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Antimicrobial and physical properties of alginate impression material incorporated with silver nanoparticles

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ABSTRACT

Background: Self-disinfecting impression materials would reduce time and energy needed for impression disinfecting process in clinic. The aim of this study was to evaluate the antimicrobial effect of alginate mixed with nanosilver solution at a concentration of 500 ppm and 1000 ppm on common oral microorganisms and assess changes in working time, setting time, and surface detail reproduction.

Materials and Methods: In this *in vitro* study, three groups were assigned. The first group was alginate, the second group was alginate mixed with 500 ppm nanosilver, and the third group was alginate mixed with 1000 ppm nanosilver. Antimicrobial effect on *Escherichia coli*, *Staphylococcus aureus,* and *Candida albicans* was studied using direct contact test in each group (n = 10). Working time (n = 10), setting time (n = 10), and surface detail reproduction (n = 10) were evaluated separately using the ISO 21563 protocol. Descriptive tables were used to describe the data. Kruskal–Wallis test used to determine significant differences in the number of colonies was counted in antimicrobial test (α = 0.05).

Results: No adverse effects observed in working time, setting time, and surface detail reproduction of alginate impressions. Alginate mixed with silver nanoparticles showed no inhibitory effect on *S. aureus* and *C. albicans*, but the number of *E. coli* colonies were counted in the group 1000 ppm was significantly lower than 500 ppm (P = 0.001).

Conclusion: Antimicrobial effect of alginate mixed with silver nanoparticles is not clinically indicated. Nevertheless, its physical features did not change significantly.

Key Words: Dental impression materials, microbiologic, nanotechnology

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INTRODUCTION

Alginate impression material has a widespread use in dentistry.[1] The oral cavity is a host to many microbial agents, and it is shown that oral fluid could mix into the impressions.[2] Therefore, they are capable of making cross infection.[3]

Many studies evaluated various methods to disinfect impression materials.[4-7] Among disinfectant agents, chlorhexidine (CHX) and quaternary ammonium salts were reported to have superior antimicrobial activity against oral microorganism.[1,5-8]

The most widespread methods for disinfecting impressions are using spray and immersing. However, these methods are time-consuming and have negative effect on mechanical properties of alginate.[1]

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Silver and its derivatives are employed as antimicrobial agent, and especially nanosilver products have been used in various medical applications.[9-11] Therefore, incorporation of nanosilver particles in alginate impression material may be useful.

Despite the widespread use of nanosilver products, few studies have examined the effect of nanosilver on antimicrobial and mechanical properties of impression materials. Therefore, the null hypothesis of this study was that adding of nanosilver particles to alginate has no effect on mechanical and antimicrobial properties of the materials.

MATERIALS AND METHODS

In this *in vitro* study, three groups were assigned. The first group was alginate, the second group was alginate mixed with 500 ppm nanosilver particles, and the third group was alginate mixed with 1000 ppm nanosilver particles. Varying concentrations (500 and 1000 ppm) of silver nanoparticles (prepared in School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran) of 90–100 nm size were added to a dust-free alginate (Iralgin, Golchi, Iran) and its properties were evaluated.

Preweighed alginate powder and silver nanoparticles were dispended in a container and mixed uniformly. Alginate without silver nanoparticles was tested as control group.

Alginate with or without nanoparticles was prepared by mixing powder with a premeasured volume of deionized water as recommended by the manufacturer (23 g powder with 50 ml water).

Alginates were mixed for 45 s using a rubber bowl and alginate mixing spatula by a single operator. At the end of the mixing time, the material was filled into an orthodontic gypsum mold of 30 ± 0.2-mm internal diameter and 16 ± 0.1-mm height. Working time was measured using indentation methods. Seventy seconds after mixing the alginate, a needle (weighed 50 g) was released. Using Universal testing Machine device (SANTAM, Iran), the amount of remained alginate under the needle was measured. This measurement should not be <0.25 mm according to the ISO 21563 protocol.[12] This process was performed ten times for each group [Figure 1]. To measure primary setting time similar to the working time, the mold was fulfilled with alginate and 5 s before the manufacturer's instruction (2 min and 30 s), and the rod was placed in contact with the surface of alginate. This procedure was repeated at 10 s intervals until the impression material no longer adhered to the rod (n = 10). To evaluate surface details reproduction, a grooved mold was used [Figure 2]. Fifteen seconds before termination of working time, the mold was fulfilled by alginate and a glass plate was placed on top of this mixture. The surface of specimens was examined by stereomicroscope (Olympus, Leica, EZ4D, Italy) and reproduction of 20-μ groove was considered acceptable (n = 6 for each group). The results were reported according to Culhaoglu *et al*.'s rating.[13]

- Rating 1: Well defined, sharp detail, continuous line
- Rating 2: Continuous line but with loss of sharpness
- Rating 3: Poor detail or loss of continuity of the line
- Rating 4: Marginally or not discernible.

Figure 1: Designed device to measure working time.

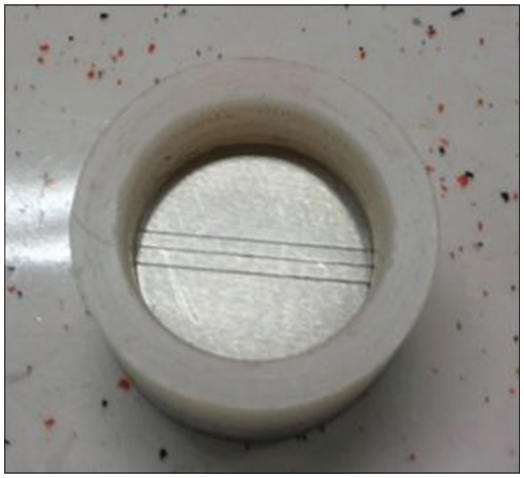

Figure 2: The mold for evaluating surface detail reproduction.

Antimicrobial activity on *Escherichia coli*, *Staphylococcus aureus,* and *Candida albicans* assessed using direct contact test. In each group, 10 alginate specimens were prepared in molds (2-cm diameter and 0.7-cm height). All the specimens were placed in autoclavable container and were sterilized in 121°C and 15 Psi. After that, 100 µl from the bacterial or fungal solution were added and remained for 1 h. The lawn cultures of all the three microorganisms tested were made with a bacterial or yeast suspension, matching the turbidity of a 0.5 McFarland standard. After 1 h, the solution was collected from alginate specimens by a sampler and was cultured in a blood agar medium and incubated at 37°C for 24 h. Then, the number of colonies was counted. Data were analyzed by Kruskal–Wallis test using SPSS software (ver. 16, IBM, New York, USA), and the statistical significance level was 0.05 ($\alpha = 0.05$).

RESULTS

Working time

The mean measured depths for control, 500 ppm nanosilver incorporated alginate, and 1000 ppm nanosilver incorporated groups were 15.82 ± 0.13 mm, 15.89 ± 0.08, and 15.93 ± 0.03, respectively [Table 1].

According to ISO 21563, the measured depth >15.75 mm is standard. Therefore, in all groups, the working time was standard.

Setting time

As shown in Table 1, the mean measured setting time for control, 500 ppm nanosilver, and 1000 ppm nanosilver groups was 161 ± 9.16 s, 166 ± 5.38 s, and 157 ± 7.48 s, respectively. According to ISO 21563,[12] the setting time 20% more or less than the time mentioned by manufacturer is acceptable (120–180 s). Therefore, all specimens showed acceptable setting time.

Surface detail reproduction

Six samples were prepared for each group. In all specimens, the 20-µm line was evident by stereomicroscope. None of the specimens showed rating 4 [Table 2].

Antimicrobial activity

To test antimicrobial activity, direct contact test was used for *E. coli*, *S. aureus,* and *C. albicans*.

There were no significant differences in *S. aureus* count among groups ($P = 0.617$). Furthermore, *C. albicans* count showed no significant differences among groups ($P = 0.086$). However, there were statistically significant differences in *E. coli* count among groups ($P = 0.008$). *Post hoc* test showed that the counted colonies in the concentration of 1000 ppm nanosilver were significantly < 500 ppm nanosilver concentration ($P = 0.008$) [Table 3].

DISCUSSION

The null hypotheses were rejected; adding of nanosilver particles to alginate had effect on mechanical and antimicrobial properties of the materials.

One way against cross infection in dental office is disinfection of impression materials. It has been reported that bacillus tuberculosis, hepatitis B, herpes virus, and other microorganisms could transfer by impression materials. Surface disinfection of impressions may inhibit this cross infection.[14] Spraying and immersing the impressions are the most

Table 1: Penetration depth of needle and setting times in groups

Group	Mean±SD	
	Penetration depth (mm)	Setting time (s)
Control	15.82±0.13	161±9.16
500 ppm	15.89±0.08	166±5.38
1000 ppm	15.93±0.03	157±7.48

SD: Standard deviation

Table 2: Surface reproduction details of samples

Groups	*n*	Control	500 ppm	1000 ppm
Rating	1	1	2	1
	2	1	2	2
	3	1	1	3
	4	1	2	2
	5	1	1	2
	6	1	1	1

Table 3: Comparison of counted colonies among the groups

Microorganism	Groups	Mean±SD	Kruskal–Wallis test χ^2, *P*
Staphylococcus aureus	Control	40.00±37.00	0.964, 0.617
	500 ppm	33.10±26.24	
	1000 ppm	47.39±0.72	
Candida albicans	Control	26.00±21.66	4.90, 0.086
	500 ppm	36.00±27.57	
	1000 ppm	12.29±16.00	
Escherichia coli	Control	122.75±125.18	9.70, 0.008
	500 ppm	122.89±81.19	
	1000 ppm	26.10±31.02	

P<0.05 is statistically significant. SD: Standard deviation

common methods to disinfect these materials.[1] However, these methods may have negative effects on physical properties of impressions.[1,14] It has been shown that incorporation of disinfectant agents into the impression materials is an effective method.[4,5,15]

Recently, nanoparticles, especially nanosilver as an antimicrobial agent, have been widely used.[11,16] In this study, we added two concentrations of nanosilver particles (500 ppm and 1000 ppm) and then antimicrobial and mechanical properties were evaluated. The antimicrobial activity was measured using direct contact test. The advantage of this method to disc diffusion and agar well method is that the procedure is more similar to clinical condition. In our study, three microorganisms *S. aureus* (g+), *E. coli* (g−), and *C. albicans* (fungi) were evaluated. Furthermore, Ginjupalli *et al*.[7] in their study examined these three microorganisms.

Our results showed that nanosilver addition to alginate has no adverse effect on its mechanical properties; however, there was no significant difference in counted colonies of *S. aureus* and *C. albicans* among the groups. Although for *E. coli,* there were significant less colonies in 1000 ppm nanosilver group in comparison with 500 ppm group. However, even existence of one colony indicates the inability of nanosilver with concentrations of 1000 ppm and 500 ppm to hinder the microorganisms. Ginjupalli *et al*.[7] showed that alginate incorporated by silver nanoparticles had a proper antimicrobial effect on *S. aureus*, *E. coli,* and *C. albicans*.

These differences may be as a result of the difference in the methods. Ginjupalli *et al*.[7] used disc diffusion method; however, we used direct contact test to count the colonies. Moreover, Ginjupalli *et al*.[7] and Jafari *et al*.[4] showed that the silver-incorporated irreversible hydrocolloids exhibited dose-dependent antimicrobial activity. However, in our study, it was true only for *E. coli*. Sondi and Salopek-Sondi showed that nanosilver particles have a limited use as antimicrobial agent.[17]

Flanagan *et al*.[18] and Kollu *et al*.[14] showed that CHX-incorporated alginate was capable of destroying 99% of microorganism. Consequently, it can be said that this method is better than incorporation of silver nanoparticles.

CONCLUSION

Incorporation of silver nanoparticles in concentrations of 500 ppm and 1000 ppm did not have the ability to completely hinder growth of *E. coli*, *S. aureus,* and *C. albicans* existed on the surface of alginate. Nevertheless, its physical properties (working time, setting time, and reproduction of surface details) did not change significantly.

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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.

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