EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Brussels, 25 May 2021

COST 054/21

DECISION

Subject: Memorandum of Understanding for the implementation of the COST Action "Therapeutical applications of Cold Plasmas" (PlasTHER) CA20114

The COST Member Countries will find attached the Memorandum of Understanding for the COST Action Therapeutical applications of Cold Plasmas approved by the Committee of Senior Officials through written procedure on 25 May 2021.

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MEMORANDUM OF UNDERSTANDING

For the implementation of a COST Action designated as

COST Action CA20114
THERAPEUTICAL APPLICATIONS OF COLD PLASMAS (PlasTHER)

The COST Members through the present Memorandum of Understanding (MoU) wish to undertake joint activities of mutual interest and declare their common intention to participate in the COST Action, referred to above and described in the Technical Annex of this MoU.

The Action will be carried out in accordance with the set of COST Implementation Rules approved by the Committee of Senior Officials (CSO), or any document amending or replacing them.

The main aim and objective of the Action is to exploit the possibilities of atmospheric pressure plasmas in medicine to share, develop and consolidate suitable therapies to make Europe's science and healthcare world leaders in this field. This will associate gaining and sharing fundamental knowledge that will improve the performance of the therapies. This will be achieved through the specific objectives detailed in the Technical Annex.

The present MoU enters into force on the date of the approval of the COST Action by the CSO.

OVERVIEW

Summary
Despite scientific and technological progress in the medical field, the treatments available today are still not completely effective concerning the fight against cancer, tissue regeneration and repair or drug-resistant pathogens, including newly emerging infections. Besides, some of the currently associated therapies associate high economic and/or societal costs. In this sense, Cold Atmospheric Plasmas have emerged as a powerful technique involving a vast number of reactive species (molecules, atoms, ions, electrons, photons, UV & visible radiation) which have demonstrated to affect cells through complex biochemical procedures, opening a great window of opportunity in the novel area known as Plasma Medicine.

This has led to an exponential increase in the research in different areas of plasma medicine, including cancer, tissue regeneration and repair and antimicrobial action which are the focus of this MedPlasma COST Action. However, many challenges still threaten this promising field to move forward, such as clarification of the mechanisms involved in the therapeutic action of plasmas and plasma-conditioned liquids, insufficient standardization, or an urgent need for enhanced dialogue and interaction between scientists (plasma experts, biologists), medical doctors or industry among others. In these circumstances, this MedPlasma COST Action aims at establishing a synergistic network that articulates researchers, the medical community, industry or patient associations, among others, and coordinate the European activity in this domain to foster the leadership of Europe in this emerging field.

Areas of Expertise Relevant for the Action	**Keywords**
● Medical engineering: Medical engineering and technology ● Physical Sciences: Gas and plasma physics (theory) ● Materials engineering: Gas and plasma physics for materials engineering applications	● Cold Plasmas ● Cancer ● Decontamination ● Wound Healing ● Tissue regeneration

Specific Objectives
To achieve the main objective described in this MoU, the following specific objectives shall be accomplished:

Research Coordination
● To monitor state-of-the-art of CAPs applied to medicine by exchanging scientific and technological knowledge between the network and beyond.
● To harmonise the protocols followed to ensure reliability of inter-laboratory comparisons.
● To establish a roadmap of the fundamental scientific issues regarding the biological mechanisms of CAP and PTL still unknown in the field to foster advance in a coordinated manner.
● To systematically collect and merge knowledge from the Network on the generation of RONS by CAP in different liquids compatible with parenteral administration, their stability and response of biological systems, to identify the most relevant cocktail of RONS from plasmas for each application.
● To investigate possible synergies of CAPs or PTLs treatment with classical therapies (pharmacology, radiotherapy, surgery, etc.) in lab and clinical scale.
● To gather best practices and convert these into recommended standardised methods and multidisciplinary guidelines to evaluate and compare the effectiveness of CAP treatments in selected fields in a laboratory setting, with possibilities to extrapolate these methods to clinical tests.

● To establish an approach for a risk / safety analysis for using CAP in the different medical fields proposed, weighing potential adverse effects versus long-term savings and benefits.
● To use the know-how and expertise of the network to stimulate development and optimisation of standard CAPs and PTLs systems and the most suitable application scenarios for decontamination that avoid bacteria and viruses resistance
● To consult and support policy and healthcare sector with regard to the advance and application of CAPs in selected medicine fields as well as to introduce a European regulatory and ethics system for CAP therapies.
● To disseminate knowledge on CAP medicine innovations and laboratory and clinic tests to relevant stakeholders such as health professionals, companies, policy makers and patients.

Capacity Building
● Articulation of a shared vision of the challenges to be met and of emerging needs arising in the field of the Action, allowing to identify knowledge gaps and establish a consensus roadmap to foster and coordinate excellence and innovative scientific research in the area.
● To promote collaborative interdisciplinary research (especially between medical doctors, pharmacists, dentists, chemical engineers, physicists, engineers and technologists, biologists and toxicologists) to achieve breakthroughs in promoting and proposing an improvement of CAP and PTL technologies applied to medical fields.
● To facilitate and favour diversity (i.e. including under-represented gender, young people, emerging groups and actors from ITC) in the Action topic.
● To organize and prioritize research lines within and between working groups to avoid duplications and promote synergisms.
● To attract and facilitate the mobility, the multidisciplinary training and participation in research excellence programs (i.e. Marie Skłodowska-Curie actions, ERC StG, etc.) of Early Career Investigators (ECI) using COST tools (STSM & Training schools), to pave the way for their future research leadership.
● To evaluate legislation and other requirements in force (opportunities and constraints) in developing new CAP technologies and in testing them in laboratory and clinical stages.
● To increase knowledge and promote the benefits of CAP therapies to various users groups and to the general public for wider economic and social benefit.
● To provide excellent interdisciplinary training for PhD students and Early Career Investigators (ECIs), especially from participating Inclusiveness Target Countries (ITC) using COST tools and to encourage their participation to the Action.
● To provide professional training for all scientists and engineers associated with the PlasTHER COST Action, with a particular focus on enhancing knowledge transfer to industry, an acknowledged weakness of the European research area.
● To stimulate connection and collaboration between research institutes, industry and healthcare system and to improve technology transfer on CAP technologies in medical fields.

TECHNICAL ANNEX

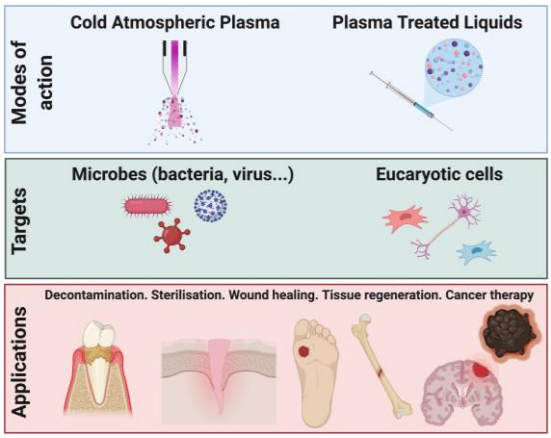
1. S&T EXCELLENCE

1.1 Soundness of the Challenge

1.1.1 DESCRIPTION OF THE STATE-OF-THE-ART

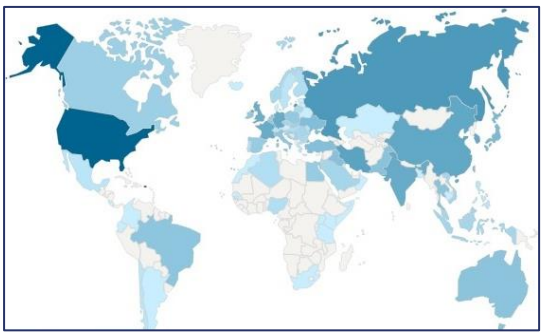
This Action is concerned with the **medical and biomedical** applications of **cold atmospheric plasmas (CAPs)**. *Plasma medicine* is an interdisciplinary field that emerged in the last two decades as an innovative field between physics, chemistry, engineering and life sciences, which combines plasma technology with clinical medicine and bioengineering with the final aim to use CAPs in the clinics for human and veterinary therapeutic applications[1,2]. A **plasma is an ionized gas**, composed of free electrons, ions, radicals, excited atoms and molecules, neutral molecules, electromagnetic fields and UV-Vis radiation with no overall electrical charge[3,2]. The features of CAP, such as the extremely high concentration of chemically reactive species while maintaining the bulk temperature close to room temperature, makes it an ideal tool for applications in many fields like agriculture, environment, manufacturing and medicine[4,5,6,7]. Moreover, the reactive species, in particular, reactive oxygen and nitrogen species (RONS - ie. O, $^1O_2$, O_3, $\cdot OH$, $\cdot O_2H$, $\cdot O_2^-$, $\cdot O_3^-$, $\cdot NO$, $\cdot NO_2$), can diffuse from the gas phase to a solution/biological medium, generating less reactive and longer-lived secondary species with high potential for biological applications[2,8,9,10]. This has led **to two possible modes of action of plasmas**: i) direct CAP treatment of the biological target or ii) CAP treatment of biocompatible and biologically relevant liquids (plasma treated liquids - PTLs) that can allow minimally invasive therapy in the target site. On the one hand, direct CAP treatment of living tissues involves the suite of synergistic effects derived of all the above-mentioned plasma components on cells. On the other hand, in PTLs, the RONS generated in the liquid phase are the predominant players[2,3,11,12].

The **huge potential of plasmas in different pathologies requiring urgent solutions** (ie. microbial/viral disinfection, tissue regeneration or cancer) has been put forward in the last years. It is a pressing matter to push this field forward to provide for alternative and improved therapeutic solutions at low cost.



Part of the action of CAP can be explained thanks to redox biology, that can be used as scientific basis to explain the biological effects related to CAP-generated RONS[13]. Briefly, the two general molecular mechanisms of the RONS signalling are (i) alterations of the intracellular redox state and (ii) oxidative modification of proteins involved in multiple signalling pathways. According to this, CAP treatment can affect all physiological processes in the human or animal body, where RONS play an important role, such as regulation of blood coagulation, vascular contraction, nerve impulse transmission, angiogenesis, inflammation, and immune system response. Besides, at the cellular level, CAP-derived RONS can alter molecular signalling pathways related to cell-to-cell adhesion, synthesis of growth factors, cell differentiation, division, migration, and apoptosis[14]. This has led to two main capabilities of CAPs which we want to exploit in the current PlasTHER COST Action:

i) the **antimicrobial action** on prokaryotic cells (bacteria), viruses, yeasts, fungi and prions to decontaminate biological tissues such as skin (related to acceleration of wound healing, bacterial-based skin diseases, etc.) and sterilize surfaces, materials or devices (prostheses, implants, etc.);
ii) their effect on eukaryotic cells and **living tissues** in the human and animal body for therapeutic purposes including stimulation of **tissue regeneration & wound healing** (diabetic leg healing, ulcers, burns, etc.) which might have implications in cosmetics (skin regeneration, scar treatment, etc.) and important applications in **cancer therapy** (melanoma, glioblastoma, colon cancer, etc.).



In the last two decades, the great interest raised has led many research groups worldwide to work in this direction (see left Figure, darker blue designating higher number of papers published from each country). While **plasma decontamination** is not a new concept in itself[15,16,17], the development of atmospheric pressure plasma jets promoted the exploration of novel potential applications, especially on living tissues[18]. The increasing incidence of bacteria with resistance to most antibiotics and the emergence of new unknown pathogens whose transmission is most probably airborne (i.e. SARS-CoV-2) means that finding novel ways of attacking infection is increasingly crucial. In this regard, recent research proved also the effectiveness of CAP in inactivation of biofilms, overcoming their acquired resistance to antibiotics[19]. From another point of view, a fundamental finding in this area was the discovery that under specific conditions, CAP can kill or inactivate harmful microorganisms infecting skin, without causing damages on patient's tissue, thereby allowing to accelerate wound healing and treat pathogen-based skin diseases[20,21]. Moreover, CAP proved to be very effective against bio non-cellular infection-transmitting agents that are resistant to more conventional techniques like the prions, which are held responsible for neurodegenerative diseases like transmissible spongiform encephalopathy or Alzheimer's disease, respectively[22,23]. For all these reasons, it is fundamental to incorporate CAP decontamination in current therapies to improve the benefits on the patients and on society in general. However, the state of research is currently of emerging fields and, in spite of the large knowledge on the biological sterilisation role of plasma treatment, there is a lack of scientific insight into the basic molecular mechanisms that are involved on the biological effect on living cells and tissues.

It has recently been demonstrated that the controlled exposure of mammalian cells to different conditions of CAP can lead either to stimulation or inhibition of cellular functions such as cell proliferation, tissue regeneration, cell detachment, apoptosis, and necrosis[24]. In this sense, different atmospheric pressure plasma jets have been recently commercialized and have demonstrated their effectiveness at **supporting the healing of non-infected acute wounds** (kINPen, PlasmDERM, SteriPlas, Plason, PlasmaCare)[25,26,27]. Up to date, these plasma jets have also been applied in the treatment of long-lasting chronic and infected wounds, particularly in cases where conventional treatment fails[28], with proof *in vitro*[27,29], and *in vivo* with studies in animals[28], that led to the initiation of the first clinical trials. In diabetic patients with chronic leg and venous ulcers, plasma-treated patients experienced accelerated wound healing[18]. These results suggest that wound healing may be accelerated due to the simultaneous stimulation of tissue regeneration and angiogenesis, besides the antibacterial action of plasmas already discussed. The results observed in such studies enable to envision CAP technology on its way to becoming a clinical routine for wound healing and skin treatment. From another point of view, the regenerative potential of CAP on skin is currently being explored in the anti-aging and skin wellness industry. Therefore, plasma technology represents a promising therapeutic approach for wound healing, but also opens new possibilities in the cosmetics field with important economic implications. This capacity of plasmas to **stimulate tissue regeneration** and repair can also be exploited for other tissues[30], opening new avenues that deserve further exploration.

Besides the two areas described, an emerging topic in the last years that will also be focus of this Action is the use of CAP in **cancer therapy**. Cancer is a leading cause of death worldwide and despite the

enormous amount of research and rapid developments seen during the past decade, cancer treatment is still challenging. In this sense, one key aspect which is attracting increasing attention is the ability of CAPs to selectively kill cancer cells **without damaging the surrounding tissues**. This would avoid undesirable side effects as those usually found in common anticancer therapies (ie. chemotherapy and radiotherapy). This has fostered the interest for the anticancer applications of CAPs, with an exponential increase in the number of publications in the last 5 years[31]. The anticancer effects of CAP have been, at least in part, related to the RONS generated by plasmas, and that are important mediators in stem cell biology. In fact, high levels of RONS have long been suggested to be detrimental to mediating oxidative stress, and adding high amounts of exogenous RONS can induce cell cycle arrest and higher doses lead to the induction of apoptotic and/or necrotic cell death. In this context, cancer cells are metabolically more active than healthy cells, thereby generating higher amount of intrinsic RONS. For this reason, delivering low plasma concentrations with exogenous RONS, allows to surpass the toxicity threshold in cancer cells activating apoptosis without affecting normal cells of the surrounding healthy tissue[32]. Interestingly, many studies demonstrated that indirect treatment using PTLs exert very similar effects compared to direct CAP treatment[33]. This may find applications in tumors that are harder to reach and an injection of PTLs may be an alternative. This brings forth one important issue, as trying to understand how to catalog the PTLs is not straightforward and matter of debate.

However, **this promising field has still to face many challenges** to really move forward, such as lack of standardization aimed at comparability of different CAP sources - the clinical use of this innovative therapy requires the development of standardized reliable protocols in order to compare the results between future clinical trials-, or insufficient dialogue between plasma experts (essentially physicists, chemists or engineers) and biologists, veterinarians and medical doctors that slows down the information exchange and therefore the publication of results. The **huge European potential** stemming from different groups that designed and used different CAP devices and working conditions employed can be coordinated as one the final aims of this proposed action. These considerations suggest that biomedical applications of CAP are about to become a major field of research in many advanced economies, with vigorous research programmes and major medical and economic impact, reflected in large user communities and substantial industrial activity. In these circumstances, the launching of a COST Action designed to coordinate European activity in this domain and promote the development of a coherent European research programme is timely, and may enable Europe to assume a leading position as this field evolves. In particular, the research supported by this Action has strong interest on shedding light on the role of plasma technology on several biomedical applications of great importance to society: antimicrobial potential, skin treatment and cancer therapy.

1.1.1. DESCRIPTION OF THE CHALLENGE (MAIN AIM)

Providing healthcare at tolerable cost and reducing side effects is one of the greatest challenges facing the world in this century. Technologies that may offer **enhanced quality of care at reduced cost**, such as CAP technology, will be of immense societal and economical value. Encouraged by the current state of the art, the **ambition of PlasTHER COST Action is** to move beyond the fundamentals involved in the medical action of CAPs and put together the suitable environment through leading European scientific teams and relevant medical and industrial actors. This will allow to push the different areas of Plasma Medicine closer to the market and clinics, implementing novel therapies, reverting in an improvement in quality of life of the patient and reduced costs. The advances and advantages brought forward by CAPs in medicine can have a critical impact in society, (ie. economic benefits and benefits for healthcare and patients through novel therapies for humans and animals), as will be detailed later. Thus, the **main aim** of the **PlasTHER COST Action** is to exploit the **unprecedented possibilities of atmospheric pressure plasmas in medicine** to share, develop and consolidate suitable **therapies currently under investigation to make Europe's science and healthcare world leaders in this field**. This will be associated with gaining and sharing fundamental knowledge that will lead to improving the performance of the therapies for the benefit of the patients.

In this regard, the explosion in research in the last 5 years in different areas of Plasma Medicine - particularly sterilisation and decontamination, wound healing and cancer treatment - urgently requires

the harmonisation of protocols, and the gathering the knowledge generated in a common direction to allow all the efforts and public investment done up to now in basic and applied science to be translated into benefits for the society and the healthcare system. Suitable articulation of the powerful scientific background generated should allow raising the general level of awareness (especially in Europe) of the potential of this technology and allow Europe lead the race for Plasma Medicine, where Asia and USA are strong competitors.

This will be done through four different axes (focusing simultaneously in the basic scientific fundamentals, as well in the application and translation of the therapy):

i. Creation of a strongly interdisciplinary research network breaking the classical research field borders
ii. Sharing the latest advances in the basic mechanisms regarding the action of plasmas, associated to the most suitable CAP devices and operational conditions for efficient therapies
iii. Generation of harmonised protocols within the community in the different therapeutic areas involved, and definition of a roadmap for each of them
iv. Broad dissemination of the results to all stakeholders

1.2 Progress beyond the state-of-the-art

1.2.1 APPROACH TO THE CHALLENGE AND PROGRESS BEYOND THE STATE-OF-THE-ART

To describe the approach to the challenge and the innovation brought by the Action, a point-by-point SWOT (Strengths, Weaknesses, Opportunities and Threats) analysis has been performed.

STRENGTHS of the area of research proposed in the PlasTHER COST Action are:
- Europe holds a leading position in Plasma therapy, closely followed by USA, Japan and China.
- The two approaches possible (direct CAP treatment or use of PTL) open a wide variety of therapeutic possibilities.
- The technologies required to produce and sustain typical CAP sources are generally much simpler, cheaper and more user-friendly, than those in other medical devices and/or treatments.
- CAP treatment for medical applications does not require the use of chemicals and shows few side effects. This is a great benefit since all the drawbacks related to the use of drugs are avoided in terms of costs, safety, environmental sustainability and benefits for the patient.

Current **WEAKNESSES** of this area of research are:
- Lack of harmonization and standardization in the field. Typically, each laboratory has developed their own plasma sources and protocols.
- Limited involvement of medical doctors and veterinarians in the field to evaluate the novel therapies.
- Lack of articulation / coordination for researchers and medical doctors in the field in Europe to advance in the fundamental scientific issues that are still unsolved (ie. In the last Int. Conference on Plasma Medicine -ICPM6 there were more than 400 participants working in the field).
- No established regulatory system to help to transfer all the excellent results of basic and applied research to the clinics and to the market.

The strengths detected can create several **OPPORTUNITIES** that are summarized here:
- The creation of an established European Network would consolidate the European leading position in plasma medicine and set is as main reference for the field worldwide.
- R+D+I results can be maximized through the coordination and generation of synergies among the responsible agents.
- The world of health has realized the importance of incorporation of novel advanced technologies to respond to many challenges and new problems that arise. They are now indissoluble.
- The CAP technology applied to medicine can contribute facing challenges in an ageing society, with emergence of new diseases and pandemics, and leading a healthy life.

- The use of CAPs and PTLs represents a breakthrough in the way of treating a number of diseases.
- The therapies proposed have a number of benefits for the patient and healthcare (ie. no antimicrobial resistance to therapies, reduction of chemicals, lack of secondary effects)
- Research and applications of CAPs is inherently a multidisciplinary field which implies collaboration of experts with very different backgrounds (physics, chemistry, biology, medicine, …).

Some **THREATS** in the field that may hamper its development:

- USA and Asia are quickly progressing and may soon take the lead, as they are intensively seeking funding to articulate networks in Plasma Medicine.
- Research budget cutoffs in Europe due to the COVID-19 pandemics may cut the wings of a field that holds promise of great socio-economic and environmental profits.

This diagnostic analysis has allowed the **PlasTHER COST Action** to elaborate a plan of action to approach the current challenge, and generate tools that facilitate and significantly accelerate the advance of science in Plasma Medicine related technologies towards the clinics and the patient. The Action proposed will allow to **progress beyond the state of the art** in the fundamentals of the area, as putting together the most advanced knowledge from the different European scientists, in conjunction with medical doctors and veterinarians will allow to unveil current aspects that are needed to advance the therapies forward. Indeed, in spite of the encouraging results already obtained in different areas (ie. wound healing and cancer treatment), the underlying mechanisms in plasma treatment are still far from understood. While most studies report that RONS formed in the plasma phase play a crucial role in their biological effects, the complexity of the system requires a more in-depth investigation. Furthermore, there is preliminary evidence that the immune system is also positively affected by CAP treatment and plays a role in the therapies embraced by plasma therapy. It is unclear whether PTLs also stimulate the immune system. This multi-faceted and global perspective is an innovative approach provided by the PlasTHER COST Action that can surely provide an advance on the fundamentals that will undoubtedly revert in the progress towards implementation of the proposed technologies. Besides, the fundamentals of decontamination have been described more with CAPs than with PTLs, but the latter can now play a significant role in a variety of emerging applications in the current COVID-19 pandemic or future ones.

1.2.2 OBJECTIVES

The **main aim** of the Action is **to create a broad and strongly interdisciplinary European research network coordinating the plasma therapy research for human and veterinary medicine.** This means to establish a leading position for Europe in exploiting this important new field and encompasses the pushing forward the knowledge relevant to medical and biomedical applications of atmospheric pressure plasmas within Europe, and to raise the general level of awareness (especially in Europe) of the potential of this technology.

1.2.2.1 Research Coordination Objectives

The **general objective** of the **PlasTHER COST Action** is to exploit the **unprecedented possibilities of CAP in medicine** to develop and investigate suitable **therapies** by creating a multidisciplinary network, that will include researchers, healthcare professionals, patient associations, companies and regulatory agents, actively involved in the study, application and regulation of CAPs applied to medicine. This will allow gaining fundamental knowledge and improving the performance of the therapies. This encompasses the following specific objectives:

Sci01. To monitor state-of-the-art of CAPs applied to medicine by exchanging scientific and technological knowledge between the network and beyond.
Sci02. To harmonise the protocols followed to ensure reliability of inter-laboratory comparisons.
Sci03. To establish a roadmap of the fundamental scientific issues regarding the biological mechanisms of CAP and PTL still unknown in the field to foster advance in a coordinated manner.
Sci03. To use the know-how and expertise of the network to stimulate development and optimization of standard CAP sources for specific therapeutic applications in each selected field and teach and learn

about the requirements to transfer plasma sources to therapeutic applications in particular through the involvement of industrial partners at an early stage.
Sci04. To systematically collect and merge knowledge from the Network on the generation of RONS by CAP in different liquids compatible with parenteral administration, and of their stability and the response of biological systems (tissue, skin, tumors) these liquids, to identify the most relevant cocktail of RONS from plasmas for each application.
Sci05. To investigate possible synergies of CAPs or PTLs treatment with classical therapies (pharmacology, radiotherapy, surgery, etc.) in lab and clinical scale.
Sci06. To gather best practices and convert these into recommended standardised methods and multidisciplinary guidelines to evaluate and compare the effectiveness of CAP treatments in selected fields in a laboratory setting, with possibilities to extrapolate these methods to clinical tests.
Sci07. To establish an approach for a risk / safety analysis for using CAP in the different medical fields proposed, weighing potential adverse effects versus long-term savings and benefits.
Sci08. To use the know-how and expertise of the network to stimulate development and optimisation of standard CAPs and PTLs systems and the most suitable application scenarios for decontamination that avoid bacteria and viruses resistance
Sci09. To consult and support policy and healthcare sector with regard to the advance and application of CAPs in selected medicine fields as well as to introduce a European regulatory and ethics system for CAP therapies.
Sci10. To disseminate knowledge on CAP medicine innovations and laboratory and clinic tests to relevant stakeholders such as health professionals, companies, policy makers and patients.

1.2.2.2 Capacity-building Objectives

The core capacity-building objective is to gather a critical mass of interacting actors all over Europe to place it at the forefront of scientific interdisciplinary research in the context CAP applied to medicine. Specific capacity-building objectives are:

Net01. Articulation of a shared vision of the challenges to be met and of emerging needs arising in the field of the Action, allowing to identify knowledge gaps and establish a consensus roadmap to foster and coordinate excellence and innovative scientific research in the area.
Net02. To promote collaborative interdisciplinary research (especially between medical doctors, pharmacists, dentists, chemical engineers, physicists, engineers and technologists, biologists and toxicologists) to achieve breakthroughs in promoting and proposing an improvement of CAP and PTL technologies applied to medical fields.
Net03. To organize and prioritize research lines within and between working groups to avoid duplications and promote synergisms.
Net04. To facilitate and favour diversity (i.e. including under-represented gender, young people, emerging groups and actors from ITC) in the Action topic. This has been already carefully taken into account in the co-proposer team of the Action.
Net06. To attract and facilitate the mobility, the multidisciplinary training and participation in research excellence programs (i.e. Marie Skłodowska-Curie actions, ERC StG, etc.) of Early Career Investigators (ECI) using COST tools (STSM & Training schools), to pave the way for their future research leadership.
Net07. To evaluate legislation and other requirements in force (opportunities and constraints) in developing new CAP technologies and in testing them in laboratory and clinical stages.
Net08. To increase knowledge and promote the benefits CAP therapies to various users groups and to the general public for wider economic and social benefit.
Net09. To provide excellent interdisciplinary training for PhD students and Early Career Investigators (ECIs), especially from participating Inclusiveness Target Countries (ITC) using COST tools and to encourage their participation to the Action.
Net10. To provide professional training for all scientists and engineers associated with the PLASTHER COST Action, with a particular focus on enhancing knowledge transfer to industry, an acknowledged weakness of the European research area.
Net11. To stimulate connection and collaboration between research institutes, industry and healthcare system and to improve technology transfer on CAP technologies in medical fields

2. NETWORKING EXCELLENCE

2.1. Added value of networking in S&T Excellence

2.1.1. ADDED VALUE IN RELATION TO EXISTING EFFORTS AT EUROPEAN AND/OR INTERNATIONAL LEVEL

Despite the great prospects of the field, there is currently no network articulating researchers, medical doctors and industry around Plasma Medicine. Up to now plasma medicine has been focused on human medicine, although the concept of the COST Action Network is broader and better targeted. The current activities in the field worldwide are the following:

- International Society for Plasma Medicine (ISPM). Founded in 2009, its main purpose is the organization of the biennial International Conference on Plasma Medicine (ICPM), which has grown exponentially in the last years. It also recognizes excellence in the area of Plasma Medicine through the early career award in Plasma Medicine and the Plasma Medicine award.
- International Workshop on Plasma for Cancer Treatment (IWPCT) (Started in 2014/annual)
- International meeting on Plasma Cosmetic Science (Started in 2019/biennial)
- During the 2020 pandemics the aforementioned conferences have been suspended/postponed, a new online meeting was created to keep the community connected: the International Low Temperature Plasma Community (ILTPC) (although it is not focused on plasma medicine only).
- A few projects are ongoing in fields related to Plasma Medicine, but really very few on the fields proposed (one ERC - APACHE in the field of plasma and cancer).

Pushing the field forward in these uncertain times is a challenge, and the creation of a network thanks to the COST Action will undoubtedly provide an added value through:

- Collecting and sharing knowledge and infrastructures
- Standardization of plasma sources, characterization methods, and creation of therapeutic standards.
- Creation of an European-wide community instead of scattered groups and/or initiatives. Bridging different backgrounds under a common mission will allow to achieve a status of broadest expertise.
- Preparation and participation to international projects
- Training of the next scientific generations with interdisciplinary background.
- Although different European companies already commercialize medical plasma devices, the Action will push the field forward and ease the knowledge transfer from academia to the healthcare system and industry, and allow academic efforts to be better focused in a more exploitable pathway.

2.2. ADDED VALUE OF NETWORKING IN IMPACT

2.2.1. SECURING THE CRITICAL MASS AND EXPERTISE

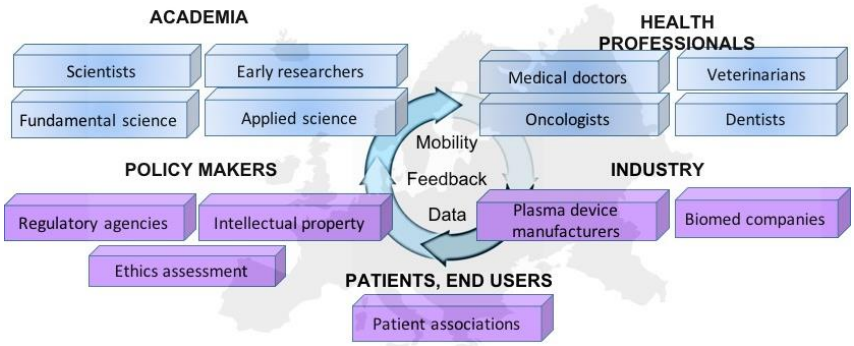
To meet the objectives of PlasTHER, the co-proposers constitute a critical mass of specialists of varied backgrounds (physics, chemists, biologist, engineers, medical doctors, etc…) and coming from various sectors (academia, company, healthcare) that will be consolidated on implementation of the Action.

The starting co-proposers Network includes 210 COST countries (14 of which ITC), and among the participants, all of them working in fields related to plasma medicine, with close to 40% with medicine as core expertise (medical doctors, veterinarians, dentists), some companies, and government organisations, in addition to top expert researchers in the field. Considering this, the future community of the COST Action will be able to start working at full capacity since the beginning to meet the objectives of the Action. Nevertheless, inclusion of new members will be possible and boosted during all the duration of the Action, in particular to fill any gaps that will be identified. Inclusion of new participants will be facilitated by the tools made available by the COST system. Top level workshops, conferences and training schools will attract many scientists and companies within Europe and worldwide. High

number of short-term scientific missions (STSMs) will be designed especially for early stage researchers to consolidate the collaborations within members of the Action.

The existence of the Network itself and the role of main European actor that it will assume, regarding collecting and spreading knowledge about CAP and PTL applied to medicine, as well as defining common standards and guidelines for all working in this field, will be an important attraction point for all entities that have not been reached yet.

2.2.2. INVOLVEMENT OF STAKEHOLDERS



The Action will reach a broad group of stakeholders from diverse fields, as represented in the figure, most of them already involved as co-proposers. The main ones are: 1) **Scientists**, Technologists and members of the Academic cluster and Early Career Investigators (ECIs); 2) **Healthcare professionals** such as medical doctors (surgeons, clinicians, oncologists, dermatologists, dentists...) and veterinarians; 3) Companies from **industrial sector** of plasma devices & medical equipment manufacturing, biotechnology, or companies involved in the development of drugs and those involved in animals breeding; 4) **Policy makers**, mainly IP strategy institutions, ethics and regulatory agencies; 5) **Patient associations** & end users.

Privileged feedback between scientists and healthcare professionals will be promoted through this Action by exclusive exchange platforms and workspaces. Both communities will be in charge of the knowledge creation and innovation part, leading jointly the research in *Plasma therapy* field from fundamental science to *in vivo* applications.

Assessment from Regulatory and Ethics agencies will be sought as well as assessment on IP protection and technology transfer for future exploitation of the results and also as support of the proposed Action through the data management plan (Section 3.2.2). Indeed, inclusion of the **industrial sector** is of capital importance for technology transfer of the findings of PlasTHER. Companies with high degree of innovation and well-recognized/established manufacturers will be the main industrial targets of the proposed Action. Finally, in a minor part, **patient associations** or other end users apart from healthcare professionals (ie. farmers) will be involved in the Action to have a feedback the final link in the chain. These three last groups are maybe the most challenging stakeholders to involve in the Action but are of great importance and some of them are already involved as co-proposers.

2.2.3. MUTUAL BENEFITS OF THE INVOLVEMENT OF SECONDARY PROPOSERS FROM NEAR NEIGHBOUR OR INTERNATIONAL PARTNER COUNTRIES OR INTERNATIONAL ORGANISATIONS

This proposal will benefit from the participation of researchers from IPCs, specifically from USA, Canada Japan and Korea, as well from the Russian Federation as NNC. Participation of other partners from other NNCs and IPCs, as well as international organizations will be welcomed and encouraged during the Action. Their inclusion will help spread the **visibility and outcomes of the Action worldwide, increasing the recognition of Europe** as leader in the field. The reciprocal benefits are clear, as this will allow sharing knowledge, establishing new collaborations, possibility to apply for international funding, as well as socioeconomic and technological impact. This is relevant also from the point of view

of regulation/ethics/policies, which are not limited to Europe and thus participation from international actors will be of significant importance.

3. IMPACT

3.1. IMPACT TO SCIENCE, SOCIETY AND COMPETITIVENESS, AND POTENTIAL FOR INNOVATION/BREAK-THROUGHS

3.1.1. SCIENTIFIC, TECHNOLOGICAL, AND/OR SOCIOECONOMIC IMPACTS (INCLUDING POTENTIAL INNOVATIONS AND/OR BREAKTHROUGHS)

According to survey among 148 German companies (2004) predicted the field of maximum growth of future plasma applications to be medical technology, biotechnology and pharmacy, respectively. This is extrapolable to the whole of Europe, and thus this field of research has immense potential and could have a high impact on the surface treatment and decontamination of implantable devices as well as other medical tools. As the PlasTHER Action proposed will be developed in different axes, it can impact on different areas:

Impact in decontamination
Benefits to society. New viruses and bacterial resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases. The actual increase in frequency and severity of Health care-associated infections (HCAIs) is especially linked to antibiotic resistances: the US Center for Disease Control and Prevention identifies that nearly 1.7 million hospitalized patients annually acquire Health care-associated infections (HCAIs) while being treated for other health issues and that more than 98,000 patients (one in 17) die due to these. Antibiotic-resistant infections kill around 37.000 patients in Europe each year with an economic impact of 7 billion euro. While only 20-30% of HCAIs are considered to be preventable by intensive hygiene and control programmes, there's a strong need for chemo-physical technologies to contain their spread.
Benefits to economy. The global antiseptics and disinfectants market size was valued at USD 16.75 billion in 2018. The global medical device cleaning market size was valued at USD 9.74 billion in 2017. Both are expected to expand at CAGR between 6.3 and 6.7 % in the following 8 years, with the subsequent window of opportunity for CAP technologies for decontamination of heat-sensitive materials (surgical materials, protective equipment such as masks etc.) or in the clinics for disinfection of wounds or in dentistry for the oral cavity.
Breakthroughs and innovations: Air sanitization with plasmas can for instance be an impactful and beneficial to address the prevention of current and future large-scale pandemics, with profound implications for improving global healthcare and alleviating the associated socioeconomic burden.

Impact in tissue regeneration
The regenerative medicine market is expected to grow from US$ 8.5 billion in 2020 to US$ 17.9 billion by 2025, at a CAGR of 15.9% during the forecast period. Therefore, the advances brought forward by a novel technology in the area such as CAP can have a huge impact, both on healthcare, wellbeing of the patients and on economics.
Moreover, regarding wound care the global market was valued at around US$ 19 Billion in 2018 and is projected to exceed US$ 33 Billion by 2026. Wound infection is a major safety concern for patients as well as for healthcare professionals globally, i.e. in chronic wounds, diabetic ulcers. The application of *Plasma Therapy* in wound healing treatment has shown efficient in complex wounds, and as it can improve the rate of wound healing, reduce the rate of infections so this would improve patient well-being and reduce hospital care costs, and the use of drugs.

Breakthroughs and innovations: While the CAP-related research on wound healing has been more outstanding, that associated specially to tissue regeneration in a variety of tissues (bone, cardiac, muscular, etc.) has been more limited, so the advances that might be brought forward by the PLASTHER Action can represent a breakthrough on the therapeutic approach to tissue regeneration.

Impacts in cancer therapy

Benefits to society. Cancer is the second leading cause of death globally, affecting more than 18 million people worldwide in 2018, and 9.6 million died from the disease. Numbers are expected to rise because of increasing life expectancy and epidemiological and demographic transitions. In fact, the global cancer therapeutics market was valued at €118.7 billion in 2018, and is expected to reach €168.6 billion by 2023, at a compound annual growth rate (CAGR) of 7.3%. Despite the enormous amount of research and rapid developments seen during the past decade, cancer treatment is still challenging and current therapies, such as chemotherapy and radiotherapy, have severe side effects. **PlasTHER aims to push forward alternative cancer treatment strategies with less associated side effects, leading to a new therapeutic option that may reduce the societal burden associated with the disease.** The benefits to the healthcare system are evident: from improvements in patient management due to lower secondary effects, reducing patient care costs, and hopefully allowing faster recovery times with shorter hospitalization periods and lower number of progress consultations.

Breakthroughs and innovations. The current treatments generally rely on the systemic delivery of drugs or therapeutic agents and their cytotoxic effect without discriminating healthy cells and tissues from cancer cells. In this regard, the plasma-based anticancer strategy would allow local administration of therapeutic agents with selective cytotoxicity, thereby killing cancer cells without affecting the healthy surrounding tissues. Therefore, our technology has the potential to develop new precision medical tools for cancer therapy, as well as, to implement novel procedures and approaches for the treatment of this disease.

3.2. MEASURES TO MAXIMISE IMPACT

3.2.1. KNOWLEDGE CREATION, TRANSFER OF KNOWLEDGE AND CAREER DEVELOPMENT

The PlasTHER Action aims to make a step forward in the fundamental biological basis for biomedical and therapeutic applications of CAP, and put together the suitable knowledge through leading European teams and relevant actors to shift the different areas of Plasma Medicine closer to the market, the patient, and the clinics. The success of the proposed Action strongly depends on the knowledge transfer from academia and research institutions to medical doctors and companies and vice versa. In this sense, the knowledge transfer will facilitate and promote cooperation in upscaling, machinery development, and technologic implementation resulting into profitable products and boost Europe's competitiveness. Therefore, the interaction of industrial partners and R&D providers is essential as it will encourage these providers to direct their research toward medical and industrial needs. To that end, PlasTHER will connect and integrate all COST members establishing dissemination and communication strategies to target different groups including: i. Young and experienced researchers and clinicians. ii. Potential investors/stakeholders. iii. Policy makers. iv. General public. v. International and regional authorities

Every effort will be made, during the course of the Action, to disseminate information about the goals of the project, results and opportunities to all members and general public trough the social media. Besides, this Action will maximize its impact at various levels, ranging from short- to long-terms, and encompassing the scientific, technological, and socioeconomic components. In this regard, PlasTHER plans to organize, promote and participate in the following actions:

- Networking to promote PhD students and other early career investigators (ECI) to have the chance to establish new contacts with internationally eminent scientists and industry researchers in these areas. Young and experienced scientists and professionals will "flow" easily between academic research and industry, through short term scientific missions (STMS).

- Encourage young researchers to orient their career development in the relevant fields and also to involve senior researchers in the management to reach the age balance. In addition, women will be encouraged to assume leading roles in the Action to promote gender balance.
- Create an ideal scenario for mobility and multidisciplinary training of ECI also by organizing training schools. This will positively impact on their career development and prepare them for future leadership in the field.
- Establishment of links and joint collaborations among proposers participating in existing projects in the field of CAP technology. Additionally, this Action can result in the submission of new international projects via coordination meetings, common conferences, workshops, training schools etc.
- Publication of relevant knowledge in peer-reviewed scientific journals and international conferences. Open access will be prioritized for maximum outreach and accessibility of results. Alternatively, open access will be provided via institutional repositories.
- Target end users (consumers), policy makers, non-profit organization by dissemination activities and conferences/workshops organized by the Action and at internationally recognized conferences.
- Invitation of Top international scientists to join the Steering Committee of the Action.

3.2.2 PLAN FOR DISSEMINATION AND/OR EXPLOITATION AND DIALOGUE WITH THE GENERAL PUBLIC OR POLICY

The **dissemination of the research outcomes of the proposed Action will be targeted to scientific audiences, the industrial sector** as well as to the **general public** through different means with the overall goal to maximize the impact and visibility of the proposed Action.
The scientific targeted audience will include: a) Researchers in materials science, chemistry, physics, engineering, tissue engineering, drug delivery, nanomedicine etc. and b) Clinicians, Medical doctors, Surgeons, Healthcare professionals, Veterinarians, Dentists, etc. The industry focused will include the biotechnology industry, Plasma device manufacturers. Innovation companies.
In that way, **promotion of the proposed Action**, and most particularly of the biannual workshop planned for the Action, will be done through other **relevant international conferences with high exposition** in the field such as the International Conference on Plasma Medicine (ICPM9) in 2023, the annual International Workshop on Plasma for Cancer Treatment (IWPCT), or the European Congresses of the Tissue Engineering and Regenerative Medicine International Society (TERMIS-EU) to be held in Manchester (UK) in 2023. To support the diffusion of the different activities of the Action, **contribution of Scientific societies** such as International Society of Plasma Medicine (ISPM), International Plasma Chemistry Society (IPCS), European Society for Biomaterials (ESB) or Tissue Engineering and Regenerative Medicine International Society (TERMIS), will be looked for. Finally, the management committee of the Action will **appeal to the different partners of the Action for diffusion and promotion of the Action activities** during national and international conferences, congresses and workshops in which they are involved in, for example, through mentioning of these activities in their presentations.
Results of research will be published in high impact factor JCR journal in the fields of plasmas, medicine (most specifically regenerative medicine), tissue engineering, biomaterials, etc. Appropriate journals such as *Biomaterials*, *Plasma Chemistry and Plasma Processing, Tissue Engineering, Journal of Tissue Engineering and Regenerative Medicine*, *Cancers, Scientific Reports,* etc. will be considered.

To optimise **the impact on the general public** and of the public's investment in research, the Action will follow the open access (OA) mandate by depositing peer-reviewed research papers/pre-prints arising from the proposed Action, in OA journals (gold access) or, once the embargo has ended, in the institution library repository (green access) as well as in common research webpages of high visibility, where appropriate. The use of social networking by creation of specific media of the Action (e.g. Twitter profile of the Action) will be used to diffuse the activity of the proposed Action as well as for raising awareness of the general public on the topic. Press dissemination will be also considered by allowing a specific session opened to press media during the workshops and congresses organized under the banner of the Action. Opening specific sessions and spaces during these workshops to the general public (students, biotechnology companies or everyone interested) with previous targeted

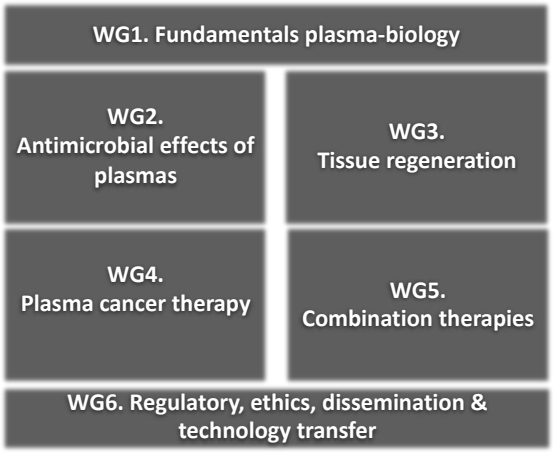
announcement will also be considered. Finally, raising awareness of the regional and international authorities regarding the promising outcomes of such a new field as Plasma Medicine will be looked for during the organization of the different workshop organized under the banner of the COST Action.
A detailed Data Management Plan will be prepared at the beginning of the cost Action after consultation of all the partners regarding the harmonization of data and metadata collections; standards for collection, processing/normalisation, sharing; storage, backup and archiving. In agreement with OA regulations, after scientific publication from part of one(s) of Action member(s), the corresponding metadata will be available for sharing. The Data Management Plan will include consideration regarding protection of the results and research findings, confidentiality, security and protection of the personal data, ensuring compliance with relevant national and European legislation and respecting the wishes of the different Action partners. IP protection, writing of the data management plan and technology transfer for future exploitation of the results will be assessed by the ethics and IP offices of the entities involved in the Action.
For the **exploitation** of the outcomes of the PlasTHER COST Action: i. first IPR protection will be envisaged with the support of the patent offices from the different universities and institutions. ii. Thanks to the involvement already from an early stage of the project of different companies, a focus will be more easily put on technology transfer of the results and findings.

4. IMPLEMENTATION

4.1. COHERENCE AND EFFECTIVENESS OF THE WORK PLAN

4.1.1. DESCRIPTION OF WORKING GROUPS, TASKS AND ACTIVITIES

As previously described, the objectives of this Action are focused on exploring the potential of cold atmospheric plasmas in novel medical and biomedical applications, to develop a basic understanding of such promising applications, and to use this knowledge to develop optimized standard protocols for each specific application. In this regard, the scientific work plan of this Action will be organized in five Working Groups (WGs) in the areas described in the Figure below:



There will be a specific WG1 devoted to investigating the fundamental interactions and mechanisms related to the biological action of plasma therapies. The other WGs are focused on specific applications aimed at combining interdisciplinary basic science knowledge of plasma with application expertise to gain insight into the fundamental biological mechanisms in the different biomedical uses of this technology. A last transversal WG6 will be devoted to regulatory, dissemination & Tech Transfer. To this end, each WG area will include a multidisciplinary network involving experts from industry, clinics, and academia. The WGs will coordinate activities that support the scientific development of the research area and highlight areas of innovation. The WGs description and their specific objectives, and tasks are presented below:

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 to advance towards a general vision and a better understanding of plasma

technology in biomedical applications. Besides, different plasma sources have already been licensed as medical devices and are 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 for 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. To this end, WG1 will have the following tasks:

Task 1.1 Identify and define the fundamental physical and chemical contributors from cold plasmas and plasma-treated liquids responsible of the biological response in the different biological actors.

Task 1.2 Gather recent (5-10 years) results and data to understand and describe the physical and biochemical interactions between CAP and PTL and i. eukaryotic cells and tissues and ii. prokaryotic cells, and particles such as viruses or prions.

Task 1.3. Define standard protocols for measuring physical and chemical characteristics of CAP and PTL and its induced effect in-vivo treatment of biological samples, to boost at EU level the market penetration of CAP sources as safe and effective medical devices with regard to their physical and biological requirements.

Task 1.4. Explore the possibility of in-situ monitoring and control of the CAP interaction with biological tissues, with the aim a process standardization.

Task 1.5. To define a roadmap of the critical fundamental points that are pending or not yet sufficiently investigated to ensure the advance of the different therapies of CAP and PTL.

WG2 – Antimicrobial effects of plasma

As discussed earlier, plasma decontamination has been shown to be promising in the treatment of medical devices and implants, as well as for 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 of time and be transmitted to others over distances >1 m. To have a better understanding of the role of plasma in decontamination processes and developing standard plasma treatment protocols for antimicrobial applications, WG2 will include the following tasks:

Task 2.1. Different plasma sources and conditions have shown some effectiveness against different bacterial biofilms. However, the variety of plasma sources, plasma conditions and biofilms makes difficult to draw any general conclusions. Special interest will be paid to virus and prions and also to the activity of plasma conditioned liquids. This task will address this issue with the following subtasks:

- **2.1.1** Elaborate and define standard protocols to produce clinically relevant bacterial biofilm/spore samples and suitable protocols for evaluating antiviral activity in a safe manner (ie. for COVID samples or prions).
- **2.1.2.** Develop standard protocols of CAP treatment including PTL to treat the standard biofilm or viral samples.
- **2.1.3.** Explore the feasibility and effectiveness of using multiple plasma sources for antimicrobial purposes.

Task 2.2 Medical devices, medical protective equipment based on medical textiles, implants and prostheses are of increasing importance. The surface sterility of such devices and prostheses play a critical role in their medical success, and in the case of protective equipment the possibility of reusing can be an important asset to generate less waste. This task will focus on assessing the antimicrobial effect of plasma treatment on different biomaterials used in this field following the standard protocols developed in Task 2.1. This includes:

2.2.1. Evaluating the standard decontamination protocols onto relevant metallic, ceramic and polymeric biomaterials with complex geometries, as well as in biological samples.

2.2.2. Developing strategies to translate scientific results towards large scale application.

Task 2.3 Human activity is the main cause for the formation of bioaerosols indoors, that could spread diseases such as influenza and respiratory syndromes. Bioaerosol exposure has become one of the major concerns for the residential, healthcare, and government sectors after the outbreaks of SARS in 2003 and influenza H1N1 viral infections in 2009 across the globe prompted worldwide attention for effective biological monitoring and control measures. Hence, increasing indoor air quality can play a pivotal role in human health. In this field, there is a need for innovative technology that could inactivate microbes and viruses in a short time and plasma has already proven to be effective in many cases and with different sources and operating conditions.

2.3.1. Evaluate the standard protocols to inactivate through CAPs bioaerosol containing bacteria, viruses, etc., in indoor and poorly ventilated spaces.

2.3.2. Develop strategies for translating scientific results towards large scale application.

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. To address these issues, WG3 will have the following tasks:

Task 3.1 Establishing standard techniques and procedures to evaluate the therapeutic effect of CAP for treatment and regeneration of tissues.

3.1.1 Evaluating and comparing experimental results conducted using different plasma sources and conditions to identify the plasma components responsible regeneration effects for each particular kind of tissue (soft tissue, mucosa, cartilage, bone...).

3.1.2 Analyzing the results to achieve synergistic outcomes and establish best practice across the partners, and understanding the main mechanisms of action, penetration depth, etc.

3.1.3 Investigating the long term effects of plasmas, safety, and suitable dose regimes to achieve suitable regeneration.

Task 3.2 Effects of CAP and PTL on wound healing, where the selectivity of the treatment is of crucial importance for clinical success. In this context, for wound decontamination, plasma treatment must destroy pathogens without affecting the surrounding patients' tissues.

3.2.1 Identifiying the most suitable models to evaluate wound healing, combining tissue regeneration and wound disinfection.

3.2.2 Comparing the experimental results obtained by all expert partners of this area to identify the common side effects of plasma exposure. With this, the PlasTHER Action will attempt to determine safe limits for plasma exposure.

3.2.3 Investigating the possible beneficial action of PTL on wound regeneration, identifying the main components of CAP and PTL responsible for its effects.

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 on studying the field of redox biology to explain and understand the molecular basis behind the biological effects of plasma in cancer treatment. In this regard, WG4 will have the following tasks:

Task 4.1 Establishing standard techniques and procedures to evaluate the therapeutic effect of CAP and PTL treatment on cancer cells. This will be essential to the effective operation the Action, but also valuable for the global scientific community. This task will include the following subtasks:

4.1.1 Elaborating standard protocols and guidelines for the evaluation and validation of the cytotoxic effect of CAP and PTL on cancer cells and relevant 3D models.

4.1.2 Compare experimental results conducted by the partners following the procedures developed in the previous subtask. The main objective here will be to correlate the characteristics of treated liquids with their anticancer potential and, to identify the specific RONS in liquids that are responsible for destroying cancer cells selectively.

4.1.3. Establishing consensus on the most suitable plasma source designs for each particular type of cancer, and for treatment of liquids. Trying to understand the regulatory classification of PTLs (Are they drugs despite their composition is not stable with time? Are they a plasma treatment (but without UV, EM, current, etc...)?

Task 4.2 Selectivity of plasma treatment is also of crucial importance for clinical success, as plasma should inhibit the metastatic process and kill cancerous cells without affecting the surrounding healthy cells and tissues. The molecular basis of this selectivity needs to be understood to avoid incidental damage on patients. This task will address this issue with the following subtasks:

4.2.1 Use the procedures developed in task 4.1 to identify the suitable “doses” CAP and PTL with selective cytotoxic effect for cancer cells.

4.2.2 To investigate the effects of PTL on the antioxidant response mechanisms, as well as the transcriptome profile to identify the gene networks regulated by CAP and PTL treatment, and correlate it to Gene Ontology a to interpret genomic and proteomic data of cancer and healthy cells.

4.2.3 Compare the experimental results obtained by all expert partners of this area to identify the common therapeutic and side effects of plasma-treated liquids exposure. With this, the Action will attempt to determine the effectiveness and safe limits of this approach, and define suitable dose regimes for different stages of the disease.

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.

Task 5.1. Combination of biomaterials or nanomedicines with plasma or PTL treatment to foster safety or promote regeneration.

Task 5.2. Combination of plasma therapies with low doses of chemotherapeutic drugs or specific metabolic inhibitors to obtain synergistic results for cancer.

Task 5.3. Combination of plasma or PTL with other drugs or biomaterials to foster tissue regeneration.

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 programmes, 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 entrepreneurial programme(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). Special focus will be on participants coming from ITC, PhD students and ECI.

Task 6.1 Organize summer/training schools and workshops, especially for ECIs. Establish a structured link between theoretical and practical knowledge allowing participants to bridge the gap between the theory, experiments and implementation. Hands-on training will be provided by industry partners and lectures from academic partners under the supervision of non-profit organizations.

Task 6.2 Activities of participants will be shared on conferences/seminars (virtual in case of traveling restrictions): Seminars will focus on high-level scientific and technological meetings with

emphasis on ground-breaking research. The aim is to promote knowledge transfer and capacity building through scientific/technological debate between young researchers and experienced scientists.

Task 6.3 Share the results of the Action with the wider community, both scientific and industrial, through a website, e-learning courses for students, social media, etc.).

Task 6.4. Discussion of possible ethical concerns regarding the technologies proposed, and approaching regulatory agents to define the main critical regulatory points towards authorisation of CAP devices and PTL for therapeutic applications.

4.1.2. DESCRIPTION OF DELIVERABLES AND TIMEFRAME

		Description of the deliverable	**Month**
WG1	**D1**	Review of the existing bioactive physical and chemical components from CAP/PTL.	18
	D2	Description of the currently known molecular interactions between CAP/PTL and eukaryotic cells or procariotes, prions, virus	24
	D3	Definition of standard protocols measuring physical and chemical characteristics of CAP and PTL and its induced effect in-vivo treatment of biological samples	24
	D4	Report identifying the fundamental and critical points that require more investigation to boost CAP- and PTL-based therapies and biomedical applications.	30
WG2	**D5**	Development of standard protocols and guidelines to characterize and test CAP and PTL for antimicrobial and antiviral purposes.	24
	D6	Report on the evaluation of standard decontamination protocols on relevant biomaterials, & potential translation of results to industrial scale.	36
	D7	Report on the evaluation of standard decontamination protocols onto pathogenic bioaerosols, and the potential translation of experimental results to industrial scale.	48
WG3	**D8**	Development of standard techniques and protocols to characterize and test CAP and PTL for skin treatment applications.	36
	D9	Report on the mechanism of action, therapeutic effect &safety of CAP and PTL therapies for tissue regeneration & decontamination in wound healing.	48
WG4	**D10**	Development of standard techniques and protocols to characterize and test CAP and PTL as cytotoxic agent in anticancer therapies.	30
	D11	Report on the mechanism of action and cytotoxic effect of CAP and PTL on cancer cells.	48
	D12	Identify and describe the common therapeutic and side effects to determine the safety of CAP and PTL-based anticancer therapies.	48
WG5	**D13**	Report on the effectiveness and safety of CAP and PTL-based therapies combined with biomaterials for skin regeneration.	36
	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.	48
	D15	Report on the potential use of CAP and PTL combined with biomaterials in the regenerative medicine field.	48
WG6	**D16**	Data management plan	4
	D17	Create content for Interdisciplinary summer/training school and workshops.	12
	D18	Conference, seminar/webinar and poster presentations.	Every 6 months
	D19	Creation of web page & dissemination through digital platforms, social media	2+ constant

4.1.3. RISK ANALYSIS AND CONTINGENCY PLANS

The work plan of PlasTHER Action is feasible as the participants involved in the different tasks have expertise and background to tackle them. However, given the fact that this Action is fully interdisciplinary, the success is directly connected to the strength of the participant and stakeholder network. In this regard, to timely mitigate any potential risks a risk analysis and contingency plan has been elaborated:

RISK DESCRIPTION	RISK PRIORITY	PROPOSED CONTINGENCY MEASURES
Low feedback from the participants	High	- In case of lack or low exchange of information / low outputs, the task will be assigned to another participant
Fulfilling the time schedule. Delayed activities, tasks and deliverables	High	- WG leaders will set deadlines and allocate duties - Periodic evaluation of indicators including nº of activities organized, degree of participation, nº of collaborative scientific publications, timely execution of deliverables, nº of posts in communication channels (web, social media, etc).
Lack of involvement of WGs Chair/Vice-Chair and low interaction within WGs	Medium	- Solving the problem with the corresponding WG Chair/Vice-Chair. MC will replace the WG Chair if required - Lack of interaction among WG will be addressed using the available COST networking tools: organization of meetings, workshops, conferences, etc. Fluent communication will be facilitated by virtual meetings and email lists.
Travelling not allowed	High	- Alternative on-line meetings, seminars and workshops will be organized. Parts of the virtual workshops will be split into small groups to favour discussion. - When possible STSM will be partially substituted (until travelling is re-allowed) by virtual bilateral meetings of short periodicity among the partners implied, to organize joint / complementary experimental to be performed in parallel in both laboratories.
Disinterest of the potential industrial partners	Medium	- Incorporate the potential end-users and industrial partners in the Action from the beginning to detect specific applications of the technology and introduce the benefits of participation. Some industrial partners are already included as co-proposers.
Unexpected R&D and technical difficulties	Medium	-Incorporate experts + increase involvement from all disciplines to provide their expertise in all scientific-technical fields of this Action - Promote proper cooperation of the Action members via regular meetings, and reports to the WG leaders
Financial deviations or lack of resources	Medium	- Regular monitoring of funds administration - Early identification with the help of the financial management staff of the institution & during the financial reporting elaboration. - Redistribution of costs and/or application for additional travel grants if necessary

4.1.4. GANTT DIAGRAM

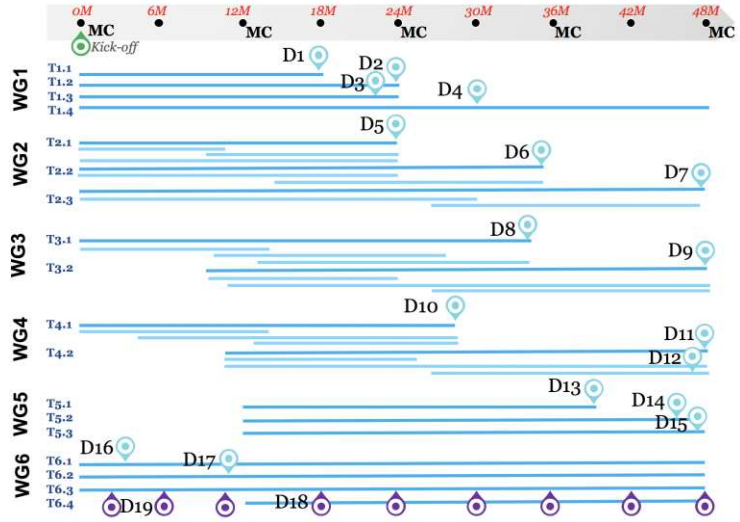


Figure. Gantt Chart. D: Deliverables. MC: Management Committee Meeting

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