

# Antimicrobial Activity of Cold Plasma Treatment on Acrylic Denture Bases: An In Vitro Evaluation



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## Abstract

**Introduction:** Cross-contamination between dental office and prosthetic laboratories is of utmost importance. The dental prosthesis could harbor tones of microorganisms harmful to health staff. It has been estimated that more than 60% of the prostheses delivered to clinics from laboratories or vice versa are contaminated with pathogenic microorganisms. Several disinfection methods and chemicals have been proposed for sanitizing prostheses. Cold plasma, or nonthermal plasma, has been introduced as a new method in medical equipment disinfection. The current study aimed to compare the antimicrobial effect of cold plasma and the routinely used alcohol-based impression disinfectant ASEPTOPRINT® on disinfecting dentures.

**Methods:** In this experimental study, 30 maxillary complete dentures were used as the microbial source. Dentures were randomly divided into 3 groups: (1) cold plasma treatment for 30 seconds, (2) cold plasma for 60 seconds, (3) ASEPTOPRINT® spray. Microbial culturing was taken before and after disinfection in each group on both Blood Agar plates and Soborou Dextrose Agar (SDA) plates. After 48 hours incubation at 37°C, the number of colonies was counted using a digital colony counter and differences between the groups were analyzed.

**Results:** A significant reduction in microbial colonies was observed after using all disinfection methods. ASEPTOPRINT® spray showed more antimicrobial effects comparing to cold plasma.

**Conclusion:** The antimicrobial effect of ASEPTOPRINT® spray solution is more than cold plasma application on both microbial and fungal microorganisms. The time of plasma application was not accompanied by any significant differences in the results.

**Keywords:** Acrylic dentures; Disinfection; Cold plasma; ASEPTOPRINT® spray.



## Introduction

Health care staff is endangered by various microorganisms due to cross-contamination in dental practice; therefore, infection control is a pivotal issue in dental procedures.<sup>1</sup>

The transmission of infection between dentists, laboratory technicians, and patients is possible when prosthetic appliances like complete dentures are fabricated, repaired, and adjusted during dental practice if appropriate disinfection is not achieved. The potential risk of contamination with the aerosols and pathogens exists during all dental procedures encompassing trimming, finishing, and polishing, so there is a need for appropriate disinfection techniques which act efficiently on a broad spectrum of microorganisms and are cost-effective, rapid and safe for human's health.<sup>2-5</sup> The disinfection of the dental impressions is strongly recommended by the health policymakers such as the Centre for Disease Control (CDC), the American Dental

Association (ADA) and the British Dental Association (BDA) to prevent the possible transmission of infectious diseases and cross-contamination.<sup>6-8</sup> Although guidelines for sterilization and disinfection have been established by these organizations, investigations have shown that proper practice is not always followed.<sup>5</sup>

Nowadays immersion in chemical disinfection agents has been mainly suggested for disinfecting dental prostheses like acrylic resin dentures. These disinfectants include a wide variety of chemicals such as 2% alkaline glutaraldehyde, 0.5% and 1% sodium hypochlorite, 3% aqueous formaldehyde, hydrogen peroxide, 1% sodium hypochlorite, 4% chlorhexidine gluconate, and 3.78% sodium perborate.<sup>9-11</sup> The effective elimination of pathogens harbored in the inner and outer surfaces of acrylic dentures is among the advantages of this method. Although affecting physical properties such as transverse strength, hardness, surface decay, and

roughness of denture base, resins have been postulated as the disadvantages of chemical agents.<sup>12-15</sup>

Disinfectants also may have disadvantages like denture staining and irritative reactions of patients' oral mucosa. It has been reported that some disinfectants such as sodium hypochlorite and glutaraldehyde indicate severe and medium cytotoxicity.<sup>16</sup> Also, some disinfectants can cause corrosion and tarnishing of denture metal ingredients. To overcome these issues, many alternative disinfection methods have been proposed.<sup>9-15</sup>

During the past two decades, cold plasma has been introduced as a new approach which has increasingly attracted researchers' attention due to its antimicrobial effects against pathogens.<sup>17</sup> It has also been used for food preservation in the food industry due to its antimicrobial properties.<sup>18,19</sup> Cold plasma is an ionized gas that generates free electrons, ions, ultraviolet photons, and free radical species which directly contact with the bacterial cell. This radiation can inactivate and kill microorganisms by damaging their DNA with its ultraviolet irradiation and free radicals.<sup>20</sup> Low-pressure plasma treatment can be an environmentally friendly approach.<sup>21</sup> The current study aimed to compare the antimicrobial effects of cold plasma and routinely used alcohol-based disinfectant spray (ASEPTOPRINT®) to find a better way for disinfecting dentures.

## Methods

### Sample Collection

In this experimental study, in order to mimic the microbial environment of an oral cavity, 30 maxillary complete dentures were used as the microbial source. After obtaining the informed consent of each patient, all dentures were referred to the prosthetic laboratory of the Dental School, Shahid Beheshti University of Medical Sciences for relining, repair or exchange.

The groups were divided as follows:

1. Disinfected by ASEPTOPRINT® spray according to the manufacturer manual (OCC, Switzerland) (Table 1).
2. Treated by cold plasma treatment for 30 seconds.
3. Treated by cold plasma treatment for 60 seconds.

Each of the maxillary complete dentures was removed and placed on a sterile Petri dish (20 x 100 mm) by the patient. Then, for microbial investigations, swab samples were collected and deposited on tubes containing 10 mL normal saline. Then, the solution was diluted by

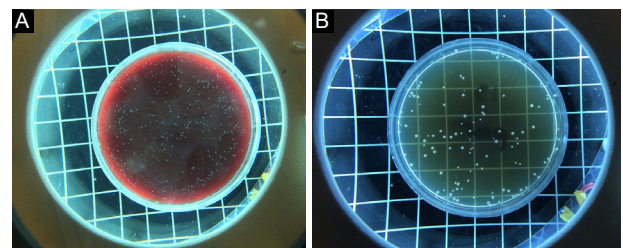
adding 90 mL of normal saline and 10 lambdas of this dilution were spread with the spread-plate technique for each sample. In order to complete the investigation of bacteria and fungi which might exist on dentures, SABOURAUD 4% dextrose agar (SDA) (Merck 1.05438, Darmstadt, Germany) and blood agar (Merck, Darmstadt, Germany) cultures were used in this study. The culture mediums were incubated at 37°C for 48-72 hours. After incubation (Figure 1), the plates were examined through a stereoscopic microscope (Stereoscopic, Nikon, Japan) under reflected light. The number of colonies was counted and reported based on the colony forming unit (CFU) via the manual colony counter (AMETEK, Inc. and Reichert, Inc., Canada).

### Disinfection Protocol

Based on the manufacturer guideline, we applied 10 mL ASEPTOPRINT® spray to each of the dentures in closed packs for 60 seconds. Then, the dentures were rinsed cautiously for 4 minutes. Finally, swab sampling was done in the same way as the previous disinfection.

### Plasma Treatment

The laboratory pulsed plasma reactor (Tehran, Iran) was used in this experiment. Cold plasma was generated by high voltage discharge in a vacuum chamber (diameter 250 mm, height 500 mm, vacuum 100 Pa) with a dielectric barrier discharge (DBD) mode and between the electrodes of the discharge condenser located on a high voltage table, where the sample was placed in glow position. With a relatively high gas flow that varied with the plasma chamber size, the plasma discharge formed



**Figure 1.** (A) Colonies on blood agar exhibit a whitish and rough surface appearance, which resembled the characteristics of *Streptococcus mutans* colonies. (B) The appearance of colonies cultivated on Sabouraud Dextrose Agar (SDA) showed yellow-white colonies with some extensions to the surroundings which mimic *Candida albicans* colonies (Observed under the Manual Colony Counter, AMETEK, Inc. and Reichert, Inc., Canada).

**Table 1.** Information Provided by the ASEPTOPRINT® Spray Manufacturer

Commercial Name	Components	Exposure time	Physical and Chemical Properties
ASEPTOPRINT® Spray CH-071046	20 g ethanol, 28 g 1-propanol, 0.056 g quaternary ammonium compounds	60 seconds	Physical state: Clear, non-viscous liquid Colour: Colourless Scent: Of alcohol, lemon pH-value: Neutral Shelf Life: 48 months

inside the chamber could be blown out of the chamber to form a brush-shaped low-temperature plasma jet, such as a low-temperature atmospheric plasma brush (Figure 2). This plasma source could operate under very low electrical power (30 W) and as a result, very low plasma temperature could be achieved. A special mesh was designed and used in order to standardize the amount of cold plasma radiation all around the dentures (Figure 2).

### Data Analysis

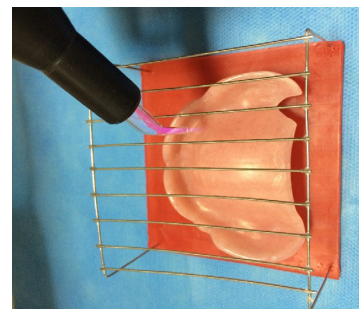
Finally, all data were analyzed using SPSS 16 (SPSS Inc., Chicago, IL, USA). Data were analyzed by conducting a one-way analysis of variance between the groups at each point of time and using the repeated-measures ANOVA for comparing within the experimental groups. Statistical significance was defined at  $P < 0.05$ .

### Results

Microbial isolation prior to the disinfection of all 30 specimens on blood agar and SDA showed a high mean of CFU without any significant difference ( $P > 0.05$ ) (Tables 2 and 3). The differences between groups before and after disinfection were significant within all three experimental groups. After disinfection via ASEPTOPRINT® Spray or cold plasma radiation (30/60 seconds), cold plasma treatment for 30 seconds represented the least antimicrobial activity and the highest CFU on both blood agar and SDA cultures. Comparisons between the groups indicated a statistically significant reduction in the number of colonies among cold plasma treatment groups (30 or 60 seconds) and ASEPTOPRINT® spray disinfection ( $P < 0.05$ ). Cold plasma treatments (30 and 60 seconds) more significantly decreased the number of colony-forming units in the SDA culture compared to the colonies of microbes which grew on blood agar (2.01 CFU vs. 1.56 CFU and 2.48 CFU vs. 1.71 CFU).

### Discussion

In the current study, we aimed to compare the efficacy



**Figure 2.** The specially designed mesh was used in order to standardize the amount of cold plasma radiation all around the dentures.

of a newly proposed method for disinfection (cold plasma treatment) with a routinely used alcohol-based disinfectant for the disinfection of the acrylic-based dentures. According to our findings, one of the advantages of this novel technique could be its ability to reduce *Candida albicans* as the main pathogen for denture stomatitis. Denture stomatitis is a frequent oral disease among geriatric patients, which mainly occurs under the host-related conditions and the imbalance of oral microflora originating from local factors such as denture wearing. The focus in the treatment of denture stomatitis is to eliminate the source of the pathogens which have been colonized in the contaminated dentures.<sup>22</sup> Therefore, the utilization of a safe protocol for disinfection without any harmful effect on the physical properties of the acrylic-based dentures is essential.<sup>23,24</sup>

In this study, it was hypothesized that cold plasma treatment could be used as an alternative method for the disinfection of the acrylic-based dentures instead of the routinely used ASEPTOPRINT® spray solution as a chemical disinfectant. Our results represented that cold plasma treatment could decrease the amount of contamination in 30 and 60 seconds. However, ASEPTOPRINT® spray was a more effective and reliable technique of disinfection compared to cold plasma treatment.

**Table 2.** The Mean Colony-Forming Units (CFU) on Blood Agar Culture at 2 Points of Time

Experimental Groups	Before Disinfection	After Disinfection
	Mean (CFU) ± Standard Deviation	Mean (CFU) ± Standard Deviation
ASEPTOPRINT® Spray (OCC, Switzerland)	5.27 ± 1.21	0.95 ± 1.23
Plasma 30 seconds	5.28 ± 0.66	3.72 ± 0.38
Plasma 60 seconds	5.13 ± 0.25	3.42 ± 0.78

**Table 3.** The Mean Colony-Forming Units (CFU) on Sabouraud Dextrose Agar (SDA) Culture at 2 Points of Time

Experimental Groups	Before Disinfection	After Disinfection
	Mean (CFU) ± Standard Deviation	Mean (CFU) ± Standard Deviation
ASEPTOPRINT® Spray (OCC, Switzerland)	4.95 ± 0.99	0.77 ± 1.25
Plasma 30 seconds	4.61 ± 0.69	2.01 ± 1.53
Plasma 60 seconds	5.15 ± 0.28	2.67 ± 1.10

According to a high microbial load of an oral cavity, blood agar and SDA culture were used to cover nearly most of the potentially wide variety of microbes including bacteria and fungi. It has been indicated that the application of cold plasma can have some antimicrobial impacts. Goree et al<sup>25</sup> showed that the 10-second application of a plasma needle could eliminate *Streptococcus mutans* in oral biofilms. In 2011, Yang et al<sup>26</sup> fabricated a novel toothbrush which irradiated cold argon plasma. They represented that this treatment could eliminate streptococcus mutans and *lactobacillus* in the collected saliva. Moreover, they indicated that 11-15 seconds of cold plasma therapy was required for the inactivation of streptococcus mutans. Nevertheless, *lactobacillus* needed more time to be thoroughly eliminated. Although the number of microorganisms was reduced in our investigation, the amount of reduction was not significant compared to the conventional method of disinfection (chemical solutions). Zamperini et al<sup>24</sup> showed that the application of ArO2 cold plasma with 70-watt power for 5 minutes could significantly decrease the amount of cultivated *Candida albicans* on artificial acrylic resin blocks. This fact can be explained by our restricted time of exposure and its physical properties.

In addition, it has been represented that cold plasma therapy can decrease the number of intra-root canal microorganisms. Jiang et al<sup>27</sup> utilized a newly designed device that irradiated cold plasma into dental root canals. Although they reported a very high antibacterial impact of this method, they concluded that the technique was highly dependent on the direct radiation. Thus, one of the possible explanations for our data is the insufficient radiation which covers the complete surface of the dentures. It seems that the utilization of a larger device may increase the effectivity due to the maintenance of appropriate, complete coverage for direct irradiation.

In conclusion, our results represented that the antimicrobial effect of ASEPTOPRINT® spray solution on both microbial and fungal microorganisms was more significant than cold plasma application. The time of plasma application did not show any significant differences in this study. Further studies are required to unravel the role of cold plasma radiation in disinfection.

### Ethical Considerations

Ethical approval was obtained from the 14th research ethics committee (2016-01-15) of Shahid Beheshti Medical University.

### Conflict of Interests

The authors declare no conflict of interest.

### Acknowledgment

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