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Plasma: From Distant Stars to Dental Chairs.

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ABSTRACT

Plasma is the fourth state of matter which comprises 99% of the universe. "***Plasmadent***" is the term applied to the plasma technology and plasma pharmacology in dentistry which represents a major paradigm shift from chemical to molecular based medium in order to treat various oral conditions. Plasma is cutting edge technology, which involves interdisciplinary partnerships in medicine, dentistry, physics, engineering, chemistry and biology. Plasmadent is evolving into a dynamic field of research. The accumulated knowledge gained in industry and medicine now lays the foundation for unique plasma applications in dentistry. Novel advances in dental technology are often sporadic; therefore, the discovery of the biological effects of plasma which are suitable for oral applications has been a major finding. The emergence of low temperature atmospheric pressure plasma is becoming a ground breaking field of research for treating a myriad of medical and dental conditions. Dental application of Cold Atmospheric Plasma (CAP) includes: dental caries management, sterilization, elimination of biofilms, root canal disinfection, increase in bond strength at the dentin composite interface and bleaching. This article is intended to provide information on the current status of plasma as an emerging field, its scope and its broad interdisciplinary approach.
Keyword*:* Plasma Dentistry, Cold Atmospheric Plasma, Non-Thermal Plasma**.**

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INTRODUCTION

Plasma is the fourth state of matter. Over 99% of the visible universe is made up of plasma. For example, the matter in stars or nebulae is plasma. It was identified by the British physicist Sir William Crookes in 1879 and was termed “plasma” by Irving Langmuir, an American chemist in 1929. When the electrons are stripped from atoms and molecules, they enter into a high energetic state called plasma [1]. If the energy dissipates, the electrons reattach and the plasma particles become a gas. Thus, plasma consists of positively and negatively charged ions and negatively charged electrons as well as radicals, neutral and excited atoms and molecules [2]. Plasma not only occurs as a natural phenomenon as seen in the universe in the form of fire and in the nuclear fusion reactions of the sun but can also be created artificially which has gained importance in the field of plasma screen television and light sources. Based on the relative temperatures of the electrons, ions and neutrals, plasma can be classified as “thermal” or “non-thermal” plasma. Thermal plasmas have electrons and the heavy particles at the same temperature, i.e., they are in thermal equilibrium with each other.

Non-thermal plasmas on the other hand have the ions and neutrals at a much lower temperature (sometimes room temperature), whereas electrons are much “hotter”. In recent years, cold (less than 40 °C at the point of application) atmospheric plasma (CAP) sources have been introduced that provide the possibility to extend plasma treatment to living tissue [3]. Non thermal Atmospheric Plasma or Low temperature plasma or cold atmospheric plasma (CAP) is characterized by a low degree of ionization at low or atmospheric pressure. CAP is known as non-thermal because it has electrons at a hotter temperature than the heavy particles that are at room temperature. Its temperature is less than 104°F at the point of application[4-6].

A relatively new area is the use of these plasmas in dental applications. Plasma treatment is potentially a unique tissue-saving technique, allowing irregular structures and narrow channels within the diseased tooth to be cleaned. Low-temperature plasma is a promising method for destroying microorganisms, an alternative to conventional methods which has numerous drawbacks. Dental applications of CAP include: dental caries, sterilization, elimination of biofilms, root canal disinfection, increase in bond strength at the dentin/composite interface and bleaching. Given the current state of knowledge, this article intends to provide an insight into the potential applications of plasma in the field of dentistry[1].

MECHANISM OF GENERATION OF COLD PLASMA

Figure 1: Schematic representation of a working model of a plasma apparatus.[8]

Plasmas can be produced by various means, e.g. radio frequency, microwave frequencies, high voltage AC or DC, etc. The main body of the device is made of a medical syringe and a needle. They are used for guiding the gas flow. The needle also serves as the electrode, which is connected to a high-voltage (HV) submicrosecond pulsed direct-current (DC) power supply (amplitudes of upto 10 kV, repetition rate of upto 10 kHz, and pulse width variable from 200 ns to dc) through a 60-kΩ ballast resistor R and a 50-pF capacitor C, where both the resistor and the capacitor are used for controlling the discharge current and the voltage on the needle. Because of the series-connected capacitor and the resistor, the discharge current is limited to a safety range for a human. It is found that, if the resistance (R) is too small or the capacitance (C) is too large, a weak electric shock can be felt when the plasma is touched by a human.[7]

The diameter of the syringe is about 6mm, and the diameter of the syringe nozzle is about 0.7mm. The needle has an inner diameter of about 200µm and a length of 3cm. Working gas such as Helium (He), Argon (Ar), or their mixtures with oxygen (O2) can be used. The gas flow rate is controlled by a mass-flow controller [7].

When working gas such as He/O2 (20%) is injected into the hollow barrel of the syringe with a flow rate of 0.4 L/min and the HV pulsed dc voltage is applied to the needle, homogeneous plasma is generated in front of the needle. A finger can directly contact with the plasma or even with the needle without any feeling of warmth or electric shock. Therefore, this device is safe for intra-oral application for the treatment of various oral lesions [9]. Some methods used to produce CAP include: Dielectric Barrier Discharge (DBD), Atmospheric Pressure Plasma Jet (APPJ), Plasma Needle, and Plasma Pencil[10].

The energy for sustaining the plasma state is usually supplied by electromagnetic field. Electrons are accelerated by the field much faster, but are less effective to transfer their energies to heat their environment than heavy ions. The plasma can remain non-thermal where the energetic electrons can lead to reactions including ionization of particles, production of reactive species, and radiation [11 -13].

The reactive species in non-thermal atmospheric air plasmas aregenerated through electron impact excitation and dissociation. These are nitrogen– and oxygen–based species such as atomic oxygen, ozone, nitric oxide (NO), nitrogen dioxide (NO2), and hydroxyl free radicals (OH¢). These active species are short living radicals in gas phase that can dissolve in liquid. After recombination/reaction the radicals are destroyed, so that no radicals remain after plasma exposure. The complex components from non-thermal plasmas achieve multi-functional treatment in oral cavity. For example, reactive oxygen species and reactive nitrogen species are regarded as a key factor for sterilization, wound healing, and tooth whitening. Concentration of the components can be controlled by the plasma operating conditions, making it possible that non-thermal plasmas be employed for various biomedical applications[14,15].

DENTAL APPLICATION OF PLASMA:

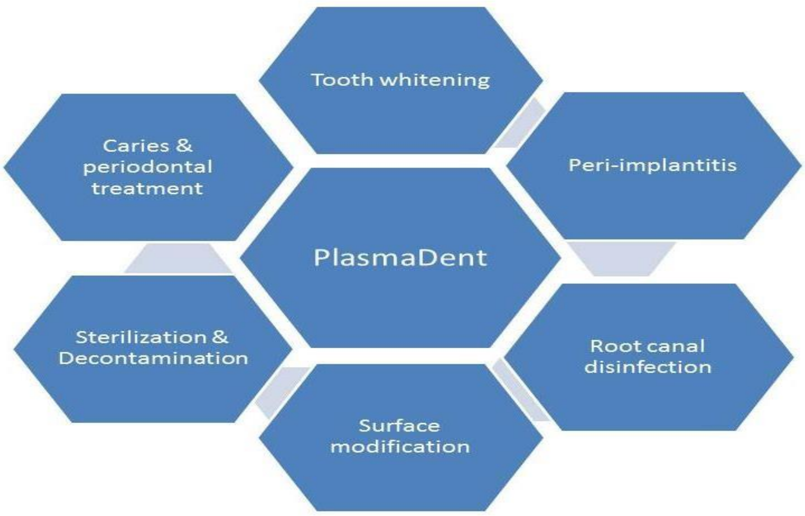

Figure 2: Diagrammatic representation of plasma application in dentistry.

Sterilization by eradication of bacteria:

The sterilization efficacy of plasma devices is influenced by gas composition, driving frequency, and bacterial strain, but plasma devices have shown to kill a higher proportion of bacteria than do conventional non-thermal methods such as UV sterilization16, 17. The bacterial cell membrane is made up of lipid bilayer which forms a protective barrier through which compounds are transported in and outside the cell. Important components of the membrane are the unsaturated fatty acids and the proteins, both are involved in transportation processes across the membrane. The unsaturated fatty acids are susceptible to attacks of OH¢. Hydroxyl radicals generated by plasma along with other free radicals destroy membrane lipids and thereby deactivate the bacteria. The same is true for the proteins as they are also susceptible to the attack of radicals when exposed to plasma [13, 17, 18]. Yang Hong Li et al. stated that plasma sterilization, with the advantage of low temperature, fastness, thoroughness and safety overcomes the deficiency of the traditional sterilization technology and may become a novel method for killing microbe. To summarize plasma has been shown to effectively sterilize instruments by removal of biofilms efficiently [19].

Plasma in dental cavities:

Yang et al. introduced and conducted a study on low-temperature atmospheric argon plasma brush for effectively deactivating Streptococcus mutans and Lactobacillus acidophilus. He concluded that about 100% bacterial elimination was achieved within 15 seconds for Streptococcus mutans and in 5 minutes for Lactobacillus acidophilus. Also, in comparison to lasers, plasmas can access small irregular cavities and fissure spaces[20]. This study was based on the known fact that plasma can treat and sterilize irregular surfaces, making them suitable for decontaminating dental cavities without drilling. Although, plasma itself is superficial, the active plasma species it produces can easily reach inside of the cavity. This approach was pioneered by Eva Stoffels, who suggested the use of plasma needles in the dental cavity on the basis of the ability of plasma to kill Escherichia coli [21]. Goree et al. provided substantial evidence that non thermal atmospheric plasmas killed Streptococcus mutans, a gram-positive cariogenic bacterium. Non-thermal atmospheric plasma brush was highly effective and efficient when used for bacterial disinfection, which is necessary in dental restorations to prevent secondary caries. Plasma treatment of dentin surfaces will prevent contamination, actively fight bacterial infections, and prepare/engineer the dentin surface for strong and durable bonding to restorative materials e.g. dental composit e[22]. Sladek et al. studied the interactions of the plasma with dental tissue using a plasma needle. He concluded that plasma is an efficient source of various radicals, which are capable of bacterial decontamination, and operates at room temperature and thus, does not cause bulk destruction of the tissue. Therefore plasma treatment is potentially a novel tissue-saving technique, allowing irregular structures and narrow channels within the diseased tooth to be cleaned [21, 23]. The lists of bacteria that can be successfully inactivated by plasma are as follows[24, 25]:

Table 1: Bacterias that can be inactivated by plasma.

Sl No.	Group	Species
1.	Streptococci	Streptococcus mutans, Streptococcus sobrinus, Streptococcus parasanguis, Streptococcus mitis 1, Streptococcus oralis, Streptococcus intermedius, Streptococcus vestibularis, Streptococcus mitis 2, Streptococcus gordonii, Streptococcus sanguis, Streptococcus anginosus
2.	Lactobacilli and Bifidobacter	Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium dentium
3.	Actinomyces	Actinomyces israelii, Actinomyces gerencseriae, Actinomyces naeslundii, Actinomyces odontolyticus, Rothia dentocariosa
4.	Microaerophile s	Actinobacillus actinomycetemcomitans, Eikenella corrodens
5.	Aerobes	Neisseria mucosa, Haemophilus parainfluenzae
6.	Anaerobes 1	Fusobacterium nucleatum ss nucleatum, Campylobacter rectus, Veillonella parvula, Capnocytophaga gingivalis, Peptostreptococcus asaccharolyticus, Gemella morbillorum, Prevotella melaninogenica, Leptotrichia buccalis, Eubacterium saburreum, Corynebacterium matruchotii, Prevotella nigrescens/intermedia
7.	Anaerobes 2	Porphyromonas gingivalis, Selenomonas noxia, Micromonas micros

Root Canal Sterilization:

Treatment of root canal infection (periapical abscess) is difficult, as it is difficult to penetrate irregular and narrow spaces, killing the pathogens and therefore infections frequently recur. Enterococcus faecalis is one of the main types of bacterium causing failure of root-canal treatment.

Lu et al. used a reliable and user-friendly plasma-jet device, which could generate plasma inside the root canal. The plasma could be touched by bare hands and directed manually by a user to place it into root canal for disinfection without causing any painful sensation. Preliminary inactivation experiment results showed that it can efficiently kill Enterococcus faecalis in several minutes [26]. Pan et al., investigated the feasibility of using a cold plasma treatment of a root canal infected with Enterococcus faecalis biofilms in-vitro. It was concluded that the cold plasma had a high efficiency in disinfecting the Enterococcus faecalis biofilms invitro in root canal treatment [27].

Intraoral diseases:

Oral candidiasis includes Candida-associated denture stomatitis, angular stomatitis, median rhomboid glossitis, and linear gingival erythema. Koban et al., and Yamazaki et al., reported the high efficiency of *Candida albicans* sterilization using various plasmas. Their result indicates the possibility that stomatitis caused by *Candida albicans* can be cured by plasma jets [28, 29].

Use of plasma in composite restorations:

Various researchers have investigated CAP in composite restorations. The plasma generates reactive species that arrive on the surface of the composite resulting in both microstructural and surface chemistry modifications that improve adhesive bonding. They observed plasma treatment increases bonding strength at the dentin/composite interface that enables it to last longer on teeth. Preliminary data has shown that plasma treatment increases bonding strength at the dentin/ composite interface by roughly 60%, and thus significantly improves composite performance, durability, and longevity [30]. Current clinical practice relies on mechanical bonding when it should rely on chemical bonding. The culprit that foils mechanical methods is a protein layer, the so-called "smear layer," which is primarily composed of type I collagen that develops at the dentin/adhesive junction. To create a porous surface that the adhesive can infiltrate, current preparation techniques etch and demineralise dentin. Interactions between demineralised dentin and adhesive gives rise to the smear layer, which actually inhibits adhesive diffusion throughout the prepared dentin surface. This protein layer may be responsible, in part, for causing premature failure of the composite restoration. It contributes to inadequate bonding that can leave exposed, unprotected collagen at the dentin-adhesive interface, allowing bacterial enzymes to enter and further degrade the interface and the tissue. Treatment with non-thermal plasma provides a unique opportunity to modify dentin surfaces in an attempt to improve the interfacial bonding of the dental-composite restoration. Non-thermal plasmas are partially ionized gases that contain highly reactive particles, including electronically excited atoms, molecules, and ionic and free-radical species, while the gas phase remains at a temperature similar to room temperature. Depending on the plasma chemistry or the gas composition, these highly reactive plasma species react with, clean, and etched surface materials, bond to various substrates, or combine to form a thin layer of plasma coating, and consequently alter the surface characteristics. Non-thermal plasmas combine exceptional chemical reactivity with a relatively mild, non-destructive character resulting from a cold gas phase. Surface treatment using non-thermal plasmas has led to enormous success in the surface engineering and processing of solid-state materials, especially in plasma cleaning/etching, surface engineering, adhesion enhancement, and biomaterial development. When utilized correctly and efficiently, non-thermal plasma is a gentle method that can be used to change the surface characteristics of the top layer of polymeric surfaces, such as collagen fibrils on the dentin surfaces, and thus enhance the surface for various types of adhesives used in composite restoration [31].

Kong et al., investigated the plasma treatment effects on dental composite restoration for improved interface properties and their results showed that atmospheric cold plasma brush (ACPB) treatment can modify the dentin surface and thus increase the dentin/adhesive interfacial bonding. The solution is to introduce bonds that depend on surface chemistry rather than surface porosity [32].

Plasma in Tooth Bleaching:

CAP can also be used to bleach teeth. Lee et al. showed that atmospheric pressure plasma in place of light sources bleached teeth by increasing the production of OH radicals and the removal of surface proteins. Furthermore it was also shown that in combination with hydrogen peroxide plasma removed stains from extracted teeth stained by either coffee or wine. Tooth whitening can also be achieved using a DC plasma jet and hydrogen peroxide. Intrinsic stains are a serious factor in tooth discoloration[33, 34]. Park et al. suggested intrinsic whitening using a low-frequency plasma source and hydrogen peroxide[35]. Another approach, by Kim et al. used liquid plasma produced by a radio frequency driven gas–liquid hybrid plasma system. In this study, the RF plasma jet was placed in deionized water and the target tooth was immersed in the water. Color changes were observed on the surface of the treated tooth after 8 min. The OH radicals were regarded as the main cause of bleaching in this work. A non thermal, atmospheric pressure, helium plasma jet device was developed to enhance the tooth bleaching effect of hydrogen peroxide (H_2O_2). Combining plasma and H_2O_2 improved the bleaching efficacy compared with using H_2O_2 alone. Tooth surface proteins were noticeably removed by plasma treatment. When a piece of tooth was added to a solution of H_2O_2 as a catalyst, production of OH after plasma treatment was 1.9 times greater than when using H_2O_2 alone. It is suggested that the improvement in tooth bleaching induced by plasma is due to the removal of tooth surface proteins and to increased OH production[36, 37].

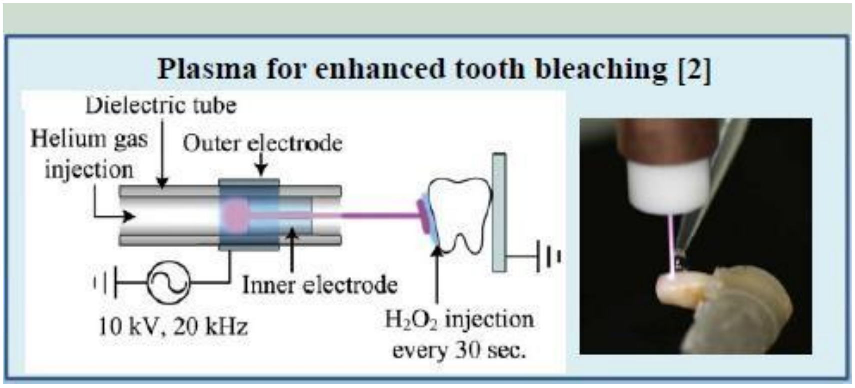

Figure 3: Schematic representation of a plasma apparatus for enhanced tooth bleaching [34].

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[38]. The tooth bleaching method using atmospheric pressure plasma shows reasonable promise of becoming practical in the future.

Effects of CAP on malignant cells:

Few studies have been performed on the effect of CAP on eukaryotic cells thus far. Eukaryotic cells are defined as cells where the genetic material is inside the nucleus. Some researchers observed either cell detachment, decrease of cell migration, apoptosis, or necrosis on several types of cells depending on the power and the time of exposure to plasma. Necrosis is defined as an unprogrammed death of cells in living tissue. This leads to inflammation by releasing intracellular content. In contrast with necrosis, apoptosis is a programmed cell death process resulting in no inflammation. Different groups have conducted in vitro experiments with fibroblasts, endothelial cells, ovarian cells, human hepatocytes, and smooth muscle cells. Some researchers observed that CAP decreases cells migration of both fibroblasts and epithelial cells by increasing integrin activation[39]. Because of the effect of CAP on mammalian cells, researchers have been interested in using it on malignant cells also. The conventional therapies for cancerous diseases are based on

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removal of the tumor, chemotherapy and/or radiation. Nevertheless some cancers remain hard to eradicate. In-vitro and in-vivo studies have been performed on the efficacy of CAP at killing cancer cells. The results of the pilot studies performed by several research groups confirmed that treatment with low-temperature plasma is able to induce several modes of cell death including apoptosis and necrosis. They also noticed decreased cell migration and induction of senescence in cancer cells[40]. Regarding the mechanism of the Atmospheric Pressure Plasma therapy on cancer cells, the hypothesis is that the ROS (reactive oxygen species) plays the main role. ROS are well known to be harmful to cells inducing apoptosis, senescence, or cell cycle arrest. Sensenig et al. proposed that ROS is the mechanism through which CAP induces apoptosis [41].

SAFETY ISSUES:

Plasma is a rich source of radicals and other active species. As we already know that free radicals have earned a bad name in biology and medicine because of their capability of causing severe cell damage, especially the ROS. The ROS family comprises radicals like oxygen (O), hydroxide (OH) and hydroperoxyl group (HO_2), peroxide anions like oxygen ions (Oi2) and hydroxide ions (HOi2), ozone and hydrogen peroxide. When the ROS level in body fluids becomes too high, various types of damage occur, known under a common name of oxidative stress. It is believed that oxidative stress bears at least partial responsibility for diseases like arteriosclerosis, cancer and respiratory problems. Moreover, high concentrations of oxygen radicals accelerate ageing of cells and tissues. On the cellular level, several effects leading to cell injury have been identified: lipid peroxidation, DNA damage and protein oxidation[41, 42].

On the other hand, free radicals have various important functions in the body. For example, macrophages generate ROS to destroy the invading bacteria, and endothelial cells (inner artery wall) produce nitric oxide (NO) to regulate the artery dilation[42].

Plasmas are often very complex mixtures; in fact, they owe their specific properties to the synergy of various components: charged particles (electrons, positive and negative ions), metastables, re-vibrationally excited molecules, active radicals and (UV) photons[42]. Therefore, it is a great challenge for the experimentalist to characterize the plasma and to tailor its properties to achieve desired benefits.

CONCLUSION

The literature on plasma sterilization has been growing substantially in the recent past. Based on the above evidence, we can say that CAP has a bright future in dentistry due to its multiple applications in various treatments. Plasma dental treatments are basically painless, drill-less making it patient friendly especially in children and under-served communities, where education and familiarity with the dentist's chair are, by definition, limited. Also due to its versatile applications it can be used in almost all the branches of dentistry. However, more studies need to be performed regarding the mechanism of action. Therefore with further research plasma technology can become a valuable tool in dentistry.

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