Plasma: Pursuit of Dentistry

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1. Introduction

Plasma, fourth state of matter where as others exist in liquid, solid and gas. Plasma, a collection of stripped particles. Plasma is stripped electrons from atoms and molecules by changing their state. As stripping electrons takes constant energy which makes plasma naturally energetic. Plasma particle become gas, If the energy dissipates and the electrons reattach. Thus, plasma consists of positively and negatively charged ions and negatively charged electrons as well as radicals, neutral and excited atoms and molecules. [1, 2].

Unlike ordinary matter, plasmas can exist in a wide range of temperatures without changing state. The aurora borealis, or northern lights, is ice cool, for instance, while the core of a distant stars white hot. Other well-known plasmas include lightning, neon signs, and fluorescent lights. Outside of a container, plasma resembles gas—the particlesdon't have a definite shape. But unlike gas, magnetic and electric fields can control plasma and shape it into useful, malleable structures.[1].

2. History

In the late 1850s, simens used first plasma discharge to create ozone, which acted as an agent to remove contaminants and toxins from water. Little research was conducted exploring the relationship between plasma and biological cells. British physicist Sir William Crookes identified in 1879. It was named "plasma" by Irving Langmuir, an American chemist in 1929. From 1960 to 1980, plasma was mainly utilized as a secondary agent to indicate biological sterilization. In 1990s, Plasma science was in its infancy but by 1997, multidisciplinary teams set out to understand the effects that plasmas had on pathogenic and nonpathogenic microorganisms, as well as develop proof of concept studies to demonstrate that plasma could be used as a decontaminant or sterilizing agent. By 1990s, plasma research has evolved at a rapid pace and extended into biomedical, environmental, aerospace, agriculture and military fields. [3,4,5,6].

Although biomedical application of plasma technology has become very popular in various fields today, it is not clear when it was first used in the field of dentistry. It is partly because the plasma has been nearly everywhere and has been related to nearly everything in reality, thus we do not readily recognize it. Perhaps the first application of plasma in dentistry occurred in the manufacturing process of dental instruments or the disinfection of them. Nevertheless, Eva Stoffel is believed to introduce the first investigation with the view of a possible therapeutic and thus medical question for dentistry. [7,8].

3. Types

Based on the temperatures of the electrons, ions and neutrals, plasma can be classified as "thermal" or "nonthermal" plasma. Thermal plasmas have electrons and the heavy particles at the same temperature. Thermal plasma has thermal equilibrium with each other.

The ions and neutrals in Non-thermal plasmas are at much lower temperature, whereas electrons are much hotter. Nonthermal plasmas are also called as cold atmospheric plasma (CAP) or low temperature plasma. In recent years, cold (less than 40 °C at the point of application) atmospheric plasma (CAP) can be used to provide the possibility to extend plasma treatment to living tissue [9]. Cold atmospheric plasma (CAP) is characterized by a low degree of ionization at low or atmospheric pressure. CAP is known as nonthermal 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.

Various types of plasma devices, such as nanosecond pulsed plasma pencils [10], radio-frequency plasma needles [11], direct-current plasma brushes [12], and plasma jets [13] have been developed for non-thermal atmospheric pressure plasma generation. The common challenge for generating these plasmas is the inhibition of the glow to arc transition at one atmosphere. Different types of discharges have used different schemes to achieve this [14]. This opens up new horizons in the field of dentistry with the size of the device becoming small enough to hold by hand.

4. Medical Application

Plasma treatments is used to improve different aspects of the therapeutic characteristics of medical implants [15,16]. Plasma processes can be used for surface coupling of antibiotic substances or for integration of metal ions into biomaterial surfaces to create implants which exhibit long-lasting antibacterial properties after implantation thereby, the often devastating effects of implant-related infections could be markedly reduced. Plasma treatment also aids in the application of therapeutic agents onto implant surfaces as well to achieve their controlled release over time. The possible applications are drug-eluting stents and vascular prostheses which release drugs to reduce blood coagulation and thrombosis as well as to prevent intima hyperplasia and restenosis.

Plasma treatment has been successful in creating implants coated with therapeutic agents which aids in the attachment of the drug molecule to the implant surface or to create a layer on top of a coating with a therapeutic compound to modulate the kinetics of its release. The current research is

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focused towards the equipment of implants with antibiotics and other compounds with antibacterial properties to prevent implant-related infections and the coating of antithrombogenic agents to prevent the formation of blood clots and thrombosis for implants with blood contact like vascular prostheses and stents. The previous studies on the drugeluting implants, like paclitaxel and everolimus [17], dexamethasone [18] or trapidil, probucol and cilostazol [19] have aimed at reducing restenosis after implantation of vascular stents, which now, can be achieved easily by plasma based approach. The equipment of implants with antibacterial properties can be achieved either by attaching antibiotic substances or by creating surfaces which release metal ions which are known to have anti-infective effects. Polyvinylchloride, a polymer which is used for endotracheal tubes and catheters, was equipped with triclosan and bronopol, compounds with immediate and persistent broadspectrum antimicrobial effects, after the surface was activated with oxygen plasma to produce more hydrophilic groups for effective coating [20]. Experiments using Staphylococcus aureus and Escherichia coli demonstrated effectiveness of surfaces. the these Similarly, polyvinylidenfluoride used for hernia meshes was modified by plasma-induced graft polymerisation of acrylic acid with subsequent binding of the antibiotic gentamycin [21].

Owing to their well-known antibacterial effects, metals like silver, copper or tin are possible alternatives to classical antibiotic compounds as an effective and sustained release from coatings is possibly easier to achieve due to their small size. Similarly, to gentamycin as mentioned before, silver has been used as a powder added to a plasma-spraved wollastonite coating on titanium implants [22-25]. Similarly, the use of copper for antibacterial implant coatings has also been studied by plasma implantation into polyethylene [26,27]. Compared to controls, the implants created by this Plasma immersion ion implantation of copper reduced the number of methicillin resistant Staphylococcus aureus cultivated on the respective surfaces [28]. Ion implantation can also be used for non-metals like fluorine which is of particular relevance for dental applications. This was examined with titanium, stainless steel and polymethyl methacrylate for fluorine alone [29] or with stainless steel for a combination of fluorine with silver [30].

Plasma also found its applications in the coating of implants with antithrombogenic agents with regard to vascular prostheses and stents which are in constant contact with blood. Thrombosis and blood clot formation are severe and potentially life-threatening complications in such cases. Classical anti-coagulants used for thrombosis prophylaxis and treatment include coumarin derivates like phenprocoumon through oral application as well as heparin for parenteral use. The Plasma-based attachment of heparin been examined for stainless steel which is used in stents.

5. Generation of Cold Plasma

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 needle. They are used for guiding the gas flow. The needle also serves as electrode, which is connected to a high-voltage (HV) submicrosecond pulsed direct-current (DC) power supply (amplitudes upto 10 kV, repetition rate upto 10 kHz, and pulse width variable from 200 ns to dc) through a 60-k Ω ballast resistor R and a 50-pF capacitor C, where both resistor and capacitor are used for controlling the discharge current and voltage on the needle. Because of the series-connected capacitor and the resistor, the discharge current is limited to asafety 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.[31]

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 200μ m 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 [31].

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 [32].

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 [33 -35].

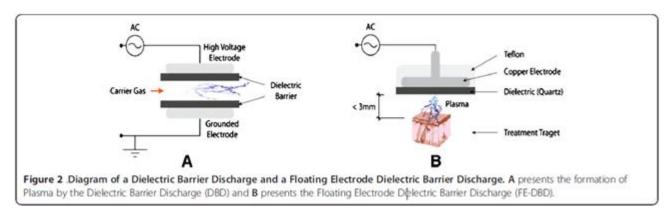
The reactive species in non-thermal atmospheric air plasmas aregenerated through electron impact excitation and dissociation. These are nitrogen- and oxygen-based species such as atomic oxygen, ozone, nitric oxide (NO), nitrogen dioxide (NO2), and hydroxyl free radicals. These active species are short 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 [36,37].

Production of CAP

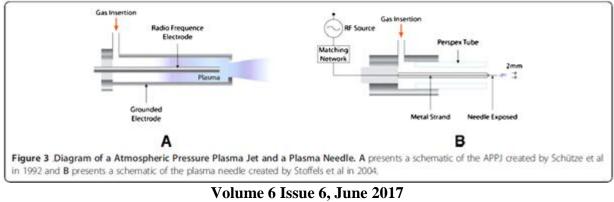
Several different types of CAP have been developed for biomedical uses. Energy is needed to produce and maintain plasma. Thermal, electric, or light energy can be used. The discharge needed to produce CAP is usually induced electrically. Some methods used to produce CAP include: Dielectric Barrier Discharge (DBD), Atmospheric Pressure Plasma Jet (APPJ), Plasma Needle, and Plasma Pencil.

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DIELECTRIC BARRIER DISCHARGE: In 1857, Siemens was first to conduct experiments on Dielectric Barrier Discharge (DBD). DBD has many applications including sterilization of living tissue, bacteria inactivation, angiogenesis, surface treatment, and excimer formation [38,39]. The dielectric barrier discharge (DBD) consists of two flat metal electrodes covered with dielectric material. A carrier gas moves between the two electrodes and is ionized to create plasma. One electrode is a high voltage electrode and the other is a grounded electrode. High voltages are necessary to produce the discharge needed to create the plasma. Alternative Current (AC) high voltages generally drive DBD"s with frequencies in the kHz range. The power consumption is between 10 and 100 W [10]. There are many variations in the configuration of the electrodes, but the concept behind them all remains the same. For example, some electrodes are cylindrical instead of being flat and sometimes the dielectric material covers only one electrode instead of both. More recently, Friedman et al. developed the floating electrode DBD (FE-DBD) [40]. It is similar to the original DBD and consists of two electrodes: an insulated high voltage electrode and an active electrode. The difference between FE-DBD and DBD is the second electrode not grounded; it's active meaning that the second electrode can be human skin, a sample, or even an organ. The powered electrode needs to be close to the surface of the second electrode (< 3mm) to create the discharge. It has been used on endothelial cells, melanoma skin cancer, and blood coagulation. It has also been used in living tissue sterilization and in deactivation of Bacillus stratosphericus (Figure 2) [41]. Plasma jets using a DBD system have also been created [42].



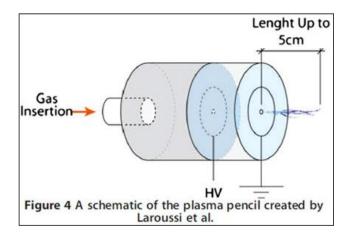
PLASMA JET-RADIO FREQUENCY Plasma Jetsisone type of plasma jet, which is employed for bacterial sterilization, is the Atmospheric Pressure Plasma Jet (APPJ) [43]. The APPJ consists of two co-axial electrodes between which feed gas (mixtures of helium, oxygen, and other gases) flows at a high rate. The outer electrode is grounded while Radio Frequency (RF) power (50-100W) at 13.56 MHz is applied to central electrode which creates a discharge. The reactive species produced exits the nozzle at high velocity and arrives to the area that is to be treated. APPJ has been used for the inactivation of several microorganisms [44-47]. Koinuma et al. developed the earliest RF cold plasma jet in 199224. The cathode is a needle electrode made of tungsten or stainless steel with 1 mm diameter connected to RF source (13.56 MHz). The needle electrode lies within a quartz tube whereas the anode electrode is grounded. Depending on the application, helium or argon was mixed with various gases. Thisgroup published several papers describing its variants and applications of the plasma jet [48,49]. In 2002, Stoffels et al. created a miniature atmospheric plasma jet that they called plasma needle [7] and created a new version in 2004 [50]. In the former version, the needle was enclosed in a box and as a result, these samples were to be placed inside box to be treated. In new version, the plasma needle consists of a 0.3 mm metal strand diameter with a sharpened tip inside of a Perspex tube. The length of entire needle is 8 cm and 1.5 cm remains uncovered by Perspex tube. The gas used most frequently is Helium due to its high thermal conductivity. The gas is then mixed with air at needle tip where micro discharge is created. Gases other than Helium are also used [51]. The diameter of the plasma glow generated is 2 mm. Microplasma is created when RF power at 13.05 MHz ranging between 10 mW and several watts is applied to the needle. Its small size enables it to be used to treat small areas where accuracy is required like in dentistry [52,53]. It has also been used to deactivate E. Coli (Figure 3) [54].



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Pulsed Direct Current-Driven Plasma Jets

Laroussi et al. developed a miniature jet that they called plasma pencil [10]. It consists of a dielectric cylindrical tube of about 2.5 cm in diameter where two disk electrodes of the same diameter as the tube are inserted. The two electrodes are separated by a gap (the distance may vary from 0.3 to 1 cm) and consist of thin copper ring attached to dielectric disk. To create the plasma, sub-microsecond high voltage pulses are applied between the two electrodes while gas is injected through holes of the electrodes. When the discharge is created, a plasma plume is launched through the hole of the outer electrode into the air. Because plasma plume (up to 5cm in length) remains at low temperature (290K), it can be touched safely. The electrical power is supplied to the electrodes by a high voltage pulse generator. The high voltage is supplied to pulse generator by DC voltage supply with variable output. The plasma pencil has been used in the treatment of E. coli, Leukemia cells, and P. Gingivalis [55]. Forster et al., Zhang et al., and Wash et al. developed a plasma jet using a DBD configuration (Figure 4) [56,57].



Plasma in Dentistry

Oral infections, including dental caries, periodontal disease, and intraoral disease, are caused by bacteria and may result in tooth destruction [58]. Although teeth brushing, fluoride uptake, antibiotics, and vaccines have been used as treatment modalities for oral disease, these conventional treatments have limitations [59]. Recently, non-thermal atmospheric plasmas have been shown to be highly efficient at killing bacteria in aninexpensive manner; therefore, the use of such plasmas could eliminatethe problems associated

with use of heat and antibiotics. Applications of plasma for sterilization of medical equipment, packaging in the food industry, implants, wound healing; blood coagulation, have been known [60-66]. This has been possible due to their high bactericidal effectiveness and partly due to their easy access into narrow and confined spaces [67-72]. As conventional methods have numerous drawbacks, lowtemperature plasma can be used as an alternative method for destroying microorganisms [67]. 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.

Sterilizaton by Eradication Of Bacteria

The sterilization efficacy of plasma devices is influenced by gascomposition, driving frequency, and bacterial strain, but plasma devices have shown to kill a higher proportion of bacteria than doconventional non-thermal methods such as sterilization [73,74]. The mechanism of plasma UV sterilization is related to the abundance of plasma components, like reactive oxygen species, ions and electrons, and UV and electromagnetic fields [75]. Also, plasma can affect not only the contacted point but also the area around it. The risk of prior transmission through surgical instruments is of both current public and professional concern. The use of plasma decontamination of surgical instruments is limited. Whittaker et al.has indicated that the use of gas plasma cleaning may be extremely beneficial in reducing the absolute amount of proteinaceous materials that may be transferred between patients when endodontic files arereused [76]. Yang Hong Li et al. stated that plasma sterilization, with the advantage of low temperature, fastness, thoroughness, safety, overcomes the deficiency of the traditional sterilization technology, and may become a novel method for killing microbe [77].Autoclaves and UV sterilizers are presently used to sterilize dental instruments. To develop a dental sterilizer which can sterilize most materials, such as metals, rubbers, and plastics, the sterilization effect of an atmospheric pressure non-thermal air plasma device was evaluatedby Su-Jin Sung et al. It was proved that the atmospheric pressure nonthermal air plasma device was effective in killing both Escherichia coli and Bacillus subtilis, and was more effective in killing Escherichia coli than the UV sterilizer [78]

Sl.no	Groups	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	Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus rhamnosus,
	Bifidobacter	Bifidobacterium dentium
3.	Actinomyces	Actinomyces israelii, Actinomycesgerencseriae, Actinomycesnaeslundii, Actinomycesodontolyticus,
		Rothiadentocariosa
4.	Microaerophiles	Actinobacillusactinomycetemcomitans, Eikenellacorrodens
5.	Aerobes	Neisseria mucosa, Haemophilusparainfluenzae
6.	Anaerobes 1	Fusobacterium nucleatumssnucleatum, Campylobacter rectus, Veillonellaparvula, Capnocytophagagingivalis,
		Peptostreptococcusasaccharolyticus, Gemellamorbillorum, Prevotellamelaninogenica, Leptotrichiabuccalis,
		Eubacteriumsaburreum, Corynebacterium matruchotii, Prevotellanigrescens/intermedia
7.	Anaerobes 2	Porphyromonasgingivalis, Selenomonasnoxia,
		Micromonas micros

Bacteria that can be inactivated by plasmas include [79,80]

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Plasma in Dental Cavities

Plasma can treat and sterilize irregular surfaces by making them suitable for decontaminating dental cavities without drilling. Although, plasma itself is superficial, the active plasma species production can easily reach inside 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 [8]. Goree et al., provided substantial evidence that nonthermal atmospheric plasmas killed Streptococcus mutans, a grampositive cariogenic bacterium [81]. Sladek et al., studied the interactions of the plasma with dental tissue using a plasma needle [8]. It is an efficient source of various radicals, which are capable of bacterial decontamination; but, itoperates at room temperature and does not cause bulk destruction of tissue. Raymond EJ et al., studied the interactions of the plasma with dental tissue using a plasma needle. Cleaning and sterilization of infected tissue in a dental cavity or in a root canal can be accomplished using mechanical or laser techniques. However, with both approaches, heating and destruction of healthy tissue can occur. A plasma needle is an efficient source of various radicals, which are capable of bacterial decontamination; however, it operates at room temperature and thus, does not cause bulk destruction of the tissue. From his research, he concluded that plasma treatment is potentially a novel tissue-saving technique, allowing irregular structures and narrow channels within the diseased tooth to be cleaned [82].

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 [83,84].

Root Canal Sterilisation:

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. When He/O2(20%) is used as working gas, the rotational and vibrational temperatures of the plasma are about 300 K and 2700 K, respectively. The peak discharge current is about 10 mA. Preliminary inactivation experiment results showed that it can efficiently kill Enterococcus faecalis, one of the main types of bacterium causing failure of root-canal treatment in several minutes [85]. Pan et al., investigated the feasibility of using a cold plasma treatment of a root canal infected with Enterococcus faecalisbiofilms in-vitro. It was concluded that the cold plasma had a high efficiency in disinfecting the Enterococcus faecalis biofilms invitro dental root canal treatment.

Use of Plasma in Composite Restorations

Preliminary data has also shown that plasmatreatment increases bonding strength at the dentin composite interface by roughly 60 percent, and with that interface-bonding enhancement to significantly improve composite performance, durability, and longevity. 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 porous surface that the adhesive can infiltrate, current preparation techniques etch and demineralize dentin. Interactions between demineralized dentin and adhesive gives rise to the smear layer, which 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.

Dr. Wang and his colleagues investigated the plasma treatment effects on dental composite restoration for improved interface properties and their experimental 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. According to Dr. Wang, using plasma etching promises such a result and their study waspresented at a symposium. [86].

Yavrich et al studied the effects of plasma treatment on the shear bond strength between fiber reinforced composite posts and resin composite for core buildup and concluded that plasma treatment appeared toincrease the tensile-shear bond strength betweenpost and composite. [87].

Lee et al in their study concluded that tooth bleaching is also enhanced by plasmas, mainly due to the *insitu* production of hydrogen peroxide. [88].

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 [89,90]. Park et al. suggested intrinsic whitening using a low-frequency plasma source and hydrogen peroxide [91]. 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 nonthermal, atmospheric pressure, helium plasma jet device was developed to enhance the tooth bleaching effect of hydrogen peroxide (H2O2). Combining plasma and H2O2 improved the bleaching efficacy compared with using H2O2 alone. Tooth surface proteins were noticeably removed by plasma treatment. When a piece of tooth was added to a solution of H2O2 as a catalyst,

production of OH after plasma treatment was 1.9 times greater than when using H2O2 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[92,93].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[94,95]. The tooth bleaching method using atmospheric pressure plasma shows reasonable promise of becoming practical in the future.

6. Conclusion

The literature on plasma sterilization has been growing increasingly in the recent past. Based on the above evidence, we can say that plasma has a bright future in dentistry due to its antimicrobial properties, cell death properties on cells and multiple applications in various treatments. Plasma dental treatments are basically painless, drill-less, patient-friendly especially in children, under-served communities, where education and familiarity with the dentist's chair are limited. Although plasmatechnology isn't an end-all to all the techniques weperform, it could well become a valuable tool in dentistry in near future.

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