PLASMA MEDICINE: THE GREAT PROSPECTS WHEN PHYSICS MEETS MEDICINE

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The research has demonstrated the antimicrobial properties of plasma urging the incorporation of cold atmospheric plasma (CAP) decontamination in current clinical therapies with the aim to improve the benefits on the patients and on society.

Plasma medicine is an innovative and interdisciplinary field of science which has experienced an immense international upswing in the last years. It has emerged two decades ago as a commingling of plasma technology with physics, chemistry, engineering and life science. The final aim of plasma medicine research is to introduce CAPs into clinical medicine and bioengineering fields for human and veterinary therapeutic applications [1].

What is plasma and how it works?

Gas discharge 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 net electrical charge [1] (Figure 1). The features of CAP, according to their non-equilibrium character, include the extremely high concentration of chemically reactive species and a bulk temperature close to the room temperature, which makes it an ideal tool for applications in many fields including agriculture, environment, manufacturing and most of all, medicine. The reactive species, derived from oxygen and nitrogen (RONS - *i.e.* O, $^1O_2$, O_3, $\cdot OH$, $\cdot O_2H$, $\cdot O^{2-}$, $\cdot O^{3-}$, $\cdot NO$, $\cdot NO_2$) are particularly relevant for the medical field as they can diffuse from the gas phase to a solution/biological medium, generating less reactive and longer-lived secondary species, which offer a myriad of potential biological applications [1].

Hence, this has led to the development of two approaches with regards to the putative application methods of plasmas (Figure 2):

i) a direct CAP treatment of the biological target (*e.g.* microbes, eukaryotic cells healthy or diseased and pathological tissue), which exhibits the synergetic effects derived of all the above-mentioned plasma components on cells.

ii) an indirect CAP treatment consisting on the treatment of biocompatible and biologically relevant liquids (plasma treated liquids - PTLs), which allows for minimally invasive therapy in the target site. The PTLs-based therapy mainly delivers the RONS, which have been reported to be one of the major players controlling biological processes [2,3].

Plasma-generated RONS and their biological relevance

Part of the action of CAP can be explained thanks to advances in redox biology, which can be used as the scientific basis to explain the biological effects related to CAP-generated RONS [3]. Briefly, the two general molecular mechanisms of the RONS to highlight 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. In addition, at the cellular level, CAP-derived RONS can alter molecular signalling pathways in both prokaryotic (*e.g.* bacteria) and eukaryotic cells (*e.g.* cancer cells) related to cell-to-cell adhesion, synthesis of growth factors, cell differentiation, division, migration, and apoptosis [3]. The important biological role of RONS in prokaryotic and eucaryotic cells has led to two main capabilities of CAPs targeting both type of organisms with corresponding therapeutical applications.

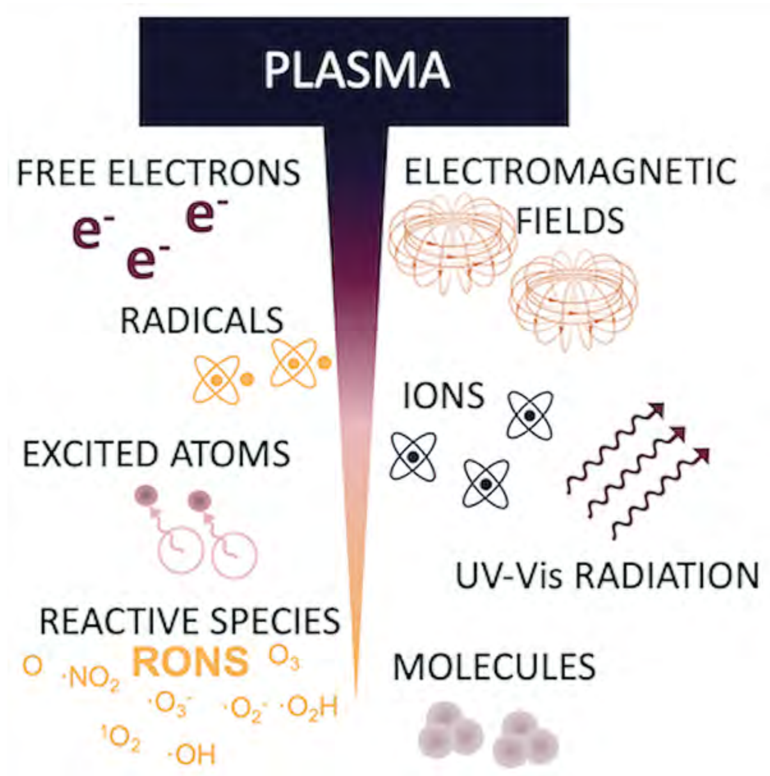
Plasma medicine: CAPs effects on prokaryotic cells

The antimicrobial properties of CAPs have been investigated for over the last two decades, anchoring the concept of plasma decontamination in this field of science. The main medical application of plasma has been focused on the sterilization of surfaces, materials and devices such as prostheses or implants [4]. Nevertheless, the increasingly growing development of atmospheric pressure plasma has promoted the exploration of novel potential applications, especially on living tissues targeting different pathogens such as bacteria, viruses, yeasts and fungi [5].

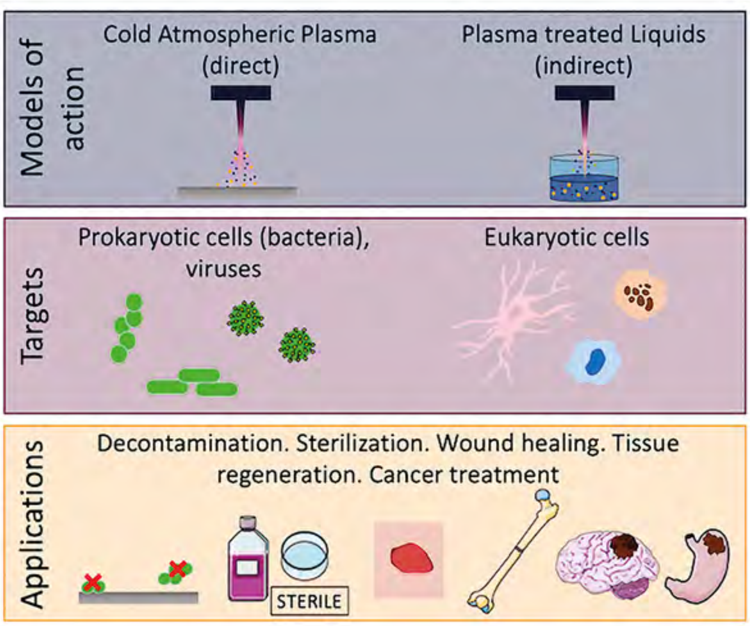
The ever-growing incidence of bacteria with resistance to most antibiotics and the emergence of new unknown pathogens whose transmission is most probably airborne (*i.e.* SARS-CoV-2) has necessitated novel solutions to overcome the handicaps of the available treatments. In this regard, recent research has proved the effectiveness of CAP for inactivation of biofilms and overcoming their acquired resistance to antibiotics [6]. From another point of view, a fundamental finding in this area has been the discovery that under specific conditions, CAP can kill or inactivate harmful microorganisms infecting skin, without causing damage to patient' tissue, thereby facilitating the acceleration of wound healing and treatment of pathogen-based skin diseases [7]. Moreover, CAP has 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 such as transmissible spongiform encephalopathy or Alzheimer's disease, respectively [8]. The research has demonstrated the antimicrobial properties of plasma medicine urging the incorporation of CAP decontamination in current clinical therapies with the aim to improve the welfare on patients and on society.

Plasma medicine: CAPs effects on eukaryotic cells

The recent advances in plasma medicine have shown that CAPs can exhibit the effects on eukaryotic cells and living tissues in the human and animal body evidencing the versatility of plasma treatment. Specifically, it has 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 [9]. This has opened the door to new therapeutical applications such as tissue • • •



▾ **FIG. 1:** Plasma components include free electrons, ions, radicals, excited atoms and molecules, neutral molecules, electromagnetic fields and UV-Vis radiation with no overall electrical charge.

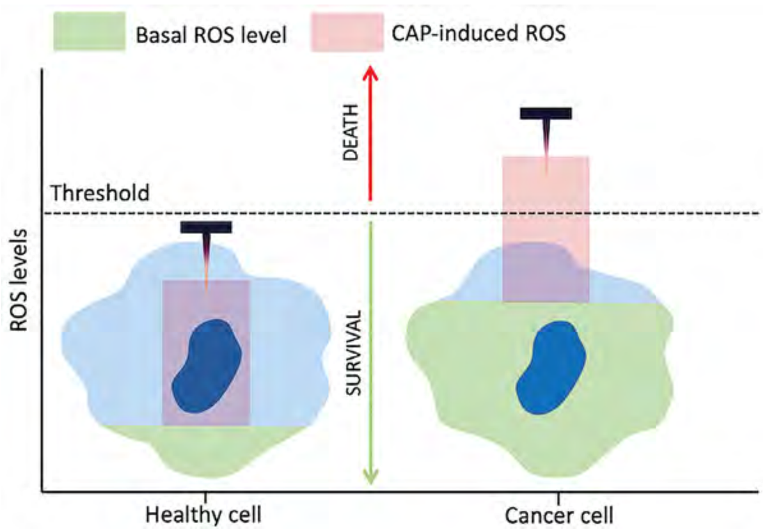


▲ **FIG. 2:** Models of action, targets and applications of cold atmospheric plasmas (CAPs).

• • • regeneration and wound healing (*e.g.* diabetic leg healing, ulcers, burns, *etc.*), with potential implications in the cosmetics field (*e.g.* skin regeneration, scar treatment, *etc.*), as well as cancer therapy (*e.g.* melanoma, glioblastoma, colon cancer, *etc.*).

For instance, different atmospheric pressure plasma jets, which have been recently commercialized, have demonstrated their effectiveness at supporting the healing of non-infected acute wounds (kINPen, PlasmDERM, SteriPlas, Plason, PlasmaCare) [10]. To date, these plasma sources have also been applied in the treatment of long-lasting chronic and infected wounds, particularly in cases where conventional treatment have failed, with evidence *in vitro* and *in vivo* with studies in animals, that have 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 [5]. These results suggest that wound healing may be accelerated due to the simultaneous stimulation of tissue regeneration and angiogenesis. The results observed in such studies enable this vision of CAP technology on its way to becoming a clinical routine for wound healing and skin treatment. This regenerative potential of CAP on skin is currently being explored in the anti-aging and skin-wellness industry. This capacity of plasmas to stimulate tissue regeneration and repair can also be exploited for other tissues [11], opening new avenues that deserve further exploration.

▼ **FIG. 3:** Treatment with CAP delivers exogenous ROS bringing the metabolically more active cancer cells over the survival/ death threshold.



Recent advances in plasma medicine have been exploiting the potential use of CAP in cancer therapies. 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 increased attention is the ability of CAPs to selectively kill cancer cells without damaging the surrounding tissues, thus offering a less aggressive solution compared to common anticancer therapies (*i.e.* chemotherapy and radiotherapy). The anticancer effects of CAP have been, at least in part, related to the RONS generated by plasmas, which are important mediators in stem cell biology. In fact, high levels of RONS have long been suggested to be detrimental to cellular health, and adding high amounts of exogenous RONS can induce cell cycle arrest, while higher doses lead to the induction of apoptotic and/or necrotic cell death (Figure 3). In this context, cancer cells are metabolically more active than healthy cells, thereby generating higher amount of intrinsic RONS. For this reason, delivering plasma with low exogenous RONS, triggers cancer cells to surpass the toxicity threshold and activate apoptosis without affecting normal cells of the surrounding healthy tissue. Interestingly, many studies demonstrated that indirect treatment using PTLs exert very similar effects compared to direct CAP treatment what could be particularly favorable in the treatments of areas harder to reach and an injection of PTLs may be a suitable alternative [12].

Quo Vadis, plasma medicine?

Merging physics, chemistry, and engineering with medical science gave rise to plasma medicine, which aims to develop novel and innovative technologies to improve the quality of life of patients and their families. The recent advancements in the field have demonstrated the great versatility of CAP systems and their ability to induce, mainly through RONS delivery, specific biological responses in pathogens (bacteria, viruses, yeasts and fungi), cells (healthy and cancellous), and living tissues. This has opened the door to a myriad of applications on the edge of tissue engineering and regenerative medicine such as eradication of biofilms, wound-healing, treatment of neurodegenerative diseases or cancer therapy. Nevertheless, there is still much to be done including the rigorous in vivo evaluation of plasma treatments or unravelling the specific molecular mechanisms that are involved on the intra- or inter-cellular level of on living cells and tissues treated with plasmas. Thus, the research community must keep exploring the CAP-tivating versatility, feasibility and therapeutic potential of plasma medicine.

The recently initiated COST Action CA20114 PlasTHER "Therapeutical Applications of Cold Plasmas" (www.plasther.eu) is dealing with all the aforementioned challenges, with the help of a big network of experts in different areas and hopes to bring significant advances to the field forward in the coming years. ■

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