translation and clinical implementation, as evidenced by the low development rate of successful treatment strategies. Therefore, our lab implemented a state-of-the-art, 3D *in vitro* model that uniquely mimics the phenotypic and genotypic characteristics from the original patient's tumor, the so-called patient-derived organoid model (HNSCC-PDO). PDOs originate from resection fragments or biopsies obtained during surgical intervention on HNSCC patients [3]. Currently, we were able to successfully set up a HNSCC PDO bank originating from 7 patients, enabling an in-depth screening of novel therapies against a diverse panel of HNSCC characteristics. As non-thermal plasma was previously reported as ICD inducer [4], our goal was to evaluate the immunogenicity of a novel combination strategy of NTP with the R/M HNSCC first-line therapies. To validate our data previously obtained in a 3D HNSCC spheroid model, the optimized NTP-CIS combination was tested in the HNSCC-PDOs to verify our treatment efficacy in patient samples. For the analysis of these therapeutic effects, our lab employs a fully in-house developed, state-of-the-art kinetic drug screening platform 'ORBITS' [5]. In response to the technical size restriction of the DBD-plasma probe, the original 384-well microplate platform is now optimized in a 96-well format to enable NTP application. We already demonstrated that the upscaled 'multi-organoid-per-well' model can be used to evaluate PDO killing with NTP, while evaluation of immune engagement by our optimized NTP-PLAT treatment is ongoing. In summary, the PDO model is a highly advanced 3D *in vitro* model with unique potential towards clinical translation. Validation of obtained results in this model is of great scientific and medical relevance and has the potential to drastically accelerate the introduction of novel therapies, like NTP, into the clinic.

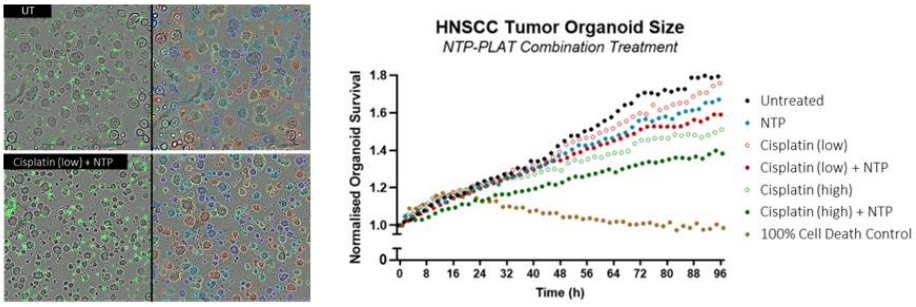

Fig 1. Live-cell imaging analysis of organoid killing for 96 hours after NTP-PLAT combination treatment in the 'patient-in-the-lab' HNSCC-PDO model. Orbits analysis masking of brightfield and cell death (green) images (left), of which organoid survival curves can be generated (right).

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WG4: Plasma cancer therapy

Combined Skin Cancer Treatment with Cold Gas Plasma and a Chromone Derivative shows Synergistic Efficacy *In Vitro* and *In Vivo*

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The potential use of cold gas plasma for cancer treatment alone or in combinational therapies has gained increasing interest. Although progress has been made towards understanding the effects of cold gas plasma on cancer cells, much still needs to be learned, especially concerning combinational therapies. Based on the recent success of small molecule-based targeted skin cancer therapies, we aimed to identify effective combinations of experimental small molecules with cold gas plasma. We confirmed a reduction in cellular metabolic activity, motility, and viability after oxidative stress induced by cold gas plasma in skin cancer cells. After screening an in-house 155-compound library using 3D tumor spheroids and high content imaging two promising chromone derivatives showing synergistic efficacy in combination with cold gas plasma were identified. Treatments of tumor organoids grown *in ovo* confirmed the principal anti-cancer effect of the selected drugs, especially when combined with cold gas plasma. In a xenograft mouse model, tumor growth was followed using caliper measurements and animal survival. While one of the two compounds (IS112) exerted severe toxicity *in vivo*, the other (Sm837) resulted in a significant synergistic anti-tumor toxicity at good tolerability. Both compounds reduced proliferation and viability and showed increased oxidative stress as well as DNA double-strand break formation in combination with cold gas plasma *in vitro*. A principal component analysis of protein phosphorylation profiles confirmed the substantial difference of the combination treatment from the monotherapies. In summary, we identified a novel compound that, combined with topical cold gas plasma-induced oxidative stress, is a promising substance to target skin cancers.

This joint research project “ONKOTHER-H” was supported by the European Social Fund (ESF), reference: ESF/14-BM-A55-0001/18 - 6/18, and the Ministry of Education, Science and Culture of Mecklenburg-Vorpommern, Germany.

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WG4: Plasma cancer therapy

UNRAVELING THE MULTIFACETED ANTITUMOR EFFECTS OF COLD ATMOSPHERIC PLASMA ON CHOLANGIOCARCINOMA

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Background and Aims: Cholangiocarcinoma (CCA) is a rare tumor of the bile ducts characterized by a poor prognosis (survival rate <5% at five years) and a rich desmoplastic stroma. To date, the only effective option is surgical resection, which can only be applied to resectable patients (<25%). As systemic palliative chemotherapies remain quite limited, it is mandatory to develop new therapeutic options against CCA, particularly local treatments targeting both the tumor and its microenvironment. In this outlook, cold atmospheric plasma (CAP) shows promises in oncology. Generated from the partial ionization of a gas, CAP generates reactive oxygen and nitrogen species that exert deleterious cellular effects leading to cell death or dysfunction.
Method: Human cell lines of CCA and of its microenvironment, namely cancer-associated fibroblasts (CAFs) and tumor-endothelial cell (TECs), were treated directly with CAP. Cytotoxicity and cell viability were determined by crystal violet assay. In tumor cells, immunogenic cell death (ICD) induction was evaluated *in vitro* by measuring the release of DAMPs (i.e ATP, Calreticulin) in the extracellular environment. Concerning the stromal compartment, alterations in phenotype and cell functions were analyzed by qPCR, WB, and live-imaging microscopy. These processes are also investigated *in vivo*, by studying the effects of CAP in an immunocompetent mouse model of CCA, as well as its impact on the mechanical properties of the tumor and its immune landscape.
Results: Our findings indicate that CAP diminishes CCA progression and appears to restore the tumor's mechanical properties, as demonstrated through in vivo ultrasound-based techniques. *In vitro*, we showed that CAP induces antitumor effects that can be direct (i.e tumor cell death) and indirect (i.e stromal cell dysfunctions, activation of immunosurveillance). As proof of the direct antitumor effects, we demonstrated that CAP-triggered oxidative stress decreases tumor cell viability and led to the release of ICD key messengers (mainly ATP and Calreticulin). Interestingly, CAP also exhibited indirect antitumor effects by modulating the tumor microenvironment, reducing CAF activation, impeding their migration, and inhibiting TEC angiogenic profiles. These findings highlight that CAP can drive the release of DAMPs by malignant cells in the tumor stroma and modulate the tumor microenvironment, which could stimulate the tumor-surrounding immune cells and promote antitumor immunity.
Conclusion: CAP opens perspectives for local treatment of CCA. In order to enhance the translational relevance of the technology for the patients, the plasma source has been miniaturized to deliver the plasma *in situ via* an endoscope (patented). In close collaboration with clinicians, feasibility and safety studies of the endoscopic plasma probe in pigs are in progress.

WG4: Plasma cancer therapy

Public and Patient Involvement (PPI) in knowledge production of plasma cancer therapy

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Since the early 1990s, there have come repeated calls to engage and involve patients and the public and to place them at the centre of healthcare. Developing stronger patient and public involvement (PPI) in the organisation and delivery of healthcare is now central to health reform across Western economies. This recognition reflects evidence that patients and the wider public can be involved and make a difference at most stages of healthcare and in service planning and delivery.

This proposal will attempt to explore the added value of PPI in knowledge production of plasma cancer therapy using the 4PI Framework as developed by NSUN.

Principles
- listening to service users and carers with respect and openness;
- creating an environment that is safe / accessible / inclusive for everyone;
- Sensitivity about language and actions.

Purpose
- collaboratively developing ways of working together;
- everyone being clear about the purpose of the involvement;
- openness to change focus with the input of different viewpoints.

Presence
- a diversity of services users and carers being involved;
- thinking about whose voices are not represented in the room and why this may be?
And taking steps to address this.
- actively making links with local community / patient advocacy groups, charities, local healthwatches, carers hubs etc.

Process
- making involvement opportunities available in different formats, languages (as appropriate) and locations;
- being aware of any bias / assumptions, and reflexive in your work.

Impact
- Better uptake of new evidence into practice;
- Improved outcomes for all;
- Valuing and sharing of different kinds of knowledge.

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WG4: Plasma cancer therapy

Comparing the induced cancer cell death pathways by direct and indirect non-thermal plasma treatment

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The ability of non-thermal plasmas (NTP) to induce cytotoxic effects and immunogenic cell death (ICD) in cancer cells, among others, has been demonstrated with a broad range of devices and treatment modalities. [1,2] Despite this, it is currently unclear whether the mechanism of action between these different NTP modalities is comparable, and this remains a long-standing challenge for the field. At the moment, the majority of studies that evaluate NTP-induced regulated cell death (RCD) were focused on apoptosis and ICD, even though these are only two of the twelve defined RCD mechanisms. These RCD mechanisms could provide a means to address the challenge to compare the different NTP modalities. [2,3] Thus, this study aims to characterize and compare the different cell death responses induced by direct and indirect NTP treatments.

Two human melanoma cell lines, A375 and SK-MEL-28, were NTP-treated either directly (DBD plasma) or indirectly (kINPen IND-LAB). To determine which RCD pathways were activated after NTP treatment, specific inhibitors were used for each pathway. Our results indicate that apoptosis, pyroptosis, and ferroptosis were activated after direct NTP treatment. In contrast, only apoptosis and ferroptosis were activated after indirect NTP treatment, with ferroptosis being the major contributor to indirect NTP-induced RCD. As there is extensive crosstalk between the cell death pathways, we further assessed the expression of key biomarkers for each RCD to verify our findings (Figure 1). We confirmed that apoptosis and ferroptosis were induced in both indirect and direct treatment. Pyroptosis was significantly upregulated after direct NTP treatment, whereas it was only minimally affected following indirect NTP treatment, confirming our previous results. In addition, necroptosis will be further explored.

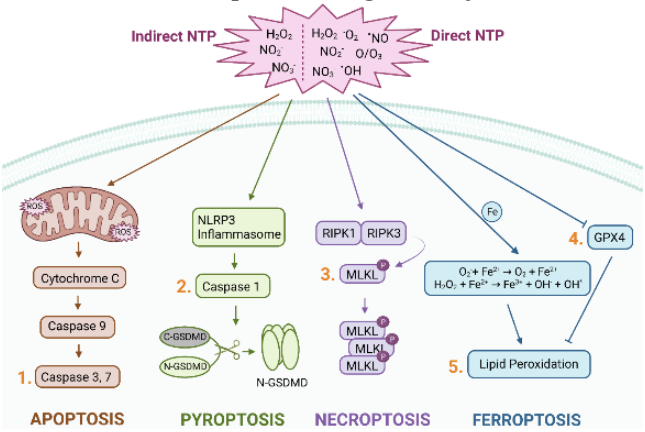

Fig. 1 Overview of the key biomarkers for each RCD pathway

Taken together, these results demonstrate that NTP can activate multiple cell death pathways independently, with variations between indirect and direct NTP treatment. Importantly, pyroptosis is exclusively induced by direct NTP treatment. This leads to greater fundamental understanding of NTP mechanisms of action in cancer and should be considered when developing therapies to minimize the risk of treatment resistance.

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WG4: Plasma cancer therapy

Effect of Plasma-induced Lipid Oxidation on the Permeability of Hemichannels for Treating Cancer Cells

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Cold atmospheric plasma (CAP) is playing an increasingly important role in biomedical applications, such as in the treatment of cancer cells. Their interesting property to induce cancer cell death is linked to the generation of reactive oxygen and nitrogen species (RONS) that permeate the cell membrane and cause oxidative stress, inducing damage to lipids, proteins, and DNA [1]. However, RONS can permeate more efficiently with the aid of transmembrane proteins, such as hemichannels. When two hemichannels of adjacent cells composed of connexin (Cx) proteins interact with each other, they form a gap junction (GJ) channel, connecting the intracellular space of these cells. Indeed, GJs have been advantageous for inducing cancer cell death via the transport of RONS to the cell interior and via the propagation of cell death induced by oxidative stress, apoptosis, and radiation [2]. Studies have shown that one of the mechanisms to control the formation and disruption of GJs is mediated by lipid oxidation pathways [3], but the underlying mechanisms are not well understood. It is well known that lipid oxidation increases the membrane permeability, but their effect on the permeability of hemichannels is still uncovered. In this study, we performed atomistic molecular dynamics simulations to evaluate how lipid oxidation influences the permeability of Cx26 hemichannels. We simulated a Cx26 hemichannel embedded into either a POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) lipid bilayer or an oxidized POPC lipid bilayer into a hydroperoxide group (POPCOOH). To simulate how RONS permeate through the membrane in the presence of the Cx26 hemichannel, $HO_2^{\bullet}$ radicals were added at the water/lipid interface. Our results demonstrate that the free energy barrier (ΔG) for water permeation across the Cx26 hemichannel is overall lower at the transmembrane region in the presence of oxidized lipids (Fig. 1A). Likewise, the $HO_2^{\bullet}$ radicals are more prone to permeate the Cx26 hemichannel in the presence of oxidized lipids (Fig. 1B). It may contribute to an intracellular accumulation of RONS to cause oxidative damage in cancer cells, improving the efficacy of CAP treatment based on GJs activity.

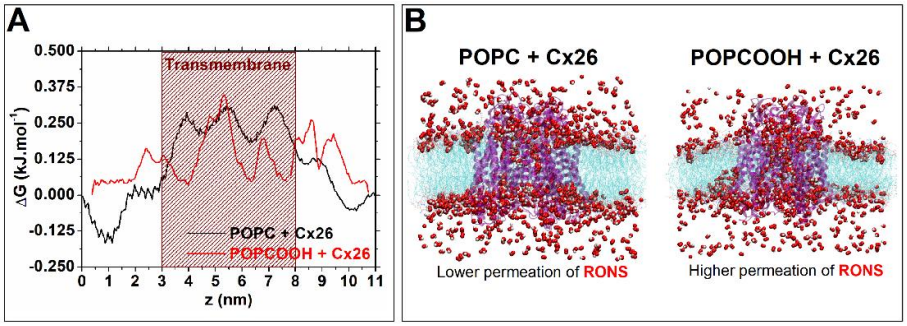

Fig. 1 Analysis of the permeation of water (**A**) and $HO_2^{\bullet}$ radicals (**B**) across the Cx26 hemichannel.

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WG4: Plasma cancer therapy

Impact of plasma-activated PBS on human prostate cancer cell line and noncancer prostatic cell line

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Patients with cancer go through difficult treatments that greatly disrupt their lives. Conventional therapies have a strong effect on different aspects of their well-being, such as their physical health, emotions, and overall quality of life. Ideally, an effective treatment modality should possess the ability to selectively target tumor cells, while minimizing harm to healthy cells, ensuring a higher level of resistance in normal tissue. Our research contributes to the growing field of plasma medicine by exploring the potential of cold plasma and plasma-treated liquids as a therapeutic modality [1].

This study focuses on the impact of indirect application of cold plasma via plasma-treated liquids, which contain long-lived reactive oxygen and nitrogen species [2]. The primary objective is to compare the effects of plasma-activated phosphate-buffered saline (PAPBS) treated by cold atmospheric plasma of streamer corona discharge on a human prostate cancer cell line PC3 and human prostatic stromal myofibroblast cell line WPMY-1, used as noncancer cells. Various durations of plasma treating of liquid were examined, alongside different incubation times of cells with plasma-activated PBS. The aim was to assess the effects of these different treatment parameters on noncancer and cancer cells. The outcomes demonstrate a selective effect observed in the targeted cancer cells. Preliminary results of direct plasma treatment by pulsed streamer corona of PC3 and WPMY-1 cells will be also shown.

These findings may pave the way for the development of innovative and more targeted treatment approaches for prostate cancer and potentially other malignancies, aiming to efficient tumor removal and minimize the detrimental impact of therapies on their lives.

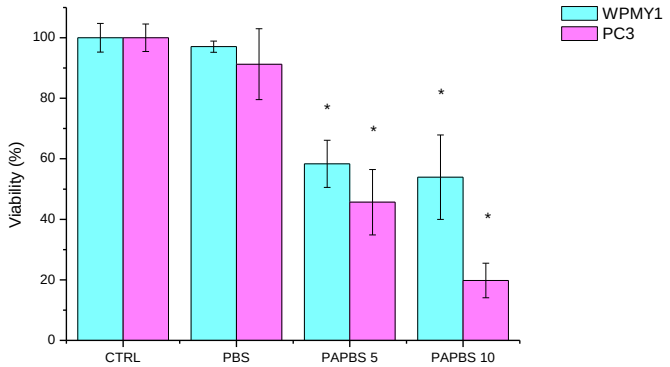

*Figure 1: Effect of PAPBS treated 5 and 10 minutes on cell viability of noncancer prostatic cell line WPMY-1 and human prostate cancer cell line PC3 measured by WST-1 assay. The time of PAPBS action was 1 hour, after which PAPBS was replaced with growth medium. Effect of PAPBS was tested compared to PBS. The significant results ($p < 0.05$) are marked with *.*

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

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WG4: Plasma cancer therapy

Cold Plasma Applications: Antineoplastic Effects in Chronic Lymphocytic Leukemia

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Cold atmospheric plasma applications in oncological diseases prevent cell proliferation and metastasis and trigger apoptosis at high doses [1]. Active plasma particles interact with the applied tissue or material, triggering the generation of ROS/RNS, which induces apoptosis [2].
In vitro and in vivo studies have shown that cold atmospheric plasma treatments (CAP) cause DNA damage of cancer cells associated with ROS/RNS production and induce apoptosis through an extrinsic mechanism [3].
Oncological diseases, which ranks second among the causes of death with 16.3%, are a general health problem. Considering the increase in cancer-related deaths and related complications, it is vital to prevent the progression of the disease with new treatment methods. Chronic lymphocytic leukemia, the most common type of leukemia, has been one of the most dynamic research areas in recent years. Chronic lymphocytic leukemia is a blood cancer characterized by the accumulation of mature CD5+ B lymphocytes in the blood, bone marrow, lymph nodes, and spleen [4**]**.
In this study, it was planned to investigate the direct and indirect effects of argon plasma source on the viability of cancer cells by applying argon plasma source to the chronic lymphocytic leukemia cell line MEC-1 cells and breast cancer cell line MCF-7, RPMI 1640 medium and antitumorigenic currant extract.

Acknowledgement: This abstract is based upon work from COST Action CA20114 PlasTHER "Therapeutical Applications of Cold Plasmas", supported by COST (European Cooperation in Science and Technology).

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WG4: Plasma cancer therapy

Osteosarcoma cell lines susceptibility to plasma treated water

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Osteosarcoma (OS) is a malignant bone tumour that occurs mainly in adolescents. It is accompanied by a high rate of pulmonary metastases and high mortality. The main challenges in OS therapy are its heterogeneity and drug resistance. The development of new therapeutic approaches is urgently needed to overcome the main mechanisms of OS growth. Cold atmospheric plasma-treated liquids (PTL) have demonstrated antiviral properties reaching cavities and tissues that direct application of other plasma sources could not. In the present work, the antitumour properties of PTL has been evaluated against different human (MG-63, 143B, SaOS-2) and mouse (MosJ) osteosarcoma cell lines. PTL was generated by employing a new plasma device [1] that allows to treat high volumes of liquid. PTL were obtained by treating 200 ml of deionized water for 30 min. Assays were performed using MTT (formazan) method to determine the viability of osteosarcoma cell lines that were exposed to PTL at different reactive oxygen and nitrogen species (RONS) concentrations and times. Among human osteosarcomas, SaOS-2 was the most vulnerable to PAM, followed by 143-B, with MG-63 being the most tolerant. Mouse osteosarcoma (MosJ) was even more resistant than human cell lines to different times and RONS concentrations. After 5 minutes treatment of the cells with 1x PBS-buffered PTL, MG-63 remained unchanged while 143B and SaOS-2 lost about 25% viability. For all cell lines, increasing exposure time and RONS concentration led to lower cell viability. Among other factors, the differences in susceptibility between cell lines could be due to the distinct mutations that characterize the original tumours. Increased intracellular oxidative stress would be responsible for triggering apoptosis and other degenerative processes in tumour cells. Research is ongoing to elucidate the mechanisms involved in the loss of cell viability caused by this PTL, and how it could be applied to the benefit of a potential treatment for osteosarcoma.

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Anticancer effects of plasma treated L-tyrosine enriched water solutions

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The last decades of research in cancer fighting clearly demonstrate how Reactive Oxygen and Nitrogen Species (RONS) are formidable tools in defeating cancer cells [1]. Liquids exposed to plasma and enriched with RONS, defined as Plasma Treated Water Solutions (PTWS), proved to be efficacious in the eradication of many cancer lines derived from aggressive tumors.[2] It has also been shown that PTWS prepared with solutions containing organic molecules address specific regulation of cancer cell death through changes in intracellular metabolites of glioblastoma cells. [3]

In this study, L-tyrosine, an amino acid with phenolic side chain, has been added to a physiological solution often used in clinical practice (Electrolyte Rehydrating III solution, SIII) to be exposed to plasma in two different experimental conditions (Air DBD and O_2 DBD). It has been found that the generation of RONS is enhanced when the plasma treatment is performed on a liquid in presence of L-tyrosine (PT-SIII-tyr) [4]. The efficacy of PT-SIII-tyr to cause tumor toxicity was evaluated in *in vitro* experiments on different cancer cell lines.

The results clearly indicate that the exposure to PTWS containing tyrosine produce stronger effects on cancer cells respect to untreated solutions resulting in an increase in intracellular ROS levels, in a reduction in cell viability and oxygen uptake, and triggers apoptosis pathways. The combination of the pro-oxidant effect of L-tyrosine and its by-products with the RONS could therefore represent a concrete way to synergistically attack tumor cells with PTWS components.

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WG4: Plasma cancer therapy

Plasma Therapy for Pancreatic Cancer

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Pancreatic cancer is the fifth most deadly cancer type and the survival rate is limited to 5 years [1]. The rate of treatment with surgery or radiotherapy and chemotherapy is very limited. Therefore, it is a type of cancer that needs to be developed in treatment areas [2]. Atmospheric plasma containing reactive oxygen and nitrogen has become a new option for therapy due to the need, and for this purpose, the cytotoxic effects of plasma-applied phosphate buffered saline (pPBS) on pancreatic stellate cell and pancreatic cancer cell lines, which contribute to the development of pancreatic cancer by causing insufficiency of treatment. A decrease in cell viability was observed in both cell lines. It also contributed to the maturation of dendritic cells, which play an important role in cell death because of their phagocytic effect [3]. In addition, the effect of plasma activated lactate solution (PAL) on pancreatic cancer cells was investigated and apoptosis was stimulated by uptake of ROS by the cells, and a decrease in the adhesion ability of pancreatic cancer cells was observed. It has been reported that H_2O_2, pyruvic acid and acetic acids formed in PAL are of great importance in terms of providing antitumor effect [4]. It is thought that more effective plasma-activated solutions (PAS) can be produced if the mechanisms underlying those seen in the efficacy of PAS are confirmed in the studies performed.

Key words: Plasma, Pancreatic Cancer, pPBS, PAL

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WG4: Plasma cancer therapy

RONS and Zn nanoparticles enriched alginate hydrosols

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The RONS enriched hydrosols and hydrogels are expected to be good candidates for the delivery of RONS in the case of different therapies, such as wound healing/disinfection or cancer therapy [1]. The present study presents the indirect plasma assisted deposition of RONS [2] and ZnO NPs into alginate hydrosols. Accordingly, the alginate solutions are prepared from RONS and ZnO NPs enriched water. RONS are deposited into the water with a surface-wave microwave discharge generated with the help of a *surfatron* wave launcher, which allows the tuning of RONS deposition in a wide concentration range [3]. 32 ml of DIW is positioned at 12 mm and 15 mm, respectively, from the edge of the discharge tube and treated with an argon discharge for 7 min under constant stirring (60-100 rpm). Under these conditions nitrite, nitrate and hydrogen peroxide of significant concentrations can be deposited into the treated water [3] as listed in Fig.1. The acidification induced by the plasma treatment is neutralized by using high reduction Zn metal powder during plasma treatment, thus assuring the stability of the deposited RONS [4]. The ZnO NPs are deposited into the filtrated RONS enriched water by laser ablation of Zn foil. The 1064 nm Nd:YAG laser is focused using a 250 mm lens on the target positioned from the lens at 125 mm distance in 20 ml solution. The energy delivered to the target surface is 320 mJ, the duration of the ablation is 60 s with 6 ns pulses at 20 Hz repetition rate. Fig. 1 shows the UV-VIS absorption spectra of the RONS and ZnO NP enriched water solutions indicating the high concentration of RONS (200-230 nm) and that of the ZnO NPs (230-400 nm). Fig 1. also shows the photoluminescence of the RONS and ZnO NP enriched 1% alginate hydrosols when excited with 351 nm.

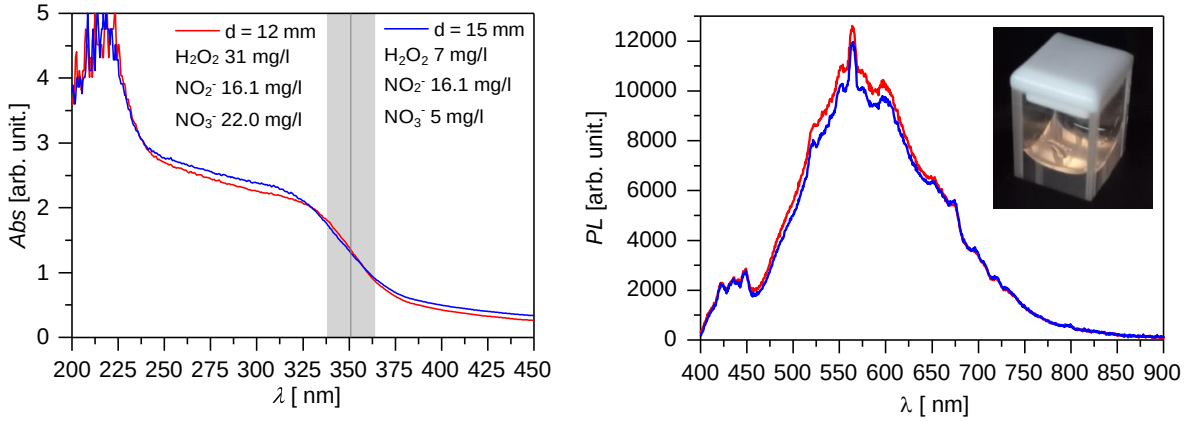

Fig. 1 UV-VIS absorption spectra a RONS and ZnO NPs enriched water and the photoluminescence of RONS and ZnO NPs enriched 1% alginate hydrogels excited with 351 nm light.

Acknowledgement: This work was supported by Hungarian NKFIH Science Founds K-132158.

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WG4: Plasma cancer therapy

The influence of cancer cells $FeCl_2$ pretreatment on the cytotoxicity of reactive chlorine species

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In this study, we evaluated the effect of reactive chlorine species (RCS) on the viability of cells, pretreated with $FeCl_2$. To produce RCS, physiological saline was treated with RF He/0.8% O_2 plasma jet operating in an ambient He/O_2 gas environment at a pressure of 200 Torr. Normal human skin keratinocytes (HaCaT) and melanoma (M21) cells were pretreated with $FeCl_2$, supplemented to cell growth medium (DMEM). The concentration of $FeCl_2$ in DMEM was varied in the range 0–400 μM. After 24h of incubation, the DMEM with $FeCl_2$ was replaced with fresh DMEM containing 0.5 mM RCS. The influence of $FeCl_2$ pretreatment on the cytotoxicity of RCS was evaluated using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay (Sigma-Aldrich), as described in [1,2]. The concentration of $FeCl_2$ did not influence the viability of healthy HaCaT nor cancer M21 cells (Fig.1).

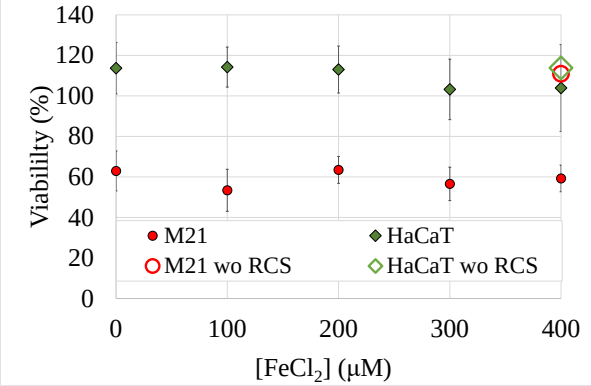

Fig. 1. Cells viability as a function of $FeCl_2$. concentration used in the pretreatment Closed symbols- following treatment by 0.5 mM RCS and open symbols- without RCS treatment, at 400 μM $FeCl_2$.

This result indicates diverse mechanisms of action of RCS compared to H_2O_2, as in the case of PAM with the main impactor H_2O_2 a similar pretreatment with $FeCl_2$ induced a clear increase in cancer cell injuries in an $FeCl_2$ concentration-dependent manner [3].

The present study was supported by the Estonian Research Council Grants no PUT1432, PSG448, PRG230, PRG1788, EAG79, Euronanomed II projects ECM-CART and iNanoGun and COST Action CA20114 PlasTher "Therapeutical Applications of Cold Plasmas", supported by COST (European Cooperation in Science and Technology). We thank Dr. Kai Kisand for providing HaCat cell line.

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WG4: Plasma cancer therapy

The effect of silica coated superparamagnetic iron oxide nanoparticles on cold plasma treated skin cancer cell lines

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Abstract: Cold atmospheric plasma (CAP) is a promising approach in anti-cancer therapy, eliminating cancer cells with high selectivity. The application of CAP for disinfection, wound healing, and cancer treatment are probably the most investigated areas of application. On the other hand, superparamagnetic iron oxide nanoparticles (SPIONs) have a dominant role in biomedicine [1]. In this study, mesoporous silica-coated SPIONs will be generated by microwave-assisted solvo-thermal route. We will test the anti-cancer efficacy of mesoporous silica-coated SPIONs in combination with cold plasma treatment. Therefore, two skin cancer cell lines will be treated with cold gas plasma (jet) followed by treatment with different concentrations of the synthesized particles. The effect of the mono and combined treatments will be assessed by measuring the metabolic activity as well as cell proliferation of treated vs. untreated cells. In this manner, we will seek to develop a novel dual cancer therapeutic method by integrating promising CAP and silica coated SPIONs and evaluate the underlying mechanism for targeting skin cancer cell lines. Through this research, we aim to make a valuable contribution to WG4. In this future work, these platform NPs will be loaded with curcumin (CUR) and doxorubicin (DOX), and will investigate their synergistic effect on the cancer lines in connection with the cold plasma treatment.

Keywords: Cold atmospheric plasma, skin cancer, SPIONs

Acknowledgement: The material characterization will be made possible by accessing the facilities at KTH Royal Institute of Technology. CAP applications on cancer cells will be made by accessing the facilities at University Medical Center Rostock via an STSM within the PlasTHER. This project has also received funding from The Scientific and Technological Research Council of Turkey (222S690-2519 TUBITAK-COST) and Istanbul University Scientific Research Projects Coordination Unit (TDK-2022-39501).

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WG4: Plasma cancer therapy

Modulating chemosensitivity of oral carcinoma to Cisplatin by combination with plasma activated medium on 3D cell models

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Oral squamous cell carcinoma (OSCC) is one of the most malignant neoplasms of the oral cavity, with a high mortality rate. Since the long-term survival rate of patients diagnosed with OSCC has remained unchanged over the past several decades, it is of utmost importance to discover new treatment modalities or enhance existing ones [1]. Since we previously demonstrated the antitumor efficacy of cold atmospheric pressure plasma (CAP) on OSCC cell lines in a two-dimensional (2D) monolayer cell model, we moved further and tried to explain the mechanism of these cytotoxic events as well as analyze the effect of plasma-activated medium (PAM) in combination with chemotherapy as a gold standard in carcinoma treatment. The modified plasma needle operating at 13.56MHz with He as working gas was used for PAM generation with the distance of 3 mm between liquid surface and the tube and applying different exposure intervals [2]. OSCC cell line (SCC25) was cultivated in 2D or 3D culture systems when regular culture medium was changed for PAM for 24 h before performing assays. PAM treatment showed cytotoxic effects on 2D-cultured OSCC by inducing apoptotic cell death through the activation of the intrinsic caspase pathway. To analyze the combined effect of cisplatin and PAM we used a 3D cell culture approach with OSCC spheroids, as this method reflects more closely the *in vivo* cellular response to chemotherapeutics [3]. When PAM was combined with the increasing concentrations of cisplatin, the results showed an almost linear dose-dependent decrease in OSCC spheroid viability. These are promising and encouraging results for a potential application of CAP in the treatment of oral carcinoma. By combining the effects of chemotherapeutics with PAM, we developed a new prospect for a possible cancer treatment in which the same or even better antitumor effects could be achieved with lower doses of cytotoxic drugs. Consequently, it means that with lower doses of chemotherapeutics, we could minimize potential side effects associated with the high-dose usage of cytostatics and improve the quality of life for patients with these malignancies.

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WG4: Plasma cancer therapy

Cold plasma as a new promising therapeutic approach in lung cancer

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The clinical outcome of Non-Small-Cell Lung Carcinoma (NSCLC) patients is still poor despite progress in treatments that now includes immunotherapy. In addition, only 30% of patients are responsive to anti-PD-1/anti-PD-L1 based therapies which involves that alternative or combinatory approaches still need to be explored.

Cold atmospheric plasma is currently investigated as an anti-tumoral physical approach. Reactive oxygen species (ROS) generated by plasma are assumed to activate cellular mechanisms such as oxidative stress, capable of initiating tumour immunogenic cell death, resulting in the activation of anti-tumoral immune responses. In the present study, we evaluated the direct anti-tumor effect of cold plasma as well as its abscopal effect, in a lung tumor model. We compared the efficacy of two plasma devices, JET source (that promotes the formation of reactive nitrogen species - N_2*, N_2^+) and POD source (that creates in addition reactive species of oxygen -especially O, OH), to induce lung tumor regression.

We first demonstrated the safety of the two plasma sources by assessing the absence of electrical hazard using targets mimicking electrical response of human body. We then demonstrated a strong direct and abscopal antitumor and anti-metastatic effects of both JET and POD plasma treatments. Mechanistically, we demonstrated that cold plasma treatments primed an adaptative immune response by inducing immunogenic cell death, that involves both $CD8^+$ T and $CD20^+$ B cells, as shown by depleting *in vivo* experiments, combined with flow cytometry and RNAseq transcriptomic analyses.

These results pave the way for using this novel physical approach to induce anti-tumor adaptive immune responses, that should be combined with immunotherapy to optimize the antitumoral treatment.

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WG4: Plasma cancer therapy

The Fourth State Matter: Potential of Cold Atmospheric Plasma Treatment in Malignant Mesothelioma

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Malignant mesothelioma is a deadly type of cancer that occurs in the mesothelium. The disease with a poor prognosis shows involvement in the mesothelial surfaces of pleura, pericardial, peritoneal cavity mesothelial surfaces and germline mutations [1,2]. The most cause of malignant mesothelioma is asbestos exposure. It has also been reported that it may occur with other mineral fibers, radiation, chronic inflammation, simian virus 40 (SV40) or idiopathic causes [2]. Incidence rates of malignant mesothelioma decrease with prevention of asbestos exposure; however, it remains a significant health burden due to the poor survival rate, resistance to chemotherapy and radiotherapy-induced mesothelioma [1,2]. There is an urgent need for new treatment approaches that are feasible and increase the survival rate. Cold atmospheric plasma (CAP) is a simple and effective strategy that can be applied in medicine, biomedicine and materials science. It has been shown that cold plasma applications can be a potential anticancer therapy method through the exogen source of ROS/RNS and various molecular signaling pathways. Several studies have been conducted on the endoscopic application of cold plasma sources, especially in anatomically difficult localizations or in large areas surrounded by healthy tissues such as body cavities. [3]. A recent study has shown promising results of cold plasma endoscopy (CPE) in the treatment of cholangiocarcinoma (CCA) in experimental models [4]. In summary, CPE in malignant mesothelioma may provide combined benefits that cannot be achieved with conventional cancer treatments as it is minimally invasive, localized, anti-cancer effect and can trigger the activation of immune cells in the tumor microenvironment.

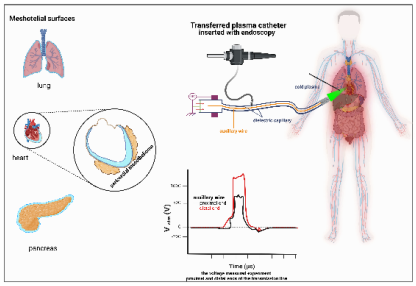

Fig. 1. Potential cancer treatment methods with endoscopy integrated CAP

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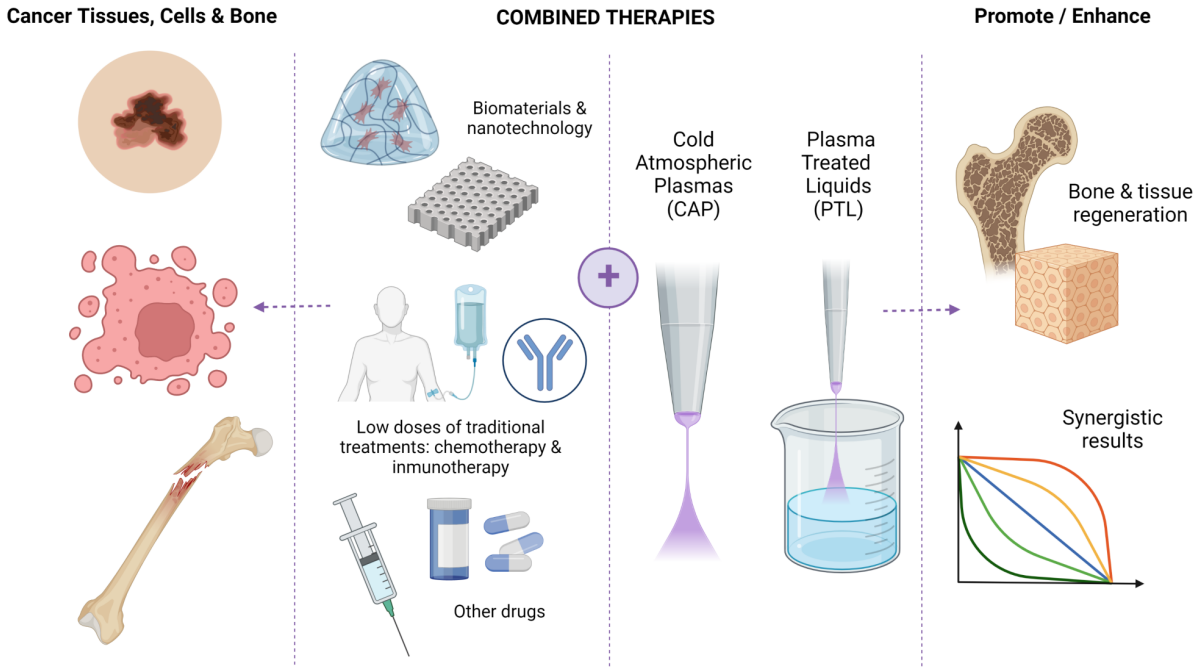
WG5 - Combination therapies

Both for tissue regeneration, skin treatment, wound healing as well as for cancer treatment, in complex cases, the use of plasma alone may not be sufficient to achieve full patient recovery, so investigation of combination therapies with ie. low doses of drugs may be an important asset.



Working Group Deliverables

D13	Report on the effectiveness and safety of CAP and PTL-based therapies combined with biomaterials for skin regeneration.
D14	Report on the effectiveness and safety of anticancer co-treatments combining CAP and PTL with low doses of chemotherapeutic drugs or specific metabolic inhibitors.
D15	Report on the potential use of CAP and PTL combined with biomaterials in the regenerative medicine field..

WG5: Combination therapies

Cold Plasma Deposition as a novel technology for targeted cancer drug delivery

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Glioblastoma multiforme (GBM) is the most common, malignant and aggressive brain cancer. Despite many innovations regarding GBM treatment, the final outcome is still very poor, making it necessary to develop new therapeutic approaches [1]. Cold Atmospheric Plasma (CAP) based technologies are being studied as new possible approaches against cancer, such as Plasma-Activated Liquids (PAL), as well as plasma deposition (PD) of new therapeutic coatings [2-3] to target the local delivery of oncology drugs to cancerous tissue.

Topotecan (TPT), a water-soluble topoisomerase I inhibitor with major cytotoxic effects during S-phase of the cell cycle, possesses potent antitumor activity. However, systemic administration of TPT is still limited for cancer types such as Glioblastoma due to low levels of blood-brain barrier crossing. For these reasons, TPT may be repurposed for local combined therapies [4]. The overall research aim is to explore the therapeutic properties of a combination between plasma based technologies and TPT on a human brain cancer cell line (U-251mg).

Combined treatments with PAW and TPT showed a reduction of the metabolic activity and cell mass, an increase of apoptotic cell death, and a reduction of the long term survival. Single applications of PAW+TPT treatments were able to inhibit cell growth as well as cell survival of glioblastoma cells, showing a cytotoxic effect in short term and an anti-proliferative effect in long term.

Evaluation of the TPT plasma deposition onto cells could reveal new avenues for a wide variety of new combinations and approaches to local drug application in tumor margin treatment. Preliminary results indicate that the plasma deposited TPT largely retains the cytotoxic features of the dissolved drug and may present a potential synergistic effect which is currently subject of further characterization.

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WG5: Combination therapies

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

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Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide. The current first-line treatment strategy in the recurrent/metastatic setting (R/M HNSCC), immunotherapy alone or in combination with platinum-based chemotherapeutics (PLAT), has limited benefits due to low response rates and severe side effects in already weakened patients [1]. Non-thermal plasma (NTP), an ionised gas containing reactive oxygen and nitrogen species, has been reported to induce immunogenic tumor cell death (ICD) [2]. Therapeutic ICD inducers, like NTP, are clinically interesting as they can engage the patient's natural cancer immunity cycle and provide long-lasting anti-tumor immunity. Therefore, the study aim was to investigate a novel combination strategy of NTP with current first-line therapies of R/M HNSCC to improve treatment efficacy and response. We optimised a micro-tissue spheroid model for several HNSCC cell lines to better recapitulate the complex processes in HNSCC and its tumor microenvironment. All experiments were performed using a microsecond-pulsed dielectric barrier discharge plasma system. After tumor kinetics were determined, combination treatments of NTP and PLAT (cisplatin) were analysed for the induction of several membrane-associated and secreted ICD markers. Immunogenicity was tested functionally with dendritic cell (DC) co-culture experiments. Our data show a significant upregulation of the cell-surface exposed ecto-calreticulin, an important 'eat-me signal' for immune cells, along with two heat-shock proteins among multiple HNSCC cell lines at 24h post treatment. In addition, combination therapy improved the release of both the early ICD marker ATP, as the late-stage factor HMGB1. Evaluating immune cell function, DC co-culture experiments demonstrated increased phagocytosis of HNSCC tumor cells of NTP-CIS combined application. These results highlight the potential of NTP to enhance treatment efficacy and tumor immunogenicity. This data was further validated in HNSCC tumor organoids, our patient-derived model that uniquely mimics the phenotypic and genotypic characteristics from the original tumour. This all together will accelerate clinical translation of the obtained results and is a pivotal step towards a rationally designed combination strategy with NTP to improve current first-line HNSCC therapies.

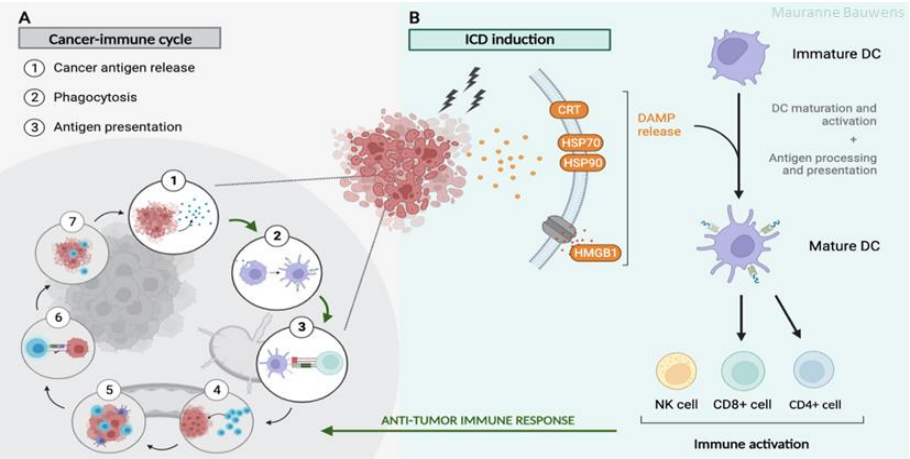

Fig. 1: ICD induction via NTP-CIS combination strategy increases tumor immunogenicity in HNSCC.

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WG5: Combination therapies

Plasma-Activated Hydrogel Therapy: An On-Demand Drug Delivery Platform for Multiple Clinical Indications

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In this talk, I will discuss a unique "plasma-materials" platform aimed to deliver therapeutic drugs effectively and plasma species safely into the diseased human tissue (Fig. 1). The novel material is composed of drug-loaded sodium polyacrylate (PAA) particles, dispersed within a polyvinyl alcohol (PVA) hydrogel matrix [1]. The composite material undergoes changes in pH and ionic strength upon plasma exposure, causing the collapse of PAA particles and release of the drug deep into the target. Results show an enhanced release of a range of proven antimicrobials (gentamicin, PHMB, Polymyxin B) for up to 14 days and effective microbial killing in biofilm models. We demonstrate the compatibility of the composite material with other therapeutics such as anticancer agents and nanoparticles, and activation by different kinds of plasma devices. The plasma-activated composite material also filters the potentially genotoxic plasma species such as hydroxyl radical to deliver only the beneficial species such as hydrogen peroxide as well as increasing oxygen tension within the tissue promoting healing.

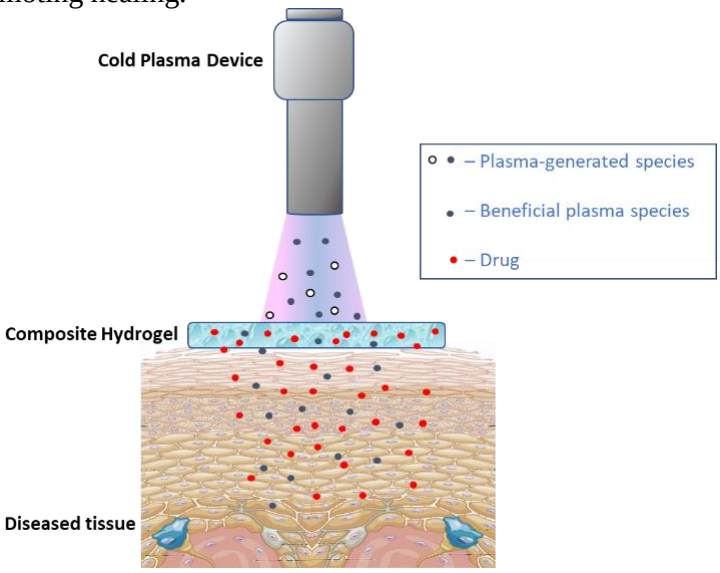

Fig. 1 Illustration of the concept of plasma-activated hydrogel therapy for a diseased tissue

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WG5: Combination therapies

Nanoporous plasma polymer films as functional layers for the delivery of reactive oxygen species

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Siloxane plasma polymer films (PPFs) are ideal candidates to functionalize catalyst layers producing reactive oxygen species (ROS). Due to their silicon organic (SiOCH) nature, the structure remains stable under the highly oxidizing conditions of the ROS atmosphere [1], whereas common organic coatings are rapidly decomposed. The catalyst and the functional PPFs can be deposited with low-pressure cold plasmas at room temperature, allowing the coating of soft materials as used for bio applications. Using hexamethyldisiloxane (HMDSO) as monomer with varying plasma operating parameters such as the applied power, the composition of the gas mixture, or the flow rate of reactants, the properties of the plasma polymer films can be varied [2]. In particular, these PPFs can be optimized for their nanoporous structure, acquiring pore volumes as high as 20%. This nanoporosity allows the infusion of small molecules such as polyethylene glycol (PEG) [3], as well as the penetration of H_2O and O_2 molecules. Therefore, nanoporous PPFs are interesting candidates for the functionalization of photocatalytic metal oxide surfaces, allowing H_2O and O_2 molecules to reach the catalytic interface while separating the metal surface from the environment. In this work, we deposited HMDSO-derived PPFs on top of metal oxide thin films of TiOx/AgOx prepared by magnetron sputtering. These metal oxides can be catalytically re-activated with visible light [1], making them interesting for medical applications avoiding UV light exposure. The antibacterial properties and the production of ROS mediated by the functionalized coatings have been tested. Thus, the production of ROS can be adjusted to inhibit bacterial growth, while avoiding potentially harmful conditions according to human levels. This non-toxic combination of metal oxides and PPFs presents a promising candidate for medical applications.

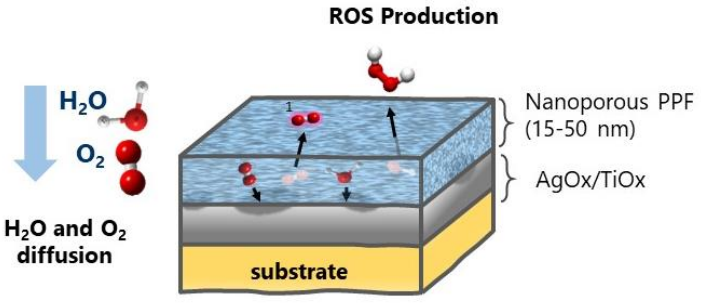

Figure. Scheme of the combination of catalytically active metal oxides and plasma polymer films (PPFs). When H_2O and O_2 molecules reach the metal surface, reactive oxygen species (ROS) are produced and delivered by the polymeric layer.

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WG5: Combination therapies

Light-Activated Antimicrobial Agents as Photocatalytic Method for Protection of Surfaces with Increased Risk of Infections

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Hospital-associated infections (HAIs) have a profound impact on patients, healthcare systems, and society as a whole. In the European Union and European Economic Area (EU/EEA) 8.9 million HAIs occur every year in acute and long-term care facilities. 1 million of the 4.1 million maternal and neonatal deaths annually worldwide may be related to unhygienic birthing practices, including lack of hand hygiene. The development and promotion of alternative, less harmful biocidal agents are crucial for the continued reduction of HAIs prevalence and the minimization of negative environmental and health impacts. In this context, **the aim** of the present study was to study and confirm a new method of photocata-lytic protection of surfaces by using light-activated photocatalytic antimicrobial paint that contains copper-doped TiO_2 anatase, active in the visible spectrum. The new composition is characterized by a wide range of analytical methods, such as UV-VIS spectroscopy, electron microscopy (SEM), X-ray powder diffraction (PXRD) or X-ray photoelectron spectroscopy (XPS). The new photocatalytic antimicrobial method uses a type of photocatalytic paint that is active in the visible spectral range and generates reactive oxygen species with inhibitory effect against all tested microbial strains. The direct impact of the results could be evaluated *in situ* by sanitizing two of the four patient area of Targoviste County Emergency Hospital, Palliative Care Sector, Romania, by applying the paint sample that included the TiO2 anatase pigment. The tests showed that the number of health-care associate infections and the frequency of their transmission decreased significantly, most of the strains being isolated from the non-sanitized salons. These results were obtained during the period of the COVID Pandemic (2020-2021) when the number of admissions and the turnover of patients increased significantly, doubling compared to the similar period 2018-2019, specifying that during the pandemic the Palliative Care section became a support section COVID.

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WG5: Combination therapies

Cold Plasma application may have the potential to be used in cancer treatment as it stimulates exosome release

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Exosomes are extracellular vesicles released from many body cells, and their size is between 30-150 nm. When a stimulus comes to the cell, it is stimulated, goes out of the cell, and transports signals and molecules to other cells. Although exosomes released from cancer cells are involved in metastasis, they can reverse these effects when a chemotherapeutic drug is loaded [1]. Our studies found that when anti-cancer drugs are loaded onto exosomes released from HeLa human cervical cancer cells, it has a faster and lower dose effect when treated with cancer cells again.

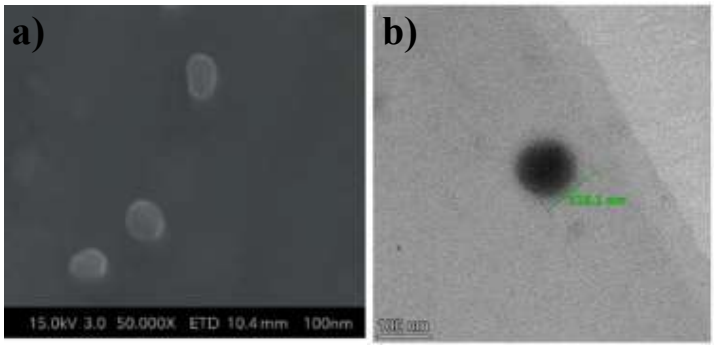

Fig. 1 (a) SEM image (b) TEM image of HeLa derived exosomes [2-3]

Exosomes are released faster from cancer cells when cold plasma is applied. If cold plasma is applied to HeLa cancer cells, the number of exosomes released increases by 32%, and the average exosome release time is shortened by 25%. Cold plasma should be applied into the cell medium in cell flasks of 75 cm^2 (each application 30 seconds, 4 times, 5kV), and exosome release should be accelerated. It causes an increase of reactive oxygen and reactive nitrogen species in cold plasma cell medium and increases cellular stress, so exosomes are released rapidly. A greater amount of drug is encapsulated in drug loading studies when the number of exosomes increases without damaging the cells. Anti-cancer potentials can be increased in exosomes produced with cold plasma, which are effective on exosomes such as docetaxel and paclitaxel, which are effective in cancer but cause toxicity.
Combined treatments with cold plasma applications will provide a new perspective in developing personalized cancer treatments for exosome-related drug delivery studies in the future.

- *Acknowledgement:* This work was supported by Aydin Adnan Menderes University Research Grant (ADU-TPF-20013, TPF-20041).

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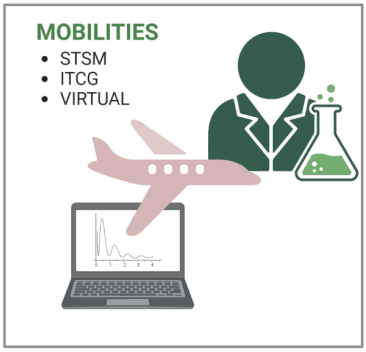
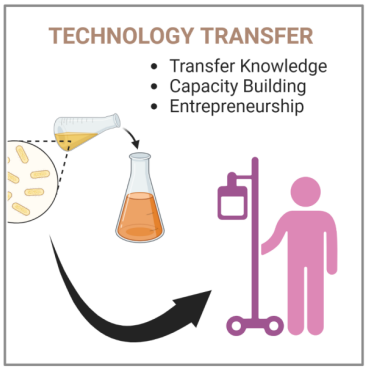
WG6 - Regulatory, ethics, dissemination & technology transfer

This WG will consolidate the multidisciplinary network actively involved in plasma medicine to facilitate scientific knowledge exchange through conferences, e-learning, video materials, seminars and training schools. It will build capacity, entrepreneurial programs, and encourage mobility for young scientists. All participants of the Action will be involved in this WG. Activities will include brainstorming, transfer of knowledge, capacity building and development of the entrepreneurial program (s) and mobility. The WG will organize workshops, training schools, conferences, online seminars, STSM, web page with member area to share confidential materials, & social media (twitter/Instagram). The special focus will be on participants coming from ITC, PhD students and ECI.





Working Group Deliverables

D16	Data management plan
D17	Create content for Interdisciplinary summer/training school and workshops.
D18	Conference, seminar/webinar and poster presentations.
D19	Creation of web page & dissemination through digital platforms, social media.

Easily accessible ex-vivo animal and electrical model for the technology transfer in plasma medicine

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Plasmas are beautiful. Anyway, alone they are mostly useless (with the exception of lighting and art). What is really interesting is the interaction of plasma with a target. The combination of plasma and target define a plasma system and an application. In plasma medicine, the final target is the human body. Nevertheless, for the development of new medical applications of plasma, it is necessary to use of other intermediate targets such as 2D and 3D in-vitro models and in-vivo animal models.
These intermediate targets are essential for the understanding of the interaction between plasma and biological tissues. However, these models also present electrical and physical properties extremely different from the one of a human body. Thus, the plasma generated on these models could have characteristics that are impossible to reproduce on a real human body [1]. This ends up potentially affecting the reliability of the results obtained on the in vitro and in vivo models and therefore the technology transfer for the treatment to patients. Anyway, since directly testing on human patients is clearly not a viable option the necessity of a suitable model arises. In a previous work we demonstrated how the use of a small and affordable electrical circuit could approach the models electrical characteristics to that of a human body [2]. In this new work we push the concept even further with the implementation of an ex-vivo animal model (Figure 1). The combination of the human body electrical circuit (HBEC) and the ex-vivo model allows to mimic both the electrical and surface characteristic of a human body.
This new model allows the fast and affordable investigation of realistic plasma conditions that could be reproduced on a real human body. Furthermore the use of HBEC makes possible to evaluate the safety of the condition according to the IEC standard for electromedical instruments [3].
This preliminary study should soon make it possible to define the appropriate parameters to be used on in vitro and animal models in order to obtain plasma conditions that are truly transferable to a patient. This new approach, based on affordable models, should be largely accessible to COST members and ease the technological transfer of plasma medical applications.

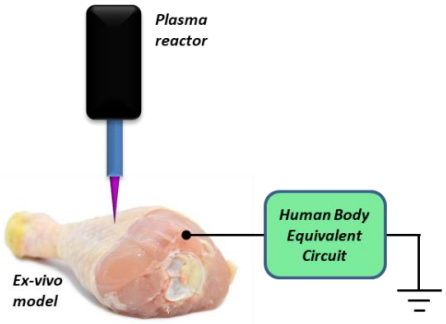

Fig. 1 Ex-vivo and electrical combined model

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WG6: Regulatory, ethics dissemination & technology transfer

Low-friction and safe coatings on suture needles via atmospheric pressure plasma polymerization

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With the objective of reducing the friction forces that are experienced during needle insertions, in our previous work [1], atmospheric pressure plasma-polymerized coatings based on different amino-silane liquid precursors were deposited on spinal needles. Decreasing these forces mitigates patients' pain, improves method accuracy and decreases bleeding and recovery times. Siloxane and amine molecules are necessary for achieving a low-friction behavior and avoiding the detachment of coatings after the injection test [1]. So, this study focuses on optimizing the low-friction capacity of plasma-polymerized coatings based on N1-(3-trimethoxysilylpropyl) diethylenetriamine (TRIAP) (the precursor that showed the best results in our previous work) on curved suture needles. The influence of plasma power, precursor gas flow and treatment time was analyzed. The evaluation of the penetration forces was carried out in accordance with the standard test method ASTM F3014-14. Coated and uncoated (control) suture needles were inserted 10 times into meat samples (as opposed to 4 insertions that were performed in the previous study). TRIAP-based coatings decreased friction forces around 30%, when compared with currently commercialized suture needles. This study also aims at the durability and toxicity of the coating; that is, ensuring that it remains stable during the puncture test, crucial for wound suture applications and to prevent lesions such as granulomas, rashes and reddening of the skin that occurs when silicone coatings are detached during needles punctures [2]. Toxicity was analyzed thought the identification of volatile compounds of the coatings by P&T-GC-MS essay. SEM and EDS analyses confirmed that the coating remained unchanged after 10 insertions. It has been noticed that the increase in punctures and the more complex curved geometry of the suture needles make it necessary to slightly increase the treatment times (compared to our previous work) to achieve a durable low-friction nature.

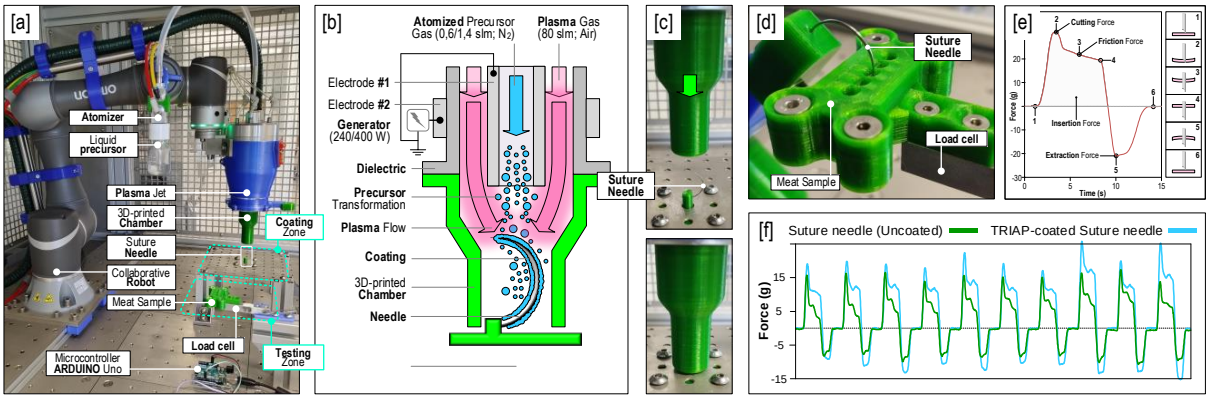

Fig. 1. [a] APPJ equipment, [b] plasma-polymerization scheme, [c] coating process, [d] close view of penetration test, [e] force diagram and [f] puncture results

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STSM - Short-term scientific missions

Short-term scientific missions (STSM) are exchange visits between researchers involved in a COST Action, allowing scientists to visit an institution or laboratory in another COST Member state/Cooperating Member or Near-Neighbour Country joining the Action.

Their aim is to foster collaboration in excellent research infrastructures and share new techniques that may not be available in a participant's home institution or laboratory.

https://www.plasther.eu/mobility-stsm/

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WG2: Antimicrobial effects of plasma

Control strategies for atmospheric pressure plasma polymerization processes to produce antimicrobial coatings

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Every year millions of people around the world suffering from debilitating bone and joint diseases experience implantation of prosthetic joints. [1] Despite implantation generally improves the patient's quality of life, at the time of implantation bacteria may adhere to the prosthetic joint surface leading to the onset of the so-called prosthetic joint infections (PJIs). [2] PJIs typically result in prolonged hospitalization for the patients with huge economic implications for the National Health Services, thus representing a major clinical issue of our times. [3] In the described context, polymerization processes assisted by atmospheric pressure (AP) plasma jets are particularly appealing since they allow to deposit antimicrobial coatings on complex 3D biomaterials (e.g. prosthetic joints) without requiring expensive vacuum systems. [4] To obtain thin films with characteristics suitable for antimicrobial purposes, a proper control of these plasma polymerization processes is highly required. Since the validity of the Yasuda parameter W/FM (W: discharge power; FM: precursor flow rate) as controlling parameter at AP has been questioned, methodologies for measuring the energy absorbed per precursor molecule (E_m) have been proposed as a more accurate alternative. [5] Nonetheless, these methodologies are nowadays limited to planar dielectric barrier discharges.

This work is focused on the development of a methodology for measuring the energy of reactions in a polymerization process assisted by an AP single electrode plasma jet with known potentialities in the production of antimicrobial coatings. [6] The E_m values are calculated through the identification and resolution of a suitable equivalent electrical circuit. To validate the methodology, the E_m values are correlated to the bond energies in the precursor molecule (hexamethyldisiloxane) and to the properties of the deposited thin films assessed by means of ATR-FTIR spectroscopy and profilometry. It is shown that the precursor fragmentation in the discharge and the coating characteristics can be successfully explained according to the obtained E_m values. Through a detailed discussion of the results, this work provides useful insights into the control of polymerization processes assisted by AP plasma jets, thus widening their applicability in the biomedical field.

Acknowledgement
The authors acknowledge the European COST Action CA20114 "Therapeutical applications of cold plasmas" (PlasTHER) for the support.

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Effects of Plasma Functionalised Water on MRSA Biofilms

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is the leading cause of nosocomial infections [1]. MRSA has an outstanding ability to acquire antibiotic-resistance genes leading to resistance to multiple antibiotic classes. Manifestations vary from minor skin infections to fatal diseases. The hypervirulent, community-acquired MRSA strain USA300 was tested in the experiments to study the antibiofilm effects of indirect plasma treatment. 24-h and 48-h old biofilms were grown in the 96-well plates for microbiological analysis, and on the stainless-steel coupons for atomic force microscopy (AFM) imaging. The AC air spark discharge was used to produce plasma functionalised water (PFW) by treating deionized water [2,3]. Single and multiple applications of PFW were compared to figure out whether the treatment in a sequential manner could increase antimicrobial efficiency. The antimicrobial effects of PFW were quantified by determining the reduction in viable cells in biofilms (CFU enumeration method), the cell metabolic activity (XTT assay), the cell vitality (live/dead staining), and the morphological changes of cells (AFM). The results showed PFW induced significant bactericidal effects, suppression of the metabolic activity of cells, and their morphological alterations. PFW caused a maximum of 3.3 $\log_{10}$ and 2.8 $\log_{10}$ reduction of 24-h and 48-h old biofilms, respectively. Indirect plasma treatment of biofilms in a sequential manner improved the overall antibacterial efficiency (Fig. 1). Besides, the different results for 24-h and 48-h old biofilms showed the importance of the biofilm composition in different growth stages that affected the antimicrobial effect of plasma.

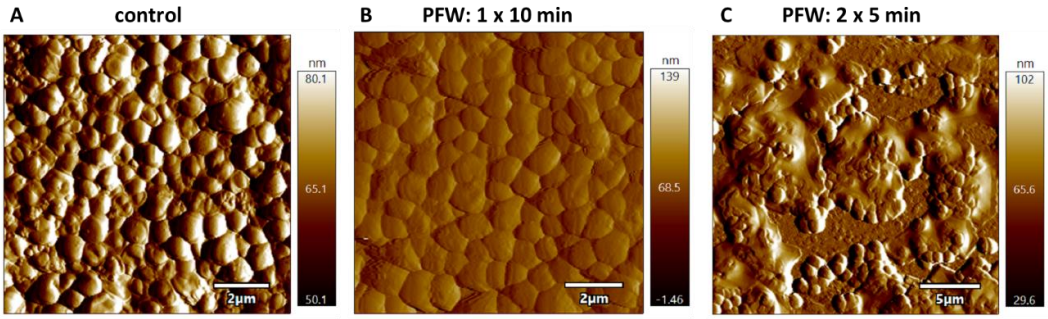

Fig. 1 Atomic force microscopy (AFM) images of MRSA 24-h old biofilms:
A – control biofilm; B – 10 min PFW treatment; C – 5 min + 5 min sequential PFW treatment.

Acknowledgement: This work was supported by COST Action CA20114 PlasTHER and by Slovak Research and Development Agency grant no. APVV-17-0382.

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WG2: Antimicrobial effects of plasma

Evaluation of the Antimicrobial efficacy of Cold Atmospheric Pressure Plasma on Surfaces

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Cold Atmospheric Pressure Plasma –CAPP- is a novel technology that is being a lot of interest among researchers. Since the maximum temperature that reach CAPP is 100ºC it can work with thermosensitive materials such as low density polyethylene –LDPE-, high density polyethylene –HDPE- and polypropylene –PP-, common plastics used in food packaging. One possible applications is the decontamination of those packaging from bacteria (*Staphylococcus spp, Listeria spp, E. coli, S. cerevisiae*). Thus, atmospheric pressure plasma has been used to decontaminate samples made of polypropylene against *S. epidermis* ATCC 12228. An effort was also put in studying how the chemistry in gas phase affects bacterial inactivation.

Samples of PP were inoculated with 20 µL of *S. epidermis* ATCC 12228 (10^7-10^8 CFU/mL). CAPP was applied changing different conditions such as duty cycle (10 % - 100 %), gap (4 cm – 20 cm), treatment time (10 min – 30 min) or fan (ON - OFF). Moreover, optical spectroscopy was performed in order to quantify NO_2 and O_3 concentration during each plasma treatment.

Among the most important results, in terms of bacterial inactivation, comparing 10 min-treatments with fan on and off, there are no statistically significant differences no matter the duty cycle studied. Low inactivation rates were achieved with 10 minutes treatments (0.32±0.27 log with 4 cm gap, fan off and NO_2 mode and 0.26±0.32 log reductions with 4 cm gap, fan off and O_3 mode). Moreover, no statistically significant differences were found comparing either NO_2 or O_3 with and without fan. On the other hand, 30-min treatments achieved higher inactivations: 1.92±0.77 log reduction for NO_2 mode, fan on and 4 cm gap and 1.60±0.53 log for the same parameters in O_3 mode. Moreover, to reach similar inactivation levels, the amount of O_3 seems to be lower than the concentration of NO_2. Finally, it could be suggested a tendency to higher inactivation when the fan is ON, regardless the plasma mode applied; besides, higher NO_2 concentration compared to O_3 concentration seems to be needed to reach the same inactivation.

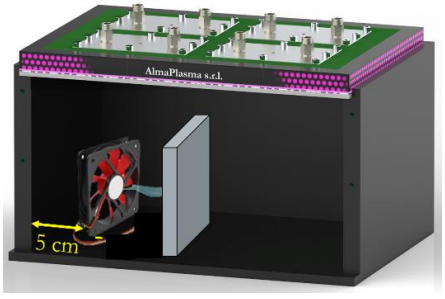

Fig. 1 Surface Dielectric Barrier Discharge (SDBD) used for treating PP samples.

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WG4: Plasma cancer therapy

Atmospheric Pressure Plasma-assisted synthesis of two types of Polypyrrole/Carbon Quantum Dot Nanoparticles for Photothermal Therapy

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Photothermal therapy (**PTT**) is one of the latest methods in treating inoperable tumors giving promising results with its quite simple and short treatment.[1] This method exploits the photosensitivity of certain nanomaterials (**NMs**); when such NMs are exposed to most often infrared light, they convert optical energy to thermal energy causing localized hyperthermia.[1,2] In recent years interesting results were obtained with carbon quantum dots (**CQDs**); due to their low toxicity, biocompatibility, chemical inertness, and low cost, CQDs seem to be promising candidates for PTT.[3] Most of these nanomaterials share the same drawbacks for their preparation such as excessive amounts of toxic solvents or acids (for synthesis and post-synthesis procedures), lengthiness of the process, and high energy consumption.[4, 5] To overcome these drawbacks, we attempted to perform atmospheric pressure plasma-assisted synthesis of two types of polypyrrole/carbon quantum dot nanoparticles for PTT, as conventionally synthesized pyrrolic CQDs have emerged as one of the most suited doped CQDs for photothermal therapy. Two sets of experiments were conducted; in the first one CQDs were previously synthesized using atmospheric pressure micro-plasma setup. These CQDs served as nucleation centers to pyrrole which was later added to the solution and plasma treated again to allow polymerization of pyrrole. In the other experiment, CQDs precursors and pyrrole were mixed together at the same time and plasma treated under same conditions. Samples were obtained at currents of 4, 6, and 8 mA from both experiments at fixed gas flow and distance between the plasma and the liquid. FT-IR spectra did not show any differences between CQDs prepared with and without pyrrole so in order to get a clearer picture on the contents of the prepared samples, XPS analysis was done. XPS analysis showed similar results from both experiments including presence of pyrrolic ring, amines, quaternary nitrogen but mostly the presence of sp^2 and sp^3 hybridized carbon as expected. From UV-VIS analysis was observed that the intensity of pyrrole's absorption peak at 205 nm drops at certain currents used in plasma treatment which is in agreement with the drop of intensity of the pyrrole peak from XPS data indicating breakdown of the pyrrolic ring to some extent. For better understanding and any further conclusions TEM, DLS and photothermal conversion efficiency analyses will be performed.

Acknowledgement
The authors would like to thank European Cooperation in Science and Technology for their grant (E-COST-GRANT-CA20114-c86cc7da) which helped us to do this research.

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WG3: Tissue regeneration

The influence of Cold Atmospheric Plasma treated hydrogels on dentinal MMPs activity

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The activity of matrix metalloproteinases (MMPs) in adhesive dentistry plays a crucial role in the longevity of dental restoration by influencing the degradation rate of resin-dentin interfaces. This fact emphasizes the need for strategies to control MMPs and preserve the integrity of the adhesive interface. In general, the chemical modifications introduced to hydrogels by plasma treatment can either enhance or hinder the enzymatic activity, leading to the potentially expected tailored effects of hydrogels for dental applications.

The aim of our study was to investigate the impact of plasma-treated hydrogels on the activity of MMPs in dentin, providing insights into their potential to be used in adhesive dentistry.

The first-class cavities were prepared on freshly extracted sound human molars, which were then cut in half, and frozen at -20°C until the day of the experiment (N=40). One part of the samples was prepared by using the standard self-etch adhesive protocol and covered by composite, to be used as a positive control (SE – control). Then, 0.5% alginate solutions (untreated hydrogels – UTH) were plasma-treated for two (PTH 2') or ten minutes (PTH 10') according to previous works [1]. All hydrogels were cross-linked using a $CaCl_2$ solution. The dentinal surfaces were covered by alginate hydrogels (untreated hydrogels – as a reference, and plasma-treated hydrogels – as experimental groups) and teeth were sealed by composite resin. The prepared tooth slices containing the dentin-adhesive or dentin-hydrogel interfaces were ground and incubated overnight with fluorescein-conjugated gelatin. The samples were subsequently examined using a confocal microscope to investigate the dentinal endogenous enzymatic activity, and the resulting data were subjected to statistical analysis ($p < 0.05$).

Results: All hydrogels (untreated and plasma-treated) reduced the MMPs activity in comparison to SE – control, used as a standard adhesive-bonding procedure. PTH 2' and PTH 10' both had an inhibitory effect on enzymatic activity. When used on the same biological sample, PTH 2' had a more prominent influence on MMPs inhibition, than PTH 10', but in general they both seem to be within the hormetic zone, indicating their potential benefits for restoration longevity. Further analysis of the specific effects of the hydrogels modified by different plasma treatment durations on the dentinal enzymatic activity, will be provided after the aging procedure (in artificial saliva at 37°C) on the given samples.

Acknowledgments: This work was carried out in the frame of a short-term scientific mission (STSM) in PlasmaMed Lab, supported by the PlasTHER COST Action CA20114.

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WG4: Plasma cancer therapy

Exploring the anticancer potential of multicomponent plasma-treated hydrogels in an *in ovo* pancreatic cancer model

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Cold Atmospheric Plasma (CAP) has emerged as a very promising technology in oncology, offering a selective strategy to eradicate cancer cells without damaging healthy tissues. It is known that plasma can generate a complex cocktail of reactive oxygen and nitrogen species (RONS) which play a key role in the targeted destruction of cancer cells. Recently, our group has demonstrated that biopolymers that form hydrogels can be used as effective vehicles for generating and releasing RONS. Based on that, we proposed plasma-treated hydrogels (PTHs) as a novel injectable platform for localized delivery plasma-derived RONS through minimally invasive procedures in cancer therapies. Previous investigations from our group demonstrated the suitability of biopolymer solutions containing alginate, hyaluronic acid, and gelatin in generating PTHs with therapeutic potential [1]. These PTHs exhibited selective cytotoxicity against cancer cells without affecting non-malignant cells *in vitro*. Building upon these encouraging findings, the present research aims to validate the PTHs-based approach using an advanced cancer model that more closely resembles the physiological characteristics of solid tumors. For this purpose, we employed the chorioallantoic membrane (CAM) model to generate vascularized tumors *in ovo* using pancreatic ductal carcinoma Mia Paca-2 cells. Then, we evaluated the therapeutic action of PTHs by assessing tumor growth, proliferation, and apoptotic levels within the tumor. Our results indicate that the composite PTH made of alginate and hyaluronic acid was able to reduce the population of cancer cells within the tumoral tissue, as well as to diminish the percentage of proliferating cancer cells.

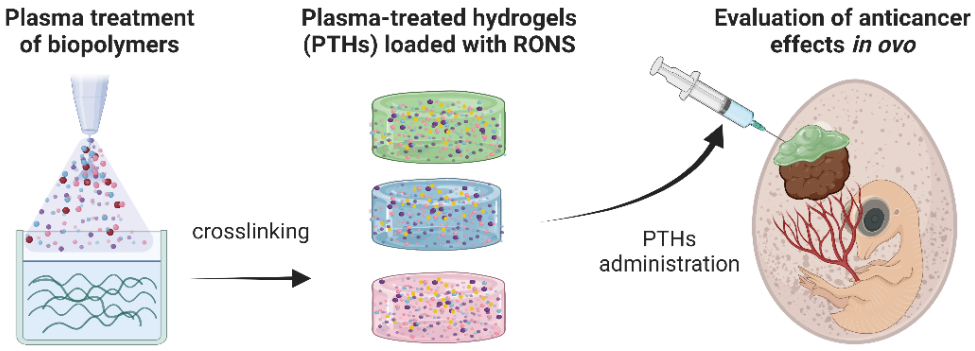

Fig. 1 Schematics of the PTHs evaluation of the therapeutic effect on pancreatic cancer tumors *in ovo*.

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WG2: Antimicrobial effects of plasma

Antimycotic effects of plasma gun tested on *C. glabrata* on various surfaces

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With the ever-increasing resistance of microbes against antimicrobials, there arise an urge to find alternative treatments for many diseases not only in the human world but also in the animal and plant kingdoms. One of the diseases that needs special attention is *otitis externa* (inflammation of the ear canal) in dogs. Otitis externa or so-called swimmer´s ear is a condition that causes inflammation of the external ear canal, which is the tube between the outer ear and the ear drum [1]. With regard to a specific application, the dog ear treatment, the plasma gun with its capillary design was tested [2]. As a model microorganism a yeast *Candida glabrata* was chosen. The preliminary tests have been done on the Petri dishes to establish the basic plasma parameters. To render this research more appropriate to real application, more complex inoculation substrates (pork skin and 3D printed models of dog ear with the ear canal) have been used. An interesting fact has been observed in the work with pork skin. The microbial cells massaged into the skin were protected by the lipid layer and were thus less susceptible to plasma treatment. The use of the plasma gun with a thin capillary allows for endoscopic treatment inside the complex structures. The results confirm the high efficiency of cold atmospheric-pressure plasma in the inhibition of the yeasts *C. glabrata* on different surfaces.

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WG5: Combination therapies

ANTIOXIDANT EFFECTS OF CAPE DERIVATIVES IN TREATING CANCER CELLS IN COMBINATION WITH DIRECT COLD ATMOSPHERIC PLASMA

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Combination therapy is a cornerstone of cancer therapy with the final aim of eliciting a synergistic interaction that allows the reduction of drug resistance and the use of lower doses, which may decrease adverse reactions [1,2]. The emerging field of plasma medicine employs cold atmospheric plasma (CAP) devices for cancer removal and more recently as emerging non-invasive anti-cancer agents capable of improving the efficacy of traditional drugs [3]. Caffeic acid phenethyl ester (CAPE), a natural compound coming from bee propolis and bearing a polyphenolic ring, is known to have anti-tumoral selective effects on different cancer cells [4].

Here we investigated the effects of two newly designed and synthesized CAPE derivatives - namely MB10 and MB14 - in combination with CAP on a human sarcoma cell model.
Their IC_{50} values, 26 µM for MB10 and 30 µM for MB14, were calculated by measuring cell viability after exposing the MG63 cell line to concentrations ranging 0-250 µM for 48 h. Then combination therapy was applied following three different strategies:

- direct CAP treatment at 10 mm distance and different exposure times (15, 30, 60, 90 seconds) before addition of the IC_{50} of the compounds;
- direct CAP treatment at 10 mm distance and different exposure times (15, 30, 60, 90 seconds) after 24 h pre-treatment with the IC_{50} of the compounds;
- indirect CAP treatment at 10 mm distance and different exposure times (15, 30, 60, 90 seconds) followed by the addition of the IC_{50} of the compounds.

The plasma was generated by an atmospheric pressure plasma jet (APPJ) using 1 L/min helium flow rate and CAPE was always used as reference compound. In all the experimental conditions, MB10, MB14 and CAPE were able to significantly revert the cytotoxicity exerted by CAP on MG63 cells.
As CAPE has a reported antioxidant activity, the effects of the three compounds on nitrites (NO_2^-) and hydrogen peroxides (H_2O_2) were investigated in DMEM without pyruvate containing 100 µM of H_2O_2 or 50 µM of NO_2^-. The concentration of NO_2^-, which was assessed by means of Griess reagent, was not significantly affected, whereas the concentration of H_2O_2, evaluated by fluorescence spectroscopy using Amplex™ Red Reagent, was significantly decreased after the addition of MB10 and CAPE at concentration comparable to or higher than IC_{50} values (40 and 80 µM).

Interestingly, a synergic cytotoxic effect towards MG63 was observed when MB14 was used at concentrations lower than its IC_{50} in combination with direct CAP treatment, thus allowing the use of lower doses for both therapeutic approaches. In conclusion, the investigated compounds, when in combination with CAP, should be used at low doses to exert anti proliferative effect on MG63 cells.

References

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