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Evaluation of antimicrobial activityof polyether impression material incorporated with copper oxide nanoparticles: an *in vitro* study

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Abstract

Elastomeric impression materials were widely utilised because they reproduce details accurately, but they also carry a lot of infectious organisms from the oral cavity. Conventional spray and immersion methods were used previously which leads to dimensional changes. So, this study aimed to evaluate the antimicrobial activity of polyether impression material incorporated with various concentrations of copper oxide nanoparticles. A total of 60 medium-body polyether impression material samples were made and divided into 2 groups based on organisms with 30 in each. The 30 specimens in each group were further divided into 5 subgroups based on the concentration of copper oxide nanoparticles. The antimicrobial activity against *S. mutans* and *C. albicans* was assessed using the Kirby-Bauer disk diffusion method. The obtained data were subjected to statistical analysis. Analysis of variance and post-hoc tests were used to identify the significant differences within and across the groups, respectively. An increase in the antimicrobial and antifungal activity was observed with an increase in the concentration of nanoparticles in the polyether impression materials. Oneway ANOVA showed significant differences (p=0.000)

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among the groups. From this study it can be concluded that the antibacterial activity of the polyether impression material was a concentration dependent.

Key words : Polyether impression material, nanoparticles, disinfection.

Dental impressions are invariably in contact with the patient's blood, saliva, and plaque, all of these can harbour harmful microorganisms. Washing the impressions under running water may reduce the microbial load by 40%. However, the models produced from these contaminated impressions may still carry infectious bacteria[1]. Therefore, it is essential to employ various sterilization and disinfection techniques to prevent the risk of spreading infectious diseases to dentists and auxiliary staff [13].

Various methods for disinfecting impression materials have been explored, including chemical disinfection, microwaving, autoclaving, and ultraviolet radiation. A range of chemical agents, such as glutaraldehyde, sodium hypochlorite, sodium metabisulfite, biguanides, quaternary ammonium salts, phenolics, and iodine compounds (iodophors), are commonly used[24]. The specific disinfection procedure required can vary depending on the type of impression material. However, some disinfection techniques have been associated with dimensional changes in the materials[26]. Consequently, there is growing interest in the development of self-disinfecting impression materials that are pre-impregnated with disinfectants[11,28]. Research suggests that these materials not only reduce bacterial contamination more effectively but also maintain better dimensional stability compared to traditional spray and immersion methods, while also saving time in the disinfection process[28]. Nanoparticles (NPs) were employed to overcome the shortcomings of traditional disinfection techniques because of their many beneficial qualities, especially their antibacterial property[7], which makes them used extensively in dentistry. Nanoparticles known for their antimicrobial activity, like silver and silver vanadate[11,4,21], titanium, chitosan[6,7], magnesium oxide[18], zirconium oxides[18], zinc oxide[12] and copper oxides[12] have been suggested for use in impression materials to overcome the shortcomings of traditional disinfectant techniques. Copper oxide nanoparticles (CuONPs) are widely known for their antimicrobial properties and low toxicity[2,8]. Alginate impression materials incorporated with various concentrations of copper oxide Nanoparticles demonstrated significant antimicrobial characteristics[12].

Elastomeric impression materials are commonly used in the making of fixed dental prosthesis impressions. Although numerous methods have been explored to disinfect polyether impressions, the results have been inconsistent[27]. However, there has been no research on the impact of incorporating copper oxide nanoparticles into polyether impression materials to enhance their antimicrobial properties. Therefore, the present study was aimed to evaluate the antibacterial efficacy of polyether impression materials after integrating various concentrations of copper oxide nanoparticles.

The materials and equipment used in this study were Polyether impression material (Medium body consistency, 3M ESPE, Impregum soft Germany -Ref 31755), Copper oxide nanoparticles with an average particle size of (80-100 nm, Vedayukt, India), Mutans-sanguis agar (Hi Media, India - Ref M977), Sabouraud Dextrose Agar (Hi Media, India - Ref MH063), *C. albicans* strains (MTCC-3017), *S. mutans* strains (MTCC-497).

A total of 60 samples of medium body polyether impression material were made. The specimens were divided into 2 groups based on organism, 6 in each(n=6) based on the concentration of the Copper oxide nanoparticles, 0.50Wt%, 1.0Wt%, 2.0Wt%, and 5.0Wt%. Impression material with no nanoparticle incorporation was considered as the control.

The equal lengths of base and catalyst pastes were dispensed on to a mixing pad and weighed. Based on the weight of the impression paste, the different concentrations (0.50 Wt%, 1.0 Wt%, 2.0 Wt%, and 5.0 Wt%) of CuONPs were weighed. The different concentrations of CuONPs were added to the base paste and mixed thoroughly for uniform dispersion of the nanoparticles. Then, the catalyst paste was mixed with the nanoparticle-modified base paste as per the manufacturer's recommended mixing time. The metal mold with the inner diameter of 6.5 mm and thickness of 3 mm was placed on a glass slab, and the impression paste modified with CuONPs was loaded. A glass plate was placed over the impression material, and a weight of one kilogram was applied to ensure that the material was evenly distributed throughout the mould. The mix was allowed to set. A total of 6 specimens (n=6) for each concentration were made.

Antimicrobial activity and antifungal were assessed using the Kirby-Bauer disk diffusion method. The impression material discs were placed on *mutans-sanguis* agar plates and sabouraud dextrose agar plates containing lawn cultures of *streptococcus mutans* (*S. mutans)* and *Candida albicans* (*C. albicans)* respectively, with the help of a sterile tweezer. The prepared Petri dishes were incubated at 37^{o} C for 24 hrs in an incubator. The antimicrobial activity was evaluated after 24 hours by calculating the zone of inhibition in millimetres (mm) around the samples.

The obtained data were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) 26.0 Version, IBM, USA. One-way ANOVA and post-hoc analysis were used for intra and inter group comparisons. The p value less than 0.5 was considered as statistically significant.

The mean zone of inhibition and standard deviation around the CuONPS-modified polyether impression material samples against the *S. mutans* and *C. albicans* are presented in Table-1. The control group showed no antimicrobial and antifungal activity compared to the modified groups. Among the modified groups, polyether impression material modified with 5.0wt% of CuONPs displayed more antimicrobial and antifungal activity and the least was observed with 0.5wt%. An increase in the zones of inhibition around the samples was observed as the concentration of NPs increased (Figures 1 and 2). However, the formation of zone of inhibitions against *S. mutans* was more compared against to the *C. albicans*. One-way ANOVA showed significant difference among the different concentrations of CuONPs modified polyether impression materials against S. *mutans* (p=0.000) and *C. albicans* (p=0.000) (Table-1).

In post hoc analysis, against *S. mutans*, the control group demonstrated significant differences with all the modified polyether impression material groups (p=0.000). However, no significant differences were observed between the modified groups (Table-2). In Post hoc analysis, against *C. albicans*, the control group displayed substantial differences with the modified groups (0.5 wt%: p=0.008, 1.0 wt%, 2.0wt%, and 5.0 wt%: p=0.000). The polyether-modified with 0.5wt% of CuONPs showed significant differences with 2.0 wt% (p=0.000) and 5.0 Wt% (p=0.000) modified groups. The polyether-modified with 1.0 wt% of CuONPs displayed significant differences with 2.0 wt% (p=0.008) and 5.0 wt% (p=0.000) modified groups (Table-2).

Table-1. Comparison of antimicrobial and antifungal activity of polyether impression materials incorporated with different concentrations of CuONPs against *mutans and C. albicans*

Concentration of nanoparticles	*S. mutans*		*C. albicans*	
	Mean ± SD#	p - Value	Mean ± SD#	p - Value
Control	0.000 ± 0.000	0.000	0.000 ± 0.000	0.000
0.5%	8.333 ± 0.816		1.583 ± 0.491	
1.0%	8.667 ± 1.211		2.250 ± 0.418	
2.0%	8.917 ± 1.625		3.833 ± 0.683	
5.0%	10.000 ± 0.894		4.417 ± 1.357	

#Standard deviation.

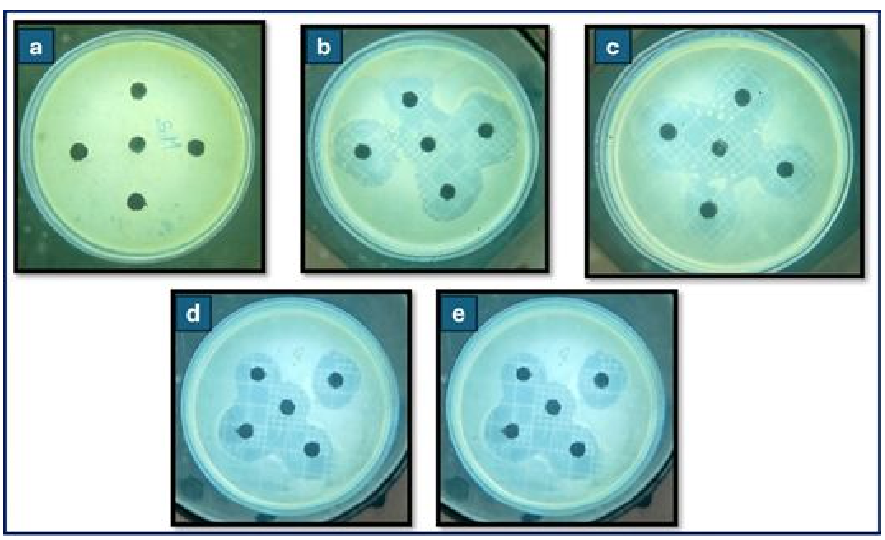

Figure 1. Antimicrobial activity of CuONPs incorporated polyether impression material against *S. mutans*. Whare, a. Control, b. modified with 0.50 wt% CuONPs, c. modified with 1.0 wt% CuONPs, d. modified with 2.0 wt% of CuONPs, and e. modified with 5.0 wt% CuONPs.

Figure 2. Antifungal activity of different concentrations of CuONPs incorporated polyether impression material against *C. albicans*.

Table-2. Pair-wise comparison (post hoc analysis) of antimicrobial activity of Polyether impression material modified with different concentrations of Nanoparticles against *S. mutans*

Concentration of nanoparticles		*S. mutans*		*C. albicans*	
		Mean Difference	p-value	Mean Difference	p-value
Control	0.5%	8.333*	0.000	1.583*	0.008
	1.0%	8.667*	0.000	2.250*	0.000
	2.0%	8.917*	0.000	3.833*	0.000
	5.0%	10.000*	0.000	4.416*	0.000
0.5%	1.0%	0.333	0.981	0.667	0.533
	2.0%	0.583	0.872	2.250*	0.000
	5.0%	1.667	0.077	2.833*	0.000
1.0%	2.0%	0.250	0.994	1.583*	0.008
	5.0%	1.333	0.217	2.166*	0.000
2.0%	5.0%	1.083	0.408	0.583	0.652

*Significant difference.

Disinfecting dental impression materials is vital for preventing cross-contamination and ensuring patient safety. Traditional methods, such as rinsing with water and using chemical disinfectants like glutaraldehyde and iodophors, can be time-consuming, may compromise the dimensional stability of the materials, and are not always fully effective in eliminating microbes[26]. Polyether impression materials, known for their excellent detail reproduction and dimensional accuracy[24], are prone to microbial contamination, posing a risk of infection transmission. Literature reported that the Incorporation of copper oxide nanoparticles (CuONPs) directly into impression materials offers a promising solution[12]. CuONPs are highly effective antimicrobial agents with low toxicity, and studies have shown they enhance antimicrobial activity against pathogens like S. *mutans* and C. *albicans*[5,15,25]. By embedding CuONPs into the impression material, continuous antimicrobial action is achieved, reducing the need for additional disinfection steps and improving overall infection control in dental practices. Hence, the present study investigated the effect of incorporating different concentrations of CuONPs into the polyether impression materials on the antimicrobial, properties.

In the present study, the antimicrobial and antifungal activity was increased with an increase in the concentration of CuONPs in the polyether impression material. These results suggest that antifungal activity increases with higher concentrations of CuONPs, with a notable increase in efficacy between 1.0 wt% and 2.0 wt%.

CuONPs exhibit notable antibacterial and antifungal properties due to several mechanisms, including the generation of Reactive Oxygen Species (ROS), disruption of bacterial cell membranes, release of Copper Ions (Cu^{2+}), and binding to DNA[14,17,29]. Upon incorporating CuONPs into polyether impression materials, these mechanisms can work synergistically to enhance the overall antimicrobial efficacy of the material. CuONPs can produce reactive oxygen species (ROS) such as hydroxyl radicals, superoxide anions, and hydrogen peroxide. These ROS can damage cellular components, including lipids, proteins, and nucleic acids, leading to oxidative stress and cell death. In Disruption of the Bacterial Cell membrane mechanism, CuONPs can interact with the bacterial cell membrane, causing physical disruption. This interaction can lead to membrane permeability changes, leakage of cellular contents, and eventual cell lysis. In the Release of Copper Ions (Cu^{2+}) method, the released copper ions in the presence of moisture or upon contact with bacterial cells, can bind to bacterial proteins and enzymes, disrupting their function. They can also interfere with essential biochemical pathways and generate additional ROS. In Binding to DNA method, Copper ions can penetrate bacterial cells and bind to DNA molecules. This binding can cause DNA denaturation, inhibiting replication and transcription, ultimately leading to cell death[14,17,29].

Several factors can affect the antimicrobial and antifungal activity of polyether impression materials containing CuONPs. These factors include concentration of nanoparticles, dispersion of nanoparticles, particle size, and surface area, interaction of nanoparticles with the polymer matrix, and the release of copper ions from the mass[19].

Generally, higher concentrations of nanoparticles lead to increased antimicrobial and antifungal activities with no much effect on physical and mechanical properties of dental materials[4,9,21,12]. Numerous researchers reported increased antimicrobial activity against *E. Coli, S. aureus, and C. albicans* with increased concentration of the nanoparticles in the impression material and denture base materials[3,21]. A similar tendency was observed in the current investigation when the concentration of nanoparticles increased. Similar to the present study, Ginjupalli *et al.*[12] reported that the incorporation of CuONPs into alginate impression materials demonstrated an increased antifungal activity against *C. albicans*.

Harikrishna. M *et al.*[20] demonstrated an increase in antimicrobial activity with an increase in the concentration of CuONPs and silver zeolite nanoparticles in the alginate impression material.

Uniform dispersion of nanoparticles within the polyether matrix ensures consistent antimicrobial activity throughout the material. Several researchers reported the agglomeration of the NPs with an increase in their concentration in the polymer matrix[10,13, 21,23]. The efficacy of the antimicrobial activity of the nanoparticles could be diminished with the increased agglomeration. However, an increase in the antimicrobial activity was observed with an increase in the concentration of the nanoparticles. The present study did not investigate the dispersion of the nanoparticles in the matrix. Research suggested that agglomeration of the nanoparticles can be prevented by treating the nanoparticles with an appropriate coupling agent to enhance the bonding between the nanoparticles and the polymer matrix[22.] The CuONPs were not treated with any surface agents in the present study.

Smaller nanoparticles with larger surface areas can enhance antimicrobial properties due to better interaction with microbial cells. Agglomeration of the nanoparticles increases the particle size and reduces the antimicrobial efficacy of dental material[13]. On the contrary, Ginjupalli *et al.*[12] reported decreased antimicrobial activity with a decrease in the nanoparticle size of 10-20 nm. The average particle size of the CuONPs used in the present study was in the range of 80-100 nm and it could have been the reason for improved antimicrobial and antifungal activity.

The chemical compatibility and bonding between CuONPs and the polyether material can influence the release and activity of nanoparticles. The increased antimicrobial activity in this study with increased nanoparticles could be attributed to the hydrophilic nature of polyether materials that can enhance the interaction between the CuONPs and microbial cells, increasing the contact and effectiveness of the antimicrobial mechanisms. The controlled release of Cu^{2+} ions also play a significant role in the antimicrobial activity against different microorganisms[14,17,29]. The polyether matrix can facilitate a controlled and sustained release of copper ions, maintaining prolonged antimicrobial activity. This is evident from the current study in which the zone of inhibitions formed against *S mutans* was more compared against to the *C. albicans*. It indicates that the CuONPs behave differently with different microorganisms.

In the present study, incorporating CuONPs into polyether impression materials enhanced their antimicrobial and antifungal properties, with higher concentrations leading to better efficacy. The modified groups did not show a significant difference between the groups. This study's findings align with similar research on metal oxide nanoparticles, underscoring the potential for CuO NPs in dental and medical applications to prevent microbial contamination[12,20]. However, future research may be focussed on treating the nanoparticles with different surface-treatment materials and analysing the dispersion of nanoparticles in the polyether matrix. The sustained antimicrobial activity may also be assessed at different time intervals and also may be focused on the effect of varying particle sizes of the nanoparticles on the antimicrobial, physical, and mechanical properties of polyether impression materials.

Within the study's limitations, the following conclusions can be drawn:

- The antimicrobial and antifungal activity of the polyether impression material increased with higher concentrations of copper oxide nanoparticles.
- The polyether impression materials modified with different concentrations of CuONPs exhibited greater effectiveness against *S. mutans* compared to *C. albicans*.
- Therefore, copper oxide nanoparticles (CuO NPs) show promise for incorporation into polyether impression materials to enhance their antimicrobial properties.

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