Original Article

Cold atmospheric plasma: Its time-dependent effects on the elimination of bacterial colony on periodontal manual scalers

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Abstract:

Background: This *in vitro* study investigated the time-dependent bactericidal effects of cold atmospheric argon plasma treatment of periodontal hand scalers as well as the scanning electron microscopic view of the scaler tip surfaces before and after plasma treatment. **Materials and Methods:** The study used 34 periodontal hand scalers which were divided into test and control groups. The scaler tips were inoculated with *Escherichia coli* and *Staphylococcus aureus* bacteria, following which the scalers in the control and test groups were subjected to conventional sterilization and argon plasma sterilization, respectively. Varying exposure times of plasma treatment were done on the test group samples to evaluate the minimum time required for complete sterilization. Subsequently, streaks were made on plate count agar using each of these instruments. The agar plates were then kept in an incubator for 24 h, following which bacterial colony count was assessed (colony-forming units/mL). Furthermore, the scanning electron microscopic (SEM) view of the scaler tip was studied before and after plasma treatment. **Results:** A complete elimination of bacterial load (Gram-positive as well as Gram-negative) from the instrument surface was achieved by the plasma exposure time of 15–20 s. SEM analysis did not show a significant difference before and after plasma treatment as not many organic residues were present on the scaler tip. **Conclusion:** Cold atmospheric pressure plasma is an efficient and time-saving method of sterilization, capable of destroying both Gram-positive and Gram-negative bacteria.

Key words:

Argon plasma, cold atmospheric plasma, *Escherichia coli,* instant sterilization, periodontal hand scalers, *Staphylococcus aureus* bactericidal, sterilization

INTRODUCTION

Plasma, as a physical system, was first observed by Sir William Crookes in 1879. He described it as the "radiant matter." Later, in 1927, the Nobel prize-winning chemist, Irving Langmuir coined the term "plasma."[1] Plasma is called the fourth state of matter and is popular among researchers due to its novel and fascinating properties. It contains charged as well as neutral particles and exhibits quasi-neutrality and collective behavior. Plasma has been put to a wide range of applications in diverse areas over the past few decades, including medicine.[2]

Plasma can be divided into high-temperature thermal plasma and low-temperature nonthermal or cold plasma. Ions and electrons of thermal plasma have the same temperature and therefore are in thermal equilibrium. On the other hand, ions have a much lower temperature than electrons in nonthermal plasma and they attain independent thermal equilibrium.[3]

Low-pressure cold plasma was used for surface decontamination purposes from as early as the 1960s and proved to be more effective compared to conventional sterilization techniques.[4] However, cold atmospheric plasma (CAP) became popular in terms of microbial load reduction only during the late 1990s.[5,6] CAP is comparatively a better alternative for applications in medicine than the plasma generated at low pressure. CAP is easier

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to use than low-pressure plasma as the former uses portable devices and thereby making it easily accessible to the affected cells and tissues. On the contrary, low-pressure plasmas use large vacuum-generating devices and are usually expensive.[4]

Plasma has a wide range of applications in the biomedical field; some of which include use in the sterilization of medical equipment and instruments, implant dentistry, blood coagulation, packaging in the food industry, etc.[7,8] These applications are mainly due to their high bactericidal efficacy as well as the ability to focus and act on confined areas.[9] In the recent years, several CAP devices have been developed, which have a working temperature range below 40°C, which makes their application feasible in living tissues. With the advent of such devices, a new horizon has opened up,[10] and researchers around the globe using CAP for pathogenic disinfection.[11] Among the available CAP devices, dielectric barrier discharge, atmospheric pressure plasma jet (APPJ), and hollow cathodes are some popular types of CAP sources.

APPJ devices [Figure 1a and b] operating at 2.45 GHz (microwave) and 10 MHz (radio frequency [RF]) were used in this study. While the former device (microwave plasma jet) was originally devised for radioactive decontamination, it can also be put to use in the destruction of pathogenic microorganisms like *Aeromonas* from a distance of 6 cm for application for a 3–4 min exposure duration.[12]

Currently, the theoretical risk of prion transmission from one patient to another through surgical instruments is a major concern in the medical field. According to various studies, it is reported that prion proteins have a high affinity to instrument structures, and conventional sterilization methods are often inadequate for their removal. In this scenario, the use of plasma therapy has proved to be effective, in removing the surface organic matter and preventing the iatrogenic transmission of diseases during dental procedures.[13,14]

During the 1960s, low-pressure CAP was first applied to decontaminate surfaces, and this treatment has been proven to be more effective in certain aspects compared to conventional sterilization. However, the effects of CAP in reducing microbial load were discovered only during the second half of the 1990s.[4]

This study aimed to evaluate the effects of cold atmospheric pressure plasma application in the elimination of bacterial colony count, as well as surface organic residues (if present) on the surfaces of dental instruments, and to compare the results to that obtained after conventional steam autoclave sterilization.

MATERIALS AND METHODS

The materials used in this experiment were periodontal hand instruments (scalers and curettes), high-frequency atmospheric pressure cold plasma devices, scanning electron microscope, and Gram-negative and Gram-positive bacterial cultures – *Escherichia coli* MG1655 (ATCC 700926) and *Staphylococcus aureus* (MTCC 740, ATCC 9144), respectively, and plate count agar.

Figure 2 shows a schematic of the typical experimental setup. Eight liters per minute argon gas was passed through the mass flow controller to the APPJ devices for cold plasma generation, and the power cable was connected to the power supply for generating plasma. During the experiment, the microwave device was fed 30 Watts of power, while the RF device was fed 20 Watts for cold plasma generation. The scaler tip was held under plasma for a different duration ranging from 2 to 20 s. In each case, as described in the subsequent section, the tip was immersed in bacterial culture, before treatment. Effects of plasma were also seen on scaler tips for a maximum of 60 s.

The periodontal hand instruments were infected with bacteria, by dipping the scaler tips into the prepared *E. coli* and *S. aureus* bacterial cultures. The instruments thus infected were then divided into two groups, namely, test and control groups, with 17 samples per group.

The instruments in the test group were subjected to CAP. These instruments were held along their handle, and the working end (scaler tip) was directed toward the plasma jet at a distance of 1–1.5 cm from the nozzle of the device from where the plasma plume was emitted [Figure 3]. The instruments in the control group were sterilized using a conventional steam autoclave (121°C at 15 lbs for 30 min). The test group instruments were subjected to different exposure times of plasma treatment (2, 5, 10, 15, and 20 s), to determine the minimum time required for the complete elimination of the microbial load. Immediately after sterilization, streaks were made on the plate count agar using each of these instruments. The agar plates were then kept in an incubator for 24 h, following which bacterial colony count was assessed (colony-forming units/mL) [Flow chart 1].

The study included 17 samples in each group. The conventional steam autoclave (121°C at 15 lbs for 30 min) method was used in the control group, while CAP treatment was used in the experimental group. The mean colony count of Gram-negative *E. coli* and Gram-positive *S. aureus* in the control group was measured only once after the completion of treatment, while in the experimental group, it was measured after 2, 5, 10, 15, and 20 s.

Statistical analysis

The data were transformed from a precoded survey form to a computer. The job of data entry, validity checks, and formation of desired results (as per the analysis plan) was done using the SPSS version 22.0 (IBM Corporation, Statistical Package for the Social Sciences, NY, USA). Microbial colony counts between the different time intervals were compared using the repeated measures ANOVA and *post hoc* Bonferroni test.

RESULTS

The mean colony count of Gram-negative *E. coli* and Gram-positive *S. aureus* in the control group, i.e., conventional steam autoclave (121°C at 15 lbs for 30 min), was 0.00 ± 0.00. The complete elimination of the bacterial population was achieved in the autoclave group[Table 1].

The mean colony count of Gram-negative *E. coli* in the experimental group after 2 s application was 4.47 × 105 ± 1.84 × 105, after 5 s application was 4.19 × 104 ± 1.02 × 104, after 10 s application was 4.06 × 103 ± 1.12 × 103 after 15 s application was 7.06 ± 2.25, and after 20 s application was 0.00 ± 0.00[Figures 4 and 5].

The complete elimination of Gram-negative *E. coli* load on the instrument surfaces was achieved after CAP exposure for 20 s [Table 2]. The *post hoc* Bonferroni test showed a significant difference between each pair of period applications. Therefore, there was a significant decrease in *E. coli* colony count on increasing the period of application.

The mean colony count of Gram-positive *S. aureus* in the experimental group after 2 s application was 1.22 × 106 ± 0.16 × 106, after 5 s application was 1.49 × 105 ± 0.29 × 105, after 10 s application was 4.68 × 103 ± 1.43 × 103, after 15 s application was 9.41 ± 3.18, and after 20 s application was 0.00 ± 0.00. The complete elimination of Gram-positive *S. aureus* load on the instrument surfaces was achieved after CAP exposure for a 20 s [Table 3]. The *post hoc* Bonferroni test showed a significant difference between each pair of period applications. Therefore, there was a significant decrease in *S. aureus* colony count on increasing the period of application.

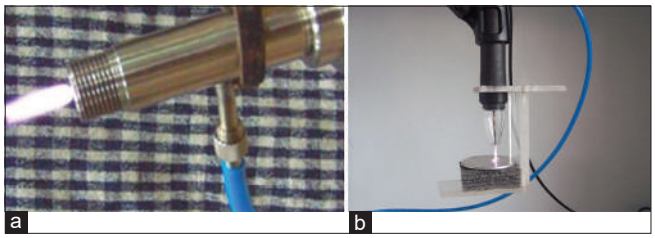


Figure 1: (a) Microwave cold plasma jet device; (b) RF cold plasma jet device. RF – Radiofrequency

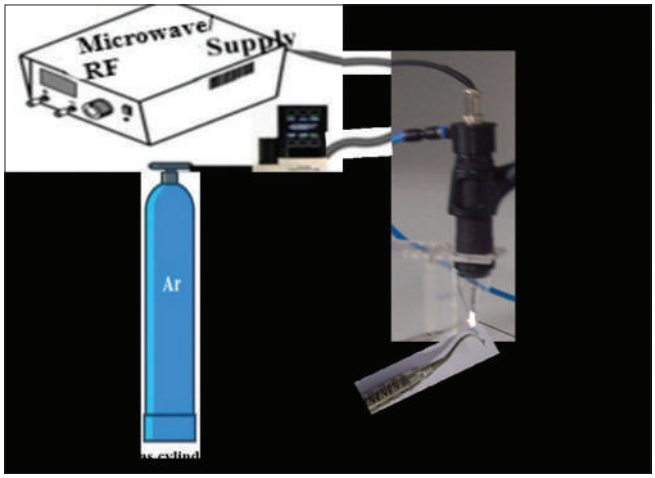


Figure 2: Schematic representation of the experimental setup; RF – Radiofrequency; Ar – Argon

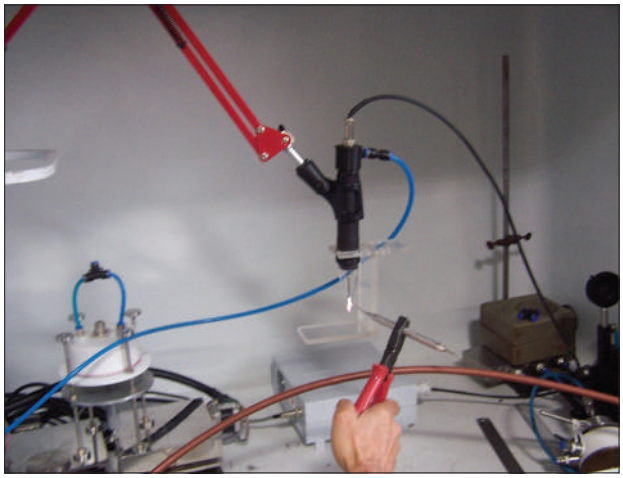


Figure 3: Scaler subjected to CAP treatment. CAP – Cold atmospheric plasma

The effectiveness of sterilization after the completion of treatment between autoclave (after 30 min) and cold plasma treatment (after 20 s) had shown nonsignificant difference between the groups. The mean level of *E. coli* and *S. aureus* was 0.00 ± 0.00, and complete sterilization had taken place in both groups.

The results showed that sterilization using CAP treatment completely eliminated the bacterial load and achieved a similar level of sterilization as conventional steam autoclave sterilization.

From an application point of view, the approximate running cost of the devices was calculated. Microwave-based APPJ uses 0.24 units of electricity, while RF APPJ uses ~ 0.16 units only in single-shift (8 h) operation. The cost of additional argon gas is ~ INR 200–250 per shift. The cost of operation per shift at the maximum will be ~ INR 250 for both devices.

DISCUSSION

The current advice as per the U. K. Department of Health on the risk of transmission of Creutzfeldt–Jakob disease utilizing surgical instruments is that the decontamination of surgical instruments

Table 1: Effect of cold plasma treatment on microbial counts

	Group	Type of application	Mean±SD	*t*	*P*
Escherichia coli	Control	Autoclave	0.00±0.00	-	-
	Experimental	Cold plasma treatment	0.00±0.00		
Staphylococcus aureus	Control	Autoclave	0.00±0.00	-	-
	Experimental	Cold plasma treatment	0.00±0.00		

P value was not computed as Standard Deviation (S.D) was 0. *t* value was not computed as the mean±SD was 0.00. SD – Standard deviation; *P* – *P* value; *t* – *t* value

Table 2: Effect of cold plasma treatment on microbial counts (*Escherichia coli*)

Time of application (s)	Mean±SD	*F*	*P*
Baseline	$3.10\times10^{6}\pm0.85\times10^{6}$	55.92	0.001**
2	$4.47\times10^{5}\pm1.84\times10^{5}$		
5	$4.19\times10^{4}\pm1.02\times10^{4}$		
10	$4.06\times10^{3}\pm1.12\times10^{3}$		
15	7.06±2.25		
20	0.00±0.00		

**Highly significant. SD – Standard deviation; *F* – F value; *P* – P value

Table 3: Effect of cold plasma treatment on microbial counts (*Staphylococcus aureus*)

Time of application (s)	Mean±SD	*F*	*P*
Baseline	$4.30\times10^{6}\pm0.95\times10^{6}$	45.65	0.001**
2	$1.22\times10^{6}\pm0.16\times10^{6}$		
5	$1.49\times10^{5}\pm0.29\times10^{5}$		
10	$4.68\times10^{3}\pm1.43\times10^{3}$		
15	9.41±3.18		
20	0.00±0.00		

**Highly significant. SD – Standard deviation; *F* – F value; P – P value

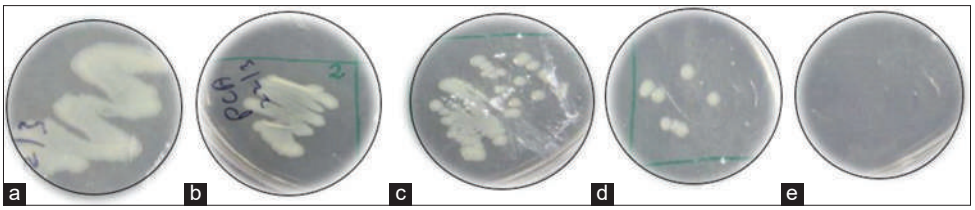


Figure 4: Agar plate counts after different exposure times of plasma; (a) Control plate (no treatment); (b) 2 s; (c) 5 s; (d) 10 s; (e) 15 s

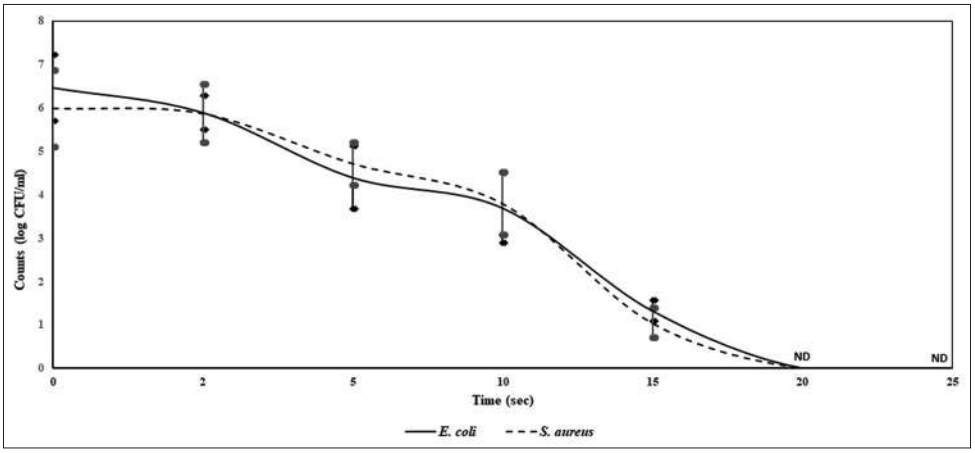


Figure 5: Effect of cold plasma treatment on microbial counts (*Escherichia coli* and *Staphylococcus aureus*) on the periodontal manual scaler; CFU – Colony forming units; E Coli – Escherichia coli; S Aureus – Staphylococcus aureus

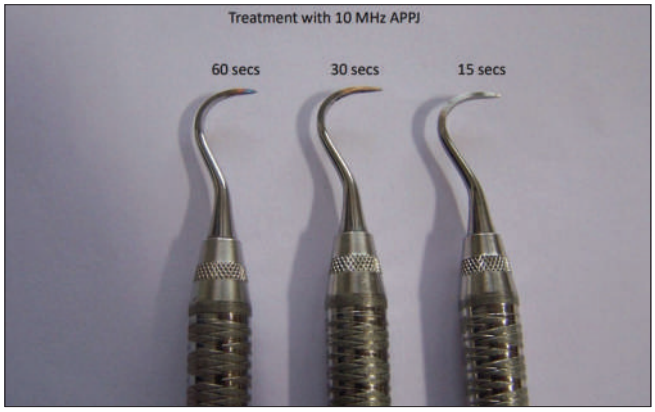


Figure 6: Color changes on scaler tips after plasma exposure

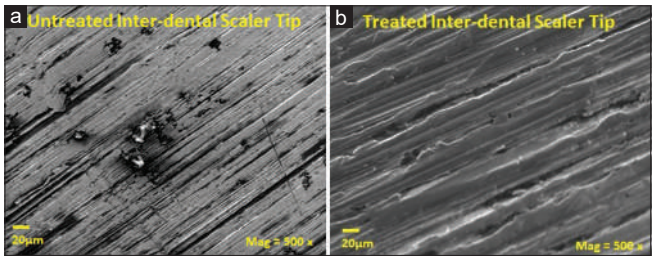


Figure 7: (a) Untreated scaler tip; (b) Plasma-treated scaler tip

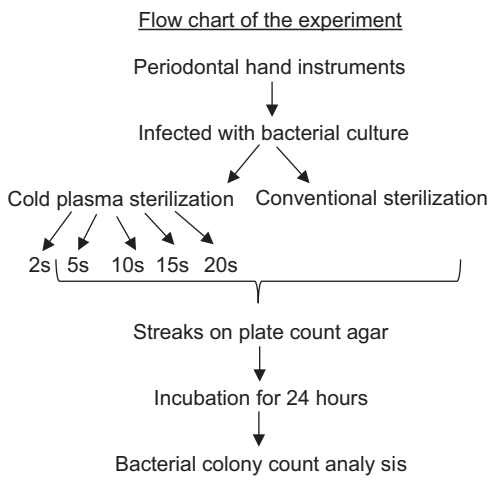
should be of the highest possible standards attainable, to minimize the risk of disease transmission.[15-17] Periodontal scaling instruments were chosen for this experiment because routine scaling and polishing treatments are typically provided in the general dental practice settings. This technique may also be referred to as prophylaxis, professional mechanical plaque removal, and periodontal instrumentation, and is practiced worldwide. These instruments come in contact with the oral mucosa, and hence, their sterilization is of utmost importance.

A study was done by Perni *et al*.[18] demonstrated the bacterial inactivation on *E. coli*, in which 2.5 log reduction was achieved after 2.5 min of CAP treatment. The findings of this experiment suggest that an exposure time of 15–20 s is sufficient to eliminate the bacterial load (Gram-positive as well as Gram-negative) from the instrument surface. Compared to the conventional steam autoclave, which uses 15–30 min at very high temperatures, plasma sterilization is thus a time-saving method of decontamination. Both the RF and microwave frequency plasma jets showed similar results with respect to their efficacy in sterilization.

The finding of this study about the bactericidal effects of CAP is consistent with that of several previous studies, such as those done by Deilmann *et al*.,[7] Sheng *et al*.,[14] and Kar *et al*.[11]

Furthermore, an exposure time of 30 s or more of a 10 MHz microwave frequency plasma jet showed color changes on the instrument tip. Plasma exposure for 15 s or lesser did not cause any color change on the instruments [Figure 6]. However, as the complete elimination of bacterial load was achieved within 20 s, a further extended exposure of plasma was not required, and thus, color changes can be avoided.

The organic residues on the morphology of the scaler surface were checked by scanning electron microscopy (SEM), before and after plasma treatment, and showed no significant differences, as they were already minimum on the scaler tips due to their relatively smooth surface architecture [Figure 7].



Flow chart 1: Methodology of the experiment.

A steady state temperature measurement of the RF APPJ device was done under floating conditions and was found to be 32.5°C after 5 min of working time, while the same for microwave APPJ was 30°C. It makes both these devices suitable for biological applications.

Only point sterilization of the instruments was obtained using this particular plasma jet device in this experiment, and a wider surface area could not be covered. The bulky armamentarium of the plasma setup (which includes the plasma gas cylinder) makes its transportation difficult, and thus, it is not easily portable. Furthermore, only one species (each) of Gram-positive and Gram-negative cultures was included in this experiment, whereas a wider spectrum of microbial species needs to be included. The above-mentioned are the limitations of this study. However, each of these limitations can be overcome as per the requirements (such as plasma devices that cover wider surface areas and armamentarium which uses atmospheric air instead of huge cylinders).

CONCLUSION

The findings of this experiment suggest that sterilization using cold atmospheric pressure plasma could potentially be a time-saving and promising method of sterilization that is capable of destroying both Gram-positive and Gram-negative bacteria. With a mere 20 s of exposure time, almost complete bacterial elimination was achieved, which was similar to the result obtained after conventional steam autoclave sterilization. Thus, it may prove beneficial as a rapid sterilization method. However, further studies that include larger sample sizes and wider microbial spectrum are required to confirm the findings of the study.

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Conflicts of interest

There are no conflicts of interest.

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