

## Prevention of Cross-contamination Risk by Disinfection of Irreversible Hydrocolloid Impression Materials with Ozonated Water

### Abstract

**Background:** Disinfection of dental impression is mandatory for preventing the cross infection between dental staff and dental laboratory. Ozone as a disinfectant can be used in various ways in dentistry. The aim of this study was to evaluate the efficacy of ozonated water for disinfection of irreversible hydrocolloid impression materials and preventing cross-contamination. **Methods:** Thirty-two circular specimens (1 cm diameter and 2 mm thickness) of irreversible hydrocolloid impression material were made. Thirty specimens were contaminated with *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 51299), and *Candida albicans* (PTCC 5027). Except control samples ( $n = 2$ ), the other specimens were immersed in ozonated water for 5 and 10 min (5 sample each group). Trypsin was used for separating microorganisms. Diluted microbial solution was analyzed microbiologically for colony-forming units (CFUs). Two-way repeated measure ANOVA and Scheffé post hoc test were used for data analysis ( $\alpha = 0.05$ ). **Results:** The number of CFUs after disinfection with ozonated water was decreased significantly (11.84%, 61.55%, and 20.27% after 5 min and 11.03%, 14.50%, and 16.99% after 10 min for *P. aeruginosa*, *S. aureus*, and *C. albicans*, respectively) ( $P < 0.001$ ). Increasing the time of disinfection resulted in significant reduction in a number of *S. aureus* only ( $P < 0.001$ ) although they did not vanish microorganisms completely. **Conclusions:** Immersion of irreversible hydrocolloid impression material in ozonated water for 10 min will not lead to complete disinfection but decreases the microorganisms to a level that can prevent infection transmission.

**Keywords:** Colony count, dental impression materials, disinfection, infection control

### Introduction

Preventing the infection transmission has been considered an important topic in dentistry for many years, especially by increasing awareness of the cross-contamination with hepatitis B and human immunodeficiency viruses.<sup>[1]</sup> Cross infection to dental staff can often occur due to the existence of microorganisms from patient's blood and saliva on dental tools as well as patient's oral impressions.<sup>[2-5]</sup> Since the impression and other prosthetic materials are the main sources that can potentially transfer the microorganisms from the patient's mouth to dental laboratory, they should be rinsed thoroughly and disinfected before delivering to the laboratory.<sup>[1,2,6-9]</sup> The impression can be disinfected through immersion or spraying with a disinfectant. The immersion method is more effective<sup>[6,8]</sup> in eliminating the microorganisms, but the spraying method reduces the distortion of the impression.<sup>[6,10,11]</sup> Rinsing with water

alone is not adequate for removing the oral pathogens.<sup>[12]</sup> Moreover, it has been shown that this treatment may increase spreading a significant number of remaining bacteria over the surface of the impression material.<sup>[6]</sup>

A variety of disinfectants were recommended for impression materials.<sup>[3,7,8,13]</sup> These disinfectants should be compatible with the available materials and have no adverse effect on the dimensional stability and surface details of the impression.

The irreversible hydrochloride material is a common impression material in dentistry. However, according to its composition, texture, and hydrophilic setting mechanism, this material can easily get contaminated compared to the elastomeric impression materials.<sup>[13,14]</sup> The common disinfection solutions for hydrochloride impression materials are glutaraldehyde and sodium hypochlorite.<sup>[10]</sup> However, using disinfecting solutions may result in dimensional changes of dental impressions,<sup>[11]</sup> and there are some

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questions regarding the potential health risks of these disinfectants for the dental personals.

Ozone, an allotropic form of oxygen, has a wide range of activity in food industry, medicine, and dentistry.<sup>[15]</sup> Furthermore, its valuable effects such as antimicrobial, antihypoxic, analgesic, and immunostimulating efficacy on biological systems have long been proven in scientific studies which allow using it in different fields of medicine.<sup>[16-18]</sup> Despite the side effects of ozone, complications caused by ozone therapy are infrequent at 0.0007 per application.<sup>[19]</sup> Moreover, ozone is a known disinfectant which has a powerful and reliable antimicrobial agent against bacteria, fungi, protozoa, and viruses.<sup>[20-22]</sup>

One of the various ways of using ozone in dentistry includes removing the microorganisms from dental unit water lines, the oral cavity, and dentures.<sup>[23]</sup> Ozone is also successfully used as a prophylactic agent before etching and the placement of dental sealants and restorations.<sup>[15,23,24]</sup> Antibacterial effect of ozone on *Staphylococcus aureus* in ultrasonic cleaning system has produced appropriate results.<sup>[25]</sup>

It has been shown that aqueous ozone has a higher biocompatibility in comparison with established antimicrobials such as chlorhexidine, sodium hypochlorite, and hydrogen peroxide.<sup>[21,26]</sup>

The aim of this study was to evaluate the preventive role of ozonated water in infection control with disinfection of irreversible hydrocolloid impression material.

## Methods

In this experimental study, 32 discs (1 cm diameter and 2 mm thickness) of irreversible hydrocolloid impression material (Alginoplast, Heraeus Kulzer, Hanau, Germany) were made. The impression material was mixed according to the manufacturer's instructions. Circular metal molds (1 cm wide and 2 mm thick) were used to create the specimens. The molds were placed on a glass slab, and the impression material was injected into the molds. Then, a second slab was inserted on top. The glass slabs, spatula, and metal molds were disinfected with 70% ethanol. After setting of the impression material, the discoid impression specimens were removed. To ensure that there is no contamination, two samples were assigned as a negative control and evaluated.

## Preparation of bacteria and yeast suspensions

In this study, two bacteria including *Pseudomonas aeruginosa* (ATCC 27853) and *S. aureus* (ATCC 51299) and one fungi *Candida albicans* (PTCC 5027) (Biomark Laboratories, Pune, India) were used. Stock culture was prepared from the bacteria and fungi samples. In this protocol, samples of *S. aureus* and *P. aeruginosa* were cultured for 24 h and *C. albicans* for 48 h in the Brain Heart Infusion Broth. The result was a thick suspension,

which was then irrigated with physiologic serum and centrifuged at 4000 RPM for 5 min.

The number of bacteria was estimated at  $1.5 \times 10^8$  and *C. albicans* at  $1 \times 10^6$  per ml.

For preparing McFarland 0.5, we used 625 nm on a spectrophotometer and optical density of 0.01.

## Specimens contamination

0.5cc (500  $\lambda$ ) of microbial suspension was transferred to small sterile tubes (121°C, 15 min, 115 Psi) through sampler. For each microbial species, 12 tubes were considered and one circular sample of impression material was placed in each of the tubes (five samples for each immersion time and 2 for control). Then, the tubes were incubated in 37°C. Based on pilot tests, the best period of time (incubation time) for exposing the specimens to bacteria to achieve maximum attachment to impression material was 60 min.

## Standardization of trypsinization protocol

Trypsin was used to separate microorganisms from the specimens. The specimens that were contaminated with *P. aeruginosa*, *S. aureus*, and *C. albicans* were tested as a pilot study to find the best trypsinization time. After contamination for 30, 60, and 120 min with bacteria and fungi, they were exposed to trypsin for 30, 60, and 120 min. Following culturing of the solution from the trypsinization, the number of colonies was calculated. With these pilot studies, the basic method for exposing the samples to microbial suspension, appropriate concentration of trypsin, and incubation time and temperature were designated (60 min for exposing the samples to microbial suspension and 120, 60, and 30 min for trypsinization for *P. aeruginosa*, *S. aureus*, and *C. albicans*, respectively).

## Samples disinfection

The disinfectant was ozonated water produced by ozone-making device (Anyzone Gold: Bionics, South Korea) with flow of 200 ppm/min. Specimens were first irrigated with water to assimilate clinical procedure. An ozone tube with a sterile sampler head was positioned in the sterilized tubes containing 50 cc sterile water and contaminated samples. Ozone tubes were adjusted to the same height in each tube. Then, the ozone making device was turned on. Disinfection time with ozone was 5 and 10 min (5 samples each).

## Culture preparation method

Specimens were put in 1 cc trypsin (37°C) 120, 60, or 30 min according to pilot tests. Impression materials pieces were removed, and the remaining suspensions were diluted to concentrations that the number of colony-forming units (CFUs) was countable (1/4 for *P. aeruginosa*, 1/16 for *S. aureus*, and 1/8 for *C. albicans*). These dilution concentrations were calculated with consecutive dilution

from 1 to 1/32 for each microbial suspension. The Mueller Hinton Agar and Sabouraud Dextrose Agar (Merck, Darmstadt, Germany) were used to culture the studied bacteria and *C. albicans*, respectively. Fifty  $\lambda$  of each diluted microorganisms were introduced onto the surface of each agar plates individually. The number of CFUs was counted after incubation of plates at 37°C for bacteria (24 h) and fungal strains (48 h).

### Statistical analysis

The mean CFU for each group was calculated. Two-way repeated measure ANOVA was used for assessing significant differences of disinfection time. Scheffe *post hoc* test was used for pairwise comparison of CFUs before and after disinfection for each microorganism. A significant level of 0.05 was used for statistical analysis ( $\alpha = 0.05$ ).

### Results

Table 1 and Figure 1 show the mean CFU count of the studied microorganisms before and after disinfection with ozonated water for 5 and 10 min ( $F = 2078.13$ ;  $P < 0.001$ ). Disinfection of specimens with ozonated water reduced the number of *P. aeruginosa*, *S. aureus*, and *C. albicans* to 11.84%, 61.55%, and 20.27% (after 5 min) and 11.03%, 14.50%, and 16.99%, respectively (after 10 min). Two-way repeated measure ANOVA showed that disinfection time significantly affected the number of

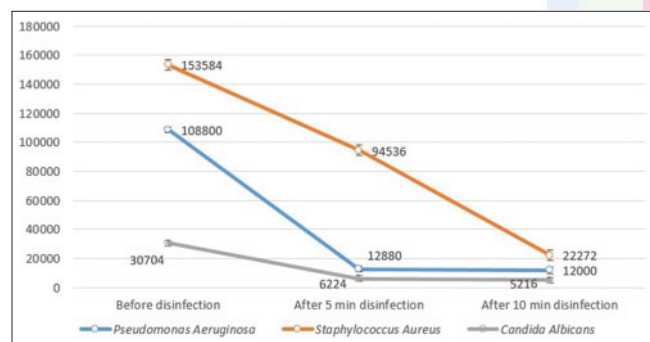


Figure 1: Mean colony-forming unit count of studied microorganisms before and after disinfection with ozonated water for 5 and 10 min

CFUs ( $F = 2078.13$ ,  $P < 0.001$ ) [Table 2]. In addition, the studied microorganisms were significantly different in the amount of attachment to the impression material. *S. aureus* had the highest and *C. albicans* had the lowest attachment to impression materials ( $F = 401.6$ ,  $P < 0.001$ ).

The interaction between disinfection time and type of microorganism showed a significant effect ( $F = 359.62$ ,  $P < 0.001$ ). Scheffe *post hoc* test showed that there is significant difference between the CFUs count before and after 5 min disinfection with ozonated water for all studied microorganisms ( $P < 0.001$ ), whereas the difference between CFUs count after 5 and 10 min disinfection was only statistically different for *S. aureus* ( $P < 0.001$ ) and  $P = 1.00$  for *P. aeruginosa* and *C. albicans* [Table 1].

### Discussion

Controlling the risk of bacteria and viruses transmission between dental clinic and laboratory by dental impressions and other prosthetic works have gathered the attention of clinic and laboratory staff.<sup>[27]</sup> To reduce the probability of cross contamination, disinfection is recommended as a part of clinical guides. However, such recommendations have been somewhat neglected and even when taken into consideration, their effects were limited due to using wrong methods. Because sterilization with heat is not an option for dental impressions and occlusal records, chemical disinfection is still the method of choice for decontamination of microorganisms.

In the present study, ozonated water was used as a disinfectant. *P. aeruginosa* (ATCC 27853) and *S. aureus* (ATCC 51299) and *C. albicans* (PTCC 5027) were the chosen microorganisms for this study. A negative control was assigned to ensure that the specimens were not contaminated earlier. For evaluating the amount of microbial attachment, various methods exist such as trypsinization, use of ultraviolet rays, sonication, and shaking the specimens.<sup>[28]</sup> Pilot tests were done to standardize the method of study and best time and concentration for trypsinization and incubation temperature were identified.

Table 1: Mean (standard deviation) and 95% confidence interval of colony-forming units of studied groups

Microorganisms	Studied groups	Mean (SD) <sup>‡</sup>	95% CI of mean	
			Lower bound	Upper bound
<i>Pseudomonas aeruginosa</i>	Before disinfection	108,800 (8627.4) <sup>a</sup>	104,516.2	113,083.8
	5 min disinfection with ozonated water	12,880 (4992.3) <sup>b</sup>	10,403.9	15,356.1
	10 min disinfection with ozonated water	12,000 (2668.0) <sup>b</sup>	10,676.9	13,323.2
<i>Staphylococcus aureus</i>	Before disinfection	153,584 (1264.9) <sup>A</sup>	152,955.2	154,212.8
	5 min disinfection with ozonated water	94,536 (14,380.5) <sup>B</sup>	87,405.3	101,666.7
	10 min disinfection with ozonated water	22,272 (324.4) <sup>C</sup>	24,221.1	22,432.3
<i>Candida albicans</i>	Before disinfection	30,704 (4253.3) <sup>*</sup>	28,594.6	32,813.4
	5 min disinfection with ozonated water	6224 (3280.7) <sup>**</sup>	4529.6	7918.4
	10 min disinfection with ozonated water	5216 (947.7) <sup>**</sup>	3521.6	5686.2

<sup>‡</sup>There is significant differences between the means, which are characterized by different characters ( $P=0.001$ ). SD=Standard deviation, CI=Confidence interval



**Table 2: Two-way repeated measure ANOVA**

Source	Sum of square	df	Mean square	F	Significant
Disinfection time	56.7×10 <sup>9</sup>	2	28.4×10 <sup>9</sup>	2078.1	0.000
Microorganism	43.9×10 <sup>9</sup>	2	22.0×10 <sup>9</sup>	401.6	0.000
Microorganism + disinfection solution	19.6×10 <sup>9</sup>	4	49.03×10 <sup>9</sup>	359.6	0.000

The recommended disinfection method for irreversible hydrochloride impression material is short-term (<10 min) immersion. If the impression's contact with water exceeds decrease in accuracy will occur due to water absorption.<sup>[8,29-31]</sup> Thus, immersion in ozonated water for 5 and 10 min was used in this study. Ozone is known as a strong antimicrobial substance against bacteria, fungi, and viruses.<sup>[20-22]</sup>

After cultivating bacteria in a solid environment, their growth is halted for a short period of time (lag phase). This helps the bacteria adapt to their culturing environment. This period is 1–2 h for a fast reproducing bacterium. For this reason, counting the bacteria is done after 24 h. For *C. albicans*, due to the slow pace of growth, the counting is carried away after 48 h.

Results of this study showed a significant decrease in a number of colonies compared to control group after immersion in ozonated water for 5 and 10 min [Figure 1]. Only the number of *S. aureus* colonies significantly differ in 5 and 10 min time periods ( $P = 0.001$ ). In addition, it does not seem that prolonging the disinfection time would cause more decrease in bacterial count. This is probably due to structural diversities between microorganisms. This study showed that even though disinfection with ozone solution for 5 and 10 min decreased the microorganism's counts, it did not eliminate them completely. In other words, concentration of 200 ppm/min ozone is not able to completely disinfect the irreversible hydrocolloid impressions.

In clinical situations, probably the number of microorganisms existing on the impression after removing from the mouth is much less than that of our study. Sofou *et al.*<sup>[13]</sup> reported that the maximum number of microorganism on the surface of impression materials is  $3.4 \times 10^4$ . Furthermore, exposure time is around 3–5 min, which is obviously less than this study. Hence, in clinical situations, it might be enough to reduce the microbial count to a level that is no longer pestiferous.

In a study done on disinfection effect of ozone on *C. albicans* attached to acrylic dentures, a decrease in the number of fungi was dependent on disinfection time in a way that after 10 min, their amount reduces to approximately 0.1%, but it did not reach zero.<sup>[20]</sup>

In this study, 200 ppm/min ozonated water was used to disinfect the irreversible hydrochloride material. It may

be more effective if a higher concentration of ozonated water is used. In addition, the present study was done on three types of microorganisms, and considering the various types of microorganisms that reside the oral environment, it would be advantageous to do a clinical study as well.

Our results prompt a researcher to ask this question: is it possible to reduce the attachment by changing the material's characteristics? The use of biosurfactant on the surface can also prevent the adhesion of microorganisms.<sup>[32,33]</sup>

## Conclusions

Despite the limitation of this study, it can be concluded that immersion in ozonated water can reduce the number of microorganisms on the surface of irreversible hydrocolloid materials and increasing the time of immersion increase the disinfection efficacy of ozonated water.

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## Conflicts of interest

There are no conflicts of interest.

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