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Original Article

Comparative Evaluation of Antifungal Efficacy of Irreversible Hydrocolloid Incorporated with Silver Zeolite and Copper Oxide Nanoparticles

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Abstract

Aim: The purpose of this study is to compare and evaluate the antifungal efficacy of two commercially available irreversible hydrocolloids incorporated with silver zeolite and copper oxide nanoparticles.

Statement of Problem: Conventional method of disinfection led to some adverse changes in dimensional properties in various alginate studies.

Objectives: The objectives of the study were as follows: • To prove the antifungal efficacy of irreversible hydrocolloids incorporated with silver zeolite nanoparticles. • To prove the antifungal efficacy of irreversible hydrocolloids incorporated with copper oxide nanoparticles. • To compare the antifungal efficacy of irreversible hydrocolloid incorporated with silver zeolite and copper oxide nanoparticles.

Materials and Methods: Two commercially available irreversible hydrocolloids were incorporated with 2.5%, 5%, and 7.5% concentrations of silver zeolite and copper oxide and antifungal efficacy is evaluable by measuring the zone of inhibition which is measured in millimeters.

Results: Addition of different types of nanoparticles in different concentrations to the commercially available irreversible hydrocolloid impression materials increased the antifungal activity significantly.

Conclusion: Alginate with silver zeolite (7.5%) showed highest antifungal efficacy compared to alginate with copper oxide.

Key words: Antifungal efficacy, Copper oxide nanoparticles, Impression material, Silver zeolite nanoparticles, Zone of inhibition

INTRODUCTION

The study of biomaterials and its properties is of immense interest in the field of research and innovative technology. One of the most widely accepted materials used in dentistry is hydrocolloid alginate impression due to its several advantages over the others. Impression material has been one of the many biomaterials that have captured the attention of many researchers. Dental impressions have a prime role in treating the patient for prosthodontic purpose. These impressions inevitably come into contact with the patient's saliva, blood, and bacterial plaque, all of which may carry pathogenic microorganisms. Contamination of impressions is major source for the transmission of diseases from patients to the dentist/dental auxiliaries.[1-7]

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Therefore, disinfecting the impressions effectively before transportation to the dental laboratory becomes indispensable. Initially, sterilization of impressions is the ideal way to avoid disease transmission, disinfection is routinely practiced. This practice is justified in view of dimensional changes that occur in the impressions due to sterilization.[7-10]

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Various disinfectants such as sodium hypochlorite, sodium metabisulfite, biguanides, iodine compounds (such as iodophors), quaternary ammonium salts, phenolic, and glutaraldehyde are used routinely. However, no single disinfectant can be selected as a universal disinfectant for all impressions, it is imperative to select a disinfectant with superior antifungal activity that does not affect recorded details. Despite the importance of disinfection of impressions, some investigations indicate that such disinfection is not regularly practiced.

Alginate is the routinely used impression material in clinical practice for recording preliminary impressions which is a major source for the transmission of diseases from patient to dentist/laboratory personnel. 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 with in the material.[11-14]

Clinical Implications

Addition of different types of nanoparticles such as silver zeolite and copper oxide will impart significant anti-activity to the impression materials without significantly affecting their properties.

Conventional immersion or spray disinfection of such materials might be avoided.

In general, these irreversible hydrocolloids are disinfected either by spray or an immersion technique using a disinfectant solution. However, both the techniques disinfect the impression only on the surface. Further, these processes may result in significant dimensional changes in the irreversible hydrocolloid impression material, leading to loss of detail. Deterioration in the surface quality and hardness of gypsum casts obtained from disinfected irreversible hydrocolloid impression materials has also been widely reported.

Hence, the duration of disinfection 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.

Self-disinfectant irreversible hydrocolloids have been developed by adding disinfectant to the irreversible hydrocolloid compositions to compensate the drawbacks associated with conventional disinfection.

The purpose of this *in vitro* study was to evaluate the antifungal activity of two commercially available irreversible hydrocolloid impression materials modified with the incorporation of silver zeolite and copper oxide nanoparticles.[15-19]

MATERIALS AND METHODS

Two commercially available irreversible hydrocolloid impression materials used in the study are Zelgan Plus and Tropicalgin. Medical grade commercially available silver zeolite and copper oxide nanoparticles was used. Varying concentrations (2.5, 5%, and 7.5%) of nanoparticles of 80–100 nm in size were added to the irreversible hydrocolloid impression materials and their antifungal properties were evaluated. *Candida albicans* ATCC 24433 were obtained from the culture collection and incubated at 37°C for 24 h. The lawn cultures of these microorganism were made with a bacterial or yeast suspension matching the turbidity of a 0.5 McFarland standard. To test the antimicrobial activity, Mueller-Hinton agar plates were incorporated with 2% glucose. After 24 h, antimicrobial activity was evaluated by measuring the zones of inhibition in millimeters (mm).

Antifungal Testing

Antimicrobial activity was assessed using the Kirby–Bauer diffusion method ($n = 3$). Concentrations of 2.5%, 5%, and 7.5% of silver zeolite and copper oxide were incorporated into alginate powder and mixed thoroughly, then water was added following manufacturer's instructions to form a smooth, homogenous mix. A poly(vinyl chloride) pipe of 3 mm diameter was taken and filled with the mix. After setting, alginate material is retrieved from the pipe, discs of 3 mm in thickness were sliced from the alginate with a sterile scalpel, placed on Mueller-Hinton agar plates containing the lawn cultures of *C. albicans*. The Petri plates containing these discs were incubated for 24 h at 37°C. After 24 h, zone of inhibition (MID) is measured from the midpoint of the sample to the border of the diameter of the sample in millimeters [Figure 1].

RESULTS

The results obtained show the antifungal activity of irreversible hydrocolloids against the selected microorganism *C. albicans*. The silver zeolite and copper oxide incorporated irreversible hydrocolloids exhibited dose-dependent antifungal activity. The mean diameter of the zone of inhibition against the tested microorganisms observed with the control and irreversible hydrocolloids incorporated with silver zeolite and copper oxide nanoparticles are presented in Table 1. The control specimens of Zelgan did exhibit a very minimal anticandidal activity but it is not significant. In contrast, Tropicalgin exhibited some activity against *C. albicans*. The anticandidal activity of irreversible hydrocolloids against *C. albicans* increased significantly with the incorporation of silver zeolite and copper oxide

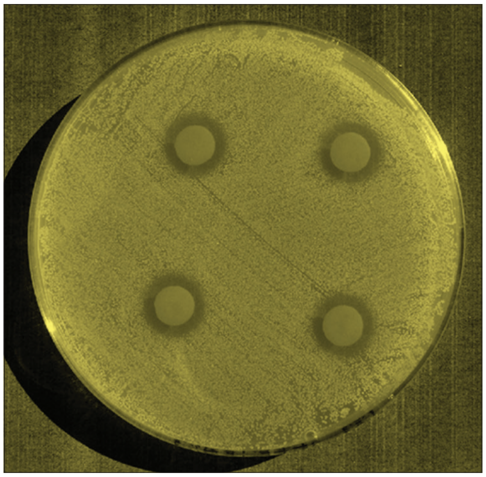
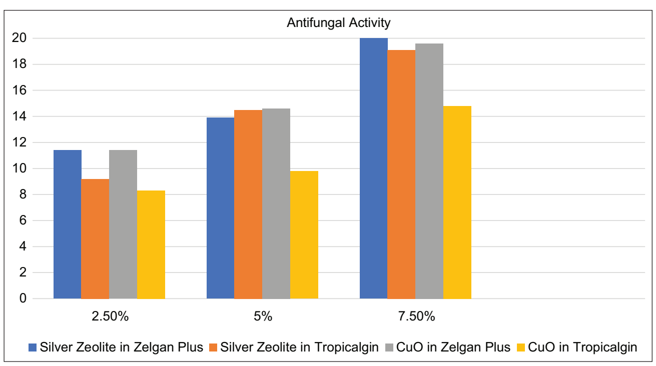


Figure 1: Alginate discs showing zone of inhibition

Table 1: Antimicrobial activity, mean diameter values, of irreversible hydrocolloids incorporated with silver zeolite and copper oxide nanoparticles (average mean diameter)

Concentration	Zelgan		Tropicalgin	
Control	Silver zeolite	Copper oxide	Silver zeolite	Copper oxide
2.5 wt%	11.4	10.2	11.4	8.3
5 wt%	13.9	14.5	14.6	9.8
7.5 wt%	20	19.5	19.6	14.8



nanoparticles ($P < 0.001$). Furthermore, a significant interaction between the concentration of silver zeolite and copper oxide nanoparticles in the irreversible hydrocolloids and zone of inhibition was found with 2-way ANOVA, indicating that increasing concentrations of silver zeolite and copper oxide nanoparticles significantly increased the antimicrobial activity of both Zelgan ($P = 0.003$) and Tropicalgin ($P < 0.001$). 2.5wt%, 5wt%, and 7.5wt% silver zeolite in Zelgan showed the mean diameter of 11.4, 13.9, 20.2.5,5 and in Tropicalgin showed 11.4, 14.6, and 19.6 mean diameter. 2.5, 5, and 7.5wt% of copper oxide in Zelgan showed 10.2, 14.5, and 19.5 mean diameter and in Tropicalgin showed 8.3, 9.3, and 14.8.

In Zelgan Plus, silver zeolite has more antimicrobial efficacy than copper oxide. In Tropicalgin, silver zeolite has more antimicrobial efficacy than copper oxide. Therefore, 7.5 wt % silver zeolite shows more zone of inhibition in Zelgan Plus and Tropicalgin compared to copper oxide.

DISCUSSION

Disinfection of impressions is of utmost importance in breaking the barrier system of transmission of infectious diseases from dental clinics to the laboratory. Conventional disinfection of impressions using spray or immersion technique has shown to be an effective means against the cross-infection. Hence, surface disinfection cannot disinfect the alginate adequately. In addition, dimensional changes associated with surface disinfection advocate for better alternative disinfection methods.

Conventional alginate without incorporation of any antifungal agents exhibits a very minute inherent anticandidal activity due to its composition. Hence, various antimicrobial agents have been incorporated into alginate composition with an aim to self-disinfect the alginate without the need for spray or immersion disinfection. The association of antifungal agents such as chlorhexidine gluconate, nystatin, and fluconazole did not yield best results.[20-22]

Among the other antimicrobial agents, increased attention is being directed at nanoparticles. Nanoparticles being inert do not alter dimensional and mechanical properties of impression material. Colloidal solution of silver has been used previously with superior antimicrobial activity against oral microorganisms but silver when used in combination showed decreased cytotoxic activity. As using silver in combination is advantageous, silver zeolite nanoparticles are preferred for the present study.

Denture stomatitis is most common opportunistic infection in edentulous patients due to decreased immunity. It is caused by *C. albicans*. Hence, antifungals used in the study were tested against *C. albicans*. Silver zeolite is an alkaline or alkaline earth metal ion complexed with crystal aluminosilicate is partially replaced with silver ion by the ion exchange method. Since these antimicrobial composites are being believed to have low toxicity for humans and the activity of the antimicrobial compound is durable, it is extensively used. Silver ion strongly interacts with the ceramics matrix and is minimally released from the matrix in deionized water. Two mechanisms are proposed for the bacterial action of silver zeolite. One is the action of silver ion itself released from zeolite and the other is that of reactive oxygen species generated from silver in the matrix. While oxygen has been reported to be necessary for the bactericidal activity of silver zeolite by some researchers, silver zeolite has also been reported to be effective on oral bacteria under anaerobic conditions by other investigators.

Silver zeolite has shown significant antimicrobial activity in various medical applications and also when incorporated into dental materials and CuO-NP has been used as antimicrobial agents in various investigations. CuO-NP has shown significant antimicrobial activity against a variety of oral microorganisms such as *Streptococcus mutans*, *Prevotella intermedia*, and *Porphyromonas gingivalis*. CuO-NP has been used in toothpastes and also as additives in dental biomaterials to impart antimicrobial activity.[23-25]

In the present study, addition of silver zeolite and CuO-NP to alginates was found to increase the antifungal activity against *C. albicans*. The observed antifungal activity was dependent on the candidal strain, alginate material, as well as on the concentration of nanoparticles.

The antifungal activity of silver zeolite and CuO-NP, like most metal based nanoparticles, can be attributed to their ability for the disruption of microbial cell membrane along with damaging the DNA or inhibiting its replication. It is also possible that zinc ions interfere with the bacterial enzymes, thereby exhibiting the antimicrobial activity.

CONCLUSION

Within the limitations of the present *in vitro* study, it can be concluded that the incorporation of silver-based nanoparticles such as silver zeolite and copper oxide nanoparticles imparted significant antifungal activity to the irreversible hydrocolloid impression materials tested. Therefore, 7.5wt% silver zeolite showed significant antifungal property.

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