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REVIEW ARTICLE

COLD PLASMA: A NEW REALM IN ENDODONTIC DISINFECTION

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ABSTRACT

Cold plasma is an emerging field with vast scope and incorporates broad interdisciplinary approach to the reader ranging from physics, chemistry, biology, and engineering. It has recently engaged medicine and dental hygiene in research. Apart from the basic plasma processes and the restrictions and requirements set by international health standards, the review focuses on plasma interaction with prokaryotic cells (bacteria), eukaryotic cells (mammalian cells), cell membranes, DNA etc. 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. The PubMed database search revealed that the reference list for cold Plasma featured 1,17,000 articles in dentistry. A forward search was undertaken for the selected articles, author names and contemporary endodontic texts. A review is presented on the key developments in the arena of plasma medicine and dentistry so as to familiarize the modern dentists with the new advances in endodontic disinfection.

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INTRODUCTION

The plasma is known as the fourth state of matter, solid, liquid or gaseous. Plasma is a (partially) ionised gas, making it electroconductive with variety of interesting attributes. In the visible universe 99% of all matter is in plasma state (McCombs *et al*., 2010). Low heat plasma that can be manipulated at the room temperature i.e low temperature atmospheric pressure plasma (LTAPP), also known as cold or nonthermal plasma has been recently developed. This kind of plasma is being widely used in plasma medicine. Plasma medicines can further be subdivided into three main fields: Plasma assisted modification of biorelevant surfaces, plasma based biodecontamination/ sterilization and direct therapeutic plasma application. Conventional sterilization techniques, e.g autoclaves, ovens and chemicals like ethylene oxide, rely on irreversible metabolic inactivation or breakdown of vital structural components of the microorganisms. On the contrary plasma sterilization operates differently because of its specific active agents, which are ultra violet photon (UV photons) and radicals (atoms or assembly of atoms with unpaired electrons, therefore chemically reactive e.g O and OH, respectively) (Michel Moison *et al*., 2002).

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An understanding of the interplay between medicine, biology and physics is needed to be able to fully realise the potential of plasma in medical and dental fields (www.campus-plasmamed.de). As plasma dentistry is a very exciting and promising field, therefore the purpose of this review is to make the dental fraternity aware of the various uses of plasma in dentistry and to provide an in-depth discussion of its properties and mechanism of action; especially in the field of Endodontics.

Historical Background

1850s : Siemens' company used plasma discharge to generate ozone, which acted as an agent to remove contaminants and toxins from water (Laroussi *et al*., 2008).
1879: English physicist sir William Crookes identified plasmas.
1929: Dr. Irving Langmuir, American physcisist was the first to apply the word 'plasma' to ionized gas.
1960s-1980s: Plasma utilized as a secondary agent to indicate biological sterilization (McCombs *et al*., 2010).
1970s-1980s: Plasma assisted sterilization system (Laroussi *et al*., 2005).
Late 1990s: Plasma research evolved at rapid pace as technology expanded in areas such as biomedical, environmental, aerospace, agriculture and the military (McCombs *et al*., 2010).

2004: Bol'shakov *et al*. published a detailed study of the effects of radio frequency (RF) oxygen plasma at reduced pressure on bacteria (Bol'shakov *et al*., 2014). The inductive mode was found to offer a better efficiency in destroying the biological matter. 2009: A Pulsed plasma dental probe was developed to disinfect root canal system during endodontic treatment (Jiang *et al*., 2009).

Mechanism of action

Basic mechanism of plasma sterilization can be studied by three following processes (Moison *et al*., 2001).

- Microorganisms genetic material destruction by UV irradiation.
- Intrinsic photodesorption causes erosion of the microorganisms atom by atom (Pelletier, 1993).
- Atomic erosion of the microorganisms, through etching. Etching stems from the adsorption of reactive species from the plasma i.e O, O_3 and $^1O_2$ and may be enhanced by *UV* photons (Michel Moison *et al*., 2002 and Pelletier, 1993).

There are two basic ways of exposing a substance to plasma:

a. Direct exposure: this occurs when samples are placed within centimetres/inches contact of the plasma plume discharge
b. Indirect or remote exposure: this occurs when materials are placed in an adjacent chamber and plasma enters and exits through a tubing system.

Sometimes spore lethality cannot be induced in a reasonable time due to insufficient penetration of UV photons in matter. This limitation depends on the number, thickness and chemical composition of the spore-protecting layers and on the location of its DNA material with respect to the surface exposed to UV radiation. Under electron microscope spores seem to be stacked, clumped or wrapped within the organic materials. Erosion of the spore surface makes it easier for the photons to reach the DNA material (Moreau *et al*., 2008).

Methods for plasma generation

Non-equilibrium atmospheric pressure plasmas can be generated using electrode materials, geometries as well as various means of excitation i.e microwave, radiofrequency and alternating current. The resistive barrier discharge (RBD) is a well known method for generation of plasma (Laroussi *et al*., 2002). The RBD works on the principles of dielectric barrier discharge (DBD) (Kanazawa *et al*., 1998). The RBD is consists of two planar electrodes in which one is covered by a layer of high resistivity material. The two electrodes are separated by a variable gap (i.e maximum 5cm) where a gas mixture is to be injected (Kanazawa *et al*., 1998). The electrodes are driven by an ac voltage of few kilovolts at the line frequency of 60 Hz. The power necessary to generate and sustain a stable discharge is in the range of 50-300 W.

Active species of electrical discharges

The role of *UV*

The high energy *UV* photons produces stronger cidal effects due to its absorption efficiency which allows them to get absorbed by DNA and other nucleic acids. In case of spores the penetration depth of *UV* photon is limited i.e one micrometer layer. In non-oxygen plasma the maximum observed depth for photochemical action was 10 micrometer in the case of polyethylene gelation or ablation. Therefore, *UV* high energy photons are more effective when dealing with small non-sporulated bacteria.

Advantages

- Cold plasma is particularly useful in hospital hygiene, in the treatment of diverse skin and infectious disease. It is contact-free, pain-less, self sterilizing and non invasive. Its application allows for the treatment of heat sensitive , non-homogenous surfaces and living tissues (Kyenam *et al*., 2006).
- It prevents the wear of instruments especially in case of implants made from thermolabile polymers which lose their mechanical integrity when other sterilization techniques are used (Lendlein and Langner, 2002).
- Cold plasma has the potential to inactivate microorganisms associated with oral diseases and wound infections (Angela *et al*., 2009).
- This technique aids the dentist to perform certain procedures without pain and shots (Smitha *et al*., 2010).

Disadvantages

- This technique is highly sensitive (Somya Govil *et al*., 2012).
- It does not work well in cases where amalgam restoration is present in the oral cavity (Somya Govil *et al*., 2012).
- Spores are difficult to inactivate due to the limited penetration depth of cold plasma (Angela *et al*., 2009).
- Equipment is expensive
- Maintenance and availability are also some issues while opting for cold plasma (Martin *et al*., 2009).

Uses

Plasma in wound sterilization

Scanning electron microscopy (SEM) have been used to visually inspect the impact that plasma exposure have on the cell morphology. Laroussi *et al*. (2009). using SEM images showed that after exposure to plasma , *E.coli* cells undergo severe morphological changes, such as lysis. He attributed such damage to one of two processes-

1) Membrane damage due to charge build up on the cells
2) Chemical attack by the radicals such as O and/or OH.

Plasma in blood coagulation

Recently it has been shown that low temperature plasma DBD treatment significantly accelerates blood coagulation without interfering with the adjacent tissue surface (Vasilets *et al*., 2008).

Plasma on cancer cells

A low power DBD does not causes direct cell destruction but can initiate apoptosis while treating melanoma cancer cells (Vasilets *et al*., 2008).

Laser induced plasma

Lasers are being widely used for cutting of tissues, blood coagulation, photodynamic cancer therapy, arterial plaque removal, dental drilling, etc (Gitomer Steven *et al*., 1991).

Plasma in wound healing

Plasma leads to generation of significant concentration of NO in an atmospheric airflow. A device named Plazon was designed by Shekhter *et al* for this purpose. It was tested in animals and also passed clinical tests in different areas of medicine. The clinical test was carried out in patients with trophic ulcers of vascular etiology and complete healing duration was reduced by 2.5 to 4 times depending on the sizes of ulcers on using plasma (Vasilets *et al*., 2008).

Plasma in food processing

The most noticeable application is the disinfection of surfaces in particular equipment, packaging, food contact surfaces or even food itself. LTAPP has the ability to destroy heat-resistant bacteria *Geobacillus sterothermophilus* and *B. cereus (Bacillus cereus* is often associated with food poisoning) (Angela *et al*., 2009).

Other uses of Plasma

- These ambient-air-temperature ionized gases could also be used as a Star Trek like protective shield around sensitive electronics-bearing devices, such as satellites; as cloaking technology for military aircraft, as a means of absorbing radar waves in order to remain hidden on enemy screens; and as components of a new generation of miniature lasers and in advanced, low-energy-consumption flourescent light tubes.

Application of cold plasma in dentistry

Cold plasma in microbial eradication

The mechanism of action of plasma sterilization is influenced by the plasma components like reactive oxygen species, ions, electrons, UV and electromagnetic fields (Mccullagh *et al*., 2007). Beside the contact point, plasma also affects the area around it (Fridman *et al*., 2008). Whittaker *et al*. indicated that using gas plasma in cleaning the root canals may be extremely beneficial in reducing the absolute amount of proteinaceous materials that may be transferred between patients on rinsing the endodontic files (Whittaker *et al*., 2004). Yang Hong Li *et al*, concluded that plasma sterilization, with its various advantage like temperature, fastness, roughness and safety, overcomes the deficiency of traditional sterilization technology and may become a novel method for killing microbes (Whittaker *et al*., 2013). Su-Jin Sung *et al*. evaluated the sterilization effect of an atmospheric pressure non thermal air plasma device and was proved that it was effective in killing both *Escherichia coli* and *Bacillus subtilis*. It was more effective in killing *Escherichia coli* than the UV sterilizer (Sung *et al*., 2013). Increased levels of resistance to a wide spectrum of conventional antifungal drugs is exhibited by *Candida* growing in biofilms (Uppuluri *et al*., 2009). Surface microdischarge (SMD) plasma technology was developed to generate plasma in ambient air (Morfill *et al*., 2009) to inactivate *C. albicans* biofilm via a contact-free disinfection procedure. The plasma is produced indirectly and is transported to the biofilm through diffusion. SMD plasma device.

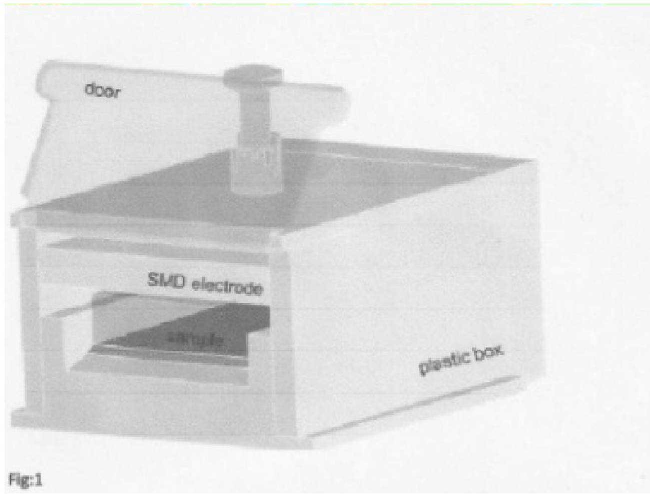

Fig. 1. Sketch of the plasma device. The device contains one SMD electrode, and the sample to be treated is placed below the electrode. In this study, the distance between the electrode and the sample was fixed at 6mm

The SMD plasma device is incorporated into a box made of plastic (Teflon and polyoxymethylene). The electrode for producing the plasma is located inside the box, as shown in Fig. A door is installed on the side of the box so that the plasma gas produced is restricted inside. The plasma device is so designed so as to 96-well plate; hence, the area that can be treated is large (9 by 13 cm). The electrode, which produces the plasma, is located above the respective samples to be treated, and the distance between the electrode and the sample is adjustable. Plasma is produced with a 0.5-mm thick Teflon plate sandwiched between a brass planar plate and a stainless steel mesh grid (line width, 2 mm; opening, 10 mm; height, 1.5 mm) which serves as the electrode. A high sinusoidal voltage of 9 kV (i.e., voltage from peak to peak) with a frequency of 1 kHz is applied between the brass plate and the mesh grid, thus producing a homogenous plasma by many microdischarges (Morfill *et al*., 2009). Electron/ion pairs and chemically reactive species (O_3, O, NO, etc.) are produced by approximately 600 chemical reactions driven by dissociation of molecules and recombination.

Cold plasma in dental caries and intra oral diseases

Plasma can decontaminate dental cavities without drilling. Eva Stoffels, suggested using plasma needles in the cavity to kill *Escherichia coli*. The active species produced by the plasma can easily reach inside the cavity even when plasma itself is superficial (Sladek *et al*., 2004). Goree *et al*., conducted a study to show that non thermal atmospheric plasmas killed

Streptococcus mutans, a gram positive cariogenic bacterium (Goree *et al*., 2006). Sladek *et al*., studied the interactions of the plasma with dental tissue using a plasma needle (Sladek *et al*., 2004). Mechanical and laser techniques can be used for cleaning and sterilization of the infected tissue in a dental cavity or in a root canal (Sladek *et al*., 2010). Koban *et al*. reported the high efficiency of *Candida albicans* sterilization using various plasmas. This resulted in the possibility of treating stomatitis caused by *Candida albicans* can be cured by plasma jets (Koban *et al*., 2010).

Plasma on Biofims

Biofilms developing on tooth surfaces and oral mucosa causes caries, periodontal diseases and oral mucositis, which may also lead to inflammation around the dental implants. Rupf *et al*. demonstrated that plasma and a non-abrasive air/water spray combination treatment is suitable for the elimination of oral biofilms from microstuctured titanium used dental implants (Koban *et al*., 2011). Koben *et al*. conducted an in vitro study to show that the treatment of dental biofilms composed of *Streptococcus mutans* with non thermal plasma was more efficient than with chlorhexidine (Koban *et al*., 2011). Jiang *et al*. developed a plasma plume at room temperature and used it to disinfect the root canals from extracted human teeth (Jiang *et al*., 2009). Two teeth were placed at a distance of 5mm from the plasma nozzle. One of them was exposed to the helium/oxygen plasma for 5 minutes, whereas the other one was exposed to the same helium/oxygen flow for five minutes, but without plasma. They observed better results in the reduction of the biofilms in the tooth treated with plasma compared with control. Schaudinn *et al*. used a plasma needle to eliminate ex vivo biofilms on root canals of extracted teeth (Schaudinn *et al*., 2013). Teeth were divided into three groups: plasma needle treated, 6% sodium hypochlorite treated and control. The authors concluded that the plasma needle was effective at killing biofilms in extracted teeth. But, using 6% sodium hypochlorite was more efficient.

Plasma in Sterilization of root canal

The compelling advantage of cold plasma is their ability to eliminate diverse microorganisms including bacteria, fungi, and viruses. Cold plasma kills microorganisms and deactivate viruses the constant bombardment of short-living reactive species and charged particles (electrons and ions), especially free radicals (Sladek *et al*., 2007). A Model RC-1, a plasma biomedical application which is especially designed and installed for root canal disinfection, has been developed and it certified safe for use (Du *et al*., 2012). The main body of the Model RC-1 is composed of a medical syringe and a needle (Fig.1A). The needle also acts as the electrode, and is connected to a high-voltage sub microsecond pulsed direct current power supply. The amplitude is set to 8 kV, the repetition rate is 8 kHz, and the pulse width was 1,600 ns. The APNPs generated by the Model RC-1 are ejected from the needle of the syringe for root canal disinfection. The APNP's working gas is usually He/O2, the flow rate of which was controlled by a mass-flow controller set at 1:0.01 L/min (Du, 2012). Lu *et al*., used a reliable and user-friendly plasma-jet device, which could generate plasma inside the root canal (Whittaker *et al*., 2004). Results have shown that it can efficiently kill *Enterococcus faecalis* (Rupf *et al*., 2010).

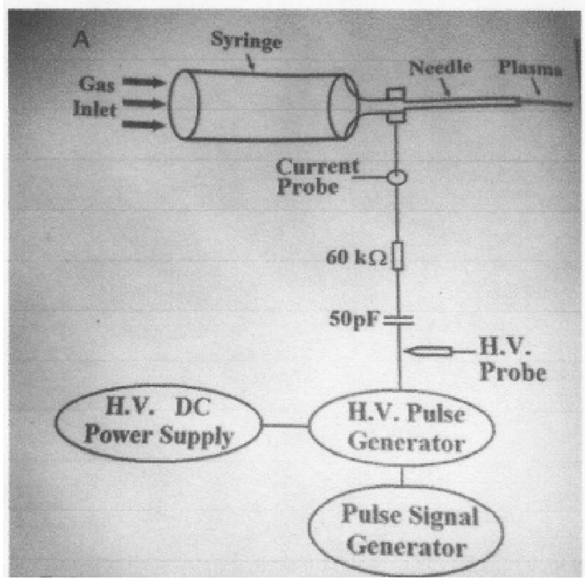

Fig. 2. (A) schematic of the Model RC-1 experiment setup

Plasma in Resin Composite restoration

Preliminary data has revealed that plasma treatment increases bonding strength at the dentin/ composite interface by roughly 60% and thereby improving composite performance, durability and longevity (Kong *et al*., 2011). Ritts *et al*. investigated a non-thermal atmospheric plasma brush on dental composite restoration (Ritts *et al*., 2011). It was observed that atmospheric cold plasma brush (ACPB) treatment could modify the dentin surface and increase dentin/adhesive interfacial bonding.

Plasma in post and core

Yavrich *et al*., conducted a study to evaluate the effects of plasma treatment on the shear bond strength between fiber reinforced composite posts and resin composite for core build-up and concluded that plasma treatment appeared to increase the tensile-shear bond strength between post and composite (Yavirach *et al*., 2009).

Plasma in tooth whitening

Lee *et al*. showed that atmospheric pressure plasma in contrast to light sources bleached teeth by increasing the production of OH radicals and by removing surface proteins (Lee *et al*., 2009). Tooth whitening can also be achieved using a DC plasma jet and hydrogen peroxide (Sun *et al*., 2010). Park *et al*. suggested intrinsic whitening using a low-frequency plasma source and hydrogen peroxide (Park *et al*., 2011). Nam *et al*. used a Plasma jet on forty extracted human molar teeth with intact crowns. They observed Cold atmospheric plasma (CAP) was the most effective for bleaching teeth and also observed that CAP does not damage the tooth due to its low temperature (Nam *et al*., 2013). Claiborne D *et al*. used a plasma plume on extracted human teeth. They observed a statistically significant increase in the whitening of the teeth after exposure to CAP + 36% hydrogen peroxide gel, compared with 36% hydrogen peroxide only, in the 10 and 20 min groups. The temperature in both treatment groups remained under 80°F throughout the study, which is below the thermal threat for vital tooth bleaching (Claiborne *et al*., 2013). In a study by Jamali *et al*. conducted a study and the results revealed that prolonged plasma treatment (without bleaching) removed some blue-stain, but the effect was small (Jamali and Evans, 2013). On the contrary, the combination of plasma treatment and bleaching removed most of the blue-stain. It was concluded that vacuum plasma pre-treatment and bleaching showed promise as a way of removing blue-stain.

Future perspective

Multidisciplinary research teams have just begun to understand the complexities of plasma. 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 affecting healthy surrounding tissues, the plasma-cellular relationship is not well understood. 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.

Conclusion

Plasma dentistry is a very promising field with numerous applications. From the above discussion an understanding is provided to fully realise the potential of plasma in medical and dental fields so as to develop this field into an exciting and important multidisciplinary research field with a huge application potential for the benefit of mankind.

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