New Journal of Physics
The open-access journal for physics

Focus on Plasma Medicine

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New Journal of Physics **11** (2009) 115011 (8pp)
Received 20 November 2009
Published 26 November 2009
Online at http://www.njp.org/
doi:10.1088/1367-2630/11/11/115011

Abstract. 'Plasma Healthcare' is an emerging interdisciplinary research topic of rapidly growing importance, exploring considerable opportunities at the interface of plasma physics, chemistry and engineering with life sciences. Some of the scientific discoveries reported so far have already demonstrated clear benefits for healthcare in areas of medicine, food safety, environmental hygiene, and cosmetics. Examples include ongoing studies of prion inactivation, chronic wound treatment and plasma-mediated cancer therapy. Current research ranges from basic physical processes, plasma chemical design, to the interaction of plasmas with (i) eukaryotic (mammalian) cells; (ii) prokaryotic (bacteria) cells, viruses, spores and fungi; (iii) DNA, lipids, proteins and cell membranes; and (iv) living human, animal and plant tissues in the presence of biofluids. Of diverse interests in this new field is the need for hospital disinfection, in particular with respect to the alarming increase in bacterial resistance to antibiotics, the concomitant needs in private practices, nursing homes etc, the applications in personal hygiene—and the enticing possibility to 'design' plasmas as possible pharmaceutical products, employing ionic as well as molecular agents for medical treatment. The 'delivery' of the reactive plasma agents occurs at the gaseous level, which means that there is no need for a carrier medium and access to the treatment surface is optimal. This focus issue provides a close look at the current state of the art in Plasma Medicine with a number of forefront research articles as well as an introductory review.

Contents

1. Introduction: plasmas in medicine

Gas plasmas represent the fourth state of matter after solid, liquid and gas, and are formed when a gas is ionized. Spanning over a vast parametric range of electron density and electron temperature, their behaviours change very significantly with profound scientific and application implications in areas such as energy, space exploration, environment, semiconductor fabrication and material modification, photonics and communication [1]. Directly relevant to this Focus Issue is low-temperature plasmas generated either at reduced or atmospheric pressures. Their electron temperature is typically a few electronvolts, sufficient to drive many reaction chemistry for modifying DNA, proteins and cell membranes, whereas their gas temperature is close to room temperature thus allowing their treatment of heat-sensitive living tissues. Biomedical application of low-temperature plasmas has been regarded as one of the most significant opportunities in plasma science [1].

Low-temperature plasmas are in general not in the local thermal equilibrium, and these non-equilibrium plasmas can occur naturally (for example in the context of astrophysics [2]) or in laboratories [3, 4]. For most laboratory plasmas, cold atmospheric plasmas (CAP), also known as atmospheric pressure glow discharges (APGDs), are of particular interest because they can be used to interact directly with living tissues without the complication of a vacuum chamber. Most current CAP plasmas are generated in helium or argon mixed with a small amount of reactive gases such as oxygen [4]–[9]; however, they can also be generated in molecular gases such as air [10, 11]. Operated at atmospheric pressure, their collision frequency is in the terahertz range and their mean-free path is a few tens of nanometres. These characters mean that ion energy is relatively low and reaction chemistry is rapid [3]. CAP offer direct delivery of biologically relevant plasma species to sample surfaces. Some of the most relevant issues in plasma medicine are

- Production of reactive atoms and molecules, in both the ground and excited states, that may modify membrane chemistry and permeability of cells;
- Production of ionic species near and even on living tissues;
- Relationship of concentration of plasma species to efficacy of plasma-mediated biological functions in order to establish dosage;
- Plasma design to effect a synergistic ‘cocktail’ of plasma agents for specific biomedical purposes;
- Fast and purpose-specific delivery of plasma agents at the atomic/molecular level;

- Safety consideration to satisfy the WHO guidelines on toxicity, UV emission, temperature, electric currents, etc.

Clearly, the use of plasmas in medicine opens up new vistas of treatment—a breakthrough that could well become as important as that which was initiated by antibiotics. This is the vision. On the practical side, the question is: what type of plasmas should be 'designed' for which purpose? And further questions: are there already any immediate (or imminent) applications in healthcare? What can plasmas 'do' that current medical treatment cannot? Where are plasmas a more economical alternative to current standards? These and many more questions are the subject of current research.

Of low-temperature plasmas, those generated at reduced pressures have been successful as a route to medical sterilization [12]–[14]. A common perception is that their use of a vacuum chamber may present an implementation barrier for treatment of living tissues as well as an economical disadvantage when compared with CAP. As low-pressure low-temperature plasmas are being advanced further, this perception may need to be revisited. Of CAP, their interaction with a biological sample such as human tissue may be divided into three general categories: *direct plasmas* (where the biological sample acts as an electrode and draws the most of the electric current through the sample) [10, 15], *indirect plasmas* (where the electric current is collected by an upstream electrode and the downstream biological sample is treated mostly by neutral species and photons) [9], and *hybrid plasmas* (where the biological sample draws part of the electric current and is treated by neutral species, charged particles and photons) [16, 17]. These three types of CAP–tissue interactions imply different compositions of the 'cocktail' of relevant plasma species as experienced by the tissue, and they also present different scaling implications when surface topologies of living tissues are considered. Plasma–tissue interactions are influenced strongly by surface conditions of tissues, and they must be made relatively insusceptible to tissue surface conditions to ensure the reproducibility and reliability of plasma treatment.

In this Focus Issue, some of the questions posed above are addressed directly, others will be touched upon more or less as a matter of passing (see also the introductory review [18]). It is clear from the publications that the broad field of 'plasma healthcare'—which incorporates medicine, food and hospital hygiene, cosmetics—is highly interdisciplinary. Expertise is needed from medicine, biology, microbiology, plasma chemistry, plasma physics and engineering. To build up teams or networks that bring all of this together for the future benefit of mankind is a challenging task—one, which we hope will be furthered and advanced through this Focus Issue.

2. Preventive medicine using plasmas

This section is entitled 'preventive medicine', because this is the most applicable common denominator for the topics summarized here. The themes cover hospital hygiene and containment of diseases, plasma bactericidal effects, plasma modification of lipid layers and plasma inactivation of proteins and prions. The main point of all these studies is to investigate whether the plasma treatment has an effect such that—if eventually employed in professionally—it would help to reduce the spread of diseases—i.e. prevent suffering and death at an early stage.

The most efficient way to overcome diseases is to prevent them. If that is not possible (the usual situation), the next most important step is containment—preventing the spread of diseases.

Accordingly, decontamination, sterilization and disinfection are major issues in healthcare, one of our most important armaments in the fight against epidemics and the spread of contagious diseases. In the case of hospital acquired infections, there are many excellent and efficient sterilizers in use in hospitals, for example those employing different fluid active agents. The obvious question is why we need another technology. Literature suggests that each disinfection with fluids takes typically a few minutes to become really effective [19]–[21]. With typically 60 patient contacts per day, medical practitioners or nurses would have to disinfect their hands for a total of 3 h each day—impossible in practice. In addition, fluids cannot readily enter into tiny skin folds or penetrate under fingernails, where pathogens may reside, and they can have side effects such as skin irritation or allergic reactions. Disinfection with plasmas, according to current research results, is fast—typically a few seconds—and is benign to the skin. Plasmas, or rather the reactive molecules produced in plasma–air–water vapour interactions, can penetrate even into the smallest skin folds and destroy bacteria, something fluids and even UV radiation cannot equal. So there is a health, a hygiene and an economic issue at stake.

Research is therefore intense into plasma sources capable of disinfecting large surfaces and human tissues in a short time, with many innovative ideas proposed, as well as detailed transport calculations and chemical models, which will influence such designs [22]–[26]. Many questions remain. Can plasmas kill viruses? Can they destroy spores, fungi, biofilms, even prions? Of course, apart from decontamination efficacy, there are other questions of great interest. Are bacteria really killed by plasmas or merely inactivated? Being essentially a 'surface effect' from the point of view of delivery of active agents, how deep plasma agents can reach into tissue?

In this Focus Issue, there are a number of reports that address some of these questions. Detailed three-dimensional atomic force microscopy measurements have shown the surface disruption of bacteria that occurs after plasma irradiation. Such measurements yield information about the mechanisms—and, most importantly, yield a direct answer to the question whether plasma irradiation kills bacteria or merely inactivates them [27]. This is consistent with similar atomic force microscopy results [28] and complements evidence of spore rupture observed using scanning electron microscopy [29]. Plasma–fluid interaction, especially with biofilms, is of great medical interest, e.g. in chronic wounds, cleansing of blood supplies, infectious diseases that are propagated by fluid transmission (e.g. sneezing), etc. Efficient destruction of immersed pathogens would be a major help to physicians. Research in this area is growing and recent advances using different techniques are reported in [30, 31], and these new results follow nicely earlier studies of biofilm inactivation using CAP [32, 33]. Also reported in this Focus issue is spore-forming bacteria inactivation [34], consolidating and expanding the understanding of earlier studies of CAP inactivation of spores [35]–[38]. This research will certainly gain in momentum as different 'tailored' plasma chemical designs become available. Finally, prion decontamination—a health issue that so far is lacking a convincing solution—is being tackled with plasma irradiation [39, 40]. First results are encouraging, a breakthrough in this area would be a spectacular success for plasma medicine.

3. Regenerative medicine using plasmas

The overall goal in the field of 'plasma medicine' points to 'plasma pharmacology', where plasmas can be 'designed' for different desired purposes and applications especially in regenerative medicine. A CAP can be 'designed' to change UV radiation, gas temperature,

electric field and especially plasma chemistry by adding, for example, different gases or catalysts or/and by changing the electron energy distribution function. So far, only first steps into the direction of 'plasma pharmacology' have been taken—this is a very ambitious and difficult topic, since it not only depends on the way the plasma is generated and delivered, but also on the organism and living tissues it is applied to.

In [41], cell biological studies are reported using an argon CAP plasma device, which is specifically 'designed' for disinfection of chronic wounds. The efficiency of this plasma is based on (a) the short-term reduction of the bacterial density in the wound area, which is achieved in roughly equal parts by the plasma-generated UV radiation and reactive species; and (b) the long-term post-irradiation inhibition of bacterial growth, which mainly results from the reactive nitrogen and oxygen species produced by the plasma without causing any negative effect on human cells. This interplay of requirements is fulfilled at certain plasma dosages or concentrations of reactive nitrogen and oxygen species, where different effects on prokaryotic and eukaryotic cells—deadly to the bacteria and supportive concerning cell regeneration in human cells—were measured. Furthermore, this Focus Issue contains a number of reports that address the extra- and intracellular chemistry, resulting from treatments with different plasma devices and which are summarized in the following.

3.1. Extracellular biochemistry

In [31], the acidification of a thin water layer on a human lipid film (modelling the hydrolipid film covering the epidermis on the human skin surface), which results from the treatment with non-thermal atmospheric pressure dielectric barrier discharge (DBD), is analysed. The measured shift in pH either results from adhesion of NO_x species on the surface or from the deposition of nitric acid by gaseous HNO_3. These preliminary observations need further investigations and might offer a first step into pH-targeted plasma therapies in dermatology by 'designing' an optimum plasma source. In [42], it was shown that an APGD torch in helium flow is capable of inducing temporary cell permeabilization. This permeabilization is achieved by plasma-generated long-lived excited helium states, which reach the cell membrane. By 'designing', i.e. inserting active agents into the plasma, the plasma-created pores (with a diameter of ∼5–7 nm) open up the possibility to transfer active pharmaceutical ingredients directly into the cell interior, where they can immediately develop their beneficial effects.

3.2. Intracellular biochemistry

Depending on the plasma source, the 'plasma design' and the plasma dosage, plasmas can be selective and therefore have an adverse (apoptotic) effect on melanoma skin cancer cells, without damaging the healthy tissue [43]. A possible explanation for plasma-induced apoptosis in cancer cells relies on the production of reactive oxygen species (ROS) by the plasma in fluid. The ROS can penetrate the cells and therefore might induce high levels of DNA damage, which as a result leads to the induction of apoptosis [44]–[46]. In [47], human melanoma cancer cell studies were performed with a low-temperature radio-frequency atmospheric plasma device, which operates with helium. The results obtained suggest that CAP inhibit the adhesion of these cells by reducing the activity of those adhesion proteins, which are important in malignant transformation and acquisition of metastatic phenotypes.

Since 2006, it is known that CAP can achieve effective blood coagulation without the need of high temperatures, depending on the applied dose [10, 48]. The cauterization effects were achieved through selective plasma stimulation of complex biochemical processes, which also take place during natural blood coagulation. According to newest research results [49], the reactive atomic oxygen, which is generated by the plasma, activates erythrocyte—platelets interactions—and therefore induces blood coagulation.

4. Conclusions and outlook

Plasma Healthcare topics are receiving increased attention, with biologists/medical researchers and plasma physicists/chemists joining forces as well as their respective engineering, technological, diagnostic and interpretative capabilities in an effort to detect and identify the basic processes—with the aim to 'design' them for specific applications, eventually. At this early stage—the field was started in earnest only a few years ago—naturally the research uses the available 'equipment'—the plasma sources that have been developed and which are available in the respective research networks. Accordingly, the results and conclusions are still specific—specific with respect to the plasma source and its operating parameters. But, as more and more data are being assembled, a picture will emerge that points in the 'right' direction for different desired applications—i.e. 'designer plasmas'—and in the long run perhaps even 'plasma pharmacy'.

Even based on the limited current level of research, there is every reason to assume that plasmas can be designed (eventually) to achieve cell permeabilization for direct molecular delivery of medical substances. Equally, by controlling cellular response, plasmas may eventually be able to generate paracrine effects that propagate far into the cellular tissue. Other plasmas may be designed to enhance cell growth, healing and regenerative effects. The beginnings have already been seen [41].

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