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Review

Plasma Medicine: A Brief Introduction

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Abstract: This mini review is to introduce the readers of *Plasma* to the field of plasma medicine. This is a multidisciplinary field of research at the intersection of physics, engineering, biology and medicine. Plasma medicine is only about two decades old, but the research community active in this emerging field has grown tremendously in the last few years. Today, research is being conducted on a number of applications including wound healing and cancer treatment. Although a lot of knowledge has been created and our understanding of the fundamental mechanisms that play important roles in the interaction between low temperature plasma and biological cells and tissues has greatly expanded, much remains to be done to get a thorough and detailed picture of all the physical and biochemical processes that enter into play.

Keywords: low temperature plasma; plasma jet; cells; tissue; apoptosis; cancer; wound healing; reactive species

1. Introduction

In the mid-1990s, experiments were conducted that showed that low temperature atmospheric pressure plasmas (LTP) can be used to inactivate bacteria [1]. Based on these results, the Physics and Electronics Directorate of the US Air Force Office of Scientific Research (AFOSR) funded a proof of principle research program in 1997 and supported such research for a number of years. The results from this research program were widely disseminated in the literature, including in peer-reviewed journals and conference proceedings, therefore attracting the attention of the plasma physics community to new and emerging applications of low temperature plasma in biology and medicine [2–8]. The goals of the AFOSR program were to apply low temperature plasmas (LTP) to treat the wounds of injured soldiers and to sterilize/disinfect both biotic and abiotic surfaces. By the early 2000s, research expanded to include eukaryotic cells when small doses of LTP were found to enhance phagocytosis, accelerate the proliferation of fibroblasts, detach mammalian cells without causing necrosis, and under some conditions, lead to apoptosis [9,10].

The above-described groundbreaking research efforts showed that nonthermal plasma can gently interact with biological cells (prokaryotes and eukaryotes) to induce certain desired outcomes. These early achievements raised great interest and paved the way for many laboratories from around the world to investigate the biomedical applications of LTP and by the end of the first decade of the 2000s, a global scientific community was established around such research activities. The field is today known by the term plasma medicine, and in the last few years a number of extensive reviews and tutorials were published (see Refs [11–18] and references therein) as well as a few books [19–21].

Today, the field of plasma medicine encompasses several applications of low temperature plasmas in biology and medicine [22–53]. These include:

- Sterilization, disinfection, and decontamination,
- plasma-aided wound healing

- plasma dentistry
- cancer applications or "plasma oncology,"
- plasma pharmacology,
- plasma treatment of implants for biocompatibility.

In the late 2000s, several LTP sources were approved for cosmetic and medical use. Examples are: in 2008 the US FDA approved the Rhytec Portrait® (plasma jet) for use in dermatology. Also in the US other plasma devices are in use today for various medical applications, such as the Bovie J-Plasma® and the Canady Helios Cold Plasma and Hybrid Plasma™ Scalpel. In Germany, the medical device certification class IIa was given to the kINPen® (plasma jet) in 2013, and the PlasmaDerm® device (CINOGY GmbH) was also approved. Figure 1 is a timeline graph showing the major milestones in the development of the field of low temperature plasma medicine.

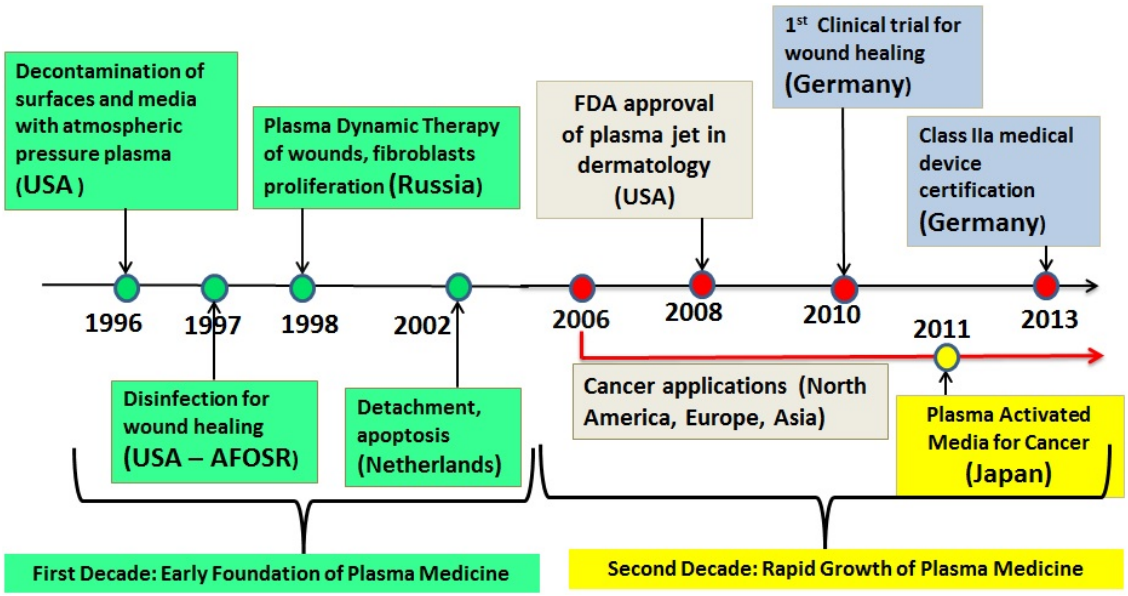


Figure 1. Timeline showing some major milestones of the new field of the biomedical applications of low temperature atmospheric pressure plasma. This timeline does not show the case of thermal (hot) plasmas, which were used for many decades in medical applications requiring heat, such as cauterization and blood coagulation.

2. LTP Takes on Hygiene and Medical Challenges

As can be seen from Figure 1 the biomedical applications of LTP started with experiments on the inactivation of bacteria on biotic and abiotic surfaces and media. Bacterial contamination proved to pose severe challenges for some industries and in the healthcare arena. The industrial challenges are mainly around the problem of food contamination and sterilization of food packaging. Several well-publicized food poisoning incidents (EHEC, *Listeria*, *Salmonella*) pointed out to consumers that the present methods employed by the food industry may not be adequate to insure food safety. The healthcare challenges are linked to nosocomial infections caused by antibiotic resistant strains of bacteria, such as Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile* (C-diff). Every year in the US, hospital acquired infections (HAI) kill thousands of patients with compromised immune systems. HAI are caused by inadequate sterilization/decontamination of instruments, surfaces, clothing, bedsheets, and personnel (nurses and doctors). In most cases, contamination by strains of bacteria resistant to the best antibiotic medications available today is the cause of HAI. LTP is therefore considered as a novel technology that can be successfully applied to help solve some of the challenges described above.

The most recent application presently receiving much attention is the use of LTP to destroy cancer cells and tumors in a selective manner [38–58]. Starting around the mid-2000s several investigators reported experiments showing that low temperature plasmas (LTP) can destroy cancerous cells in vitro. This was followed by some in vivo work showing that LTP can reduce the size of cancer tumors in animal models. The in vitro work covered a host of cancerous cell lines, which included glioblastoma, melanoma, papilloma, carcinoma, colorectal cancer, ovarian cancer, prostate cancer cells, squamous cell carcinoma, leukemia, and lung cancer. The in vivo (animal model) work can be found in [38,44,45,53].

In addition to direct plasma applications to cancer cells and tissues, investigators reported that plasma-activated media (PAM) can also be used to destroy cancer cells [38,50,54–58]. Plasma-activated medium is produced by exposing a biological liquid medium to LTP for a length of time (minutes). In this case, the plasma-generated reactive species interact with the contents of the medium and generate solvated long-lived reactive species in the liquid, such as hydrogen peroxide, H_2O_2, nitrite, NO_2^-, nitrate, NO_3^-, peroxynitrite. $ONOO^-$, and organic radicals. These molecules subsequently react with the cells and tissues causing various biological outcomes.

3. Mechanisms of Biological Action of LTP: Brief Summary

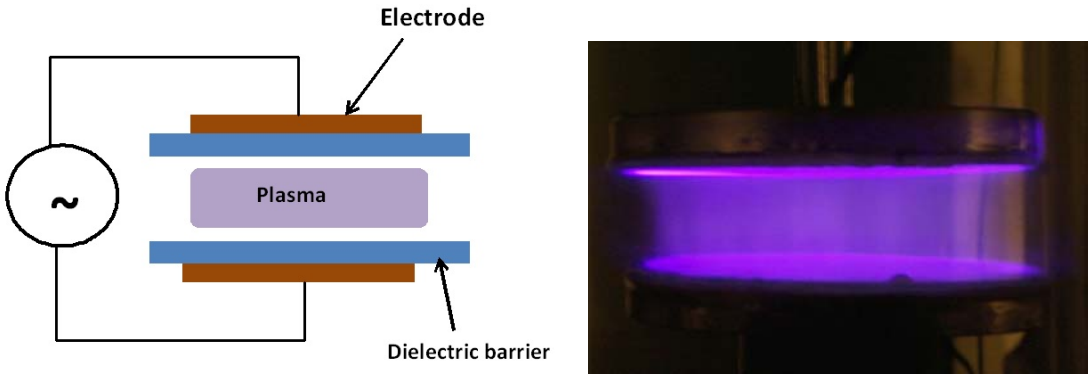
Investigators reported that the effects of LTP on biological cells (prokaryotes and eukaryotes) are mediated by reactive oxygen and nitrogen species (RONS) [11,12,59–66]. These species include hydroxyl, OH, atomic oxygen, O, singlet delta oxygen, $O_2(^1\Delta)$, superoxide, O_2^-, hydrogen peroxide, H_2O_2, and nitric oxide, NO. For example, the hydroxyl radical is known to cause the peroxidation of unsaturated fatty acids, which make up the lipids constituting the cell membrane. The biological effects of hydrogen peroxide are mediated by its strong oxidative properties affecting lipids, proteins, and DNA (single and possibly double-strand breaks). Nitric oxide, which acts as an intracellular messenger and regulator in biological functions, is known to affect the regulation of immune deficiencies, cell proliferation, induction of phagocytosis, regulation of collagen synthesis, and angiogenesis.

In cancer cells, the mechanisms of action of LTP are suspected to be related to an increase of intracellular reactive oxygen species (ROS), which can lead to cell cycle arrest at the S-phase, DNA double-strand breaks, and induction of apoptosis. Research by various groups showed that RONS generated by LTP react with cell membranes and can even penetrate the cells and induce subsequent reactions within the cells that can trigger cell-signaling cascades, which can ultimately lead to apoptosis in cancer cells [56–66]. In addition, investigators have shown that plasma-generated RONS can indeed penetrate biological tissues up to depths of more than 1 mm and therefore interact not only with the cells on the surface but with those underneath [67–72].

LTP delivers not only reactive species but it also can exhibit large enough electric fields [73–77]. The magnitudes of these electric fields are several kV/cm and they are suspected to play a role, such as in cellular electroporation, which may allow large molecules to enter the cells.

4. Two LTP Sources for Biomedical Applications: Brief Description

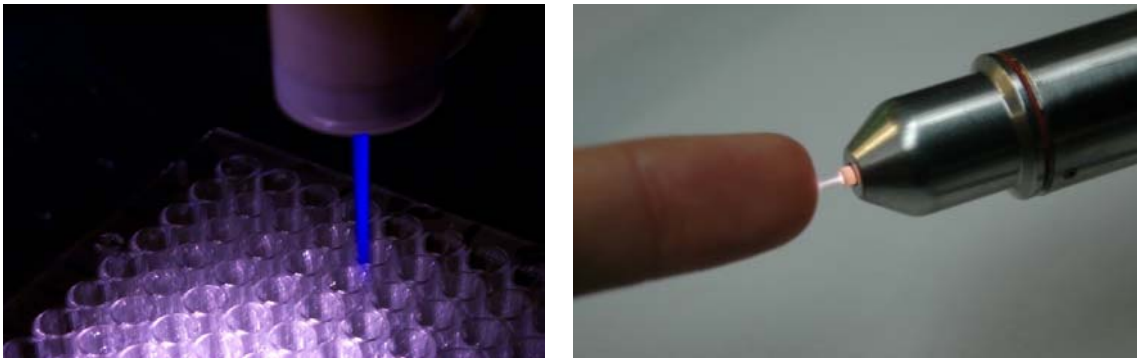
The main LTP devices used in plasma medicine research are the dielectric barrier discharge (DBD) and nonequilibrium atmospheric pressure plasma jets (N-APPJ). In fact, the DBD was the device used in the first experiments on the inactivation of bacteria [1]. The DBD uses plate electrodes covered by a dielectric (such as glass). The plasma is generated in the gap separating the electrodes by the application of high sinusoidal voltages in the kHz frequency range. Gases such helium with admixtures of oxygen or air are usually used. For more information on the working of the DBD see references [61,78,79]. Figure 2 shows a schematic of the DBD and a photograph of a diffuse plasma at atmospheric pressure generated by a DBD.



(**a**) (**b**)

Figure 2. Schematic (**a**) and a photograph (**b**) of an atmospheric pressure diffuse plasma generated by a dielectric barrier discharge (DBD). The discharge in the photo on the right is driven by kHz sinusoidal high voltage and the gas is helium with a small admixture of air. Photo taken at the author's laboratory.

Nonequilibrium atmospheric pressure plasma jets (N-APPJs) produce plasma plumes that propagate away from the confinement of electrodes and into the ambient air. The reactive species generated by the plasma can therefore safely and conveniently be transported to a target at a remote location and away from the main plasma generation area. This characteristic made N-APPJs very attractive tools for applications in biology and medicine [60,80–82]. Various power driving methods that include pulsed DC, RF, and microwave power have been used. In addition, various electrode configurations ranging from single electrode, to two-ring electrodes wrapped around the outside wall of a cylindrical dielectric body, to two-ring electrodes attached to centrally perforated dielectric disks have been used. Figure 3 shows photographs of two N-APPJs, the plasma pencil and the kINPen, which have been used extensively in plasma medicine research.



(**a**) (**b**)

Figure 3. Photographs of two plasma jets that have been used in various biomedical applications. (**a**) is the plasma pencil (ODU, Norfolk, VA, USA), and (**b**) is the kINPen (INP, Greifswald, Germany).

The plasma plumes emitted by N-APPJs turned out to be made of small plasma packets traveling at very high velocities (tens of km/s). These plasma packets came to be known as "plasma bullets" and they were independently first reported in the mid-2000s by Teschke et al and by Lu and Laroussi [83,84]. Lu and Laroussi used nanosecond-pulsed DC power while Teschke et al used RF power. The plasma bullets were subsequently researched extensively, both experimentally and by modeling, by various investigators [85–91]. Today there is agreement that the plasma bullets are guided ionization waves. To learn about these guided ionization waves in greater detail, the reader is referred to [92].