THE JOURNAL OF PROSTHETIC DENTISTRY

RESEARCH AND EDUCATION

Antimicrobial activity and properties of irreversible hydrocolloid impression materials incorporated with silver nanoparticles

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ABSTRACT

Statement of problem. Conventional spray and the immersion disinfection of irreversible hydrocolloid impression materials may lead to dimensional changes.

Purpose. The purpose of this in vitro study was to investigate the antimicrobial activity and properties of irreversible hydrocolloid impression materials incorporated with silver nanoparticles.

Materials and methods. The antimicrobial activity and properties of 2 commercially available irreversible hydrocolloid impression materials were evaluated after incorporating varying concentrations of silver nanoparticles. Antimicrobial activity was determined using the disk diffusion method. The gel strength, permanent deformation, flow, and gelation time were measured according to American Dental Association specification #18. Analysis of variance was used to identify the significant differences within and across the groups (α=.05).

Results. Adding silver nanoparticles to irreversible hydrocolloid impression materials resulted in superior antimicrobial activity without adversely affecting their properties. Adding silver nanoparticles to Zelgan significantly increased the gel strength compared with the control group, except at 5 wt%. However, the gel strength of Tropicalgin was unaffected except at 5 wt%. An increase in the permanent deformation was found with the incorporation of silver nanoparticles in both Zelgan and Tropicalgin. The flow of Zelgan increased with the incorporation of silver nanoparticles, whereas a decrease in the flow of Tropicalgin was observed at 1 wt% and 2 wt%. An increase in the gelation time of both Zelgan and Tropicalgin was observed with the incorporation of silver nanoparticles.

Conclusions. Based on this in vitro study, silver nanoparticles can be incorporated into irreversible hydrocolloid impression materials as antimicrobial agents without adversely affecting their properties. (J Prosthet Dent 2016;115:722-728)

Dental impressions inevitably come into contact with the patient's saliva, blood, and bacterial plaque, all of which may carry pathogenic microorganisms. Contaminated impressions are a source of cross infection and transmitters of diseases to the dental staff and/or laboratory personnel.[1-3] Therefore, sanitizing the impressions effectively before transportation to the dental laboratory becomes indispensable. Although, sterilizing the impressions would be the ideal way to avoid disease transmission, mere disinfection is routinely practiced. This practice is justified in view of the dimensional changes that occur in the impressions due to sterilization.[4]

Various disinfectants such as sodium hypochlorite, sodium metabisulphite, biguanides, iodine compounds (such as iodophors), quaternary ammonium salts, phenolics, and glutaraldehyde are used routinely. Because no single disinfectant can be selected as a universal disinfectant for all impressions, it is imperative to select a disinfectant with superior antimicrobial activity that does not affect the recorded details.[5] Despite the importance of disinfection of impressions,

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Clinical Implications

Adding silver nanoparticles to irreversible hydrocolloids will impart significant antimicrobial activity to the impression materials without adversely affecting their properties; as a result, the conventional immersion or spray disinfection of such materials might be avoided.

some investigations indicate that such disinfection is not regularly practiced.[6-8]

Irreversible hydrocolloid impression material is routinely used in clinical practice.[9] This material is commercially available as a powder containing soluble alginate along with calcium sulfate dihydrate and trisodium phosphate. In addition, the powder also contains diatomaceous earth as a filler. Upon mixing with water, a double decomposition reaction between the soluble alginate and calcium sulfate dihydrate results in the formation of a crosslinked calcium alginate gel.[10-12] During impression recording, the surface texture and hydrophilic nature of irreversible hydrocolloid impression material allows it to retain the maximum amount of microbial pathogens not only on the surface but also well within the material.[13]

Irreversible hydrocolloids are disinfected either by spray or an immersion technique using a disinfectant solution. However, both techniques disinfect the impression only on the surface.[14] Further, these processes may result in significant dimensional changes in the irreversible hydrocolloid impressions, leading to loss of detail.[15-21] Deterioration in the surface quality and hardness of dental gypsum casts obtained from disinfected irreversible hydrocolloid impression materials has also been widely reported.[15,22,23] As a result, the disinfection time of irreversible hydrocolloids should be short to avoid significant dimensional changes. However, such a reduction in immersion time may significantly reduce the efficacy of disinfection, especially for a porous irreversible hydrocolloid impression material.

To address the drawbacks associated with the conventional disinfection of irreversible hydrocolloids, self-disinfectant irreversible hydrocolloids have been developed by adding disinfectant to the irreversible hydrocolloid compositions. Such a process was thought to eliminate further disinfection of irreversible hydrocolloids.[11] Several water soluble and easily dispersible antimicrobial agents have been added to irreversible hydrocolloids, including chlorhexidine, quaternary ammonium salts, and didecyl dimethyl ammonium chloride.[14,24-27] One of the main advantages of self-disinfectant irreversible hydrocolloid impression materials is that they are not only disinfected on the surface but throughout the material as the disinfectant is uniformly distributed within the material. Among the disinfectant materials investigated, chlorhexidine and quaternary ammonium salts were found to exhibit superior antimicrobial properties with zones of inhibition when tested against common oral microorganisms.[14,24,25,28]

However, irreversible hydrocolloids containing chlorhexidine have been found to exhibit longer gelation times, and higher concentrations of chlorhexidine are cytotoxic to human fibroblasts.[14,27] An additional disinfection process involving conventional immersion or spraying technique is generally recommended by the manufacturers even for the self-disinfectant irreversible hydrocolloids.[4] Some investigators have reported significant changes in the properties of self-disinfectant irreversible hydrocolloids, such as gel strength, gelation time, permanent deformation, and surface detail reproduction.[14,18,29] Also the reproducibility of surface detail of self-disinfectant irreversible hydrocolloids was significantly reduced upon pouring with gypsum products.[27] Sodium hypochlorite-incorporated irreversible hydrocolloids showed faster setting and loss of surface detail.[27] In addition, some of the disinfectants added to irreversible hydrocolloids can cause tissue irritation and allergic reactions.[30] Furthermore, the efficacy of these disinfectants on drug-resistant bacteria has not been investigated.

Silver has a long history of use as a broad-spectrum antimicrobial agent and is used in the treatment of skin ulcers, burn injuries, and eye infections.[31] With the advent of nanotechnology, nanoparticles of silver have been used in various biomedical applications.[32,33] Despite this, the use of silver nanoparticles as an antimicrobial agent in impression materials has not been investigated. The purpose of this in vitro study was to evaluate the antimicrobial activity and properties of 2 commercially available irreversible hydrocolloid impression materials modified with the incorporation of silver nanoparticles. The hypothesis was that incorporating silver nanoparticles into irreversible hydrocolloids would impart antimicrobial activity to the materials without significantly affecting their properties.

MATERIAL AND METHODS

The 2 commercial irreversible hydrocolloids studied were a dust-free alginate (Zelgan Plus; Dentsply India Pvt Ltd) and a color-changing alginate (Tropicalgin; Zhermack SpA). Varying concentrations (0.5, 1, 2, and 5 wt%) of silver nanoparticles (Nano Labs) of 80 to 100 nm in size were added to these irreversible hydrocolloids, and their properties were evaluated.

Preweighed irreversible hydrocolloid powder and silver nanoparticles were dispensed into a container and tumbled to mix the powders uniformly. Irreversible

hydrocolloids without silver nanoparticles were tested as control groups. Irreversible hydrocolloid specimens with or without nanoparticles were prepared by mixing the powder with a premeasured volume of deionized water as recommended by the manufacturer. All materials were mixed for 45 seconds using a rubber bowl and alginate mixing spatula by a single operator (K.G.) to standardize the manipulative variables. At the end of the mixing time, the material was filled into a polyvinyl chloride mold of 30 mm internal diameter and 16 mm height on a flat glass slab. Immediately after filling the mold, a second mold of 15 mm internal diameter and 19 mm height was forced into the irreversible hydrocolloid mix in the first mold until it extruded onto the top. Subsequently, a flat glass plate was pressed on top of the second mold to remove excess material, and the material was left in the mold until it set; the specimens were then retrieved and tested.

Antimicrobial activity was assessed using the Kirby Bauer disk diffusion method (n=3). Disks of 3 mm in thickness were sliced from the irreversible hydrocolloid specimens with a sterile scalpel, placed on Mueller Hinton agar plates containing lawn cultures of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), or *Candida albicans* (ATCC 24433) and incubated at 37°C for 24 hours. The lawn cultures of all 3 microbes tested were made with a bacterial or yeast suspension matching the turbidity of a 0.5 McFarland standard. To test anticandidal activity, Mueller Hinton agar plates were incorporated with 2% glucose. After 24 hours, antimicrobial activity was evaluated by measuring the zones of inhibition in millimeters (mm).

Gelation time was measured using the indentation method as described by Lemon et al.[34] Sixty seconds after the start of mixing, the flat end of a polished polymethyl methacrylate rod of 6 mm in diameter and 10 cm in length was placed in contact with the surface of the irreversible hydrocolloid mix and withdrawn immediately. This procedure was repeated at 5-second intervals until the impression material no longer adhered to the rod. The gelation time was established from the beginning of mixing until the material no longer adhered to the rod (n=5).

Flow was determined as described by Wang et al.[14] A standard 0.5 mL of irreversible hydrocolloid mix (n=3) was injected onto a glass plate (15×15×2 mm) with a disposable syringe. Another glass plate was placed on top of this mix, and a load of 14.7 N was placed on the upper plate for 5 seconds. The diameter of the impression disk formed was measured at 3 different places, and the average diameter was considered as flow.

Gel strength was measured using a universal testing machine (Model 3366; Instron Corp) under compressive loading as described in a previous investigation.[35] Six minutes from the start of mixing, the specimens (n=10) were placed on the bottom plate of the universal testing machine and were stressed at a rate of 10 mm/min. The maximum compressive load reported during the test divided by area of the specimen was considered as the gel strength.

Permanent deformation was measured according to a previously reported method with a few modifications.[35] Six minutes from the start of mixing, specimens (n=5) were placed in the universal testing machine and were subjected to 10% deformation. The deformation force was maintained for 15 seconds, after which the load was removed and the specimens allowed to recover for 30 seconds. At the end of the recovery time, the length of the specimen was measured, and the percentage change in the length of the specimen was reported as permanent deformation.

The data were analyzed with 1-way ANOVA, and significant differences between the control and test groups were compared with the Dunnett test (α=.05).

RESULTS

Figure 1 shows the antimicrobial activity of irreversible hydrocolloids against the selected microorganisms. The silver-incorporated irreversible hydrocolloids exhibited dose-dependent antimicrobial activity. The mean diameter and standard deviation of the zone of inhibition against the tested microorganisms observed with the control and irreversible hydrocolloids incorporated with silver nanoparticles are presented in Table 1. The control specimens of Zelgan did not exhibit any antibacterial activity, although some anticandidal effect was observed. In contrast, Tropicalgin exhibited some activity against all the organisms tested. The antimicrobial activity of irreversible hydrocolloids against all the microorganisms tested increased significantly with the incorporation of silver nanoparticles (P<.001). Furthermore, a significant interaction between the concentration of silver nanoparticles in the irreversible hydrocolloids and zone of inhibition was found with 2-way ANOVA, indicating that increasing concentrations of silver nanoparticles significantly increased the antimicrobial activity of both Zelgan (P=.003) and Tropicalgin (P<.001).

Figure 2 shows the gelation time of irreversible hydrocolloids incorporated with varying concentrations of silver nanoparticles. An increase in the gelation time compared with the control group was observed with Zelgan incorporated with silver nanoparticles at all concentrations (P<.001). However, the increase in gelation time was not statistically significant at 2 wt%. Tropicalgin incorporated with silver nanoparticles showed an increase in gelation time at all concentrations (P<.001).

As seen in Figure 3, the flow of Zelgan incorporated with silver nanoparticles was significantly higher compared with the control group at all concentrations

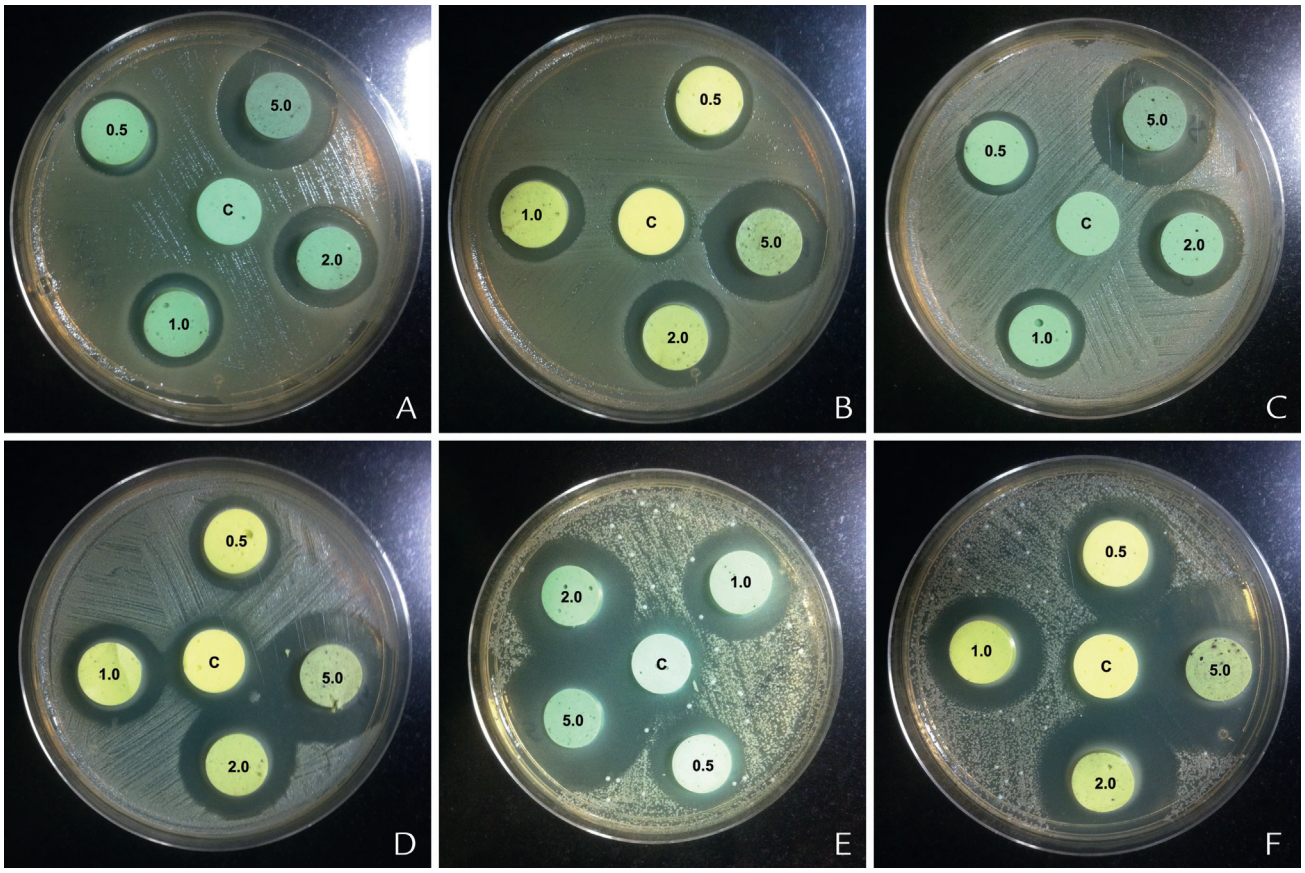

Figure 1. Antimicrobial/antifungal activity. Numerical values on disks represent weight percentage of silver nanoparticles and C represents control group. A, Zelgan incorporated with silver nanoparticles against *Escherichia coli*. B, Tropicalgin incorporated with silver nanoparticles against *E. coli*. C, Zelgan incorporated with silver nanoparticles against *Staphylococcus aureus*. D, Tropicalgin incorporated with silver nanoparticles against *S. aureus*. E, Zelgan incorporated with silver nanoparticles against *Candida albicans*. F, Tropicalgin incorporated with silver nanoparticles against *C. albicans*.

Table 1. Antimicrobial activity, in millimeters, of irreversible hydrocolloids incorporated with silver nanoparticles (mean ±SD)

	Zelgan			Tropicalgin		
Concentration	***E. coli***	***S. aureus***	***C. albicans***	***E. coli***	***S. aureus***	***C. albicans***
Control	0.00 ±0.00	0.00 ±0.00	8.00 ±1.73	4.00 ±1.00	5.67 ±1.53	5.67 ±0.58
0.5 wt%	1.67 ±0.58	4.33 ±0.58	10.33 ±1.15	5.67 ±0.58	6.67 ±0.58	9.33 ±1.15
1 wt%	6.33 ±0.58	7.00 ±0.00	13.33 ±1.53	7.67 ±0.58	9.33 ±0.58	12.33 ±0.58
2 wt%	9.00 ±0.00	9.67 ±0.58	16.33 ±1.15	9.33 ±0.58	11.67 ±1.15	16.67 ±1.15
5 wt%	11.67 ±0.58	14.67 ±0.58	23.00 ±1.00	13.33 ±0.58	17.67 ±0.58	23.67 ±0.58

E. coli, Escherichia coli; S. aureus, Staphylococcus aureus; C. albicans, Candida albicans.

(P<.001). However, the flow of Tropicalgin was unaffected by the incorporation of silver nanoparticles at 0.5 wt% (P=.988) and 5 wt% (P=.213), whereas a significant decrease in the flow was observed at 1 wt% and 2 wt% of silver nanoparticles (P<.001).

Figure 4 shows the gel strength of irreversible hydrocolloids incorporated with silver nanoparticles. Adding silver nanoparticles to Zelgan significantly increased the gel strength compared with the control group (P<.001). However, the increase in strength was not statistically significant at 5 wt% concentration (P=.076). The gel strength of Tropicalgin, however, was unaffected by the silver nanoparticles except at 5 wt%, where a significant decrease in the gel strength was observed compared with the control group (P=.007).

The effect of silver nanoparticles on the permanent deformation of irreversible hydrocolloids is presented in Figure 5. The permanent deformation observed in the control group and 0.5 wt% silver nanoparticles groups were not significantly different for either Zelgan (P=.163) or Tropicalgin (P=1.00). A significant increase in permanent deformation was observed at all other concentrations both for Zelgan (P=.002) and Tropicalgin (P<.001).

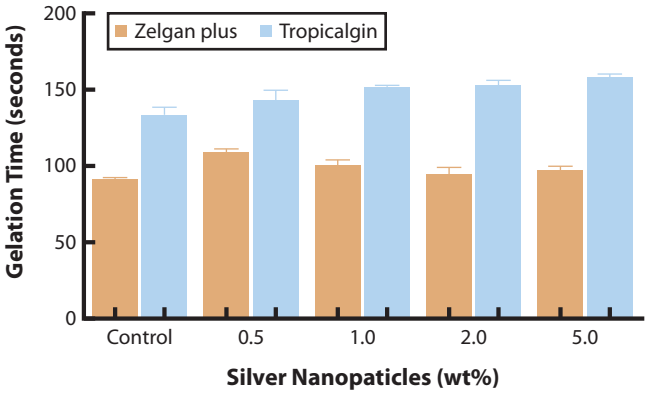

Figure 2. Gelation time (mean ±SD) of irreversible hydrocolloid impression materials incorporated with silver nanoparticles.

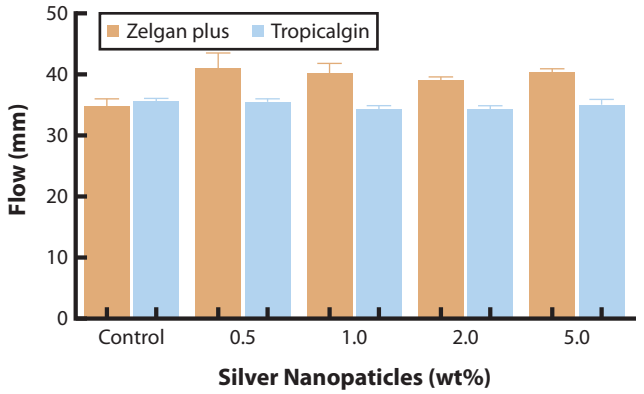

Figure 3. Flow (mean ±SD) of irreversible hydrocolloid impression materials incorporated with silver nanoparticles.

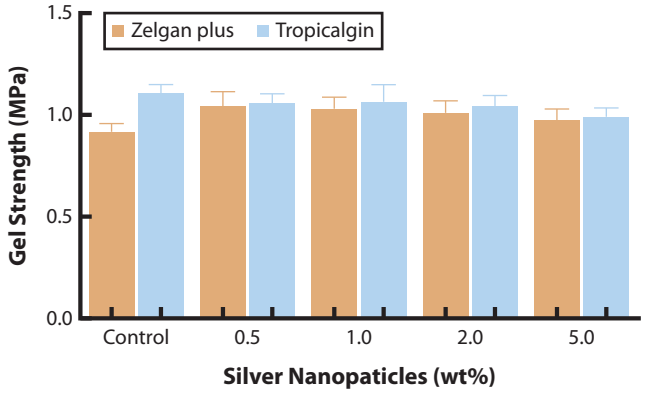

Figure 4. Gel strength (mean ±SD) of irreversible hydrocolloid impression materials incorporated with silver nanoparticles.

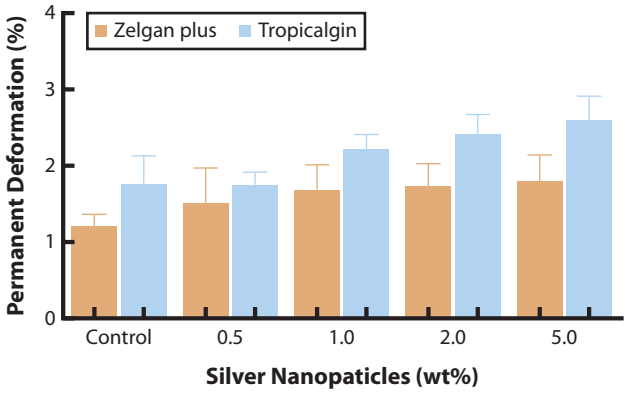

Figure 5. Permanent deformation (mean ±SD) of irreversible hydrocolloid impression materials incorporated with silver nanoparticles.

DISCUSSION

The disinfection of impressions provides an effective barrier against cross infection in dental clinical conditions. The hydrophilic nature and porous structure of irreversible hydrocolloids lead to maximum retention of microorganisms both on the surface and within the material. Irreversible hydrocolloids themselves do not have significant antimicrobial activity, and the conventional spray or immersion techniques only disinfect the impression surface. Disinfectant incorporated into the irreversible hydrocolloid, however, provides an effective disinfection throughout the material.[13,28,36] The use of nanoparticles, silver in particular, as antimicrobial agents has been used increasingly for various biomedical applications.[31]

In the present study, the antimicrobial activity was measured using disk diffusion method, which is widely used for measuring antimicrobial susceptibility. In the process of evaluating antimicrobial activity, *S. aureus* and *E. coli*, belonging to gram-positive and gram-negative bacterial species, respectively, are widely used. For dental applications, *C. albicans* is well known to be associated with fungal infections and so in the present study these 3 organisms were selected.

Silver nanoparticles have a high surface area and are nontoxic to the human body at low concentrations.[37,38] Even at higher concentrations, incorporating silver may not increase the incidence of tissue irritation or toxicity because of its low percutaneous absorption and the limited contact time with the tissues.[39]

Silver nanoparticles were found to exhibit antibacterial action against several species of bacteria, including some multidrug-resistant bacteria.[37,38,40] They also exhibit antiviral and fungicidal properties.[38] The antibacterial action of silver is attributed to the ability of positively charged silver ions to interact with negatively charged bacterial cell membranes. This causes pits in the cell wall, leading to increased cell wall permeability and cell death.[33] Silver adheres to bacterial DNA, RNA, bacterial proteins, and the thiol groups of bacterial enzymes,[39] inhibits cell division, and damages the cellular content of the bacteria. The antibacterial action against *S. aureus* and *E. coli* was due to the inactivation of lactate dehydrogenase and increased protein leakage from the cell walls.[41]

Gelation time refers to the formation and crosslinking of calcium alginate chains. The gelation time of irreversible hydrocolloids was found to increase with the increasing concentration of silver nanoparticles. The increase in gelation time indicates a delay in the formation of calcium alginate gel. A similar increase in gelation time was observed with the incorporation of disinfectant into the irreversible hydrocolloid.[14,27] The flow of irreversible hydrocolloids refers to the ability of irreversible hydrocolloid to deform under applied load. A longer gelation time of irreversible hydrocolloids provides a longer time for flow and so irreversible hydrocolloids with silver nanoparticles, especially Zelgan, showed higher flow. However, in the case of Tropicalgin, no significant increase in flow was observed despite the longer gelation times observed during the study.

Silver nanoparticles in Zelgan improved the gel strength, whereas the gel strength of Tropicalgin decreased. The observed difference in the gel strength between the irreversible hydrocolloids could be due to compositional differences and the differences in the water-to-powder ratio. Tropicalgin requires less water (15 g powder to 30 mL water) for mixing compared with Zelgan (15 g powder to 33.7 mL water), indicating that the gel network of Tropicalgin is denser and so has a higher gel strength. At lower concentrations, silver nanoparticles reinforced the brush heap structure, whereas at higher concentrations, the strength decreased because a large number of reactive ingredients were replaced with silver nanoparticles, leading to the formation of a weaker gel network.[10]

The permanent deformation of both irreversible hydrocolloids increased with the incorporation of silver nanoparticles in a dose-dependent manner. However, the increase in the permanent deformation in irreversible hydrocolloid impression materials did not exceed the maximum permanent deformation of 3% as required by the American Dental Association specification.

The addition of silver nanoparticles to irreversible hydrocolloids resulted in dose-dependent antimicrobial activity. However, changes in their gel strength, permanent deformation, flow, and gelation time were noted. The observed changes in gelation time can be considered of minor clinical significance, whereas increased flow would improve detail reproduction. An increase in or no effect on the gel strength was observed at most concentrations along with an increase in permanent deformation. However, the increase in permanent deformation was well within the requirements for clinical use. As a result, addition of silver nanoparticles to irreversible hydrocolloids can be considered to impart antimicrobial activity. Further investigations on the effect of silver nanoparticles on detail reproduction and biocompatibility are needed. In addition, a detailed investigation on the effect of silver nanoparticle size on the antimicrobial activity and properties of irreversible hydrocolloids is warranted.

CONCLUSION

Within the limitations of the present in vitro study, it can be concluded that the incorporation of silver nanoparticles imparted significant antimicrobial activity to the irreversible hydrocolloid impression materials tested. Furthermore, the incorporation of silver nanoparticles into irreversible hydrocolloid impression materials did affect some of their properties, depending on the type of irreversible hydrocolloid impression material.

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Acknowledgments
The authors thank Mr Vaikunta Holla for his assistance during the study.