ORIGINAL RESEARCH

Comparative Evaluation of Ozonated Water and 0.5% Sodium Hypochlorite for their Effect of Disinfection and Surface Wettability on Polyvinyl Siloxane Impression Material

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Abstract

Aim: To evaluate and compare the disinfection of polyvinyl siloxane (PVS) impression material using ozonated water and 0.5% sodium hypochlorite (NaOCl) and their effect on surface wettability.

Materials and methods: Fifty circular disks of PVS impression material were fabricated. The samples were divided into three groups group A (20), group B (20), and group C (10). The samples from group A (20) were treated with ozonated water at room temperature for 10 minutes, and samples from group B (20) were treated with NaOCl (0.5%) at room temperature for 10 minutes. Samples of group C (10) were neither contaminated nor disinfected and were used as a control for microbial enumeration and surface wettability. The contact angle goniometer was used to determine the surface wettability using the sessile droplet technique. The plate count technique was used for microbial enumeration.

Results: The treatment group with 0.5% NaOCl showed greater contact angle values which indicate decreased surface wettability, while the treatment group with ozonated water showed comparatively lesser contact angle values indicative of a very slight change in surface wettability of PVS impression material.

Ozonated water and 0.5% NaOCl showed negligible colony-forming unit (CFU) count indicative of inhibition of bacterial colonies of *Streptococcus mutans*, *Lactobacillus salivarius*, and *Staphylococcus aureus* on PVS impression material.

Conclusion: Treatment with ozonated water can significantly reduce microbial count on PVS impression material without a substantial alteration in surface wettability.

Clinical significance: Along with the inactivation of microorganisms, disinfection procedures must guarantee that the hydrophilicity of the impression material remains unaltered to facilitate complete surface detail reproduction on the poured casts. As a consequence, more research is necessary to assess the impact of ozonated water on surface properties of various impression materials.

Keywords: Colony count, Dental impression, Disinfection, Infection control, Ozone, Sodium hypochlorite, *Staphylococcus aureus*, *Streptococcus mutans*, Wettability.

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Introduction

The Glossary of Prosthodontic Terms Ninth Edition defines dental impression as a negative likeness or copy in reverse of the surface of an object, an imprint of the teeth and adjacent structures for use in dentistry.[1] Contaminated impressions and casts become tools for the transmission of both bacteria and viruses between clinics and dental laboratories.[2] Disinfection of dental impressions is a necessary procedure that protects dental employees from infections caused by contact with microorganisms like viruses such as hepatitis B, hepatitis C, herpes, and HIV, as well as *Mycobacterium tuberculosis*.[3]

The British Dental Association, in the Health Technical Memorandum 01-05, recommends disinfection and decontamination of dental impressions before dispatching them to the processing labs.[4] Microorganisms are not removed completely from impressions by rinsing them with water. Five different microbial growths were seen in 77% of impressions cleaned merely with water in research by Sofou et al.[5] As a result, before entering the laboratory's processing area, dental professional groups have set strict criteria for disinfecting the impressions with chemical agents.[6]

Disinfection solutions, on the contrary, can change the external characteristics of impressions.[6,7] The surface wettability of the impressions may be influenced by the action of disinfectants.[8] As a result, alternate cleaning processes that are less damaging to impression materials are now being investigated in dentistry.

Recently, newer materials like ozone have been studied for their effectiveness as potential disinfectants on impression materials.[9] Antimicrobials in the vapor or dissolved form are less likely than their aqueous equivalents to influence the characteristics of materials.

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Ozone affects the cell membrane, vital proteins, unsaturated lipids, and the intracellular enzymes of microorganisms.[10] It is an ecologically beneficial sanitizer since ozone decomposes to oxygen and leaves no hazardous residues.[11]

The bactericidal impact of gaseous ozone is related to the oxidization of cellular membranes, which results in increased membrane permeability, cell content leakage, and microbial lysis.[10] Ozone can also enter the cell through damaged membranes, causing harm to intracellular components and impairing their function.[10] This action of cell lysis is quicker as it does not involve membrane penetration and thus can never cause microbial resistance.[11]

The previous studies have shown that silicone materials exhibiting higher hydrophilicity show greater wettability and uniform coating with plaster slurry, thus producing dental casts with a smaller number of voids.[12] As a result, contact angle measurement is used to assess the influence of individual agents on the hydrophilicity of PVS impressions (using contact angle goniometer). The goal of this research was to compare the antibacterial effectiveness of ozonated water (1 mg/L at a flow rate of 4 L/hour) and 0.5% aqueous solution of NaOCl on polymerized PVS impression specimens (3M, Express XT Light Body impression material).

Materials and Methods

The study was carried out at Bharati Vidyapeeth Dental College and Hospital, Pune, Maharashtra, India (December 2020–March 2022).

Preparation of Samples

Fifty circular disks of PVS impression material (3M, Express XT Light Body impression material) were fabricated of specified dimensions and shape (cylindrical disk with 15 mm diameter and 3 mm height) with the help of a prefabricated metal mold. The PVS impression material was allowed to set for 3–4 minutes. The specimens were carefully removed from the mold after polymerization to obtain the sample (Fig. 1).

- The samples were divided into three groups: group A (20), group B (20), and group C (10).
- The samples from group A (20) were subdivided into two groups: group A1 (10) for surface wettability and group A2 (10) for microbial enumeration.
- The samples from group B (20) were subdivided into two groups: group B1 (10) for surface wettability and group B2 (10) for microbial enumeration.
- The samples from group C (10) were used as a control for microbial enumeration and surface wettability.

Preparation of 0.5% NaOCl

Commercially available 5% NaOCl (Prime Dental Products Pvt Ltd) and distilled water were used for the preparation of 0.5% concentration of NaOCl. An amount of 1 L of 5% NaOCl was mixed with 4 L of distilled water to obtain a 0.5% optimum concentration of the solution.

Preparation of Ozonated Water

Ozone gas was produced from a medical oxygen cylinder using a medical grade ozone generator (ADC Diagnostic Center, Navi Mumbai). The flow rate of air at the inlet was adjusted to 4 L/hour. A total of 10 minutes duration was required to reach the value of 1 mg/L concentration, which was verified by the iodometric technique.

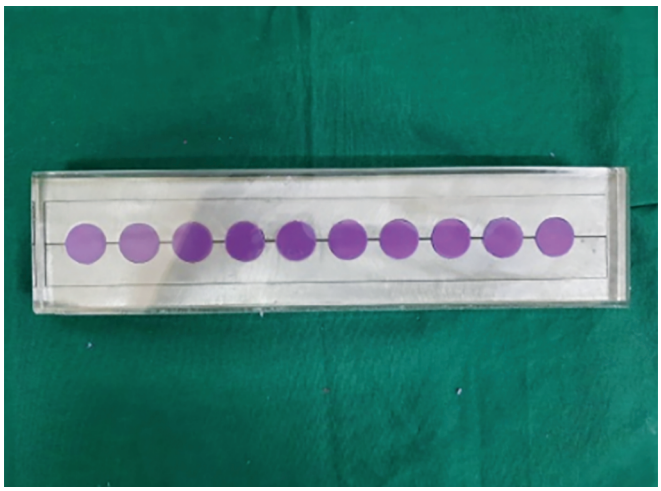


Fig. 1: Preparation of PVS samples (cylindrical disk with 15 mm diameter and 3 mm height) using a prefabricated metal mold

Standardization of Inoculum

Lyophilized cultures of *S. mutans*, *S. aureus*, and *L. salivarius* were supplied by CSIR-National Chemical Laboratory (Pune). Tryptone soya broth was used for incubation at 37°C for 12 hours.

The same quantities of culture were mixed in a tube to make a cocktail of these microorganisms. The centrifugation was done at 3600 rpm for 10 minutes. To maintain cell density, a 0.5 McFarland turbidity level was employed. The suspension thus obtained was used for inoculation.

Contamination of Samples

A total of 10 samples were arranged on sterile glass plate each, that is, group A2 (n = 10) and group B2 (n = 10), respectively. A total of 10 µL of 10^6 cells/mL of cell suspension was added to the surface with a micropipette.

Disinfection of Specimens

In groups A1 and A2, the samples were treated with ozonated water with a concentration of 1 mg/L at room temperature for 10 minutes, followed by 10 seconds of distilled water rinsing.

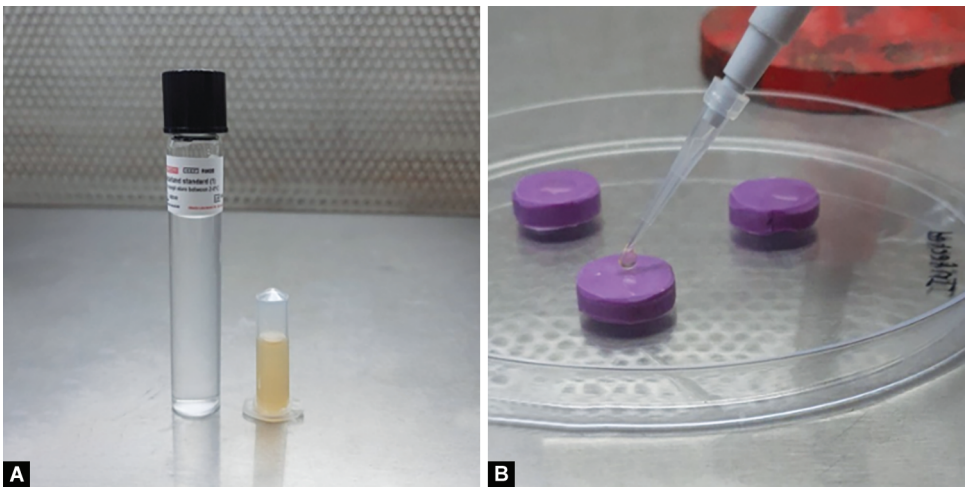
In NaOCl groups, that is, B1 and B2, the specimens were treated with 0.5% NaOCl (pH 7.5) at room temperature for 10 minutes, followed by 10 seconds of distilled water rinsing (Fig. 2).

Microbial Testing

On the inoculated specimens, microbiological enumeration was performed using the plate count technique for *S. aureus*, *S. mutans*, and *L. salivarius,* respectively. Samples were moved into centrifuge tubes having neutralization broth (10 mL) and vortexed (60 seconds). Microbes that survived on disks were measured with the help of enumeration media plates. Plates were inoculated and incubated for 48 hours at 37°C. All three types of colonies were identified on the enumeration media, and colony count was done for each disk (Fig. 3).

Measuring Surface Wettability

The sessile droplet method was used to determine wettability using a contact angle goniometer (Department of Physics, Pune University). A 6 µL droplet of distilled water was allowed to settle on the sample. Capturing of the pictures was done 15 seconds



Figs 2A and B: (A) Cell suspension adjusted to 0.5 McFarland turbidity standard; (B) Contamination of samples using micropipette

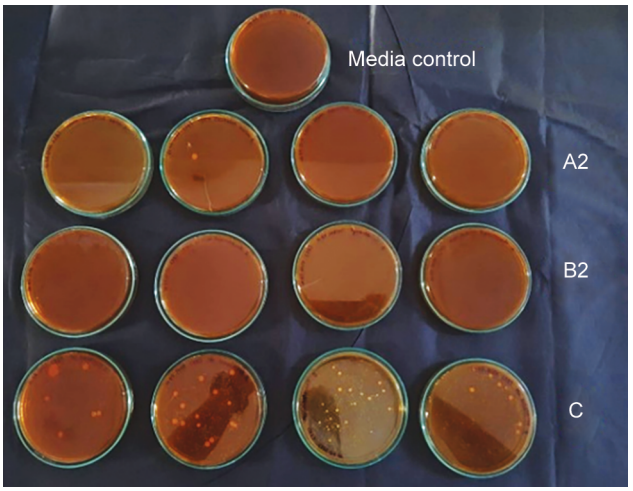


Fig. 3: Microbial enumeration, group A2 CFU count after disinfection using ozonated water, group B2 CFU count after disinfection using 0.5% NaOCl, group C CFU count before disinfection

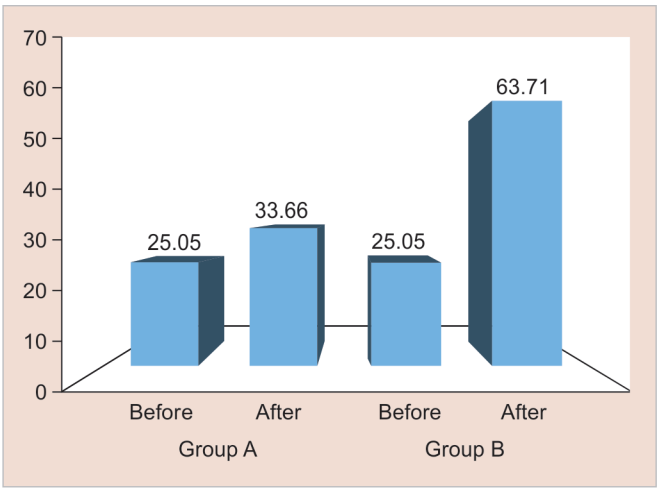


Fig. 4: Bar diagram representing mean contact angles of group A1 and group B1, before disinfection and after disinfection with ozonated water and 0.5% NaOCl

after drop deposition. Contact angles were determined from the captured images.

Statistical Analysis

The sample size was estimated to be 10 per group, and simple random sampling was used for the allocation of groups. Once the data were obtained, it was tabulated and entered into the Excel sheet, after which it was run through a Statistical Package for the Social Sciences (SPSS) software to check for normality. Mean and standard deviation was calculated for CFU of studied groups and contact angle. The normality of data was assessed using Shapiro–Wilk test. The data followed the normal curve.

Results

The mean values of contact angle for groups A1, B1, and C are 33.66, 63.71, and 25.05, respectively. Paired *t*-test was carried out to test significance in group A1 and group B1, and the *p*-value was <0.05 in group A1 and group B1. Hence, we concluded that there is a significant change in group A1 and group B1 (Fig. 4).

An unpaired *t*-test was carried out for comparison between group A1 and group B1, and the *p*-value was <0.05. Hence, we concluded that there is a significant difference between group A and group B.

Further, we observed that the mean difference for group B1 is greater than group A1. Hence, we concluded that the mean change observed in group B1 is more than in group A1.

As a result, the treatment group with 0.5% NaOCl (group B1) showed greater contact angle values which indicate decreased surface wettability, while the treatment group with ozonated water (group A1) showed comparatively lesser contact angle values indicative of a very slight change in surface wettability of PVS impression material (Table 1).

The mean values of CFU count before disinfection (group C) was 0.082516665, after disinfection with ozonated water (group A2) was 0.00958218, and after disinfection with 0.5% NaOCl (group B2) was 00381385, respectively. Paired *t*-test was carried out to test significance in group A and group B, and the *p*-value was <0.05 in group A and group B. Hence, we concluded that there was a significant change in group A and group B.

Table 1: Values of microbial enumeration obtained using CFU count method, measured before disinfection (group C), after disinfection with ozonated water (group A2), and after disinfection with 0.5% NaOCl (group B2)

Sl. no.	*Control CFU/mL (10^4)*	*CFU count before disinfection (group C)*	*CFU count after disinfection using ozonated water (group A2)*	*CFU count after disinfection using 0.5% NaOCl (group B2)*
1	0.00	5.96	0.00	0.00
2	0.00	6.08	0.03	0.00
3	0.00	5.90	0.00	0.00
4	0.00	6.16	0.01	0.00
5	0.00	6.11	0.01	0.01
6	0.00	5.97	0.00	0.00
7	0.00	6.01	0.02	0.00
8	0.00	5.95	0.00	0.01
9	0.00	5.91	0.00	0.00
10	0.00	6.04	0.00	0.00
Average	0.00	0. 082516665	0. 00958218	0. 00381385

Cultures used: mixture of *S. mutans*, *L. salivarius*, and *S. aureus* (1:1:1) with an OD 600 nm of 0.5

An unpaired *t*-test was carried out for comparison between group A and group B, and the *p*-value was >0.05. Hence, we concluded that there was no significant difference between group A and group B.

As a result, both the treatment groups, that is, ozonated water (group A2) and 0.5% NaOCl (group B2), showed negligible CFU count indicative of inhibition of bacterial colonies of *S. mutans*, *L. salivarius*, *S. aureus* on PVS impression material.

Discussion

Impressions are a possible cause for transmission of infections from clinics to dental labs. Powell et al.[13] discovered harmful germs in 67% of items used in dentistry, which also included impressions.

To reduce the risk of cross-contamination, most dentists depend on their undergraduate basic educational experience in the area of infection control. Microorganisms are not totally removed from impressions by rinsing them with water. Until 1991, the recommended method of disinfection was washing the dental impressions under running tap water. Washing the impressions with running tap water, on the contrary, only removes 40% of bacteria, viruses, and fungus, resulting in a higher risk of infection.[14] Hence the use of chemical disinfectants was advocated. Disinfection solutions, on the contrary, can change the surface properties of imprint materials.[7] Chemical action of disinfectant solutions on the surface of impressions can hamper the wettability of the material.[8] As a result, alternate disinfection processes that are less damaging to impression materials are currently being investigated in dentistry.

The intrinsic hydrophobic nature of PVS impression materials causes difficulty in replicating moist oral tissues; also, wetting of impressions using plaster slurry becomes tough.[15] Producers thus tried improving the wettability of PVS by adding surfactants to its chemical composition.[16,17]

However, hydrophilized PVS wettability has been shown to get altered by aqueous disinfectant exposure. Milward and Waters[8] stated that disinfection with the immersion method significantly decreased the surface wettability of PVS impression material. Blalock et al.[18] stated that the greater the duration of exposure to chemical disinfectants more will be the contact angle formed with PVS impression material.

Lately, techniques such as ozone gas or ozonated water have been studied for the disinfection of impressions.[19] Antimicrobial agents in the gaseous or dissolved state are less likely than their aqueous equivalents to change the characteristics of materials.[20]

Ozone affects the cell membrane, vital proteins, unsaturated lipids, and the intracellular enzymes of microorganisms.[21] Using ozonated water to treat PVS impression materials under optimal conditions may induce bacterial viability reductions equivalent to those observed with disinfectants. Sharma and Hudson[21] found that treatment with ozone gas for 20 minutes reduced the bacterial counts by more than 3 log. As observed in our study, gram-positive bacteria showed greater structural resistance to ozonated water than gram-negative bacteria.

The bactericidal impact of gaseous ozone is related to oxidization of cellular membranes, which results in increased membrane permeability, cell content leakage, and microbial lysis.[22] Ozone can also enter the cell through damaged membranes, causing harm to intracellular components and impairing their function.[22] This action of cell lysis is quicker as it does not involve membrane penetration and thus can never cause microbial resistance.[22]

Ozone has gained a lot of interest recently as an alternate oxidant for low-temperature, damage-free silicon oxidation.[23] Contact angle assessed on samples showed that ozonated water had no effect on the material's hydrophilicity due to a change in polarity caused by oxidation of H^+ ions to OH^- ions.[24]

The study was planned in which 50 PVS (3M, Express XT Light Body impression material) impression disks of 15 mm diameter and 3 mm height were prepared using a prefabricated metal mold. Similar to a study done by Celebi et al.[25]

On the inoculated specimens, microbiological enumeration was done using the plate count technique using Baird-Parker agar for *S. aureus*, *S. mutans*, and *L. salivarius*. The results showed that the mean CFU count before disinfection was 6.0090×10^4/mL, while that for samples treated with ozonated water showed 0.0070×10^4/mL and 0.5% NaOCl showed 0.0020×10^4/mL. Both the treatment groups showed negligible CFU count indicative of inhibition of bacterial colonies of *S. mutans*, *L. salivarius*, and *S. aureus* on PVS impression material, similar to the results obtained by Gomes et al.[26]

A contact angle goniometer was used to test wettability using the sessile droplet method. The results showed that the mean contact angle before disinfection was 25.05 while that for samples treated with ozonated water showed 33.66° and 0.5% NaOCl

showed 66.71°. As a result, the treatment group with 0.5% NaOCl showed greater contact angle values which indicate decreased surface wettability, while the treatment group with ozonated water showed comparatively lesser contact angle values indicative of a very slight change in surface wettability of PVS impression material.

As a consequence, the null hypothesis was rejected since ozonated water produced equivalent outcomes to common disinfectants such as 0.5% NaOCl without affecting the surface properties of PVS impression material.

Limitations of the Study

In the present study, PVS impression disks were manually contaminated with specified microbes, that is, *S. mutans*, *S. aureus*, and *L. salivarius*; actual patient impressions were not used. The actual patient impressions may contain a greater number of microbial species in vegetative and spore state, which may show competitive inhibition/mutualism/commensalism.

In the given study, the surface of the prepared samples was smooth and regular, dissimilar to that of dental impressions, which often show undercuts and surface irregularities, thus affecting the effectiveness of surface disinfection.

Here, both the disinfectant solutions were prepared using distilled water which may not be readily available in clinical practice. Also, the prepared ozonated water had a shelf life of only 40 minutes at room temperature; hence its immediate utilization was necessary to achieve desired disinfection.

Scope of the Study

The present study will provide a baseline for future *in vivo* studies on disinfection of actual patient impressions using ozonated water. There are a few aspects of disinfection of dental impressions which can be further investigated. In addition to microbial inactivation, disinfectants may change surface properties.[6,7] As a result, alternate cleaning processes that are less damaging to impression materials should be studied. Also, methods for improving the shelf-life and effectiveness of ozonated water and the possibility of packaging of predetermined concentration solution should be considered.

Conclusion

On light body hydrophilized PVS impression material, treatment using ozonated water can considerably reduce viable bacterial populations without compromising surface wettability. Because of its favorable influence on the impression material's surface wettability, ozonated water treatment has been recognized as a viable approach for disinfecting polymerized PVS impression materials.

References

1. The glossary of prosthodontic terms. J Prosthet Dent 1999;81(1):39–110. DOI: 10.1016/j.prosdent.2005.03.013
2. Lewis DL, Arens M, Harllee R, et al. Risks of infection with blood- and saliva-borne pathogens from internally contaminated impressions and models. Trends Tech Contemp Dent Lab 1995;12(5):30–34. PMID: 9587266.
3. Hemalatha R, Ganapathy D. Disinfection of dental impression—a current overview. Int J Pharm Sci Res 2016;7(8):661–664.
4. Whitworth CL, Palmer NOA. Decontamination in primary care dentistry. J Infect Prev 2010;11(6):200–204. DOI: 10.1177/1757177410385368
5. Sofou A, Larsen T, Fiehn N-E, et al. Contamination level of alginate impressions arriving at a dental laboratory. Clin Oral Investig 2002;6(3):161–165. DOI: 10.1007/s00784-002-0173-4
6. Kotha SB, Ramakrishnaiah R, Divakar DD, et al. Effect of disinfection and sterilization on the tensile strength, surface roughness, and wettability of elastomers. J Investig Clin Dent 2017;8(4). DOI: 10.1111/jicd.12244
7. Martin N, Martin MV, Jedynakiewicz NM. The dimensional stability of dental impression materials following immersion in disinfecting solutions. Dent Mater 2007;23(6):760–768. DOI: 10.1016/j.dental.2007.01.004
8. Milward PJ, Waters MG. The effect of disinfection and a wetting agent on the wettability of addition-polymerized silicone impression materials. J Prosthet Dent 2001;86(2):165–167. DOI: 10.1067/mpr.2001.116774
9. Achen M, Yousef AE. Efficacy of ozone against *Escherichia coli* O157:H7 on apples. J Food Sci 2001;66(9):1380–1384. DOI: 10.1111/j.1365-2621.2001.tb15218.x
10. Ishizaki K, Sawadaishi K, Miura K, et al. Effect of ozone on plasmid DNA of *Escherichia coli in situ*. Water Res 1987;21(1):823–827. DOI: 10.1016/0043-1354(87)90158-8
11. Chuwa C, Vaidya D, Kathuria D, et al. Ozone (O3): an emerging technology in the food industry. Food Nutr J 2020;5(2):224. DOI: 10.29011/2575-7091.100124
12. Panichuttra R, Jones RM, Goodacre C, et al. Hydrophilic poly(vinyl siloxane) impression materials: dimensional accuracy, wettability, and effect on gypsum hardness. Int J Prosthodont 1991;4(3):240–248. PMID: 1810315.
13. Powell GL, Runnells RD, Saxon BA, et al. The presence and identification of organisms transmitted to dental laboratories. J Prosthet Dent 1990;64(2):235–237. DOI: 10.1016/0022-3913(90)90185-f
14. McNeill MR, Coulter WA, Hussey DL. Disinfection of irreversible hydrocolloid impressions: a comparative study. Int J Prosthodont 1992;5(6):563–567. PMID: 1339136.
15. Khatri M, Mantri SS, Deogade SC, et al. Effect of chemical disinfection on surface detail reproduction and dimensional stability of a new vinyl polyether silicone elastomeric impression material. Contemp Clin Dent 2020;11(1):10–14. DOI: 10.4103/ccd.ccd_9_19
16. Rupp F, Axmann D, Jacobi A, et al. Hydrophilicity of elastomeric non-aqueous impression materials during setting. Dent Mater 2005;21(2):94–102. DOI: 10.1016/j.dental.2004.02.006
17. Yuan Y, Lee TR, Gianangelo B, et al. Contact angle and wetting properties. In: Surface Science Techniques. 2013;11(2):3–34.
18. Blalock JS, Cooper JR, Rueggeberg FA. The effect of chlorine-based disinfectant on wettability of a vinyl polysiloxane impression material. J Prosthet Dent 2010;104(5):333–341. DOI: 10.1016/S0022-3913(10)60151-5
19. Azarpazhooh A, Limeback H. The application of ozone in dentistry: a systematic review of literature. J Dent 2008;36(2):104–116. DOI: 10.1016/j.jdent.2007.11.008
20. Aslam R, Alam MS, Saeed PA. Sanitization potential of ozone and its role in postharvest quality management of fruits and vegetables. Food Eng Rev 2020;12(1):1–6. DOI: 10.1007/s12393-019-09204-0
21. Sharma M, Hudson JB. Ozone gas is an effective and practical antibacterial agent. Am J Infect Control 2008;36(8):559–563. DOI: 10.1016/j.ajic.2007.10.021
22. Nagayoshi M, Fukuizumi T, Kitamura C, et al. Efficacy of ozone on survival and permeability of oral microorganisms. Oral Microbiol Immunol 2004;19(4):240–246. DOI: 10.1111/j.1399-302X.2004.00146.x
23. Fink CK, Nakamura K, Ichimura S, et al. Silicon oxidation by ozone. J Phys Condens Matter 2009;21(18):183001. DOI: 10.1088/0953-8984/21/18/183001
24. Karg M, Lokare KS, Limberg C, et al. Atomic layer deposition of silica on carbon nanotubes. Chem Mater 2017;29(11):4920–4931. DOI: 10.1021/acs.chemmater.7b01165
25. Celebi H, Büyükerkmen EB, Torlak E. Disinfection of polyvinyl siloxane impression material by gaseous ozone. J Prosthet Dent 2018;120(1):138–143. DOI: 10.1016/j.prosdent.2017.09.003
26. Gomes BP, Ferraz CC, Vianna ME, et al. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of *Enterococcus faecalis*. Int Endod J 2001;34(6):424–428. DOI: 10.1046/j.1365-2591.2001.00410.x