

Original Article

A comparative study on the antimicrobial activity of irreversible hydrocolloid mixed with silver nanoparticles and chlorhexidine

Azadeh Farhang Nia¹, Mahdi Ataei¹, Habib Zeighami²

¹Department of Prosthodontics, Dental School, Zanjan University of Medical Sciences, ²Pharmaceutical Biotechnology Research Center, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

ABSTRACT

Background: Impressions taken from patients have the potential of cross-transmission of infection among dentistry personnel. The present study aimed to compare the antimicrobial activity of chlorhexidine (CHX) and silver nanoparticles (AgNPs) combined with irreversible hydrocolloid.

Materials and Methods: This experimental study examined the *in vitro* antimicrobial effects of irreversible hydrocolloid mixed with silver nanoparticles and chlorhexidine using four groups, namely CHX (0.2%) solution and mouthwash mixed with irreversible hydrocolloid Groups 1 and 2), AgNPs (0.1 and 0.2%) (Groups 3 and 4), and specimens mixed with distilled water as a control group (Group 5) on bacterial strains, namely, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus epidermidis* through disc diffusion method. There were three replications per bacterial species. As data were not normally distributed, Kruskal–Wallis test was used at a significance level of 0.05.

Results: No antimicrobial activity was observed in the control groups. In *S. aureus*, CHX mouthwash had the highest antimicrobial activity, and AgNPs 0.1% and 0.2% groups had lower antimicrobial activity, and there was a significant difference between the two concentrations of AgNPs ($P < 0.05$). In *E. faecalis*, the effects of CHX compounds and AgNPs 0.2% were similar to each other and were higher than the effect of AgNPs 0.1% ($P < 0.05$). In *E. coli*, CHX compounds exhibited the highest efficacy relative to other materials ($P < 0.05$), and the AgNPs had no effect. In *P. aeruginosa*, AgNPs showed the highest growth inhibition zone, which had a significant difference compared to other materials ($P \leq 0.01$), whereas the CHX compounds were not effective. In *S. epidermidis*, the effect of CHX compounds was similar to one another and was higher than the effect of AgNPs ($P \leq 0.01$).

Conclusion: According to our observations, the antimicrobial activity of AgNPs at 0.1 and 0.2% against five tested bacterial strains was similar to those of pure CHX 0.2% solution and CHX 0.2% mouthwash.

Key Words: Alginate, chlorhexidine, disinfection, nanoparticles, silver

Received: November 2018
Accepted: June 2019

Address for correspondence:
Dr. Mahdi Ataei,
School of Dentistry, Zanjan
University of Medical
Sciences, Mahdavi Street,
4513956111 Zanjan, Iran.
E-mail: drataeimahdi@
gmail.com

INTRODUCTION

Dental impression materials may carry pathogenic microorganisms because of direct contact with the blood, saliva, and bacterial plaque of patients, which

can transmit infectious diseases to dentistry and/or laboratory personnel.^[1-3] Irreversible hydrocolloid is usually used to record primary impressions in

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Nia AF, Ataei M, Zeighami H. A comparative study on the antimicrobial activity of irreversible hydrocolloid mixed with silver nanoparticles and chlorhexidine. Dent Res J 2020;17:120-5.

Access this article online



Website: www.drj.ir
www.drjjournal.net
www.ncbi.nlm.nih.gov/pmc/journals/1480

dentistry.^[4] While impression recording, the surface tissue and hydrophilic nature of the irreversible hydrocolloid allow maintaining maximum microbial pathogens not only on the surface but also inside the material.^[5] Research has shown that a large number of impressions have been so far sent to laboratories without passing through any kind of disinfection process.^[6-8] It has been shown that a combination of disinfectants with irreversible hydrocolloid powder provides an additional disinfection method with no adverse effects in dimensional stability and surface accuracy of the impression.^[9] Chlorhexidine (CHX) and silver nanoparticles (AgNPs) are two disinfectants that have been demonstrated to be effective in combination with irreversible hydrocolloid while maintaining physical properties of the impression.^[10-12] The main aim of the current study is to determine the selective material with the most effective antimicrobial activity, when mixed with irreversible hydrocolloid. The null hypothesis of the study was that there is no difference of antimicrobial effect between CHX and AgNPs.

MATERIALS AND METHODS

In this experimental study the irreversible hydrocolloid impression powder (Lascod Kromopan type A, Florence, Italy) was mixed in a ratio of 20 mL liquid with 9 g of the powder according to the manufacturer's instruction. AgNPs (US Research, USA, No. 4-22-7440) were used at concentrations of 0.2% and 0.1% with a size range of 5–8 nm. CHX was used in the following two forms:

1. CHX 2% solution (Stalowa Wola, Cerkamed, Poland) diluted to a 0.2 solution
2. Alcohol-free CHX 0.2% mouthwash (Hexadiene, Donyaye Behdasht pharmaceutical Company, Tehran, Iran).

In this study, the antimicrobial activity of five substances in combination with irreversible hydrocolloids was studied, which included the following groups:

1. Irreversible hydrocolloid mixed with CHX 0.2% solution (CHXs)
2. Irreversible hydrocolloid mixed with CHX 0.2% mouthwash (CHXm)
3. Irreversible hydrocolloid mixed with AgNPs 0.1% (AgN1)
4. Irreversible hydrocolloid mixed with AgNPs 0.2% (AGN2)

5. Irreversible hydrocolloid mixed with sterile distilled water only as a control group (C).

Preparation of discs

The irreversible hydrocolloid impression powder used for the specimens had already been weighed and found to be of equal weights. Nine grams of irreversible hydrocolloid powder was put in a plastic bowl and 20 ml of the study solution was added to the bowl to prepare the discs of each study group. The mixture was stirred by a user for 45 s using an alginate spatula. Thereafter, the materials were filled in an impression cast with 15-mm internal diameter and 19-mm height placed on a glass slap. The impression cast was then compressed from above by a smooth glass slap to remove extra materials, which was left to set the material, followed by cutting discs of 4 mm thick and 15 mm in diameter by a sterile blade [Figure 1]. Three discs were obtained from each of the five study groups per test bacterium (i.e., a total of 15 discs were obtained from each of the five groups).

Evaluation of antimicrobial effect

The antimicrobial activity was measured by disc diffusion method in Mueller–Hinton agar medium (Merck, Darmstadt, Germany). The standard bacterial strains, namely, *Staphylococcus aureus* (ATCC 20739), *Staphylococcus epidermidis* (ATCC 14990), *Enterococcus faecalis* (PTCC 1237), *Pseudomonas aeruginosa* (Pao1), and *Escherichia coli* (ATCC 35218), were obtained as lyophilized ampoule from the Iranian Research Organization for Science and Technology. The lyophilized ampoules were broken by observing aseptic standards and microbiological methods to prepare a suspension containing the target microorganism, which was cultured and incubated to grow. McFarland 0.5 standard was used to have similar bacterial concentrations. Afterward, a very little amount of precultured bacteria was collected using a sterile swab, mixed in sterile saline, and compared with the opacity of 0.5 McFarland standard. More saline was added in case of a higher opacity, and if it was more transparent, a little amount of bacteria

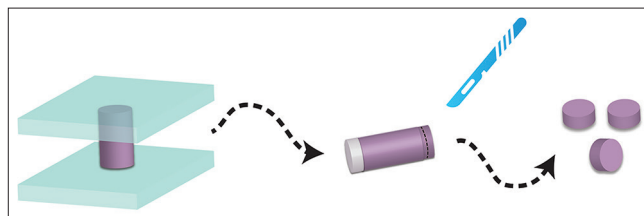


Figure 1: Schematic view of disc preparation.

was added to reach the opacity of 0.5 McFarland. Similar dilutions of bacterial strains were prepared and inoculated in the media. Each individual microbe was cultured in three separate plates to ensure the data. After that, the test discs were placed in the peripheral of the plates containing microbe-infected Mueller–Hinton agar medium. The control samples were placed at the central part of the plates.

The plates containing microorganisms and the discs were incubated at 37°C for 24 h. The inhibition zone diameter for individual microorganisms was measured with a ruler in millimeter by a user. The diameter of the shortest path was considered in the examination.

Data were assigned appropriate codes and were analyzed by SPSS statistics 24 software (I. B. M. Company, New York, U. S. A.). Mean values with standard deviations were reported for continuous data in descriptive statistics. As data were not normally distributed, Kruskal–Wallis test was used at a significance level of 0.05.

RESULTS

Following incubation, growth inhibition zone (GIZ) was obtained for assessing the antimicrobial activity of each bacterial strain [Figure 2]. Table 1 summarizes the GIZs observed in the tested samples. All control groups of the bacterial strains exhibited no antimicrobial activity. In *S. aureus*, the greatest and lowest GIZs were recorded in CHX 0.2% mouthwash and AgNPs 0.1% groups, respectively. Both CHX mouthwash and CHX solution displayed an uppermost effectiveness with a statistically significant difference ($P < 0.05$). A lower antimicrobial activity was detected with AgNPs 0.1% and 0.2% than those of the other two groups, with a statistically significant difference between the two concentrations ($P < 0.05$).

CHX compounds showed the largest GIZs in the case of *E. faecalis*, upon which both CHX solution and

mouthwash presented a fully similar effect ($P = 1$) followed by AgNPs 0.2% with maximum effectiveness. No statistically significant differences were found between these three groups ($P > 0.1$). However, all the three groups were statistically significantly different from AgNPs 0.1% group with lowermost effectiveness ($P < 0.05$).

In *E. coli*, GIZs were of the highest size as a result of CHX compounds with the greatest effectiveness compared to the other substances ($P < 0.05$), while their effects were quite similar to one another ($P = 1$). Both concentrations of AgNPs showed no antibacterial property against this bacterial strain.

AgNPs represented the largest GIZ in *P. aeruginosa*, which had statistically significant differences with the other substances ($P \leq 0.01$), but both concentrations of AgNPs were not statistically significantly different ($P = 1$). CHX compounds exerted no antimicrobial impacts on this bacterial strain.

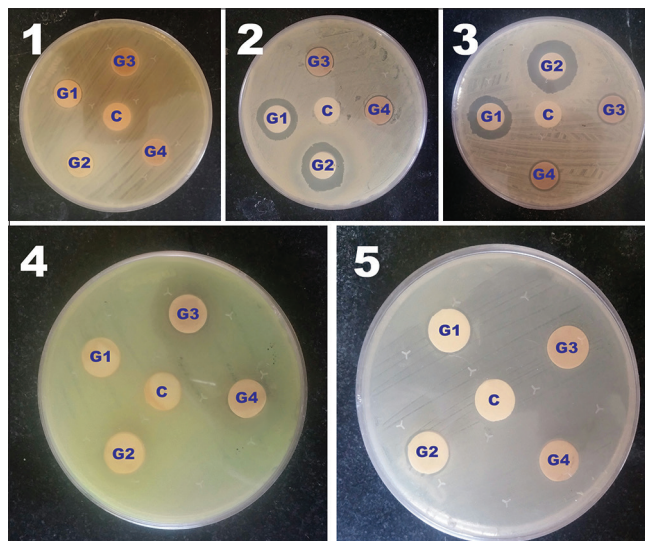


Figure 2: Growth inhibition zone for individual bacterial strains: (1) *Escherichia coli*, (2) *Staphylococcus aureus*, (3) *Staphylococcus epidermidis*, (4) *Pseudomonas aeruginosa*, and (5) *Enterococcus faecalis* under the effects of 0.2% chlorhexidine solution (G1), chlorhexidine mouthwash (G2), 0.1% silver nanoparticles (G3), 0.2% silver nanoparticles (G4), and control group (c).

Table 1: Mean±standard deviation diameter of inhibition zone (mm) for each bacterial species and group

Species	CHX 0.2% solution	CHX 0.2% mouthwash	AgNPs 0.1%	AgNPs 0.2%	Control
<i>Staphylococcus aureus</i>	24.33±0.57	27.66±1.52	17.00±0.00	18.33±0.57	0
<i>Enterococcus faecalis</i>	19.00±0.00	19.00±0.00	17.00±0.00	18.33±0.57	0
<i>Escherichia coli</i>	16.00±0.00	16.00±0.00	0	0	0
<i>Pseudomonas aeruginosa</i>	0	0	17.33±0.57	18.00±1.00	0
<i>Staphylococcus epidermidis</i>	25.33±0.57	27.33±1.15	17.33±0.57	17.00±0.57	0

CHX: Chlorhexidine; AgNPs: Silver nanoparticles

CHX mouthwash led to the greatest GIZ size in *S. epidermidis*. With almost similar effects ($P > 0.05$), both CHX mouthwash and solution were more effective than AgNPs ($P \leq 0.01$). Moreover, AgNPs at concentrations of 0.1% and 0.2% were not statistically significantly different ($P > 0.1$). Overall, the antibacterial activities of CHX solution and mouthwash on the five tested microorganisms were not statistically significantly different from those of AgNPs at concentrations of 0.1% and 0.2% ($P > 0.1$).

DISCUSSION

Based on the study results, our null hypothesis was that there is no difference of antimicrobial effect between CHX and AgNPs. Our results indicated that all the tested materials showed antibacterial effects on *S. aureus*, *E. faecalis*, and *S. epidermidis*. However, CHX compounds and AgNPs revealed no antibacterial impacts on *P. aeruginosa* and *E. coli*, respectively. Even so, when all GIZs were examined among the five experimental groups, no significant differences were observed among CHX 0.2% mouthwash, CHX 0.2% solution, and both AgNP 0.1% and 0.2% groups. Nonetheless, all the tested agents were significantly different from those of the control group. CHX mouthwash exhibited the highest antibacterial effect on *S. aureus*, even higher than its pure solution, with AgNPs 0.1% and 0.2% showing the least influences. The different influences of CHX mouthwash and solution on *S. aureus* can be attributed to the ingredients of CHX mouthwash used herein, which consist of glycerin, methyl paraben, paraben profile, and C.116035 flavor. Because paraben is an antimicrobial preservative, it could have sensitized most of the *S. aureus* strains to CHX mouthwash compared to pure CHX solution.^[13]

CHX compounds and AgNPs 0.2% displayed the highest and lowest antibacterial activities against *E. faecalis*, respectively.

The uppermost antibacterial activity toward *S. epidermidis* was recorded in both CHX mouthwash and solution, whereas AgNPs 0.1% and 0.2% showed the lowermost impacts.

As noted above, CHX compounds presented no antibacterial activities against *P. aeruginosa*, whereas AgNPs at concentrations of 0.1% and 0.2% similarly affected this strain. In line with our results, Wang *et al.*^[10] reported a high resistance of *P. aeruginosa* toward CHX treatment, although they used relatively

lower concentrations than those applied in this study. Such a difference in the response of *P. aeruginosa* to CHX can be attributed to different strains of this bacterium, some of which are more CHX resistant. The present research, however, demonstrated a high antibacterial resistance of *P. aeruginosa* to CHX compared to the other bacterial strains examined. CHX binds to the bacterial cellular membrane, thereby causing intracellular matrix leakage, inhibition of respiratory system, and cytoplasmic coagulation in bacteria. Gram-negative bacteria are more resistant to CHX than Gram-positive ones; in particular, *P. aeruginosa* that has acquired an improved natural resistance to this agent owing to its outer membrane. In addition, the presence of flow pumps (that expel any potentially damaging agent out of the cell) can account for more resistance of this strain toward disinfectants. *P. aeruginosa* can generally resist CHX even at high concentrations.^[14]

Contrary to the former strain, AgNPs showed no antibacterial activity against *E. coli*. This strain was highly affected by the antibacterial activities of both CHX solution and mouthwash while showing almost similar effects. The antibacterial effect of AgNPs on *E. coli* has been reported in some studies.^[11,15,16] The discrepant reports are attributable to differences in the size, concentration, and shape of the used nanoparticles.^[17,18] Furthermore, *E. coli* is a Gram-negative species with a robust polysaccharide cellular wall, which is more resistant to AgNP infiltration into the cell by taking a longer exposure time. Durán *et al.* have also presented evidence that mutant strains of this bacterium are six times more resistant to AgNPs because of lacking OMPG and OMPF prions, and that *E. coli* has a rapid evolutionary ability to resist AgNPs.^[19] The differential mechanism in various strains of this bacterium can explain the different observations reported on *E. coli*.

As mentioned, *E. coli* is also Gram negative similar to *P. aeruginosa*; hence, it is expected that AgNPs and CHX yield similar outcomes on these two bacteria. Inconsistent results, however, can be explained by the unique characteristics of individual bacterial species.

Results of studies by Ginjupalli *et al.* and Penden Wangchuk *et al.* suggest a high antibacterial activity of AgNPs.^[11,12]

Kollu *et al.* reported a minimum inhibitory concentration of 0.02–0.05 for CHX and found that 0.01% CHX solution was sufficient to inhibit the

growth of most microbes present in the irreversible hydrocolloid (before impression forming).^[20] Casemiro *et al.* found a better antimicrobial effect of 0.02% CHX than the other used agents,^[21] which is in agreement with a high antimicrobial activity of 0.02% CHX observed herein.

Kangarlou Haghighi *et al.* compared the antimicrobial activity of AgNPs-containing solution with those of sodium hypochlorite and CHX against *E. faecalis*, *Candida albicans*, *E. coli*, and *P. aeruginosa*. Their results suggested a rather similar antimicrobial effectiveness of AgNPs and CHX,^[15] corroborating our observations. Jafari *et al.*, on the other hand, noticed that AgNPs at 1000 ppm were more effective on all the tested microorganisms compared to other agents used (CHX 2%).^[16] The inconsistent results can be ascribed to differently sized NPs and various bacterial strains used in the two studies.

This study is the first one to examine the antimicrobial activities of AgNPs and CHX compounds with similar concentrations in combination with irreversible hydrocolloid impression material. The two materials showed comparatively identical antimicrobial activities at similar concentrations, with no difference between AgNP concentrations at 0.1% and 0.2%. CHX mouthwash is widely available at a lower price than the other materials tested. Based on the research by Wang *et al.* and Amalan *et al.*, which unveiled the satisfactory physical properties of irreversible hydrocolloid in combination with CHX,^[10,22] our research findings, which showed the strong antimicrobial effect of this substance, and the low cost and availability of this substance as a mouthwash, it is recommended to conduct clinical trials to clinically analyze irreversible hydrocolloid in combination with this substance. Besides, contrary to CHX, both AgNP powder and solution are available. Hence, alginate-producing factories can integrate this material in irreversible hydrocolloid ingredients to induce its antimicrobial properties. Nonetheless, further investigations should scrutinize the effects of size, concentration, and shape of AgNPs as well as its long-term interaction on the physical properties of impression material.

Despite what mentioned above, the present study suffers from some limitations as follows:

It was not possible to determine the antimicrobial activities of samples against harmful and communicable pathogens (e.g., tuberculosis and hepatitis) likely present in the mouth ambiance.

Another study limitation was its *in vitro* nature, the results of which cannot be generalized to *in vivo* conditions. However, the current results may be considered as a base for *in vivo* examinations.

CONCLUSION

Overall, the antibacterial activities of 0.2% CHX compounds against the five tested microorganisms were similar to those of AgNPs at concentrations of 0.1% and 0.2%. CHX mouthwash and pure solution acted similarly in relation to one another.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial, in this article.

REFERENCES

- Jennings KJ, Samaranyake LP. The persistence of microorganisms on impression materials following disinfection. *Int J Prosthodont* 1991;4:382-7.
- Samaranyake LP, Hunjan M, Jennings KJ. Carriage of oral flora on irreversible hydrocolloid and elastomeric impression materials. *J Prosthet Dent* 1991;65:244-9.
- Leung RL, Schonfeld SE. Gypsum casts as a potential source of microbial cross-contamination. *J Prosthet Dent* 1983;49:210-1.
- Rubel BS. Impression materials: A comparative review of impression materials most commonly used in restorative dentistry. *Dent Clin North Am* 2007;51:629-42, vi.
- Cserna A, Crist RL, Adams AB, Dunning DG. Irreversible hydrocolloids: A comparison of antimicrobial efficacy. *J Prosthet Dent* 1994;71:387-9.
- Jagger DC, Huggett R, Harrison A. Cross-infection control in dental laboratories. *Br Dent J* 1995;179:93-6.
- Watkinson AC. Disinfection of impressions in UK dental schools. *Br Dent J* 1988;164:22-3.
- Blair FM, Wassell RW. A survey of the methods of disinfection of dental impressions used in dental hospitals in the United Kingdom. *Br Dent J* 1996;180:369-75.
- Ismail HA, Asfour H, Shikho SA. A self-disinfecting irreversible hydrocolloid impression material mixed with povidone iodine powder. *Eur J Dent* 2016;10:507-11.
- Wang J, Wan Q, Chao Y, Chen Y. A self-disinfecting irreversible hydrocolloid impression material mixed with chlorhexidine solution. *Angle Orthod* 2007;77:894-900.
- Ginjumpalli K, Alla RK, Tellapragada C, Gupta L, Upadhy Perampalli N. Antimicrobial activity and properties of irreversible hydrocolloid impression materials incorporated with silver nanoparticles. *J Prosthet Dent* 2016;115:722-8.
- Penden Wangchuk N, Nisalak P, Thaweboon B, Thawboon S,

- Sawaengkit P. Antimicrobial Property of Hydrocolloid Impression Material Incorporated with Silver Nanoparticles Against *Staphylococcus Aureus*. MATEC Web Conf 2017;95:01001.
13. Bargiota E, Rico-Munoz E, Davidson PM. Lethal effect of methyl and propyl parabens as related to *Staphylococcus aureus* lipid composition. Int J Food Microbiol 1987;4:257-66.
 14. Thomas L, Maillard JY, Lambert RJ, Russell AD. Development of resistance to chlorhexidine diacetate in *Pseudomonas aeruginosa* and the effect of a “residual” concentration. J Hosp Infect 2000;46:297-303.
 15. Kangarlou Haghighi A, Tashfam B, Nasseri M, Dianat O, Taheri S. *In vitro* comparison of antibacterial efficacy of a new irrigation solution containing nanosilver with sodium hypochlorite and chlorhexidine. J Dent School Shahid Beheshti Univ Med Sci 2013;31:1-7.
 16. Jafari A, Bakhtiari R, Nia JR, Mehrabadi JF, Yousefi B. Antimicrobial activity of irreversible hydrocolloid impression against oral microorganisms. J Basic Appl Sci Res 2013;6:397-401.
 17. Naghsh N, Safari M, Hajmehrabani P. Investigating the effect of silver nanoparticles on *E. coli* growth. Qom Univ Med Sci J 2012;6:65-8.
 18. Salmani M. Survey of silver nanoparticles antibacterial activity against gram-positive and gram-negative bacteria *in vitro*. Toloobehdasht 2017;16:74-84.
 19. Durán N, Durán M, de Jesus MB, Seabra AB, Fávaro WJ, Nakazato G. Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity. Nanomedicine 2016;12:789-99.
 20. Kollu S, Hedge V, Pentapati K. Efficacy of chlorhexidine in reduction of microbial contamination in commercially available alginate materials – *In Vitro* study. Global J Med Res 2013;13 (2):19-23.
 21. Casemiro LA, Pires-de-Souza Fde C, Panzeri H, Martins CH, Ito IY. *In vitro* antimicrobial activity of irreversible hydrocolloid impressions against 12 oral microorganisms. Braz Oral Res 2007;21:323-9.
 22. Amalan A, Ginjupalli K, Upadhya N. Evaluation of properties of irreversible hydrocolloid impression materials mixed with disinfectant liquids. Dent Res J (Isfahan) 2013;10:65-73.