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Editorial

Special Issue “Plasma Technology for Biomedical Applications”

Emilio Martines [1,2]

[1] Consorzio RFX, 35127 Padova, Italy; emilio.martines@igi.cnr.it
[2] Istituto per la Scienza e Tecnologia dei Plasmi del CNR, 35127 Padova, Italy

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1. Introduction

The use of plasmas for biomedical applications in encountering a growing interest, especially in the framework of so-called “plasma medicine”, which aims at exploiting the action of low-power, atmospheric pressure plasmas for therapeutic purposes [1–4]. Several applications have already reached the stage of clinical trials, while others are on their way, a large set of different plasma sources able to work at atmospheric pressure with low dissipated power have been created, and some of them are already certified as medical devices. From the scientific viewpoint, action mechanisms for the interaction of plasmas with cells, tissues and pathogens are being elucidated, although this is a slower process which still requires great efforts. Furthermore, the indirect action through the use of plasma-treated liquids is also being explored, presenting promising possibilities. Finally, one should mention the possibility of plasma-cell interactions not directly related to a therapeutic action of the plasma, but of great importance for facilitating other therapeutic approaches, such as plasma-mediated gene transfection and drug penetration.

The plasmas used in this kind of applications have two main requirements: To be produced at atmospheric pressure, and to keep the treated substrate at temperatures below 37 °C. These two requirements imply that we are dealing with cold atmospheric plasmas (CAP), where only the electrons have a high temperature (of the order of 1 eV, that is 11,600 K), while ions and the neutral molecules are at or near room temperature. These are weakly ionized plasmas, where most of the gas molecules are neutral, and electron-neutral collisions are the main drive of transport processes. In order to keep the power deposition, and thus the gas heating, to low values, a method for limiting the current needs to be employed in the plasma generation, so as to avoid transition to an electric arc: The two most widespread approaches are the dielectric barrier discharge (DBD), where a dielectric layer separating the electrodes rapidly extinguishes the current when enough charge deposits on it, and the radiofrequency (RF) discharge, where the voltage is reversed very fast, at frequencies above 1 MHz [5].

This special issue was launched to collect the latest advancements in this exciting and interdisciplinary field of research. There were 13 papers submitted, of which 11 papers were accepted. When looking back to this special issue, various topics have been addressed: Mechanisms of interaction of plasma with substrate (two papers), technologies for production of plasma-activated water (two papers), and applications to cancer treatment (three papers), disinfection (two papers), regenerative medicine (one paper) and dentistry (one paper).

2. Interaction of Plasma with Substrate

There are two papers in this special issue dealing with the problem of the interaction of the plasma with the substrate, and in particular with substrates composed of living tissues. The first one, by Schweigert and co-workers, deals with a problem which has gained considerable interest in the last few years, that is the effect on the plasma-substrate interaction of the substrate grounding condition [6]. The study was performed both experimentally, using DBD sources operating in helium and argon with cylindrical and planar geometries to treat cancer cells in vitro, and through 2D simulations. It was shown that a metal grounded target positioned below the plate with cells and medium led to an increase of the electric field over the plasma-medium interface, resulting in higher electron energy and density and OH-radical production rate. As a consequence, the ability of killing cancer cells was enhanced, pointing to the importance of grounding to achieve relevant biological effects.

In the second paper, Cordaro and co-workers analyzed the performance of a plasma source, based on the helium DBD jet concept, designed for non-thermal blood coagulation [7]. They demonstrated that the plasma action indeed accelerates platelet aggregation and fibrin formation, thus inducing coagulation, while at higher powers and for longer treatment times also harmful effects appear, such as red cells lysis, with destruction of collagen fibers and dehydration of muscle fibers. In carrying out this task, it was ascertained that the main sample heating mechanism was due to the electric current flowing to the sample. This led to the conclusion that a power deposition evaluation performed on sample targets (as could be prescribed, for example, in a technical norm) could not be representative of what happens when the plasma is applied to actual living tissues. In agreement with the previous paper, this also implies that the grounding condition of the substrate is an important issue.

3. Production of Plasma-Activated Water

The indirect treatment realized using water or other liquids (most often, cell culture medium) previously treated with the plasma is an important part of plasma medicine studies. A wealth of devices have been proposed to perform this treatment in an efficient way [8].

In the first paper of this section, a new device based on a low-current arc formed in ambient air is proposed [9]. The idea (already exploited also by other authors) is that, being the treatment indirect, the requirement of low thermal load holding for direct plasma treatments can be relaxed, so that the plasma used to treat liquids does not need to strictly be a CAP. The authors demonstrated the possibility of generating a stable discharge, even when using liquids that have low electrical conductivity, and confirmed the possibility of treating a continuously flowing liquid. The concentration of reactive oxygen and nitrogen species in water after treatment using the low-current arc object of this study was two orders of magnitude higher than that of water treated using conventional CAP under similar conditions. Strong bactericidal effects of the treated water were demonstrated on *Escherichia coli* cultures.

The second paper, by Schmidt and co-workers, uses a different approach, that is an inductively limited discharge, to treat large volumes of water, overcoming the limitation of few millilitres associated to the most usual approaches [10]. The proposed technique uses high voltage leakage transformers for discharge current limitation to avoid arcing. The authors treated tap water, saline solution and distilled water, and observed that, except for tap water, the treated liquids became acidic and the conductivity increased. In all liquids, distinct nitrification was observed. The microbiological studies showed that physiological saline solution and tap water became antimicrobial. The authors concluded that with the proposed, portable setup, significant volumes of plasma-treated water can be easily produced in less than 30 min.

4. Cancer Treatment

There are several intriguing evidences that the reactive oxygen species (ROS) originated in the plasma can kill cancer cells selectively, preserving healthy ones. This has been attributed, in analogy to other redox therapies, to the faster formation and loss level of these species in cancer cells as compared to healthy ones, leading to higher baseline concentrations. The plasma action will raise ROS concentration in both cell types, but only in cancer cells this will go beyond the threshold leading to apoptosis [11]. Reactive nitrogen species are also considered to be important in determining the plasma action, although their role is less understood [12].

In the paper of Hasse and co-workers, a pre-clinical study on the use of argon plasma as adjuvant therapy on progressive head and neck cancer is described [13]. The plasma was produced by the certified medical device kINPen MED, operating at radiofrequency with an argon flow. The response of healthy and tumour cells of head and neck cancer to CAP exposure was addressed. In addition, tissue samples from 10 patients with histologically proven cancers of the maxillofacial region were treated and then investigated for induction of apoptosis and secreted proteins with antitumour activity. The viability of cancer cells was found to be strongly reduced by the plasma action, although no clear selectivity of cancer cells could be observed. However, induction of apoptosis was superior in tumour tissue than in healthy mucosal tissue. Furthermore, CAP treatment significantly decreased cell motility in squamous cell carcinoma cells only but not in non-malignant keratinocytes. Overall, these results point to CAP treatment as a promising adjuvant treatment option to eliminate minimal residual cancer cells after radical surgery of carcinoma.

The antitumour effect of the reactive species generated by the plasma can be displayed through direct plasma application, as in the previous study, or through the application of liquids previously treated with the plasma. This is the theme of the second paper of this section, by Nguyen and co-workers, which shows that plasma-activated medium can be stored for up to six months in a freezer and then exhibit cytocidal effects on human cervical cancer HeLa cells, similar to those of a direct plasma treatment [14]. The treated medium was Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and antibiotics, and the treatment was performed with a micro plasma-jet nozzle operating in air flow. The cytocidal effect was attributed to H_2O_2 and nitrite/nitrate formed in the liquid by the plasma action.

Apart from the previoulsy mentioned mechanism, a new paradigm is also taking momentum as a possible way of inducing cancer cell death through the plasma action. This is the immunogenic cancer cell death, which proposes stimulation of an immune response against apoptotic tumor cells, and is the theme of the third paper, by Rodder and co-workers [15]. In this work, the role of plasma-treated murine melanoma cells in modulating murine immune cells' activation and marker profile was investigated. The results indicate a tumor-static action in terms of metabolic activity and cell motility and a negligible protective effect of protein present during the treatment. A role of plasma-mediated activation of splenic immune cells and a modulation of inflammatory parameters, in agreement with a pro-immunogenic role of plasma treatment, were also observed.

5. Disinfection

The ability of atmospheric pressure plasma to kill bacteria, either by disruption of the cell envelope or through more subtle effects [16], is well known, and indeed is the first effect which has been invoked for utilization in medical practice [17].

The first paper of the special issue related to CAP disinfection properties is a review, by Gupta and co-workers, concerning the effectiveness of this technique on biofilms [18]. While most studies in this field test the plasma action against bacteria cultures in planktonic form, in real life applications bacteria are often found in the form of biofilms, that is groups of microorganisms adhered to a substrate within a self-produced matrix of extracellular polymeric substance, mostly composed of water, polysaccharides, proteins, and extracellular DNA. Biofilms are much more resilient to antibiotics and antiseptics, thanks to the fact that the extracellular polymeric substance forms a physical barrier, responsible for limiting the transport of chemicals into and out of the biofilm, and are usually challenging to eradicate. The ability to significantly affect biofilms is thus a crucial property to be demonstrated if plasma-based disinfection is to be brought to the market. The paper of Gupta et al. reviews the existing literature on biofilm eradication through CAP, concluding that the technology appears to be promising, but further effort is required in developing (and certifying, I would add) plasma sources adequate for use in real world environments.

The second paper, by Liu and co-workers, is aimed at fully understanding the bio-decontamination process in a reduced-pressure oxygen plasma, using *Escherichia coli* as the target microorganism [19]. This study does not completely fall into the plasma medicine realm, as defined in the introduction, in the sense that it makes use of low-pressure conditions. It is however very interesting, as it makes a thorough comparison of the role of different agents, that is UV radiation, charged species and free radicals, in the decontamination process, and thus offers valuable information also for processes operating at atmospheric pressure. In particular, the authors found that the essential effectiveness on *E. coli* of the oxygen plasma can to be attributed to the intense etching action of charged species, that is electrons and ions, on the bacilli materials. Lipid peroxidation in the cell membrane by oxygen radicals plays a major role only during the initial phase (< 40 s), and is then restrained by the effect of charged particles. The function of UV radiation is to assist in the whole process, resulting in slight damage and rupture of DNA. This study, while confirming the marginal role of UV radiation, adds another bit to the ongoing debate about the importance of charged species in the deactivation process.

6. Regenerative Medicine

The beneficial effects of plasma exposure in regenerative processes, such as wound healing, is one of the most advanced applications of plasma medicine. Holganza and co-workers have studied the effects of the exposure to a helium plasma produced in a DBD plasma jet on tadpoles of *Xenopus laevis*, in relation to developmental effects such as tail regeneration and metamorphosis [20]. The effect of plasma treatment following tail amputation was investigated. The experiment confirmed previous observations about the fact that the plasma treatment accelerates tail regeneration while slowing down the metamorphic progress, the latter possibly indicating the physiological cost of enhanced regeneration involving metabolic machinery at the cellular and organelle level. These effects, associated to higher oxidative stress, were linked to increase in Ca^{2+} content during wound healing, possibly derived from extracellular stores such as the endoplasmatic reticulum. Additionally, adherens junctions between epidermal cells of the tail and reduction of intercellular spaces following plasma exposure were observed, indicating adaptive changes in order to maintain skin integrity.

7. Dentistry

The paper by Shahmohammadi Beni and co-workers [21] adds to the application for which the first plasma medicine tool was originally designed [17], that is the use of a CAP to perform dental treatments in the oral cavity [22,23]. The authors have investigated numerically the transport by convection and diffusion of OH radicals and of hydrogen peroxide (H_2O_2) generated by CAP over treated teeth. This is important, as OH radical and hydrogen peroxide are two of the most important reactive species generated by the plasma, in terms of biological effects. The model used by the authors consists of an equation for the carrier gas motion, based on the level set method, that is a conceptual framework allowing to perform numerical computations involving curves and surfaces on a fixed Cartesian grid without having to parametrize these objects, and transport equations giving the evolution of the OH radical concentration and of the H_2O_2 concentration. The equations were solved in a realistic geometry model of the mandibular jaw and of the space between it and the plasma source. The simulation results allowed a realistic evaluation of the deposition of the two active species on the different teeth of the simulated jaw. Overall, apart from the scientific merit of the specific results, this code appears to be a valuable tool for carefully assessing the actual deposition of active chemical species (possibly including also other species) in a complex geometry, thus allowing to optimize the design of the plasma source, and could be possibly extended also to different environments and plasma treatments.

8. Conclusions

As a matter of fact, it is clear that the strongly interdisciplinary plasma medicine community is evolving towards a higher level of scientific depth and analysis detail, while at the same time progressing towards bringing applications from the laboratory to the patient. This is crucial to fulfill the expectations created by this new discipline, which is foreseen to soon become, at least in some contexts, part of the tools routinely available to the practitioner.

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