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Plasma in Dentistry: Novel Technology beyond Lasers

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ABSTRACT

Mechanical and chemical methods have been used to treat oral conditions since ages. Recently laser technology has been added to the existing armamentarium. But all these aforementioned techniques have a common disadvantage of unwanted and uncontrolled damage to the adjacent healthy -hard and soft tissues. The emergence of 'Plasma Technology' has proved to be a ground breaking treatment modality for a myriad of dental conditions overcoming the disadvantages of the currently used modalities. This fourth state of matter contains free moving charge carriers and has various medical and dental applications. This novel tissue-saving technique is painless and is effective in both wet and dry environment. In dentistry, it can be used to prepare and disinfect dental cavities and root canals, for disinfection and sterilisation, for bleaching and for non-inflammatory tissue modifications. This review on plasma technology aims to highlight the current status and clinical applications of this emerging field in dentistry.

Keywords: Cold atmospheric plasma; Wound Healing; sterilization; Implants; plasma jet

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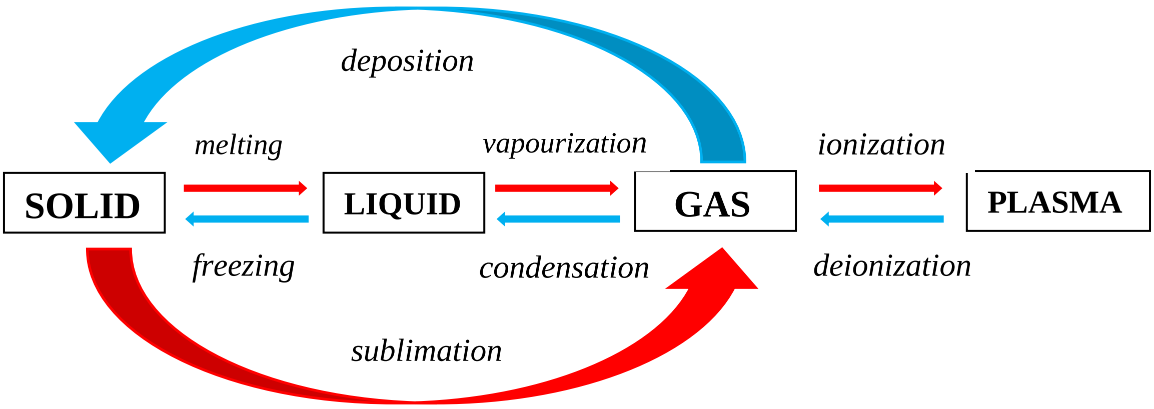
INTRODUCTION

Plasma has been recognized as the fourth state of matter other three being the solid, liquid, and gas as shown in Fig.1. Plasma is form of an ionized gas which consists of ozone, ions, radicals, ultraviolet radiations and electric fields[1].Plasma was identified by William Crookes in 1879[2]and was first named "plasma" by Langmuirin1929. Plasma makes around 99% of the visible universe. Research in Plasma had gained momentum recently and its applications have extended into the fields of biomedicine, environment, aerospace, agriculture and military [3].

Figure 1: Transition among the four states of matter



Plasma has been basically classified into: thermal and non-thermal (cold atmospheric) plasma. This distinction depends on the relative value of temperature of ions (Ti) to electron temperature (Te) present in the plasma (Table 1). In thermal plasma, electrons and heavy particles like neutrals and ions are at the same high temperature. In cold plasma, heavy particles are at a lower temperature than electrons [4]. Although the average temperature of electrons in cold plasma is much higher than the room temperature, their number and mass is very less as compared with atoms and molecules. Therefore, the overall temperature increase in non-thermal plasma is small and hence it is called Cold plasma. Various gases used for plasma production are Nitrogen, Argon, Heliox (a mixture of helium and oxygen) and air.

Table 1: Temperature of Hot and Cold plasma

Low-temperature cold plasmas	**High-temperature Hot plasmas**
$T_i \approx T \approx 300$ K $T_i << T_e \leq 10^5$ K	$T_i \approx T_e > 10^6$ K

[*Relative value of ion temperature (Ti) and electron temperature (Te) present in the plasma*]

Non-thermal plasma can produce plasma at body temperature [5]. This permits medical use of cold plasma to humans by small plasma hand devices [6]. Plasma medicine has its applications in various fields like in the treatment of gingivitis, periodontitis [7], peri-implantitis [8] and caries and denture stomatitis[9],dermatological diseases and chronic wounds[10].Most of the applications are based on antimicrobial property of plasma.

Production of Plasma:

Plasma can be produced at low, atmospheric and high air pressure and their temperatures could be different. While thermal plasmas are natural phenomena, non-thermal plasmas are artificially made and their composition and temperature are adjustable. Non-thermal atmospheric pressure plasma (NTAPP), is sometimes called cold atmospheric plasma (CAP) or low-temperature atmospheric pressure plasma [11].

Energy in form of heat, light or electricity is required in production and maintenance of plasma. Most commonly, electricity is used to induce the discharge needed to produce CAP. Methods commonly used to produce Cold atmospheric plasma are: Dielectric Barrier Discharge (DBD), Atmospheric Pressure Plasma Jet, Plasma Needle and Plasma Pencil [4].

Dielectric Barrier Discharge (DBD) by Siemens -1857:

This plasma-producing device is made of two flat metal electrodes. These electrodes have a covering of a dielectric material as shown in Fig. 2. One electrode is grounded and the other one is at high voltage. A gas is made to pass between the two electrodes through gas inlet, which gets ionized to form plasma. DBD is driven by Alternative current (AC) and high voltages. Power consumption is between10 and 100W [12-13].

Floating electrode Dielectric Barrier Discharge:

It was developed by Frid man et al [14] and is similar to the conventional Dielectric Barrier Discharges (DBDs) with two electrodes, only difference being that none of the electrodes is grounded in FE-DBD as shown in Fig. 3. One of the two electrodes is connected to high voltage while the other can be human skin, cells in growth media or an organ. It is non-thermal and operates at room temperature and pressure. It has been utilized on endothelial cells, for treating melanoma skin cancer, for blood coagulation without damaging surrounding tissue [15], living tissue sterilization (e.g. skin) and deactivation of microbes like Bacillus stratosphericus[16].

Figure 2: Formation of Plasma by Dielectric Barrier Discharge

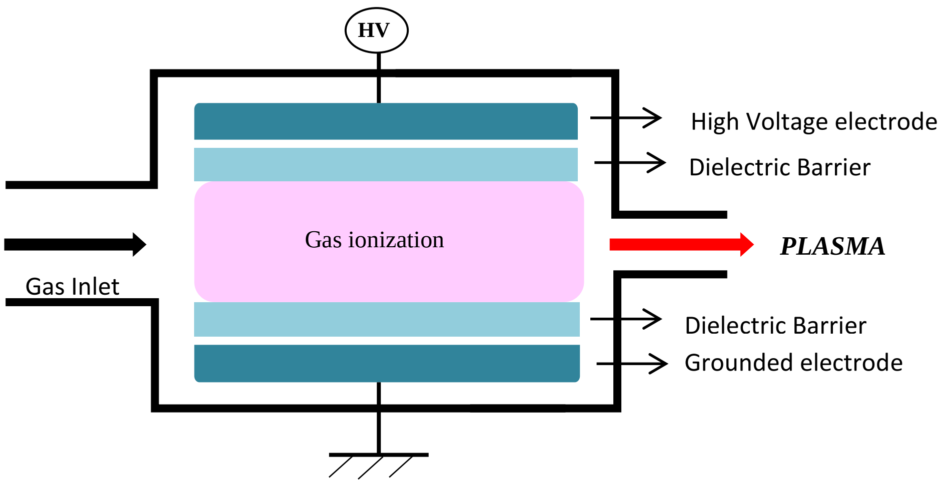
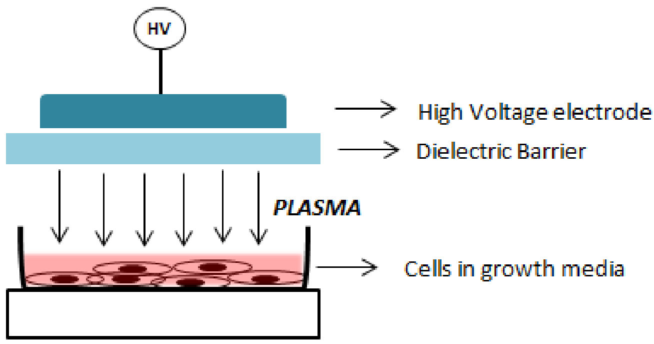


Figure 3: Floating Electrode Dielectric Barrier Discharge



Plasma jets:

Radio frequency plasma jets:

The earliest Radio Frequency Cold Plasma Jet was developed by Koinuma et al in 1992[17]. A common example of Radio frequency plasma jets is Atmospheric Pressure Plasma Jet (APPJ) [18].It has two electrodes placed coaxially between which a mixture of different gases like oxygen, helium and other gases flows at a high rate. Helium or argon can be mixed with various gases, depending on the application of plasma. The outer electrode is grounded. A radio Frequency is applied to the central electrode and a discharge is created which produces reactive species. These reactive species exit as plasma through the nozzle at high velocity and reach to the treatment area. It can also be used for the inactivation of several microorganisms [19].

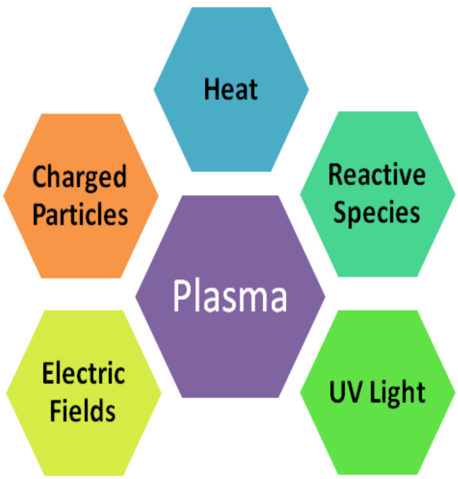
Stoffel set al [5] designed plasma needle**,** which was a miniature form of atmospheric plasma jet in which the needle was placed in a box. Hence, the samples to be treated had to be put inside the box.

Later in 2004[20] they created a newer version of plasma needle, which consisted of a metal strand, 0.3 mm in diameter with a tip inside a Perspex tube. Due to its high thermal conductivity Helium gas was used most frequently. At the needle tip, the gas was mixed with air where a micro discharge was created. This generated a plasma glow of 2 mm in diameter. This small size of plasma generated by plasma needle enables to treat small areas like oral lesions in dentistry, where accuracy is required.

Pulsed directs currents driven plasma jets:

In 2005, Laroussi et al designed a miniature plasma jet and called it plasma pencil [21]**.**It is used to treat E. coli, P. gingival is and Leukemia cells[22-24].It comprises of a dielectric cylindrical tube of diameter 2.5cmin which two disk electrodes of the diameter same as that of the cylindrical tube are inserted. The gap between two electrodes can vary from 0.3 to 1 cm. There is an application of high voltage pulses between the two electrodes for sub-microsecond and a gas is injected. This creates a discharge and a plasma plume is generated which passes out through a small opening in the outer electrode as plasma jet. This plasma plume is at low temperature (290K) and hence can be safely touched.

Figure 4: Components of Plasma



Components of Plasma:

The main components of plasma that are responsible for its effects are: reactive species, charged particles, UV photons excited atoms and molecules as shown in Fig.4. The biological effects of different components of plasma have been summarized in Table 2.

Table 2: Different components of plasma and their effects

S. No.	Components of plasma	Effect
1.	Reactive oxidative/Neutral species (ROS) like Oxygen, Hydroxyl Radicals, and Nitrogen Dioxide.	• Major component for Sterilization. • Forms major mechanism in bacteria deactivation. • Cause morphological changes in bacteria, membrane depolarization, lipid peroxidation, and DNA damage in a dose dependent manner.
2.	Charged particles	Sterilization process: • Bacterial cells membrane rupture • Release of cellular contents in surrounding medium
3.	UV radiation	UV radiation have a possible but not significant role in plasma sterilization and inactivation of spores at atmospheric pressure. Further studies are required.

Therapeutic plasma application:

Disinfection and sterilization of materials and tissues:

Conventional methods of sterilization and disinfection need high temperatures or chemical substances in high concentrations which may cause damage of equipments especially those made of rubber, plastic or any corrosive metal. But with cold plasma sterilization can be achieved without any damage to heat-sensitive materials or chemically reactive surfaces [25].Plasma helps in attaining rapid but gentle disinfection of tissues via inactivation of various pathogens. Low temperature plasma also aids in the removal of biofilms from medical implants and teeth [26]. In future plasma devices can prove to be a fast and efficient sterilization method for hospitals and nursing homes.

Wound Healing:

Plasma treatment can influence healing of wounds by reducing colonization of bacteria. Plasma has direct effects on epidermal and dermal cells[27, 28].It is proved to be effective against gram positive and gram negative bacteria, spores, multi-resistant bacteria such as MRSA and fungi like Candida albicans [29-31].By combating infections caused by bacteria and fungi, plasma treatment reduce is comfort and accelerates recovery.

Skin diseases and Cosmetic applications:

Plasma medicine can be used in treatment of wrinkles, atopic dermatitis and other pruritic diseases, actinic keratosis, seborrheic keratosis, viral papilloma, scars, sun-burns and skin pigmentation, for skin rejuvenation [32] and for tooth bleaching.

Blood coagulation:

High-temperature plasma can also be used for hemostasis, for sclerosing angiodysplasias and tumor ablation. [33,34].

Other Applications:

Plasma therapy has been also used in treatment of cutaneous leishmaniasis, impetigo contagiosa, folliculitis, ecthyma and fungal infections. In dentistry, it can be used to treat periodontal infections, chronic gingivitis and infected root canals (which is due to its ability to enter in microscopically small channels). It may also prove useful for blood cleansing, cancer treatment in near future.

APPLICATIONSIN DENTISTRY

Heat and chemical agents destroy microorganisms but at the same time they cause damage to the human tissues. Non-thermal atmospheric pressure plasmas have proved to be highly efficient in disinfection and sterilization in a cost effective way and tissue friendly way. Inactivation of microorganisms by plasma is brought about by both physical means like reactive species, free radicals and UV photons and biological mechanisms such as DNA and cell membrane damage.[25].

Thus, it can be used in treatment of various oral infections, dental caries and periodontal infections and for dental bleaching, biofilm removal, dental instrument sterilization and composite restoration [35].

Sterilization of Instruments:

Autoclaves are most commonly used to sterilize dental instruments which can damage metals, rubber, and plastics. Recent studies have shown that UV sterilizers and plasma technology can also prove to be efficient for sterilization. Plasma devices can be used to sterilize all type of dental instruments without damage as it does not attain high temperatures. Plasma devices have proved to be better than non-thermal conventional methods such as UV irradiation as they have shown to destroy higher number of bacteria than the latter [36, 37].The action of plasma sterilization is based on its constituents like UV radiation, reactive species, ions and electrons[38]. Also, plasma treatment not only sterilizes the point of contact but also the area around it. Yang Hong Li et al. stated that sterilization with plasma may form a novel way to destroy microbes in future due to its advantages of low temperature, rapidity, thoroughness and safety. [39] Idlibi et al inoculated discs of titanium with biofilms and treated them with methods including Cold atmospheric plasma, laser, air-abrasion and chlorhexidine[40].They concluded that quantity of the biofilms was reduced maximum with CAP treatment among all the treatments. Rupf et al. in 2012 used a Cold plasma jet to destroy biofilms on micro-structured titanium[41]and observed that a plasma and air/water spray combination removed the biofilm completely and results were superior to chlorhexidine biofilm removal.

Deactivation of Biofilm:

Biofilms develop on all hard and soft tissue surfaces including prosthesis and result in caries, periodontal diseases, oral mucositis and other oral diseases including peri-implantitis. Rupf et al. (2011) showed that plasma treatment along with a non-abrasive air and water spray can eliminate oral biofilms on micro-structured titanium which is used for dental implants [42]. Koban et al demonstrated that the removal of biofilms using non-thermal plasma gave better results as compared to chlorhexidine treatment *in vitro*[8].CAP has also proved efficient in destroying biofilms in root canals. Jiang et al. disinfected root canals of extracted teeth using plasma plume at room temperature[43].

In vitro studies have demonstrated disinfection of Streptococcus mutans grown on agar plates successfully[44].The substantial reduction of oral micro-organisms adherent to dentin slices was also reported with the use of plasma[45].The sterilization effect was suggested to be due to reactive oxygen species[46].

Treatment of Oral Candidiasis:

Candida infections of oral cavity include - angular stomatitis, median rhomboid glossitis, candida-associated denture stomatitis, and linear gingival erythema etc. According to studies conducted by Koban et al. and Yamazaki et al. plasma treatment can cause efficient *Candida albicans* sterilization and can possibly be used to cure stomatitis caused by *Candida albicans*[9, 46].

Dental caries:

Plasma treatment can be used in sterilization of irregular surfaces. This makes plasma a suitable alternative for decontamination of dental cavities. Eva Stoffels et al. for the first time suggested that plasma needles can be used in the dental cavities because of their ability to kill *Escherichia coli* [47]. Goree et al. showed that non-thermal atmospheric plasma scan kill cariogenic bacteria - *Streptococcus mutans*[44].Sladek et al. used plasma needle to treat dental tissue and concluded that plasma treatment leads to sterilization of irregular structures, narrow channels and openings of diseased tooth [47]. Subsequently, Yang et al. used a low-temperature atmospheric argon plasma brush and found that it can deactivate *S. mutans* and *Lactobacillus acidophilus* effectively.[48].Plasmas can reach and decontaminate small irregular cavities and fissure spaces which are inaccessible to lasers.

Root canal disinfection:

The root canal system in human tooth is very complex and has various ramifications and irregularities, along with minute dentinal tubules [49]. Studies have shown that microbes can enter and reach up to a depth of 500–1000 μm in dentinal tubule.[50].

Lu et al. generated plasma inside the root canal using a plasma-jet device [51]and found that *E. faecalis,* which one of the main bacteria leading to failure of root-canal treatment, can be destroyed efficiently. Pan et al. also studied the use of plasma in disinfection of a root canals infected with *E. faecalis* and found high efficiency of cold plasma in disinfecting root canals[52]

Use of CAP for tooth whitening:

Tooth bleaching has become a highly in-demand esthetic dental treatment in today's dentistry. The most widely used bleaching material is (H_2O_2) [53]. In-office bleaching systems usually use 30–44% Hydrogen peroxide bleaching gel and a high-intensity light source [54, 55] which has proved to be effective and safe.

Lee et al. found that bleaching of teeth caused by atmospheric pressure plasma was due to increase in production of OH radicals and surface proteins removal. [56]. Park et al. used a low-frequency plasma source and hydrogen peroxide for intrinsic tooth whitening [57]. Nam et al. conducted a study using a Plasma jet on 40 extracted human molar teeth. They divided forty teeth randomly into 4 groups (n=10). Teeth in four groups were treated with Carbamide peroxide + Plasma Arc Lamp (PAC), Carbamide peroxide + CAP, Carbamide peroxide + diode laser and Carbamide Peroxide alone (control) respectively. In their study, CAP proved to be most effective in bleaching teeth without damaging the tooth due to its low temperature [58].

Thus Plasma treatment was suggested to be complementary to the traditional method as it helps in effective bleaching without any thermal damage[56, 57].

Surface Modification of the implant surface:

Over the years, dental implants have become a standard procedure in replacement of missing teeth with high success rate. Implant surface treatment has received a considerable attention as it plays a major role in successful implant osseointegration. Studies have shown that treatment of titanium implant surface with plasma is capable of improving cell adhesion by changing surface roughness and wettability [59, 60]. Recently, Giro et al [61] have conducted a study in which a chair side non-thermal atmospheric pressure plasma treatment was given to implants immediately before placement. They found that plasma treatment reduced the contact angle and supported the spread of osteoblastic cells. Plasma treatment improves osseointegration without leaving any residues after treatment. Zirconia implants which have better esthetic properties than titanium implants have also demonstrated increase in hydrophilicity and enhanced osseointegration after plasma treatment[62].

CONCLUSION

Plasma technology is an evolving and dynamic field of research integrating various fields of medicine, dentistry, physics, chemistry and biology. Although still not popular among dentists, it has shown promises at various fronts of dentistry. In vitro and in vivo studies of Cold Atmospheric Plasma have shown encouraging results in tooth whitening, biofilm deactivation, instrument sterilization and root canal disinfection. Still literature discussing the role of *Plasma in Dentistry* is scarce and it requires many randomized controlled trials to prove its efficacy and establish as new frontier beyond laser. Further research on mechanism of action and interaction of plasma with tissues and its effects at cellular and molecular level are needed, to have a better insight of this futuristic technology.

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