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REVIEW ARTICLE

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New discoveries and directions for medical, dental and dental hygiene research: low temperature atmospheric pressure plasma

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McCombs GB, Darby ML. New discoveries and directions for medical, dental and dental hygiene research: low temperature atmospheric plasma.

Abstract: The study of plasma integrates physics, chemistry, biology, and engineering, and has recently engaged medicine and dental hygiene in research efforts. The study of plasma holds promise for a myriad of applications ranging from lasers and electronics, hazardous waste management, decontamination, sterilization and disinfection of foods, soil, water, instruments, to medical uses in wound healing and treating certain types of tumours and cancers. Plasma represents a new state-of-the-art sterilization and disinfection treatment for certain oral and enviornmental pathogens, heat-sensitive materials, contaminated medical waste, hard and soft surfaces, and ventilation systems may assist health care facilities in the management of various health concerns. The role that Low Temperature Atmospheric Pressure Plasma (LTAPP) could play in the inactivation of pathogenic microorganisms might prove to be a new, faster, noncorrosive, more economical alternative, as well as support green healthcare.

Key words: cold plasma; disinfection; low temperature atmospheric pressure plasma

What is atmospheric plasma?

The universe contains four forms of naturally occurring matter: solids, liquids, gases and plasmas (see Box 1). Hearing the word plasma most people think of plasma televisions, neon and fluorescent lights bulbs, computer screens or the clear component of blood that remains after centrifuging off blood cells and platelets.

Box 1. Terms used to describe the four states of matter

- Solid – Objects that resist deformation and changes of volume. A solid has atoms or molecules that are 'fixed' or packed closely together. If sufficient force is applied, properties can be disrupted, causing permanent deformation.
- Liquid – Substance that consists of loose particles that can freely form a distinct surface at the boundaries of its bulk material and a free surface where the liquid is not constrained by a container.
- Gas – A collection of particles (molecules, atoms, ions, electrons, etc.) without definite shape or volume and which is in more or less random motion.
- Plasma – A collection of freely moving charged ions and electrons. In the sciences, plasma is considered an ionized gas. Plasma is categorized into two types, hot and cold.

Known as the fourth state of matter, plasma is the least recognized, yet most prevalent form, making up over 99% of the universe. Plasmas represent a 'cosmic soup' of fixed and freely moving molecules, atoms, electrons and ions in various densities and temperatures that range from cool to very hot (Fig. 1). Plasmas are conductive and respond to electric and magnetic fields and therefore can be an efficient source of 'energy'.

Most forms of plasma are not easy to test because they are extremely hot and difficult to manipulate, but currently, researchers are developing techniques to produce low heat plasmas that can be manipulated at room temperature and atmospheric pressure. Dense, very high temperature 'superheated' plasmas are associated with lightning, stars and the sun. In contrast, low temperature, less dense forms of plasma are interrelated with solar and stellar winds and auroras. Likewise, the glow of plasma cells is responsible for the coloured light phenomena in the sky referred to as *aurora borealis* in the northern hemisphere and *aurora australis* in the southern hemisphere.

One of the distinct features that make plasma technology so appealing to scientists is that energy is used to accomplish a goal instead of liquid chemicals or heat. Plasma chemistry is driven by electrons; consequently, the utilization of plasma has the potential to transform present day products, devices and services, as well as support a more 'green' environment.

Historical perspective

The English physicist Sir William Crookes identified plasmas in 1879, although it was an American physicist, Dr Irving Langmuir, who first applied the word 'plasma' to ionized gas in 1929. In the late 1850s, the Siemens' company used plasma discharge to generate ozone, which acted as an agent to remove contaminants and toxins from water (1). Nevertheless, for the next 100 years, little research was conducted exploring the relationship between plasma and biological cells (1). From the 1960s to 1980s, plasmas were mainly utilized as a secondary agent to indicate biological sterilization, yet diminutive cause and effect knowledge was advanced (1). It was not until the mid-1990s that scientists made considerable progress in cold plasma technology (1, 2). As the news of plasma science spread, visionary researchers took notice and began to explore various ways to utilize plasma's unique properties.

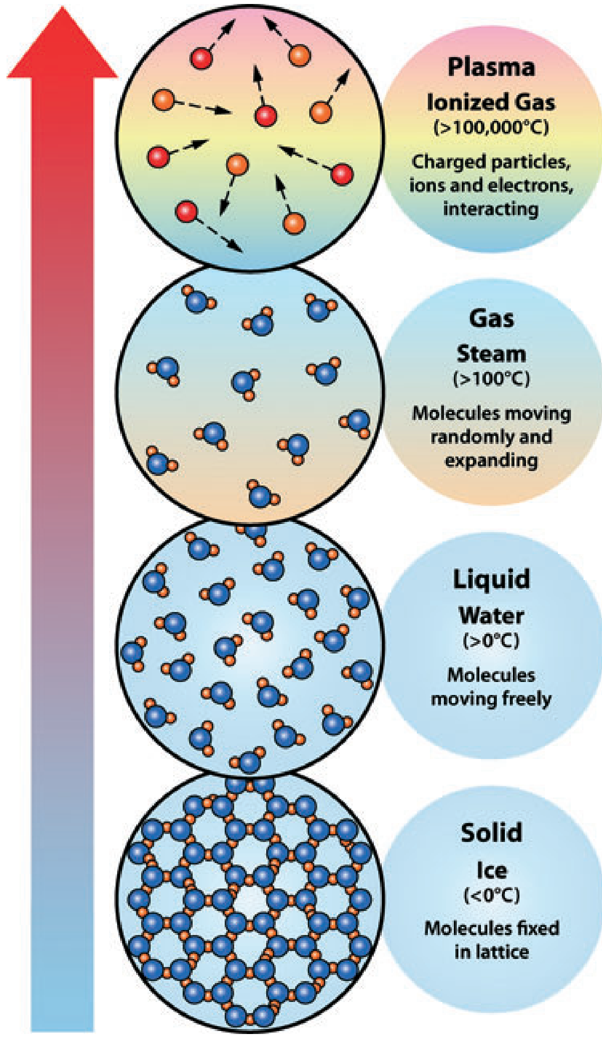

Fig. 1. Four states of matter (diagram courtesy of G. McCombs and D. Emminger, Old Dominion University).

Plasma science was in its infancy in the 1990s, but by 1997, multidisciplinary teams set out to understand the effects that plasmas had on pathogenic and non-pathogenic microorganisms, as well as develop proof of concept studies to demonstrate that plasma could be used as a decontaminant or sterilizing agent (1). Since the late 1990s, plasma research has evolved at a rapid pace as technology expanded into areas such as biomedical, environmental, aerospace, agriculture and the military.

Emerging research

The newfield of ''plasma medicine'' has been created which integrates physics, chemistry, biology and engineering and has recently engaged medicine and dentistry in research efforts. What has emerged from this scientific collaboration is plasma technology that holds promise for a myriad of parallel applications including lasers, electronics, hazardous waste management, decontamination, sterilization and disinfection, as well as wound healing and treating certain types of tumours and cancers.

The majority of the latest plasma research has focused on one form, referred to as low temperature atmospheric pressure plasma (LTAPP), also known as 'cold' or 'non-thermal' plasma. Because of plasma's unique ability to destroy harmful microorganisms by disrupting the cell wall using highly reactive free radicals, LTAPP has the potential to kill harmful bacteria that are in air, liquids or on a variety of hard and soft surfaces, without the use of heat, chemicals or pressure (2, 3).

Currently, there are two basic ways to expose a substance to plasma:

- *Direct exposure* occurs when samples are placed within centimetres/inches contact of the plasma plume discharge (see Figs 2 and 3).
- *Indirect or remote exposure* occurs when materials are placed in an adjacent chamber and plasma enters and exits through a tubing system (see Fig. 4).

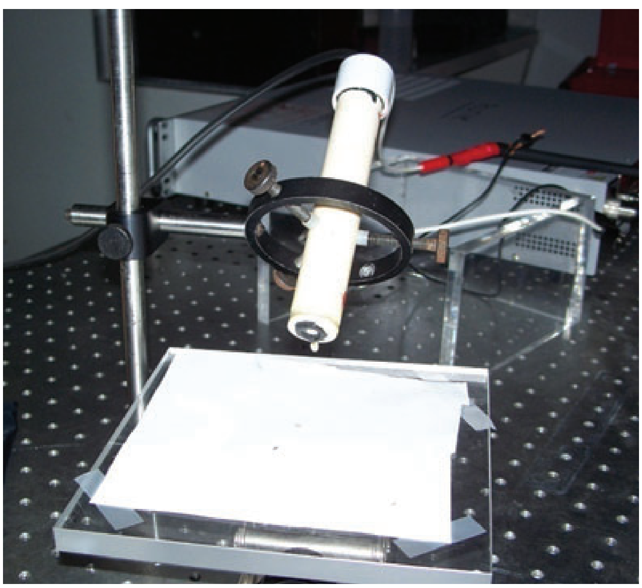

Fig. 2. Plasma pencil.

Biological decontamination

Cold plasma has the potential to ensure that food products are free of dangerous bacteria such as *Escherichia coli*, *Bacillus subilis* and *Salmonella typhimurium*. Plasma scientists are investigating ways to utilize plasma's germicidal effect on a multiplicity of hard and soft substances in addition to creating food packaging that resists microbial growth. For instance, researchers at the Food Research Institute, University of Wisconsin, Madison, are exploring ways to make foods safer by creating anti-fouling, stainless steel and rubber surfaces that, when exposed to low temperature plasma, can inhibit bacterial attachment and biofilm formation (http://www.wisc.edu/fri/newsltr/fri172nwsltr.pdf). Investigators have determined that when certain food-borne pathogens are exposed to plasmas' glow, they

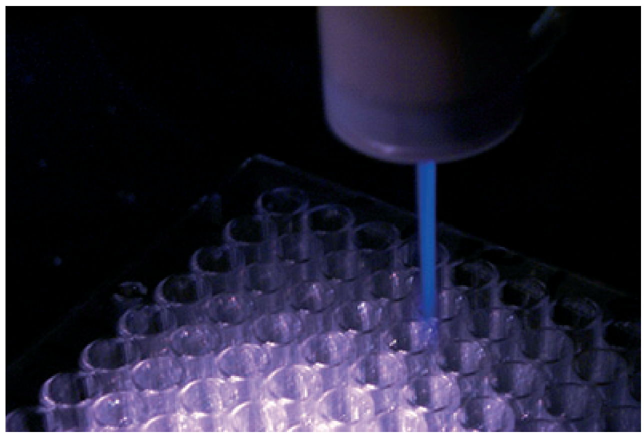

Fig. 3. Plasma pencil plume (photo courtesy M. Laroussi, Old Dominion University).

Fig. 4. Plasma-based chamber prototype.

were reduced significantly. One apparatus developed at the university is a white ceramic block, which resembles a shower head and features a grid of over 200 circles. Each circle houses an electrode that, when activated, produces a controlled uniform flow of plasma. This device can be mounted so that plasma is emitted downward towards any surface, object or substance which needs decontamination, even on a moving conveyer belt (http://www.foodproductiondaily.com/Quality-Safety/Cold-plasma-devices-developed-to-kill-food-pathogens). This type of technology has tremendous potential for agricultural and meat processing plants as they seek to control and prevent food-borne diseases that account for millions of illnesses and hospitalizations annually.

Experiments are ongoing to evaluate plasma's effects on biological contamination in fresh and salt water. Although potable water decontamination is a priority, researchers are looking at ways to reduce or eliminate contaminates in water that can negatively affect the food products grown, washed and packaged. In particular, drinking and irrigation water may serve as a medium to spread potentially harmful microorganisms to animal, plants and eventually to humans. For example, problems associated with contaminated spinach grown in large agricultural areas have left many people gravely ill or dead. For these reasons, researchers have initiated studies to determine if plasma could be used to decontaminate a variety of water, soil and food-borne microorganisms, which in turn would help protect the health of the public (4–6).

Worldwide intermittent outbreaks of an airborne illness called Legionnaires' disease are not uncommon. Legionnaires' disease is a life threatening respiratory infection caused by the bacterium *Legionella pneumophila,* which flourishes in heating and air conditioning systems in places like hospitals, nursing homes, prisons, hotels and cruise ships. People usually acquire Legionnaires' disease by breathing contaminated water 'mist' flowing from heating/air conditioning sources. As LTAPP operates at or near room temperature, researchers are looking to design an apparatus that could generate a uniform plasma glow discharge for destroying lethal airborne pathogens. If investigators are successful at utilizing LTAPP to eradicate airborne vectors, this technology may prove beneficial as a room sterilization technique to help prevent the spread of disease through ventilation systems.

Removing biological contaminants from people represents an even greater unresolved issue in light of biological and chemical warfare. During the Persian Gulf War, officials feared that biological weapons would be used against US soldiers and civilians. Although that threat never materialized, the military is re-evaluating their traditional scheme of decontamination, with an emulsion of water and bleach, and testing non-thermal plasma as a method to ensure that equipment, uniforms and personnel are safe. Furthermore, the aerospace industry is considering ways to utilize plasma to decontaminate astronauts and equipment used in space exploration. Not only does the aerospace industry worry about taking contaminants into space, but is also concerned about preventing alien species from hitchhiking back to earth.

Biomedical research

Scientists are looking for ways to integrate plasma technology into numerous areas of medicine. Plasma has the potential to kill pathogenic microorganisms by breaking open or disrupting the cell wall without causing damage to surrounding tissues through non-thermal means (4–6). As plasma is activated by electrons, the plume emitted generates no heat and can be safely handled. Researchers at Drexel University's Plasma Institute have demonstrated the non-toxic direct sterilization capabilities of low temperature plasma on pathogenic bacteria in mice and on cadaver skin (http://plasma.mem.drexel.edu/mri/). Scientists continue to investigate plasma's therapeutic effects to treat certain types of cancers and tumours, burns, ulcers and other dermatological disorders, as well as to induce blood coagulation during surgery. Plasma medicine has a significant potential for diabetic patients, specifically in the management of life threatening diabetic ulcers, which are the most common cause of lower extremity amputation.

In healthcare, plasma technology represents a novel and less toxic method to treat infectious waste, sterilize equipment and decontaminate surfaces. Currently, researchers are investigating ways to utilize LTAPP as an alternative to conventional sterilization methods such as high pressure steam, ethylene oxide gas or dry heat. High pressure steam, although widely used, is not applicable for certain heat-sensitive materials such as powders, plastics and polymers. Moreover, ethylene oxide is a toxic gas that, if not managed correctly presents health risks to healthcare workers. Cold plasma, on the other hand, possesses none of these drawbacks and may provide a practical environmentally safe alternative. Sterilization achieved through LTAPP may perhaps prove to be a safe, effective method for destroying harmful microorganisms without damaging the medium itself.

Dental and dental hygiene research

At Old Dominion University, Laroussi and colleagues at the Laser and Plasma Engineering Institute, in collaboration with the Dental Hygiene Research Center, are undertaking experiments to explore LTAPP sterilization and decontamination techniques. Foundational research conducted in a plasma-based chamber observed morphological changes in bacterial cells from plasma exposures (Fig. 4). Research conducted by Morris *et al.* (7) revealed that LTAPP has the ability to destroy heat-resistant bacteria *Geobacillus sterothermophilus* and *B. cereus*. *G. sterothermophilus* was specifically selected for testing because it is highly resistant to moist-heat sterilization methods and is present on biological indicator test strips used to verify sterility; *Bacillus cereus* was chosen because it is often associated with food poisoning (7). Data analysis suggests that *G. sterothermophilus* vegetative cells and *B. cereus* vegetative cells and spores exposed to LTAPP were significantly destroyed, in a matter of seconds, yet *G. sterothermophilus* spores were not significantly inactivated. The fact that spores develop an encapsulated protective coating and vegetative cells are actively dividing and growing may help explain this finding (7, 8). Nevertheless, results from this research laid the ground work for expanding plasma technology into various applications for decontamination and sterilization.

Advances in plasma technology and the need for a more focused plasma exposure led to the development of the ''plasma pencil'' (Fig. 2; 5, 9, 10). In 2005, Laroussi developed a way to project LTAPP's germ-killing capabilities into small, hard-to-reach areas. The plasma pencil, which looks like a handheld miniature light, is about the size of a power toothbrush and was specifically designed to provide a focused thin plume of pulsating plasma energy to a small target area (Fig. 3). The germicidal capacity of the plasma pencil can be directed into small cavities or narrow crevices, which holds great promise for destroying pathogenic oral microorganisms associated with dental caries and periodontal disease.

Plasma scientists are evaluating the effects LTAPP has on *Streptococcus mutans*, the leading cause of tooth decay. Sladek *et al.* (11) conducted research to assess the antimicrobial activity of non-thermal atmospheric plasma on *S. mutans* compared to chlorhexidine digluconate mouthrinse (0.2%), with promising results. *In vitro* studies suggest that the antimicrobial effects of non-thermal plasma have the potential to destroy *S. mutans* and prevent regrowth (11–13).

Researchers at Old Dominion University have stepped up scientific efforts to include testing the plasma pencil on *S. mutans*, *P. gingivalis*, *Treponema denticola*, *Tannerella forsthensis* and other oral pathogens. Initial *in vitro* testing on *S. mutans* revealed that the plasma pencil was capable of inactivating this microorganism in a matter of seconds (13). This work, although preliminary, is promising not only for the management of dental caries, but also for parallel applications i.e., oral biofilm control, mouth and periodontal pocket disinfection and oral malodor. Researchers continue to investigate ways to use plasma's energy to penetrate oral biofilms and keep them from attaching, proliferating and recolonizing, as they seek to find non-chemical ways to destroy oral and environmental pathogens.

Future of cold plasma technology

Multidisciplinary research teams have just begun to understand the complexities of plasma as they delve into new therapeutic and commercial applications. Translational research is needed to determine the effect that LTAPP has on prokaryotic and eukaryotic cells. Although plasma technology has the potential to destroy pathogens without adversely effecting healthy surrounding tissues, the plasma-cellular relationship is not well understood (4–6, 8, 14). Researchers postulate that after short plasma exposure, the outer bacterial cell membrane ruptures, which causes leakage of the cytoplasm, where as longer plasma exposure triggers cell fragmentation, both resulting in cell death (5, 6). To date, no clear conclusions are advanced and the effects LTAPP has on human and non-human cells require further exploration. Yet, the overall possibilities for plasma applications bode well for both industry and biomedical fields.

In dentistry, plasma researchers envision a small table-top focused delivery system that can be utilized in the treatment and prevention of dental caries and periodontal disease. If a thin plume of LTAPP can be effectively projected into a carious lesion or an infected periodontal pocket, it is possible to kill pathogenic oral microorganisms that are often difficult to reach through traditional mechanical and chemotherapeutic methods.

Perhaps in the future, healthcare professionals will routinely utilize LTAPP to reduce or eliminate environmental and surface pathogens. LTAPP may represent a new state-of-the-art sterilization and disinfection treatment for certain heat-sensitive materials, contaminated medical waste, hard surfaces, and ventilation systems which may assist healthcare facilities in the management of various contaminants. The role LTAPP could play in the inactivation of pathogenic microorganisms might prove to be a faster, non-corrosive, more economical alternative, as well as support green healthcare (10, 14).

Although it would be difficult to include all aspects of plasma science in this paper, it was the authors' intent to create an awareness of plasma technology and the emerging field of plasma medicine. Plasma technology is well positioned for improving the way dental professionals prevent, treat and manage oral health and other health-related concerns. Plasma science encompasses immense diversity with potential applications in medicine, dentistry, dental hygiene, agriculture, military, environmental and aerospace fields, just to name a few. The significance of developing safe, efficient and environmentally friendly technology that can destroy pathogenic microorganisms cannot be understated and may well propel this once futuristic science into an everyday reality.

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