

Applications Of Cold Atmospheric Pressure Plasma In Dentistry- A Review

ABSTRACT

Plasma is one of the most dominant state of matter in the universe, which was identified by Sir William Crooke in 1879. It is an electrically conducting medium that responds to electric and magnetic fields. It implies studies concerning the direct action of low temperature, the effect of low atmospheric plasma pressure on body tissues for various non invasive therapeutic treatments or diagnostic purpose. Plasma consists of large quantities of highly reactive species, such as ions, energetic electrons, excited atoms and molecules, ultraviolet photons in the meta-stable state and active radicals. Research has revealed promising and successful medical applications of these non thermal cold plasma- Reactive oxygen species. The purpose of this review article is to highlight the various dental applications of cold atmospheric pressure plasma (CAPP) which includes- dental caries prevention by bacterial inactivation; prevention of oral cancer by selective destruction of the tumour cells and damaged tissue repair effects; for tooth bleaching or whitening procedures; in restorative dentistry for placement of composite restorations; in Endodontic therapy for root canal disinfection and for the treatment of Oral candidiasis.

Keywords: Cold Atmospheric Pressure Plasma; Antimicrobial Agent; Plasma Medicine; Dentistry

1. INTRODUCTION

Apart from the three states of matter namely solid, liquid and gaseous state, Plasma belongs to the metastable state of matter comprised of a gaseous mixture of high energy protons, electrons, reactive oxygen species, high energy ultraviolet photons at varying densities and different temperatures [1].

Gas molecules can be heated or exposed to strong electromagnetic field to as to generate plasma radiation. Plasma is produced by passing high intensity irradiation such ultraviolet rays, microwaves through gaseous molecules like oxygen, nitrogen, helium and argon in the presence of an electrical field [2]. Plasma technology forms the basis for the working of computes, mobile phone and various display panels. The successful biomedical applications of plasma irradiation has been proved through research. The first application of plasma in dentistry occurred in the manufacture of dental instruments while disinfecting them. Eva Stoffels was the first to investigate the therapeutic applications of plasma in dentistry which can be subdivided into two principal approaches: one is the use of plasma technology for the

treatment of surfaces, materials or devices, and the other is the direct plasma application on the human body for therapeutic purposes like tooth bleaching, root canal disinfection etc [3].

2. CLASSIFICATION OF PLASMA

On the basis of relative temperatures of the ions, neutrons, and electrons, plasmas are categorized as “thermal” or “non-thermal”.

2.1 Thermal Plasma:

This represents thermally balanced high intensity electrons and ions, a part of the natural phenomena. Hot plasma techniques in the form of electrosurgery and coagulation have been used in medical sciences for a long time to achieve hemostasis.

2.2 Non Thermal Plasma (NTP):

Low temperature plasma is also called as cold plasma , is used in modification of biomaterial surfaces. It is created by low conversion of a compound into gas followed by applying ionization energy in the form of heat, direct or alternating electric current , radiation or laser light. Oxygen, nitrogen, hydrogen or argon are the commonly used plasma gas sources [4].

3 GENERATION OF PLASMA:

In the last decade, atmospheric pressure plasmas have become a very attractive tool for material processing applications because they are generated in an open environment and can be easily implemented in online processing. The deleterious effects of hot atmospheric plasma include permanent necrosis of healthy tissues and damage to heat sensitive materials [5] [Figure 1].

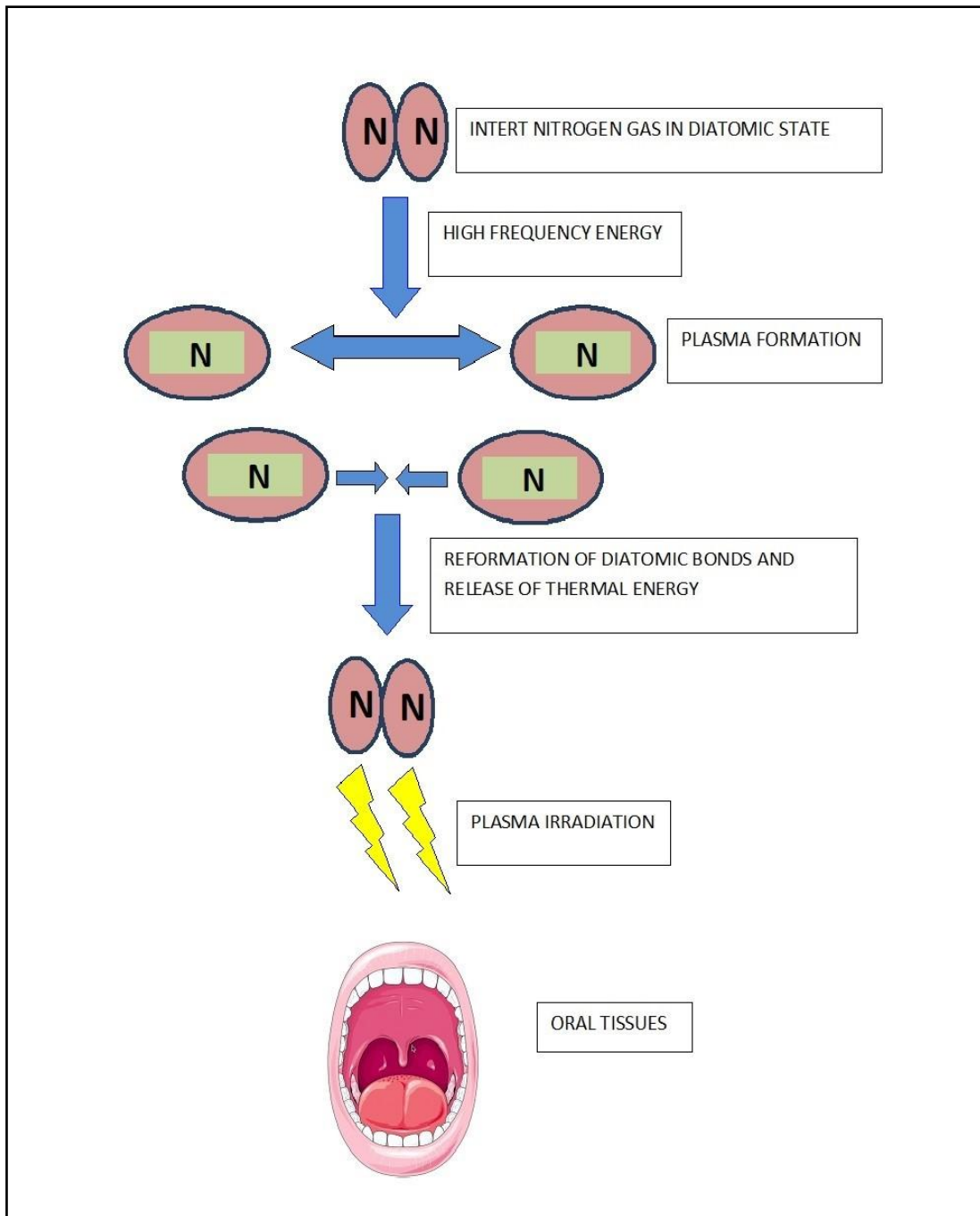


Figure 1 - Nitrogen gas being acted upon by high frequency energy to cause dissociation of gas by stripping of electrons and generation of plasma

4 DEVICES PRODUCING PLASMA:

Electric energy in the form of direct current or alternating current, microwaves and high frequency radio-waves can be used for generating plasma. When the electrons in plasma are displaced from their equilibrium positions, strong electric fields are created between the

negatively charged layers and the background positive layers. These electric fields tend to restore the initial neutral condition by bringing the particles back to their original positions. As a result, the more mobile electrons oscillate with a certain frequency known as the plasma frequency. When these oscillations propagate, the electron plasma wave, also known as the Langmuir waves are created. This is a high-frequency wave which is electrostatic in nature.

4.1 DIELECTRIC BARRIER DISCHARGE

Experiments on Dielectric Barrier Discharge (DBD) was first conducted by Siemens in 1857. DBD has many applications including: sterilization of living tissue, bacteria inactivation, angiogenesis, surface treatment and excimer formation [6].

The dielectric barrier discharge (DBD) consists of two flat metal electrodes that are covered with dielectric material. The two electrodes used include an high voltage electrode (that generates alternating current with power consumption upto 100 W) and a grounded electrode. Plasma is created by the movement of a carrier gas between these two electrodes [6]. The concept of the floating electrode DBD (FE-DBD) was introduced by Friedman et al. This employs the use of an insulated high voltage electrode and an active electrode. In this device the second electrode is active (it can be human skin, a sample, and even an organ) and it is not grounded. The distance between the two electrodes must be less than 3 mm to create the discharge. This device is effective on endothelial cells hence has various biomedical applications like haemorrhage control and treatment of melanoma [7].

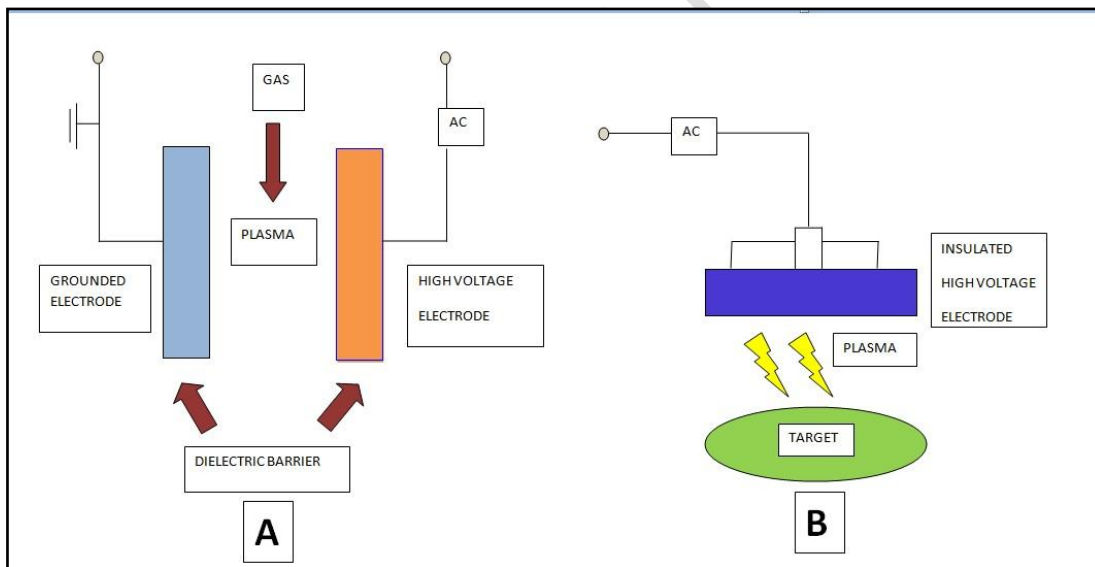


Figure 2: Dielectric Barrier Discharge & Floating Electrode Dielectric Barrier Discharge. (A) Formation of Plasma by the dielectric barrier (DBD); (B) Floating electrode dielectric barrier discharge (FE-DBD).

The temperature of the gas in DBD is close to room temperature, which makes it adequate for biological applications. However FE-DBD cannot be used for plasma irradiation inside root canals and internal organs.

Atmospheric pressure plasma jets (APPJs) represents plasma irradiation created by dielectric enclosure (tube or syringe). The expelled plasma is called as plasma plume that is directed towards the target tissue. Figure 2 is a schematic representation of the APPJ

concept. More details about APPJs and their characteristics can be found in some recent research papers [8].

Depending on the jet's operating conditions, the plasma plume tip can be maintained below 40°C, enabling the contact with living tissues without any risk of burns and electric shock. Thus, cold atmospheric pressure plasma (CAPP), such as FE-DBD and APPJ, have been appointed as the most promising tools for biomedical and hospital applications [7,8].

5. MECHANISM OF ACTION

The mechanism of action of plasma is based on release of free radicals and reactive species (e.g., reactive oxygen and nitrogen species, i.e., ROS and RNS), Metastables and UV Light. These radicals control the cell redox signaling pathway. Electrons get accelerated by the presence of an electromagnetic field at a very faster rate than heavy ions. Accelerated electrons results into ionization of particles, radiation, and creation of reactive species. Common gas sources used for the production of plasma are argon, hydrogen, oxygen, or nitrogen. It is useful for wound sterilization and wound healing, causes cell death of tumour cells, but their high concentration damages surrounding healthy cells. Research has showed that non-thermal plasma generates ozone. This ozone in aqueous media further generates biologically active ROS and RNS [9].

6. APPLICATIONS OF PLASMA IN DENTISTRY

Multiple dental applications have emerged because a new version of plasma technology, the "non-thermal atmospheric plasma's," permits surface preparation in open air at room temperature. One of the important features of non-thermal plasma is the abundant production of reactive oxygen species, reactive nitrogen species, meta-stables in low gas temperature along with charged particles, radiation [10].

The dental applications of physical plasma can be sub divided into two principal approaches:

A) **Surface Treatment** of materials or devices to enhance specific qualities for subsequent special applications including disinfection. Surface treatments made use of NTAPP in the form of chairside applications. These include the following:

- Surface modification of Implants
- Plasma Cleaning and sterilization of Dental Instruments
- Enhancing adhesive qualities

B) **Direct Plasma Application** in the human body for therapeutic purposes includes the following:

- Microbicidal activities
- Treatment of Dental caries
- Root canal disinfection
- Tooth Bleaching
- Oncology
- Intra Oral Lesions

6.1 SURFACE TREATMENTS OF PLASMA

6.1.1 MODIFICATION OF THE IMPLANT SURFACE TO IMPROVE OSSEOINTEGRATION:

The modification of implant surface is being carried out using plasma technology to improve osseointegration. Since the implant surface is the first part to interact with the host attempts are made to hasten the early host-to-implant response focussing on rapid bone healing. Plasma treatment is capable of improving cell adhesion by changing surface roughness and wettability. A chairside operating NTAPP immediately prior to implant placement was also reported, which stated that plasma treatment reduced the contact angle and supported the spread of osteoblastic cells [11].

6.1.2 FIBRE REINFORCED COMPOSITE POST:

Aesthetic Dentistry employs the use of Fiber-reinforced composite (FRC) posts for rehabilitation and for core build up of grossly decayed teeth. Plasma irradiation enables to achieve "monoblock", reliable adhesion between FRC posts and resin composites is essential. However, the highly cured and cross-linked matrix of FRC posts disturbs effective adhesion to resin cements or resin composite core materials. The surface treatment of fibre posts with plasma improves the hydrophilicity of epoxy polymers due to oxygen containing functional groups which improve the humectation that is wetting of post surface along with changing the chemical composition of the surface. Plasma treatment has various effects on the surfaces of polymers, such as removal of organic contaminants and weak boundary layers by cleaning and ablation; degradation of the polymer chain; formation of radicals on the surfaces; creation of a thin crosslinking layer; and formation of chemical groups on the stabilized surfaces. These effects result in acid-base interactions and covalent linkages [12].

6.1.3 WOUND STERILIZATION:

Investigation has been done using scanning electron microscope (SEM) to visually inspect the impact of plasma exposure to the microbial morphology. Laroussi et al showed that after exposure to plasma, E.coli (Gram negative) cells underwent severe morphological changes such as lysis. While in B.Subtilis (Gram positive) showed no morphological changes in the cell while there is reduced cell viability causing cell un-culture [13] [Figure 3].

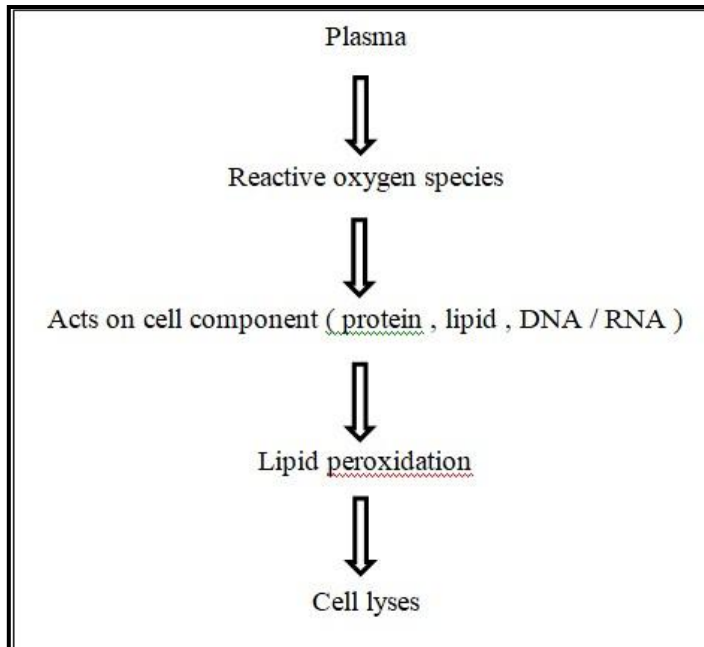


Figure 3 – Schematic representation of the effect of plasma irradiation on bacterial cells.

6.1.4 STERILIZATION OF DENTAL INSTRUMENTS :

Recent reports of contamination on sterilized endodontic files are of utmost concern due to close contact of these instruments with peripheral tissues. Rare group of fatal human diseases include: familial , sporadic & acquired Creutzfeldt – Jakob disease (CJD). It is characterized by accumulation of an abnormal form of prion protein in the central nervous system which is also seen in the dental pulp . Thus there is an increased risk of CJD transmission in humans by dental instruments such as endodontic files, which comes in intimate contact with the peripheral tissues. In this condition use of gas plasma irradiation is extremely beneficial in reducing the proteinaceous materials which can be transferred between endodontic files & patients, when the instruments are reused [14,15].

6.1.5 PLASMA CLEANING:

The mechanism of plasma based sterilization is directly related to the production of ROS, composition of the carrier gas, frequency of the plasma irradiation and the virulence of the bacterial strain. Plasma irradiation has proven to be more effective than conventional non-thermal methods such as UV sterilization, as the former leaves no residue. Its advantages include: lack of toxic residue, reduced turnover time, and applicability on moisture-sensitive instruments. In one experiment, using diamond burs and silicone impression materials, the colony – forming unit was significantly reduced for both E. coli and B. subtilis after treatment with atmospheric pressure non-thermal air plasma. Cross-contamination between the dental instruments and the patients; and transmission of infection from dental impressions to the lab technicians can be prevented by exposure to high intensity plasma free radicals [16].

6.1.6 ENHANCING ADHESIVE QUALITIES:

Adhesion is the critical factor in improving the performance of dental composites. Optimal adhesion is achieved with good wettability when the adhesive material spreads impulsively across the entire adherend surface. Plasma irradiation enhance adhesive bonding by creating micro-structural and chemical modifications on the etched enamel and dentin

surface forming a thin plasma coating improving bonding with composites. Atmospheric cold plasma brush (ACPB) treatment can enhance adhesive qualities [17].

6.2 DIRECT APPLICATIONS:

6.2.1 MICROBICIDAL ACTION:

Biofilms formed over the tooth surface lead to dental caries, gingival and periodontal diseases, and oral mucositis. These biofilms can also affect dental implant by causing peri-mucositis and peri-implantitis. NTP has the ability to destroy biofilm matrix without causing any damage to the oral tissue and also the transmission of heat to the dental pulp is minimal. Koban et al. in his in-vitro study found that NTP is more efficient in killing of bacteria present in the dental biofilm than chlorhexidine [18]. The bacterial cell membrane is made up of lipid bilayer, 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 Hydroxyl ions. Hydroxyl radicals generated by plasma along with other free radicals destroy membrane lipids and thereby destroy the bacteria.

6.2.2 DENTAL CARIES :

Plasma can treat and sterilize irregular surfaces; making them suitable for decontaminating the dental cavities without drilling. The plasma releases radicals that helps in bacterial decontaminating which doesn't cause bulk destruction of tissue. Smith et al., studied the interactions of the plasma with dental tissue using a plasma needle. Plasma irradiation reaches the entire depth of the prepared cavity very easily. Firing low plasma temperature beams on dentin, reduces the amount of caries producing bacteria [19].

6.2.3 CAVITY PREPARATION:

Cold plasma has great potential for use in dentistry as it is vibration free, leading to lesser pain perception by the patient. It is helpful in dealing with patients suffering from anxiety and fear of the drill use for cavity preparation prior to the restoration of teeth and removal of necrotic, infected, and non-remineralizable tissues. Plasma can treat and sterilize irregular surfaces by making them suitable for decontaminating dental cavities without drilling. Eva Stoffels proposed plasma irradiation through plasma needles has bacteriocidal effect on *Escherichia coli* causing cell wall membrane lysis and bacterial DNA damage [19].

Advantages of plasma needle compared to conventional cavity preparation includes the following: bacteriocidal action, minimal tooth preparation preventing bulk removal of tooth structure, easy access and disinfection of small pit and fissure carious lesions and low temperature of operation thereby temperature increase in the pulp is prevented.

Argon plasma brush discovered by Yang et al. was found to be very effective in deactivating *Streptococcus mutans* within 15 seconds and *Lactobacillus acidophilus* in 5 minutes[20].

6.2.4 ROOT CANAL DISINFECTION:

The root canal system is known for its anatomical complexities such as accessory canals, lateral canals, isthmuses, ramifications, apical deltas, canal curvatures etc. It has been reported that bacteria such as *Enterococcus faecalis* can invade the dentinal tubules as deep as 500–1000µm. Eliminating the residual micro-organisms especially within the biofilm is the challenging task and clinical investigations showed that persistent endodontic infections with failure of endodontic therapy occurred when conventional disinfections were performed.

Pan et al proved that the cold plasma had a high efficiency in disinfecting the *Enterococcus faecalis* biofilms in his in-vitro study [21].

NTP as a gas phase has the capability of reaching deep into the complex canal system establishing direct contact with bacteria which is impossible with conventional methods. Several studies suggested that ROS plays the most crucial roles in bacterial inactivation. Lu et al. developed a plasma jet device, named „Model RC-1“, using a medical syringe and a needle. The device was powered by an 8 kV, 500 ns pulsed direct current at a pulse frequency of 10 kHz. The gas mixture of 80% He and 20% O₂ was injected into the syringe at a flow rate of 0.4 slm. The needle served as an electrode and had an inner diameter of ~200 μm. Therefore, the needle could be inserted into the root canal and generated a very narrow plasma plume producing effective root canal disinfection [22].

6.2.5 TOOTH WHITENING:

Bleaching with Hydrogen peroxide (H₂O₂) is one the treatment procedures performed in regular clinical dental practice. NTP is recommended for teeth bleaching.

Lee et al proved that application of an atmospheric pressure plasma jet device resulted in the release of hydroxyl radicals and removed the surface proteins thereby improving the efficacy of hydrogen peroxide.

Park et al. suggested removal of intrinsic stains using a low-frequency plasma source and hydrogen peroxide [23]. Another approach, by Kim et al used liquid plasma produced by radio frequency (RF) driven gas–liquid hybrid plasma system [24]. Hamid et al. investigated the efficacy of tooth bleaching using non- thermal atmospheric pressure plasma (NAPP) with 15% carbamide peroxide including 5.4% hydrogen peroxide, as compared with conventional light sources. It was observed that the NAPP has a greater capability for effective tooth bleaching than conventional light sources with a low concentration of hydrogen peroxide without causing thermal damage [25].

6.2.6 ORAL ONCOLOGY:

In addition to surgical approaches, the stereotactic body radiotherapy associated with smart drug delivery systems (SDDSs) have been proposed to oral cancer therapy. Immunotherapy has also advanced considerably in the last years. However, those new discoveries involve expensive treatments and ultra-expensive drugs. For this reason, the search for alternative is needed.

The application of CAPP in oral oncology is due to its ability to generate ROS and RNS causing selective destruction of the malignant tumour cells.

The role of oxidation/reduction potential is already understood as key factor for the progression and establishment of the disease based on the HOCl or the ·NO/ONOO– signalling pathway. In this way, NO and nitrite therapies have already been applied as anti-cancer agent based on their effects on cancer cells and on catalase-dependent apoptotic pathways, which are implied in development and regression of the disease [26,27].

A list of the functions of the CAP mechanisms in cancerous cells:

1. Activating P53
2. Activating inhibitor P21CDK
3. Phase cell cycle arrest at G₂ / M and S phase
4. Release of ROS, DNA destruction and preventing cell replication.

5. Stimulating apoptosis through mitochondrial ROS production and turn the mitochondria
6. Decrease in mitochondrial membrane potential, decreases mitochondrial enzyme activity and a decrease in cellular respiration in cancerous cells.
7. The concentration changes of ROS, NO and intracellular fluid lipid peroxide.

Han et al reported that N2-CAPP induced DNA damage in SCC-25 oral cancer cells [28]. The effect on head and neck squamous cell carcinoma (HNSCC) was also detected [27]. CAPP can also be an alternative for the treatment of oral lichen planus, a precancerous lesion. Interestingly, cancer cells such as SCC-15 and HNSCC were more sensitive to CAPP when compared to non-cancer cells lines.

6.2.7 TREATMENT OF ORAL CANDIDIASIS

Oral candidiasis is an opportunistic disease with high prevalence in immune-compromised patients. Reports on refractory cases of oro-pharyngeal candidiasis are increasing and the treatment of these cases is challenging due to the increase of antifungal resistance . Proton ATPases, efflux pumps, adherence, morphogenesis, and resistance to oxidative stress have posed as new targets to the development of novel antifungal agents. CAPP showed modulatory antifungal effects on *Candida albicans* virulence factors, such as adhesion, filamentation and suppression of ergosterol biosynthesis which was scientifically proved on murine model in vivo [29].

7. LIMITATIONS:

CAP also has limitations as this is a new technology; safety of the equipment is of utmost concern.

1. Difficulty in portability and transporting the instrument for dental use.
2. High technique sensitivity.
3. Efficiency is compromised by old previous amalgam restoration.
4. Spores cannot be inactivated due to the limited depth of penetration of cold plasma.
5. High Cost of the equipment, challenges in marketing, difficulty in maintenance and availability are some issues of major concern.

8. CONCLUSION:

The scope CAP in dentistry is enormous due to its multiple applications and microbicidal properties. It can be used in almost all the branches of dentistry. However, further investigations are necessary to gain in depth knowledge about the mechanism. Plasma dental treatments are painless, drill-less and patient-friendly.

A deeper understanding of biological mechanisms of plasma- cell interaction mechanisms is being pursued.

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UNDER PEER REVIEW