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REVIEW ARTICLE

PLASMA: A NOVEL BIOENGINEERING CONCEPT-A REVIEW

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ABSTRACT

Plasma is the fourth state of matter which comprises 99% of the universe. Plasma is cutting edge technology, which involves interdisciplinary partnerships in Medicine, Dentistry, Physics, Engineering, Chemistry and Biology. 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. Plasmadent is evolving into a dynamic field of research. Plasma technologies are very important in the modern Dentistry; we will try to focus on these technologies, their scopes and its broad interdisciplinary approach.

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INTRODUCTION

Plasma, referred to as the fourth state of matter. The other states of matter are solid liquid and gas. (Smitha *et al.*, 2010) Physical Plasma is defined as a gas in which part of the particles are present in ionized form. This is achieved by heating a gas which leads to the dissociation of the molecular bonds and subsequently ionization of the free atoms. Thus, Plasma consists of positively and negatively charged ions and negatively charged electrons as well as radicals, neutral and excited atoms and molecules (Mike Martin, 2009; Kong *et al.*, 2009) 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". (Mike Martin, 2009; Kong *et al.*, 2009) 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 (Kong *et al.*, 2009).

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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 (Roth, 1995; Roth, 2001; Hippler *et al.*, 2008). *Sir William Crookes* identified the fourth state of matter in 1879, but it was not called "Plasma" until Irving Langmuir, an American chemist, applied the name in 1929. As the most common form of matter, making up more than 99 percent of the visible universe, Plasma is a collection of stripped particles. When electrons are stripped from atoms and molecules, that particles change state and become plasma. Plasmas are naturally energetic because stripping electrons takes constant energy. If the energy dissipates, the electrons reattach and the plasma particles become a gas once again. (Roth, 1995). 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 star is white hot. Other well-known Plasmas include lightning, neon signs, and fluorescent lights. Outside of a container, plasma resembles gas—the particles don't have a definite shape.

But unlike gas, magnetic and electric fields can control Plasma and shape it into useful, malleable structures. (Mike Martin, 2009)

Mechanism of 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 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) sub microsecond pulsed direct-current (dc) power supply (amplitudes of up to 10 kV, repetition rate of up to 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 of R is too small or the capacitance of C is too large, there is feeling of weak electric shock when the plasma is touched by a human. 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 He, Ar, or their mixtures with O2 can be used. The gas flow rate is controlled by a mass-flow controller12. 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. 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 the application of root-canal disinfection. (Conrads *et al.,* 2000; Hoffmann *et al.,* 2013; Lerouge *et al*., 2001; Moisan *et al.,* 2001)

Methods of Production

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. Usually, the discharge needed to produce CAP is induced electrically. Some methods used to produce CAP include: Dielectric Barrier Discharge (DBD), Atmospheric Pressure Plasma Jet (APPJ), Plasma Needle, and Plasma Pencil. (Conrads *et al.,* 2000; Hoffmann *et al.,* 2013)

Applications in Dentistry

Plasmas have been used extensively for fabricating semiconductor devices, modifying the surfaces of materials, sterilization, and other applications. (Mike Martin, 2009) Dental applications have emerged because a new version of Plasma technology, so called "non-thermal atmospheric Plasmas," permits surface preparation in open air at room temperature. A high-tech company focusing on Medical devices in Dental, Orthopedic, and Cardiovascular areas has developed a hand held Plasma apparatus that can be used by dentist for multiple Dental clinical applications. The apparatus applies small amounts of electricity to a non-toxic gas through a "narrow slit chamber" to generate Plasma with a brush-like shape. They have created a tiny Plasma scrub brush that resembles a white-hot flame. But this flame is cool to the touch. It glides across the surface of the tooth, killing bacteria and preparing the dentin and enamel, and it limits the acid etching, mechanical whirring, and phobias typically associated with a drill in the mouth. (Moisan *et al.,* 2001)

Sterilization by eradication of bacteria: (Laroussi, 2002; Whittaker *et al.,* 2004; Sung *et al.,* 2013 YangHong *et al.,* 2013)

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 sterilization .The mechanism of Plasma sterilization is related to the abundance of Plasma components, like reactive oxygen species, ions and electrons, and UV and electromagnetic fields. Also, Plasma can affect not only the contacted point but also the area around it. Plasma sterilization has been used to treat Dental diseases. 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. *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. 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 evaluated by *Su-Jin Sung et al.* It was proved that the atmospheric pressure non thermal 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.

Plasma in Dental cavities: (Kong *et al.,* 2009; Sladek *et al.,* 2004; Goree *et al.,* 2006; Sladek and Stoffels, 2005)

Goree et al. provided substantial evidence that non thermal atmospheric Plasmas killed *Streptococcus mutans*, a gram-positive cariogenic bacterium. *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. *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.

Effects of CAP on malignant cells: (Laroussi, 2002; Socransky *et al.,* 1994; Sladek *et al.,* 2006; Sensenig *et al.,* 2011)

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. 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 removal of the tumor, chemotherapy and/or radiation. Nevertheless some cancers remain hard to eradicate. 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.

Biofilms 1, (Smitha and Chaitanya Babu, 2010; Sladek *et al.,* 2006; Socransky *et al.,* 1994)

Biofilms develop on tooth and oral mucosa, cause caries, periodontal diseases, and oral mucositis, which can also lead to inflammation around Dental implants. *Rupf et al.* demonstrated that combination treatment with plasma and a non-abrasive air/water spray is suitable for the elimination of oral biofilms from microstructured titanium used in dental implants. *Koban et al.* showed that the treatment of dental biofilms composed of *Streptococcus mutans* with non-thermal plasma was more efficient than the treatment with chlorhexdine *in vitro* .CAP was also effective in destroying biofilms either on root canals or on dental slices. *Jiang et al.* developed a plasma plume at room temperature. They used it to disinfect root canals from extracted human teeth.

Root Canal Sterilization: (Sensenig *et al.,* 2011; Koban *et al.,* 2010)

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. 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.

Intraoral diseases: (Yamazaki *et al*., 2011; Ohl and Schröder, 2008)

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.

Use of plasma in composite restorations: (Smitha and Chaitanya Babu, 2010; Roth, 2001; Laroussi, 2002; Kong *et al*., 2011)

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. Current clinical practice relies on mechanical bonding when it should rely on chemical bonding. The smear layer which is a 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. *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. *Ritts et al.* investigated a non-thermal atmospheric plasma brush on Dental composite restoration . It was observed that atmospheric cold plasma brush (ACPB) treatment could modify the dentin surface and increase dentin/adhesive interfacial bonding.

Post and Core

Yavirach 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 increased the tensile-shear bond strength between post and composite (Sun *et al.,* 2010)

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. *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_2improved 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. **(**Sun *et al.,* 2010; Park *et al.,* 2011; Kim *et al*., 2012; Nam *et al*., 2013; Claiborne *et al.,* 2013**)**

Use of plasma in Sports Medicine

Biologic Engineering is a rapidly evolving field in Sports Medicine and Orthopedic surgery. The ideal biologic tool would be effective, simple to use, inexpensive, safe and available immediately at the point of care. Platelet rich plasma (PRP) meets many of these criteria. Platelet rich plasma (PRP) is a powerful new biologic tool in sports medicine. PRP is a fraction of autologous whole blood containing and increased number of platelets and a wide variety of cytokines such as platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and transforming growth factor beta-1 (TGF-B1), fibroblast growth factor (FGF), Insulin-like growth factor-1 (IGF-1) among many others.[1,5,21]

Platelet rich plasma in implant dentistry (Claiborne *et al*., 2013; Allan Mishra *et al*., 2012)

Platelet-rich plasma (PRP) in dental and oral surgery: from the wound healing to bone regeneration Implant dentistry entails surgical reconstruction of localized alveolar defects to improve quality and quantity of the host bone using ewer materials with better osseous regenerative properties (Osseoconduction & Osseoinduction). Guided bone regeneration is an accepted surgical method to help achieve this goal. Platelet rich plasma (PRP) is rich in growth factors like a) PDGF b) TGFβ c) PDEGF d) IGF1, derived from autologous blood. PRP is being used nowadays to derive these factors in high concentrations to sites requiring osseous-grafting, prior to or in conjunction with implant placement. Advantages include better handling of the graft material (alloplastic/autogenous) in the presence of an adhesive medium aiding in compaction, increased rate of collagen matrix synthesis, increased rate of bone deposition & quality of bone achieving benefits without risk of infection or disease transmission obvious ease of procurement &preparation. Since PRP is free from potential risks for patients, not difficult to obtain and use, it can be employed as a valid adjunct in many procedures in oral and Dental surgery.

Drug delivery (Nam *et al.,* 2013; Claiborne *et al.,* 2013)

Plasma surface modification provides sufficient adherence to metallic and polymeric materials for the binding of drug molecules. The bioabsorbable materials can act as drug carriers by controlling the release rate of the drug initially loaded in an application for drug delivery systems.

Tissue engineering

Artificial materials are of growing importance in medicine and biology. A modern scientific interdisciplinary field known as tissue engineering has been developed to design artificial biocompatible materials to substitute irreversibly damaged tissues and organs. Cells can sense the physical properties and chemical composition of these materials and regulate their behavior accordingly (Bačáková *et al.* 2004). (Nam *et al.,* 2013; Claiborne *et al.,* 2013; Allan Mishra *et al.,* 2012)

Conclusion

The plasma brush may serve as an extremely valuable tool among dentists in Less-serviced communities, where individuals do not readily obtain proper dental care. Plasma will create a revolution in dental treatment; procedures without shots and pain, teeth whitening without sensitivity, painless cavity preparations can change a lot for patients, dentists and might represent a solution in dental phobia, many studies focus on this technology and how to make the best benefits from it.

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