

# Low temperature atmospheric plasma for microbial decontamination

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Low temperature Plasma (LTP) is a high-energy gas that is created when an electrical current is passed through a gas. Until recently, plasmas could only be created at relatively high temperatures in a vacuum and the use of plasma on sensitive materials such as human tissue, food products, medical devices and the packaging industry, was therefore impractical. However, over the last few years technological breakthroughs have made it possible to produce low temperature plasmas under atmospheric conditions providing many advantages. Plasma discharges with a higher oxygen concentration have been associated with increased levels of microbial survival inhibition due to oxygen based active species, atomic oxygen and ozone. Low temperature plasmas have shown success in decontamination of a wide range of microorganisms including bacteria, fungi and algae and has even shown success in damaging bacterial spores. These factors have brought this exciting new, emerging technology to the forefront of novel antimicrobial techniques.

**Keywords** non thermal plasma; decontamination; antimicrobial; cold plasma

## 1. What is Plasma ?

Although plasma was first described by Sir William Crookes in 1879 [1]. The name “plasma” was first used by Irving Langmuir in 1926 who described this state of matter as: “... near the electrodes, where there are *sheaths* containing very few electrons, the ionized gas contains ions and electrons in about equal numbers so that the resultant space charge is very small. We shall use the name *plasma* to describe this region containing balanced charges of ions and electrons...” [2]. Later, the definition was broadened to define a state of matter in which a significant number of atom and/or molecules are electrically charged or ionised. Plasma is considered as a distinct state of matter due to its properties; it does not have a regular shape or volume and it can form filaments and/or beams under magnetic fields. Depending on the method of generation used, the plasma can display a broad spectrum of states ranging from extreme nonequilibrium to almost complete thermal equilibrium.

Plasma can be found in the form of natural phenomena such stars and lightening or man-made as in the production of fluorescent and neon lights, plasma television etc. The research areas of plasma technology is fast growing and has been particularly studied for its use on bio-medical materials and devices [3, 4, 5], surface modification of textiles [6], removal of chemicals on surfaces of devices manufactured from heat sensitive materials [7], water sterilization [8] and more recently wound healing and food decontamination [9-10].

### 1.1. Non Thermal Atmospheric Plasma

Plasmas can be divided into two groups depending on the method of generation as non-thermal plasma (NTP), and thermal plasma (TP). NTP consists of gas molecules with moderate temperatures and electrons with higher temperatures whereas in TP the electrons and gas temperatures are several thousands of Kelvin and these species are found in equilibrium [11]. NTP is also known as cold and non-equilibrium plasma with regards to the energy level, temperature and ionic density.

An important aspect in the use of NTP for decontamination, is the ability to be effective, without affecting the material being decontaminated. This is possible due to the weakly ionized nature of the cold plasma discharge. The antimicrobial efficacy of NTP has been related to the specific type of plasma technology used including; the power level used to generate the plasma, the gas mixture in the plasma emitter, the intensity and length of exposure, design of the system, flow rate and pressure.

Cold or non thermal Plasma (NTP) is a high-energy state of a gas when an electrical current is allowed to pass due to high voltages. Until recently, plasmas could only be created in vacuum systems making its use for decontamination or sterilization of sensitive materials impossible. However, over the last few years technological developments have made it possible to produce cold plasma under atmospheric pressures providing cost effective systems [12].

The ability of plasma to work at moderate operating temperatures has shown to result in minimal to almost no thermal damage to materials being processed. This property has opened up the possibility of using NTP for disinfection and sterilization of heat sensitive materials where thermal plasma can not be used [13-15]. To date several types of NTP's that work at atmospheric pressure have been described for biodecontamination purposes ( Fig.1), radio Frequency (RF) torches, dielectric barrier discharges (DBD), corona discharges and gliding arc discharge plasmas. The general advantage of these techniques is their capability to decontaminate heat sensitive materials at both atmospheric pressures and at moderately low temperatures. Furthermore, when NTP's are applied with a gas an abundant variety of reactive species is produced combining photonic, electric and chemical events [16]

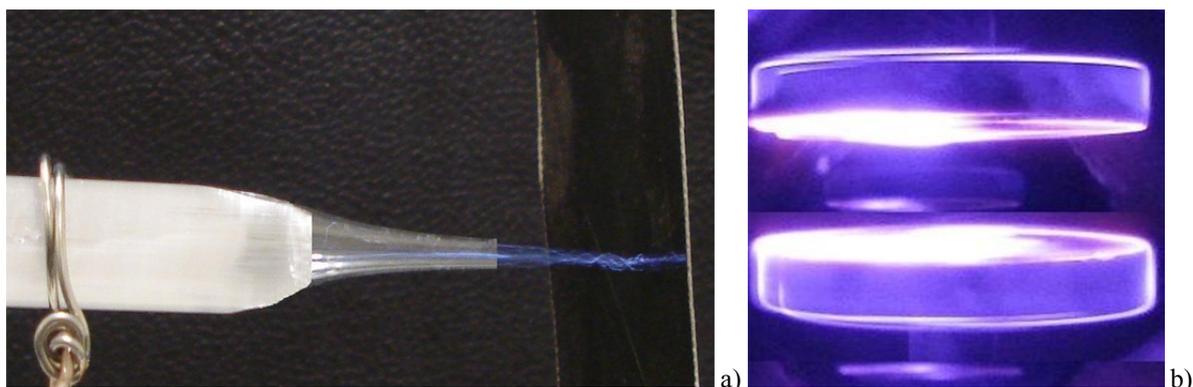


Fig. 1 a) the atmospheric pressure plasma torch, b) the dielectric barrier plasma discharge

## 2. Decontamination and sterilization mechanism

Atmospheric pressure NTP has been observed to have a killing effect on many kinds of microorganisms and is therefore becoming the technology of choice for decontamination purposes. Studies have outlined three basic mechanisms attributed to cell death by plasma. These are: cell surface etching induced by reactive species formed during plasma generation, volatilization of compounds and intrinsic photodesorption of ultraviolet (UV) photons and the destruction of genetic material. These deactivation mechanisms cause several reactions in bacterial cells including lipid peroxidation of poly-unsaturated fatty acids, oxidation of amino acids and DNA oxidation [17-19].

Although a sterilizing effect can be caused by UV radiation produced which acts by disturbing the genetic material of microorganisms [20], several studies have shown that the UV radiation produced by NTP's does not play a major part in inactivation of microorganisms [21-23].

Instead it is the effect of reactive species produced by NTP's that are believed to be the major cause of microbial cell death. The reactive species in non-thermal atmospheric plasmas have been found to be nitrogen and oxygen based species such as atomic oxygen, ozone, nitrogen oxide and hydroxyl. These active species play a crucial role in the microbial inactivation mechanism, since they directly interact with the bacterial membrane. These active species have short lives in the gas phase thereby disappearing in milliseconds after they are produced and can also dissolve in liquids [8].

The main chemical reactions leading to the production of reactive species in atmospheric plasma discharges are shown in Table 1. [24]. These reactive species have higher sterilization and chemical potentials than conventional chemical agents giving plasma its potential antimicrobial effect [18].

Table 1 Possible chemical reactions in the atmospheric plasma discharges

$e + O_2 \rightarrow 2O + e$
$O + O_2 \rightarrow O_3$
$O_3 + H_2O \rightarrow O_2 + H_2O_2$
$H_2O_2 \rightarrow 2 OH\cdot$
$e + H_2O \rightarrow H\cdot + OH\cdot + e$
$e + N_2 \rightarrow N\cdot + N\cdot + e$
$e + N_2 \rightarrow N_2\cdot + e$
$e + NO \rightarrow N\cdot + O\cdot + e$
$2H_2O \rightarrow H_2O_2 + H_2$
$OH\cdot, O_3 + M \rightarrow [\text{decomposed } M]$ where M is a macromolecular compound

Plasma discharges with a higher oxygen concentration have been associated with increased levels of microbial inhibition due to higher levels of oxygen based active species such as atomic oxygen and ozone [23]. Damage to membrane proteins and/or lipids occur because of oxidative stress. The active species formed during plasma discharge, hydroxyl (OH), singlet oxygen, ozone, and radicals can initiate lipid peroxidation thus producing shorter chain fatty acyl compounds. These products include alkanes, ketones, epoxides, and aldehydes [25-27]. The short chains of

charged fatty acids have a lower ability to rotate within the membrane and increase the fluidity of the membrane [27-28] which can result in distraction of structural membrane integrity.

The rates and biocidal effects of NTP have shown great variance based on the system used, material being treated, the bacterial strains and species. It is known that vegetative cells tend to be more susceptible to plasma application than spores, as is generally true of most sterilizing systems. Differences in efficacy of such systems have also been demonstrated regarding gram-positive and gram-negative bacteria [29], where gram-negative species have been seen to be more susceptible to plasma treatment than gram positive ones. This can be explained by the higher susceptibility of the Gram negative, thinner murein layer (~ 2 nm) compared to gram-positive bacteria (~ 15 nm) to the plasma produced reactive oxygen species.

The ability of non-thermal atmospheric plasmas to inactivate vegetative cells, spores, yeast, fungi [8] and biofilm formers [30-32] is clearly evident in the literature with the use of various NTP systems. The inactivation of *Streptococcus mutans* [33] and *Escherichia coli* [34] with the use of microplasmas further confirms the potential of this type of plasma in the areas of microsurgery and dentistry. The successful inactivation of *Listeria monocytogenes* from processed meat surfaces [35] and on cheese [36] by way of novel atmospheric plasma jet has added to the knowledge for the use of NTP's in food processing. Moreover, the successful inactivation and destruction of *S. aureus*, *Pseudomonas aureginosa* biofilms with gas-discharge plasmas [30,37] has provided a possible solution for the application of this technology to these sometimes, treatment resistant and less susceptible populations. Moreover, the use of plasma discharge has shown to be useful against spore forming, *Bacillus pumilus* and atmospheric plasma applied to *Bacillus subtilis* [38-39].

However, despite the obvious potential of atmospheric NTP technology the inactivation mechanisms of these different plasma systems are not yet fully understood. The variance in plasma source, process parameters and process gases used, results in differences in the produced reactive species, thus making inactivation mechanisms difficult to compare.

### 3. NTP applications

Cold plasma can be successfully used for the decontamination of products where micro-organisms are externally located. The ability of plasma to flow around objects, eliminating any 'shadow effects' does not occur ensuring all parts of the contaminated products are treated. NTP could also be used to disinfect surfaces before packaging or included as part of the packaging process [40]. Plasmas generated by electric discharge, similar to those used in fluorescent lighting tubes, have very efficient (80%) conversion rates from electricity to plasma. Although no actual detailed feasibility studies have been carried out, it is thought that the energy consumption of NTP would be similar to existing UV systems and therefore treatments would be highly cost-effective.

NTP, was first observed to be capable of killing bacteria by Laroussi [41] in 1996. Since then, NTP has been studied against a broad spectrum of microorganisms including those related to contamination of medical [42], oral [43], food and agricultural products [44-46]. The research and application areas of this technology are growing every day especially for plasma-based processing techniques and novel systems are being developed and patented worldwide.

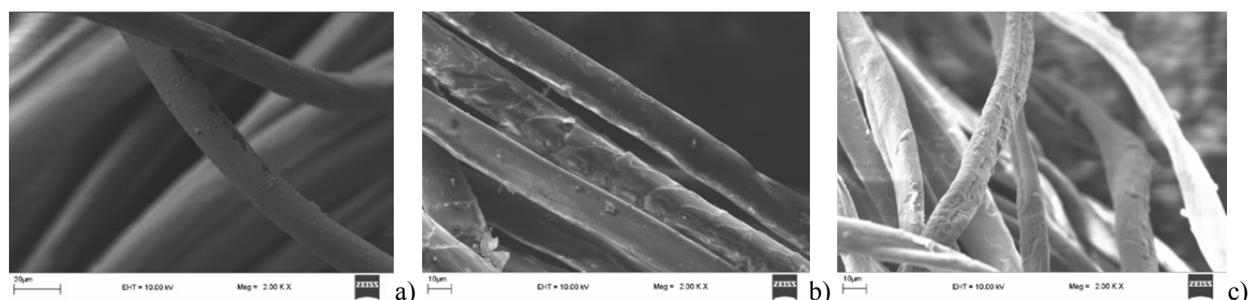
#### 3.1. Surface modification for antimicrobial properties

Plasma, as a very reactive material, can be used to modify the surface of a certain substrate typically known as plasma activation or plasma modification. This entails depositing chemical materials (plasma polymerisation or plasma grafting) to impart some desired properties or removing substances (plasma cleaning or plasma etching), which were previously deposited on the substrate [47]. Such techniques have been applied in the production of antimicrobial textiles and heat sensitive surfaces such as medical equipment [48].

The use of plasma for surface modification has been studied on many kinds of textiles including wool, nylon, cotton, woven and non woven textiles. The effect of such treatments have been successful in producing textiles that have antimicrobial properties against a variety of microorganisms including *E. coli* and *Staphylococcus aureus* [7, 49].

Research has shown that silver and copper provide antimicrobial properties when coated on textiles by a variety of different methods such as soaking, sol-gel coating, chemical washing, atmospheric and vacuum coating [50-51]. When the amount of copper inside a bacterial cell increases, hydrogen peroxide is generated due to the resulting oxidative stress. Under these conditions, copper participates in the Fenton-type chemical reaction causing oxidative damage to cells [52]. Silver ions have been shown to react with the thiol groups in enzymes and inactivate them, leading to cell death. Silver ions have also been shown to interact with DNA to enhance pyrimidine dimerization by the photodynamic reaction and possibly prevent DNA replication [53].

The antimicrobial property of titanium oxide TiO<sub>2</sub> has also been examined [54]. The inhibitory activity of TiO<sub>2</sub> is due to the photocatalytic generation of strong oxidizing power when illuminated with UV light. The generation of active free hydroxyl radicals (-OH) by photo-excited TiO<sub>2</sub> particles is believed to be responsible for its antibacterial activity [55]. Fig.2 represents electron micrographs (SEM) of cotton textile fibers coated with copper (Cu), silver (Ag) and titanium (Ti) respectively.



**Fig. 2** SEM of cotton fibers coated by plasma coating technology with a) Cu, b) Ag and c) Ti.

Plasma technology operating at atmospheric pressure can be used for de-sizing, functionalizing, and designing of surface properties of textile fibers [56]. Dielectric barrier discharge is broadly used for atmospheric pressure treatment of large movable materials since the surfaces of textile fibers can be modified with dielectric barrier discharge without modifying the bulk properties. The influence of this type of plasma treatment has been studied with regards to the absorption of silver nanoparticles on textile has been tested and very encouraging results obtained resulting in an antibacterial effect against *E. coli* [56].

### 3.2. Sterilization of water

NTP has been used for the sterilization of water from a wide range of bacteria, fungi and yeasts [8]. It has been shown that the plasma corona discharge systems are able to efficiently sterilize water from the majority of microorganisms tested including bacteria (*E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, yeast (*Candida albicans*), fungi (*Aspergillus niger*) and green algae [21]. Studies on water decontamination have also investigated the timely development of pulsed ultraviolet light (PUV) and pulsed-plasma gas-discharge (PPGD) technologies for the destruction of harmful chlorine resistant *Cryptosporidium* oocysts, which have caused waterborne illness outbreaks worldwide. [57]. Another investigation on drinking water demonstrated that a low energy input of 75 J/L of water was required to obtain a one-log reduction in *E. coli* concentration. It was also found that water treated with plasma had a prolonged resistance to *E. coli* even hours after treatment [58]. The potential sterilizing effect of an atmospheric low temperature plasma corona discharge system that works on both AC and DC power, on water inoculated with various microorganisms and algae has also been shown [8].

### 3.3. Food processing

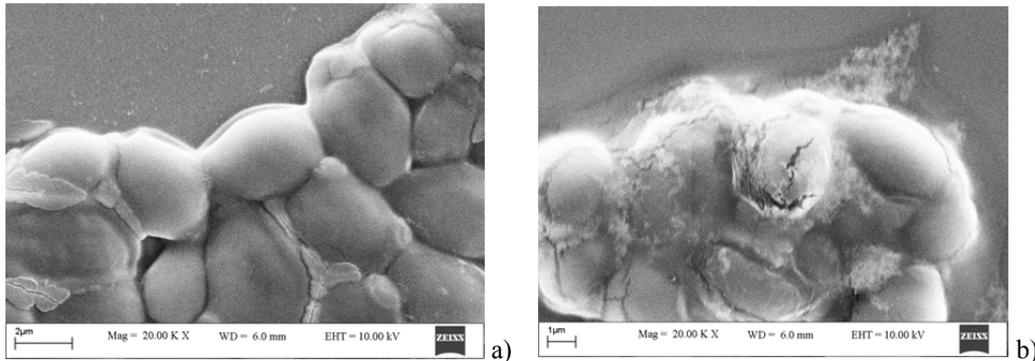
Studies to date on plasma have shown its capability to injure and inactivate pathogenic bacteria on inert food contact surfaces, solid foods and in liquids [59-63]. These include decontamination of fruits [62], vegetables [64] and milk [63]. Recent investigations on the bacteriocidal effects include the evaluation of the inactivation of food-borne pathogens seeded onto thin films of agar, treated with NTP [65], and sprayed onto the surface of heat sensitive polyethylene terephthalate (PET) foils. A 5 log reduction in *E. coli* adsorbed on the surface of almonds, was observed using a DB system [61-62]. Other studies have focused on the inactivation efficiency of cold plasma with respect to contaminated pericarps of mangos, melons [66], bell peppers [67], almonds [62], eggs [68], Cheese and meat products [36], and milk [63]. Although these are very limited preliminary studies, the potential of this technology in food processing is being recognised.

To date, plasma systems are not commercially available as a sterilizing tool in the food industry, mainly due to lack of research on the damaging effects of plasma discharge on foods' chemical components. The important aspects of this technology are still immature, particularly with respect to its use with food. We do not know how cold plasma inactivates spores or how the cold plasma interacts with the food or packaging materials, or the feasibility of the plasma for large-scale commercial production. The application of NTP to food products still needs to be studied in depth, in order to supply a basis for the use of this technology in food products.

### 3.4. Plasma medicine

NTP has been related to a variety of procedures in the emerging area of "plasma medicine" for surgical equipment sterilization [49] accelerated wound healing [69] and sterilization of hospital surfaces [7]. Cold plasma discharges in medical and decontamination applications are operated at atmospheric pressure, eliminating the need for a vacuum chamber which can increase costs. Commonly, an inert gas, such as helium or argon is used. The ionization of these gases produces an ultraviolet emission which assists in decontamination. The flowing carrier gas also removes debris and decomposition products, and helps cool the substrate, minimizing damage to it.

Plasma decontamination of tooth surfaces and the biocidal effect on pathogens in root canals have also been studied [70]. *Candida albicans* commonly infects oral mucosal surfaces. Studies have shown a plasma torch that operates at atmospheric pressure can efficiently inactivate this yeast (Fig.3) (unpublished data).



**Fig. 3** a) *C. albicans* (untreated-control) b) inactivated *C. albicans* following 5 min by plasma torch system developed at Yeditepe University, Turkey.

Plasma sterilisation of dialysis-tubes in their packaging is also being studied, as a procedure for reducing the risk of infection for patients with kidney failure. Sterilization of stents and blood has also been explored (unpublished data). A further contribution to medical progress is in the decontamination of tablets in order to reduce the amount of spoilage during production. Plasma applications in medicine goes even further. NTP's for wound healing are currently under investigation. Cold plasma treatment is a contact-free and painless procedure to sterilize wounds and promote wound healing. The costs are comparable to or lower than those of standard antimicrobial wound treatment [69]. Mechanisms of blood coagulation by direct contact of nonthermal atmospheric pressure dielectric barrier discharge (DBD) plasma has been investigated [71]. It was observed that no significant changes occurred to the pH or  $\text{Ca}^{2+}$  concentration of blood during discharge treatment. Thermal effects and electric field effects were also shown to be negligible.

Atmospheric pressure (direct and indirect) plasma treatment has also been used in dermatology [72]. In indirect plasma treatment, the plasma is produced in a small cavity and ejected by a gas flow onto the skin in the form of an effluent. In direct plasma treatment, the skin itself acts as the counter electrode. Advantageous features of direct plasma treatment include the higher plasma density as well as the induced high frequency electric current onto the skin. Following plasma treatment, anti-inflammatory, anti-puritic, antimicrobial, tissue stimulation, stimulation of microcirculation and other therapeutic effects have been achieved in a single treatment. This is due to the combined action of ultraviolet radiation, reactive oxygen species (e.g. ozone), reactive nitrogen species, and electric fields. The use of direct plasma treatment for skin disinfection in atopic eczema (superinfected dermatitis), in modulating the epidermal barrier as well as in chronic wound treatment has been demonstrated as very efficient with no side effects [71].

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