Time effect and aliquot concentration in *Streptococcus mutans* elimination by plasma needle

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Abstract: Atmospheric plasma needle systems are being intensively studied with a view to potential applications in medicine. The aim of this *in vitro* study is the improved elimination of *Streptococcus Mutans* (*S. mutans*) bacteria. A 5 ml volume of Luria-Bertani culture medium has been inoculated with a test bacterial population and incubated during 24 hours, followed by ten dilutions producing aliquots at 20, 50 and 100 micro l per dilution. Each aliquot is deposited on a paper filter and then exposed to a 2 W RF room pressure helium plasma needle discharge at a 1.5 l.p.m. rate for 1, 3, 5 or 7 minutes. Each sample paper is placed in a test tube, again containing Luria-Bertani fluid, in order to develop a new bacterium colony after a 24h incubation period. The plasma needle lethality has been evaluated from absorbance studies by means of a 6305 Jeway spectrophotometer at a 600 nm wavelength, indicating a clear correlation with exposure time. These studies validate the high disinfection efficacy of the plasma needle.

1. Introduction

The plasma medicine concept is emerging worldwide with promising applications such as bacterial inactivation [1,2], dental cavity [3], surface sterilization [4,5], live skin treatment [6,7], blood coagulation [8] or tissue cauterization [9]. Such applications require a wide variety of plasma sources: plasma needle [10], atmospheric-pressure plasma plume [11], floating-electrode dielectric barrier discharge (FE-DBD) [12], atmospheric-pressure glow discharge torch [13], microhollow cathode discharge air plasma jet [14], microwave plasma torch [15], helium plasma jets [3], dielectric barrier discharge (DBD) [16] and nanosecond plasma gun [17], among many others. Provided that all these plasma devices are operated in room air, their use can easily overcome the limitations imposed by the currently available vacuum-based plasma facilities. More importantly, atmospheric plasma can generate active chemical species, which can be propelled toward the surface to be treated. The short life of these species is desirable as they should not remain after the treatment is completed.
The plasma produced by a plasma needle is restricted to the tip of the jet which cannot contact directly the samples. The plasma needle consists of a thin wire (~μm in diameter) with a sharpened tip powered by radio frequency (RF 13.56 MHz). Helium (He) is typically used as a source gas to ignite and sustain stable plasma. The needle has various advantageous features: no pump is required, the discharge is localized into a region in the order of hundreds of micrometers to millimeters, and the neutral gas temperature is usually not far from the room one. Another important factor is that the process is operated in an open environment.

Dental caries is an infectious pathologic process that destroys irreversibly the dental hard core tissue. Several micro-organisms are associated with caries production, of which the *Streptococcus mutans* or *S. mutans* [18] is the most active, given its ability to adhere to the tooth surface while being the most acidic. *S. mutans* can also produce extracellular polysaccharides out of sugars and break down glycoproteins naturally found in saliva, factors of primordial importance in the development of tooth decay, as the usual *S. mutans* population in the mouth reaches $10^5$ CFU/ml [2], [10], [11] [19]. It follows the relevance of designing preventive policies directed to control this bacteria. Several researchers have worked on the inactivation of different bacteria by means of non-thermal plasma [19], [20], [21] identifying fundamental parameters such as gap distance, applied voltage, source gas flow rate, etc.

In this paper a low temperature open air helium plasma needle has been applied to the gram-positive oral *S. mutans* bacteria in order to study the effects of plasma deactivation as a function of time and aliquot concentration.

2. Experimental set-up

2.1 Plasma source

The plasma needle possesses a coaxial configuration constituted by a copper axial powered filament electrode surrounded by a ceramic tube that provides both mechanical rigidity and electric insulation. The components are assembled on a Nylamid SL® stainless steel frame which includes the gas jet and the electrode connection to a female BNC. It produces a plasma spot of ~1 mm in diameter at power levels in the order of a few hundred mW (figure 1). Previous to the treatment, a full diagnostics of the needle plasma, based on optical emission spectrometry (OES) indicated substantial UV emission in the range 300-400 nm. Active oxygen radicals (O and OH) were also detected [22].

![Figure 1. Experimental set-up](image-url)
2.2 Sample preparation
A strain of ATCC3358 *S. Mutans* was inoculated in 5 ml of Luria-Bertani (LB) culture stock, continuously stirred and then incubated for a 24 h period. Then, 1:10 dilutions of it were made, taking 20, 50 and 100 microlitre aliquots respectively, each of them being deposited on previously sterilized filter paper and subjected to the helium plasma treatment at a 2 W RF generator power level. The experiment was carried out with a previously optimized 1.5 l.p.m. gas flow for 1, 3, 5 or 7 minute periods. Afterwards, the filter papers were placed in test tubes in order to form new bacterial colonies. To this purpose, each paper was stirred for 10 minutes at 5000 rpm and then incubated, again for a 24 h period.

2.3 Colony count up
The counting up process of the new colonies was conducted by means of turbidimetry on a 6305 Jaway spectrophotometer at a 600 nm wavelength which determined the bacteria/ml content of the original undiluted sample. Every sample was diluted down to $10^{10}$ bacteria/ml and then its optical absorbance was measured against a calibrated standard. This process requires producing a turbidity plot in the 540-660 nm wavelength range on the assumption that the proportion of light scattered by the sample is directly proportional to the concentration of cells in the culture.

3. Results and discussion
In the interest of applying the plasma needle technology to dental treatments, the end jet temperature has been established around 28°C at 3 mm of the sample approximately. These results can guarantee not to cause necrosis to the dental pulp whose temperature is in the order of 37°C [24].

Some of the general results of the treatment are showed in figure 2. The control sample absorbance value lies between 0.225 and 0.187. All the treated samples showed some degree of bacterial elimination, considering the initial (control) $10^{10}$ bacteria/ml bacterial population density.

![Absorbance curves corresponding to 20, 50 and 100 microlitre aliquots of the culture after a 1, 3, 5 or 7 minute exposure to helium plasma](image)

*Figure 2.* Absorbance curves corresponding to 20, 50 and 100 microlitre aliquots of the culture after a 1, 3, 5 or 7 minute exposure to helium plasma

As to the 20 microlitre volume, the population density descends two orders of magnitude, $10^7$ to $10^5$ bacteria/ml, in 1 and 3 treatment minutes, respectively. After 5 and 7 minutes the density decayed
one order, to $10^4$ bacteria/ml. In the case of the 50 microlitre volume, the rhythm of elimination was maintained. However in a 100 microlitre concentration volume, the original $10^7$ bacteria/ml population remained unaltered after a 3 minute exposure. Only after 5 minutes did the density declined in one order of magnitude and, at 5 minutes, it reached $10^5$ bacteria/ml. In all, as expected, the bacterial elimination is favored by smaller volumes (20 and 50 microlitre) and longer exposure times (5 and 7 minutes) although the quantitative relationship among these variables is far from being monotonic, as seen in figure 2. Previously reported data [19, 20] have established typical elimination rates for *S. mutans* as $10^5$ bacteria/ml in 5 minutes.

4. Conclusions
Typical operational conditions for the elimination of *S. mutans* up to a 99.9% by means of a low temperature open air helium plasma needle have been experimentally established as a 1.5 l.p.m. gas flow ionized by a 2 W RF generator applied during 5 minutes in order to achieve a population density reduction from $10^{10}$ bacteria/ml to $10^4$ bacteria/ml. Thus, this technique can be successfully applied to higher *S. mutans* concentrations, which entails considerable expectations for the plasma needle as an effective tool in the sterilization of dental cavities.

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