Section A-Research paper

Comparative Evaluation of Antimicrobial Activity of Irreversible Hydrocolloid Impression Material Incorporated with Magnesium Oxide and Silver Nanoparticles

Dr. Anandkumar G Patil[1], Dr Sayali Hogepatil[2], Dr. Swapnil Shankargouda[3], Dr Prashant Karni[4]

Professor and Head of the Department, Department of Prosthodontics and Crown and Bridge, KAHER'S KLE V.K. Institute of Dental Sciences, Belagavi-590010. Karnataka, India

Post Graduate , Department of Prosthodontics and Crown and Bridge, KAHER'S KLE V.K. Institute of Dental Sciences, Belagavi-590010. Karnataka, India

Reader, Department of Prosthodontics and Crown and Bridge, KAHER'S KLE V.K. Institute of Dental Sciences, Belagavi-590010. Karnataka, India

Reader, Department of Prosthodontics and Crown and Bridge, KAHER'S KLE V.K. Institute of Dental Sciences, Belagavi-590010. Karnataka, India

Abstract:

Context:: Irreversible hydrocolloids are disinfected either by spray or immersion method. These two procedures clean impression only on the surface. Dental impressions interact with the patient's salivation, blood, and bacterial plaque, which are contaminated with pathogenic microbes. Silver has a long history of use as a broad-spectrum antimicrobial agent.

Aims:The main objective of the study was to compare and evaluate the antimicrobial activity of irreversible hydrocolloid impression material incorporated with 5% and 7% magnesium oxide (MgO) nanoparticles and 5% silver nanoparticles.

Settings and Design: In Vitro Study

Methods and Material:**:** The antimicrobial activity of commercially available irreversible hydrocolloid impression materials incorporated with varying concentrations of MgO nanoparticles (5 and 7 wt%)

and silver nanoparticles (5 wt%) were compared. Antimicrobial activity was assessed using the disk diffusion method by measuring the zone of inhibition.

Statistical analysis used:Kruskal-Wallis test and Mann Whitney

Results:The three organisms *E. coli*, *Candida albicans*, and *Staphylococcus aureus* weretested. MgO 7%showed an18-mmzone of inhibitionin case of *E. coli* (*p* value 0.021) and 20.67-mm zone of inhibitionin *Staph. aureus* (*p* value 0.029). For *Candida albicans*,silver nanoparticles had zone of inhibition of 18.33±0.577 mm (*p* value 0.028).

Conclusions:The incorporation of 5% silver and 7% MgO nanoparticles imparted significant antimicrobial activity to the irreversible hydrocolloid material. MgO 7% did not cause any esthetic or visible changes and showed higher antimicrobial activity in comparison to other filler materials used in this study

KEY-WORDS: MAGNESIUM OXIDE, METAL NANOPARTICLES, DENTAL IMPRESSION MATERIALS, COLLOIDS, ANTI-INFECTIVE AGENTS

INTRODUCTION:

Dental impressions come in contact with the patient's salivation, blood, and bacterial plaque, all of which may be contaminated with pathogenic microorganisms. Disinfecting the impressions successfully before transportation to the dental laboratory is vital as contaminated impressions are a potent source of cross-contamination and transmitters of ailments to the dental staff and laboratory faculty.[1]

Different disinfectants, for example, sodium hypochlorite, sodium met bisulphite, biguanides, iodine mixes, (e.g., iodophor), quaternary ammonium salts, phenolics, and glutaraldehyde are utilized routinely. Since no single disinfectant can be chosen as a widespread disinfectant for all impressions, a disinfectant with superlative antimicrobial action that does not influence the details of an impression is preferred.[2] Irreversible hydrocolloid impression material, which is available in the form of a powder containing dissolvable alginate with calcium sulphate dehydrate and disodium phosphate, is routinely used in clinical practice. The powder additionally contains diatomaceous earth as filler.[3] The surface and hydrophilic nature of irreversible hydrocolloid impression material enable restriction of microbial pathogens superficially as well asinside the material during impression recording. [1,3]

Irreversible hydrocolloids are disinfected either by spray or immersion method, which cleans the impression only on the surface. Deterioration in the surface quality and hardness of dental gypsum casts acquired from cleaned irreversible hydrocolloid impression materials has been generally reported.[4] In any case, such a decrease in inundation time may essentially decrease the adequacy of purification, particularly for a non-permeable, irreversible hydrocolloid impression material.[4] One of the fundamental characteristics of self-disinfectant irreversible hydrocolloid impression materials is that they are not just cleaned superficially but all through the material as the disinfectant is consistently incorporated inside the material.[5] A few investigators have reported critical changes in the properties of self-disinfectant irreversible hydrocolloids such as gel quality, gelation time, permanent deformation, and surface detail generation.[6-8]

Magnesium oxide (MgO) nanoparticles are a promising antibacterial agent, as there is increased protection from harsh preparing conditions. Numerous synthetic techniques such as sol–gel, aqueous, and small-scale emulsion technique have been utilized to prepare MgO nanoparticles.[1,7,9,10] The aqueous technique has been given more consideration because of its straightforwardness. 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. Silver nanoparticles have been used as a disinfecting agent in an *in vitro* study and established to have a potent antibacterial activity.[11,12] Thus, inthis in vitro study, we sought to establish the efficacy of MgO as a disinfecting agent against oral micro floras in comparison with the silver nanoparticles which are incorporated in irreversible hydrocolloid.

SUBJECTS AND METHODS:

Standard cultures and commercial colour-changing irreversible hydrocolloid (Tropicalgin; Zhermack SpA) were used in this study. MgO and silver nanoparticles were obtained from Ultra Nanotech, Bangalore. Various concentrations of MgO nanoparticles (5% and 7% by weight) and silver nanoparticles (5% by weight) were added to the hydrocolloid and its antimicrobial activity was evaluated. Hydrocolloid without nanoparticles was utilized as a control group.

Irreversible hydrocolloid powder and silver and MgO nanoparticles were pre weighed and dispensed according to the required concentration and manipulated to obtain a uniform mix. Hydrocolloid samples were prepared by mixing premeasured quantities of powder in distilled water according to the manufacturer's instructions. A single operator prepared the samples using a rubber bowl and an alginate mixing spatula to standardize the manipulative factors.

The antimicrobial effect of the alginate incorporated with nanoparticles was evaluated using Kirby-Bauer disk diffusion method.[22] The Mueller-Hinton agar culture medium was prepared and sterilized according to the manufacturer's instructions and dispensed into sterile petri dishes. Sterile needle caps were used to form standard wells (5 mm in diameter) in the culture media plates containing lawn cultures of *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* and incubated at 37 ^{o}C for 24 h. Both the modified and unmodified alginate powders were used, and sterile syringes were labelled accordingly. All the methods were performed under aseptic conditions in a laminar air flow.[23]

After gelation of the hydrocolloid, the petri dishes were incubated at 37 °C for 24 h in an incubator after which, the diameter of the zone of inhibition of hydrocolloid samples was measured and documented for all three organisms.

RESULTS:

Antimicrobial activityof irreversible hydrocolloids incorporated with MgO and silver nanoparticles.

Figure 1 illustrates the anti-microbial activities of tropical gin incorporated with MgO (5%, 7%), sliver (5%) nanoparticles, and the control group. The three organisms tested were *E. coli*, *Candida albicans,* and *Staphylococcus aureus*. In case of *E. coli*, MgO (7%) had an 18-mm zone of inhibition in comparison to that of control with 7±6.08 mm. For *Candida albicans*,silver nanoparticles had a zone of inhibition of 18.33±0.5 mm compared to that of control's which was of 8±7.0 mm. In case of *Staph aureus*, MgO (7%) had a zone of inhibition of20.67±0.577 mm in comparison to that of control which was of 11.33±0.577 mm (Table 1).

TABLE 1. Comparison of the mean distribution of the zone of inhibition between the groups using Kruskal-Wallis test

Zone of inhibition (mm)					
	Control	**MgO (7%)**	**MgO (5%)**	**Silver**	**P value**
E. coli	7±6.08	18±0	12.3±1.5	14.33±2.08	0.021*
C. albicans	8±7.0	18.33±0.5	16.33±0.577	18.33±0.577	0.028*
S. aureus	11.33±0.577	20.67±0.577	19.33±0.577	19.67±0.577	0.029*

*significant;

Table 1 shows the comparison of mean distribution of zone of inhibition among the groups using Kruskal-Wallis test. Three organisms were tested in 4 groups, *Staph aureus* had mean distribution of 20.67 mm in the MgO (7%) group which was statistically significant (*P value* of 0.029).

TABLE 2: Post-hoc (Mann-Whitney) test for comparison of control and test groups

		E coli		***Candida albicans***		***Staph aureus***	
Group	Groups	Mean Difference	P value	Mean Difference	P value	Mean Difference	P value
Control	MgO (7%)	-11.00	0.037	-10.33	0.046	-9.33	0.043
	MgO (5%)	-5.33	0.077	-8.33	0.046	-8.00	0.043
	Silver	-7.33	0.050	-10.33	0.046	-8.33	0.043
MgO (7%)	MgO (5%)	5.66	0.037	2.0	0.043	1.3	0.068
	Silver	3.66	0.037	0.00	0.50	1.0	0.09
MgO (5%)	Silver	-2.0	0.134	-2.0	0.09	-.33	0.45

The post-hoc test indicated that the mean score of MgO (7%) was significantly different from that of the control, MgO (5%), and silver groups. However, MgO (7%) was not significantly different from the silver group for Candida and *S. aureus*. Also, MgO (5%) and silver were significantly different from the control group. However, MgO (5%) did not differ significantly from control group as shown in Table 2.

DISCUSSION:

The disinfection of dental impressions reduces the risk of cross infection in the dental clinic environment.[1] The hydrophilic nature and permeable structure of irreversible hydrocolloids make them prone to carry microorganisms both superficially and inside the material. Irreversible hydrocolloids are not known to have antimicrobial activity. When a disinfectant is consolidated into the irreversible hydrocolloid, it provides complete sterilization of the material. Nanoparticles, magnesium, and silver have been utilized progressively as antimicrobial agents for different biomedical applications.[10]

In the present study, the antimicrobial activity of different nanoparticles was analyzed with the help of disk diffusion technique, which is broadly utilized for estimating antimicrobial susceptibility. *S. aureus* and *E. coli* are Gram-positive and Gram-negative bacterial species, respectively, and are commonly known as opportunistic pathogens.[10-11]*C. albicans* is mostly associated with contagious diseases. Thus, these 3 microorganisms were chosen for the present study.

Silver nanoparticles have a high surface area and are non-toxic to the human body at low concentrations. Even at higher fixations, incorporation of silver may not cause tissue irritation or toxicity in view of its low percutaneous assimilation and the constrained contact time with the tissues.[12,14,16, 17]

Ginjupalli et al used disc diffusion method in their study and showed that the silver-incorporated irreversible hydrocolloids exhibited dose-dependent antimicrobial activity.[20] In the present study, the antimicrobial outcome of the alginate incorporated with nanoparticles was evaluated using Kirby-Bauer disk diffusion method. Silver holds fast to bacterial DNA, RNA, bacterial proteins, thiol groups of bacterial enzymes and represses cell division and harms the cell structure of the microorganisms.[20] The antibacterial activity against *S. aureus and E. coli* was because of the inactivation of lactate dehydrogenase and extended protein spillage from the cell walls.[21]

In the current study, the addition of silver and MgO nanoparticles to irreversible hydrocolloids resulted in dose-dependent antimicrobial activity. However, changes in their gel strength, permanent deformation, flow, and gelation time must be studied.

The addition of silver nanoparticles in our study showed change in color which is a limitation for its application as it may hamper the surface details of the impression material after setting and make the impression difficult to examine. However, MgO nanoparticles are odorless and non-toxic. They possess high hardness, high purity, and a high melting point. MgO nanoparticles appear in a white powder form. Metal oxide nanoparticles such as ZnO, MgO, CuO, CaO, Ag_2O, and TiO_2 are another class of antimicrobial agents that have been progressively used for their antibacterial properties and potential applications in sustenance, environment, and healthcare and biomedical applications.[3,15]

A study by Panacek et al focused on nanostructured MgO which is particularly interesting due to its strong antibacterial activity, high thermal stability, and low cost it reported that the antibacterial activity of MgO nanoparticles is attributed to the production of reactive oxygen species which induce lipid peroxidation in bacteria.[18] Miri et al reported in their study that MgO nanoparticles had substantially higher antibacterial activities on Gram-positive (G+) than Gram-negative (G−) bacteria, presumably due to the differences in cell membrane structure between these organisms. MgO nanoparticles were reported to be nontoxic to human cells.[19] In the present study, it was observed that *Staph A* showed high mean distribution of 20.67 in MgO 7% group with a *p* value of 0.029, which is significant.

MgO (7%) did not cause any esthetic or visible changes like silver nanoparticles during the study and showed higher antimicrobial activity in comparison to other filler materials used in this study.

CONCLUSION

The inclusion of magnesium oxide and silver nanoparticles conferred critical antimicrobial action to the irreversible hydrocolloid impression material within the restrictions of the present in vitro investigation.

REFERENCES

1. Jennings KJ, Samaranayake LP. The persistence of microorganisms on impression materials following disinfection. Int J Prosthodont 1991;4:382-7.
2. Samaranayake LP, Hunjan M, Jennings KJ. Carriage of oral flora on irreversible hydrocolloid and elastomeric impression materials. J Prosthet Dent 1991;65:244-9.
3. Leung RL, Schonfeld SE. Gypsum casts as a potential source of microbial cross-contamination. J Prosthet Dent 1983;49:210-1.
4. Cottone JA, Young JM, Dinyarian P. Disinfection/sterilization protocols recommended by manufacturers of impression materials. Int J Prosthodont 1990;3:379-83.
5. Infection control recommendations for the dental office and the dental laboratory. ADA Council on Scientific Affairs and ADA Council on Dental Practice. J Am Dent Assoc 1996;127:672-80.
6. Yüzbasioglu E, Saraç D, Canbaz S, Saraç YS, Cengiz S. A survey of cross-infection control procedures: Knowledge and attitudes of Turkish dentists. J Appl Oral Sci 2009;17:565-9.
7. Ferreira FM, Novais VR, Simamoto Júnior PC, Soares CJ, AJ Fernandes Neto. Evaluation of knowledge about disinfection of dental impressions in several dental schools. Rev Odontol Bras Central 2010;19:285-9.
8. Muller-Bolla M, Lupi-Pégurier L, Velly AM, Bolla M. A survey of disinfection of irreversible hydrocolloid and silicone impressions in European Union dental schools: Epidemiologic study. Int J Prosthodont 2004;17:165-71.

9. Rubel BS. Impression materials: A comparative review of impression materials most commonly used in restorative dentistry. Dent Clin North Am 2007;51:629-42.
10. Nallamuthu NA, Braden M, Patel MP. Some aspects of the formulation of alginate dental impression materials-setting characteristics and mechanical properties. Dent Mater 2012;28:756-62.
11. Srivastava A, Aaisa J, Tarun TA, Ginjupalli K, Upadhya NP. Alginates: A review of compositional aspects for dental applications. Trends Biomater Artif Organs 2012;26:31-6.
12. Nandini VV, Venkatesh KV, Nair KC. Alginate impressions: A practical perspective. J Conserve Dent 2008;11:37-41.
13. Ahmad S, Tredwin CJ, Nesbit M, Moles DR. Effect of immersion disinfection with Perform-ID on alginate, an alginate alternative, an addition-cured silicone and resultant type III gypsum casts. Br Dent J 2007;202:36-7.
14. Rosen M, Touyz LZ. Influence of mixing disinfectant solutions into alginate on working time and accuracy. J Dent 1991;19:186-8.
15. Knetsch MLW, Koole LH. New strategies in the development of antimicrobial coatings: The example of increasing usage of silver and silver nanoparticles. Polymers 2011;3:340-66.
16. Martínez-Gutierrez F, Thi EP, Silverman JM, de Oliveira CC, Svenson SL, Hoek AV, et al. Antibacterial activity, inflammatory response, coagulation, and cytotoxicity effects of silver nanoparticles. Nanomedicine 2012;8:328-36.
17. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, et al. Antimicrobial effects of silver nanoparticles. Nanomedicine 2007;3:95-101.
18. Panacek A, Kvítek L, Prucek R, Kolar M, Vecerova R, Pizúrova N, et al. Silver colloid nanoparticles: Synthesis, characterization, and their antibacterial activity. J Phys Chem B 2006;110:16248-53.

19. Miri SA, Sadeghi GM, Rabiee M. Self-disinfecting reversible hydrocolloid impression gels: Effect of composition and nanosilver on characteristic properties and gelation temperature. J Res Update Polym Sci 2012;1:52-8.
20. Ginjupalli K, Alla RK, Tellapragada C, Gupta L, Perampalli NU. Antimicrobial activity and properties of irreversible hydrocolloid impression materials incorporated with silver nanoparticles. J Prosthet Dent 2016;115:722-8.
21. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for gram-negative bacteria. J Colloid Interface Sci 2004;275:177-82.
22. Gautam V, Singhal L, Arora S, Jha C, Ray P. Reliability of Kirby-Bauer disk diffusion method for detecting carbapenem resistance in Acinetobacter Baumannii-Calcoaceticus complex isolates. Antimicrob Agents Chemother 2013;57(4):2003- 4.
23. Ginjupalli K, Alla RK, Tellapragada C, Gupta L, Perampalli NU. Antimicrobial activity and properties of irreversible hydrocolloid impression materials incorporated with silver nanoparticles. J Prosthet Dent 2016;115(6):722- 8.

Acknowledgements:

Name	Role
Basic science research lab , Belagavi	Support with microbiological part of the study.

Source(s) of support: This project is self funded.

Conflicting Interest (If present, give more details): No