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Effect of copper-doped phosphate glass on dental resin material: In vitro study

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ABSTRACT

Background: This study aims to incorporate the oxide copper particles with phosphate-based glass fibers (PGF) into a poly methyl methacrylate (PMMA) resin material and investigate its antimicrobial effect. *Material and Methods:* (CuO)-containing PGF added to the PMMA resin (5, 10, 20 and 30 wt %). The antimicrobial activity was measured in the lab using the direct contact test against Klebsiella pneumonia, Staphylococcus aureus and Candida albicans. The agar diffusion test was used to measure the release of the (CuO). The results were analyzed and compared using a one-way analysis of variance, followed by the Student-Newman-Keuls-multiple-comparisons test. Differences were considered significant when the p-value was less than 0.05. *Results:* Compared to the control groups, a significant drop in *K. pneumonia*, *S. aureus*, and *C. albicans* was observed with all antimicrobial agent ratios. *Conclusion:* Adding (CuO)-containing PGF to PMMA dental resins provided a high antimicrobial effect against *K. pneumonia*, *S. aureus* bacterial and Candida species.

Keywords: Dental resin, PMMA, Copper Oxide (CuO), Phosphate glass fiber (PGF), Antimicrobial.

1. INTRODUCTION

For decades, (PMMA) resins have been widely utilized in dentistry as a temporary restoration or denture base. It has the advantages of being easy to handle, inexpensive, having pleasing esthetic and sufficient discoloration resistance (Gad et al., 2017; Naji et al., 2018). On the other hand, it has some drawbacks, including a lack of surface hardness, limited flexural strength and poor antibacterial activity (De-Castro et al., 2016).

Different microorganisms may invade the dental resin material, especially if oral hygiene is neglected. It may act as a reservoir for various microorganisms such as *Candida albicans*, *Streptococcus mutans* and *Staphylococcus aureus* species which may lead to a chronic inflammatory

reaction in the mucosa, increase the risk of dental caries, periodontal disease and infectious inflammatory diseases in general (Chen et al., 2017; Compagnoni et al., 2014; Paleari et al., 2011). On top of that, these lesions may result in increased mortality (An et al., 2018; Chen et al., 2017; Wen et al., 2016). Several therapy methods have been developed to prevent fungal and bacterial growth around the dental resin material, including using various cleansing agents (Dhamande et al., 2012), systemic or local antibiotic/antifungal agents and the inclusion of those agents into the resin material itself (Zhang et al., 2016).

Nanomaterials have advanced dramatically in recent years. In biomedical research and nanomedicine, these materials have shown various intriguing uses. It has antimicrobial activity when incorporated into dental resin material (Jangra et al., 2012). For several years, Phosphate-based glass fibers (PGF) have shown unique properties that encourage scientists to use them in tissue engineering and dental material development (Ahmed et al., 2004; Knowles, 2003; Salih et al., 2000).

Silver and copper ions have antimicrobial characteristics and can offer a localized antibacterial delivery system by adding them to PGF to treat an infection by releasing ions as the fiber glass dissolves in aqueous conditions. Many studies show the successful incorporation of copper oxide into phosphate glass fiber with excellent results that reveal solid antibacterial properties (Mulligan et al., 2003). This study incorporated different ratios of oxidized copper containing phosphate glass fiber into a PMMA resin to measure the antimicrobial effect among various bacterial species (*Klebsiella pneumonia*, *Staphylococcus aureus* and *Candida albicans*).

2. MATERIAL AND METHODS

DENTSPLY supplied conventional heat-cured PMMA. The copper oxide-containing phosphate glass fibers were made according to (Neel et al., 2005). It is a Phosphate-based glass fibers (PGF) composition of the general formula Na2O–CaO– P2O5 and 10 mol% Copper oxide (CuO).

Sample groups

CuO-containing PGF was added to PMMA powder at 5%, 10%, 20% and 30% by weight to form the following groups,
Group A: Control group, only conventional heat-cured PMMA resin.
Group B: Heat-cured PMMA resin with 5% (CuO)-containing PGF.
Group C: Heat-cured PMMA resin with 10% (CuO)-containing PGF.
Group D: Heat-cured PMMA resin with 20% (CuO)-containing PGF.
Group E: Heat-cured PMMA resin with 30% (CuO)-containing PGF.

Sample preparation

Ten specimens were fabricated for each concentration group. The tested specimens (10x2mm discs) were made between April 2022-October 2022 by adding the selected ratio of the CuO-containing PGF to the conventional heat-cured PMMA powder. The suitable monomer-to-polymer ratio was 1:2.5 by weight and 1% of benzoyl peroxide (initiator) was added to the powder according to the manufacturer's instructions. The specimens were cured in the same curing cycle according to the manufacturer's instructions, smoothed and polished using silicon carbide papers grade 240 up to 1200 grit with pumice and slurry and eventually stored in distilled water at 37 ± 1°C for 48 hours before testing.

Evaluation of the antimicrobial effect

Quantitative measurement of the antimicrobial property was determined by the direct contact test (Mirizadeh et al., 2018). Initially, the disc specimens were soaked for one week in distilled water to release the free resin monomer from the discs. The specimens were sterilized with H2O2 plasma sterilizer for 20 minutes before the assessment.

Single colonies of gram-negative bacteria (*Klebsiella pneumonia* ATCC 25922) and gram-positive bacteria (*Staphylococcus aureus* ATCC25923) were obtained from fresh agar plate cultures and inoculated in 10 mL of brain-heart infusion (BHI) broth for initial suspension. For colony-forming unit (CFU) counting, 100 µl of the initial and diluted suspensions (10^{-2}) were cultured on the appropriate media using a calibrated loop. In parallel, each specimen disc was inoculated with 40 µl of initial and diluted bacterial suspensions of *Klebsiella pneumonia* and *Staphylococcus aureus* in 15 mL of BHI broth and was incubated with agitation at 37°C for 24 hours (Figure 1).

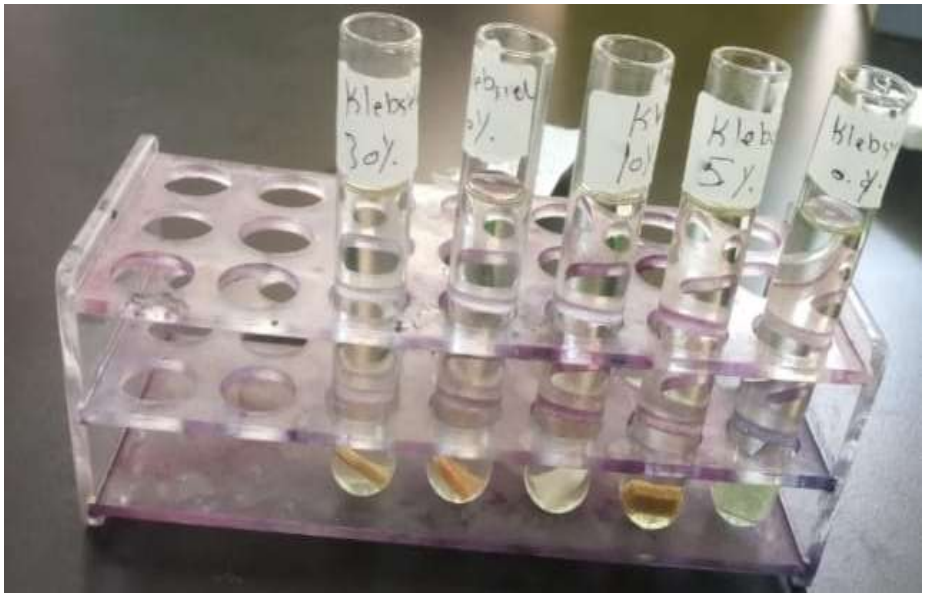

Figure 1 The specimens were inoculated with 40 μl of bacterial suspensions of *K. pneumonia* in BHI broth

On the contrary, initial and diluted suspension of *C. albicans* (ATCC 1880) on Sabouraud Dextrose agar plates were prepared and incubated with test discs at 37°C for 24 hours. Each numeration experiment was parallel with control specimens (0% antimicrobial, group A). The antimicrobial effect was determined by the reduction in colony counts and expressed as log CFU/cm2 for each sample (Figure 2).

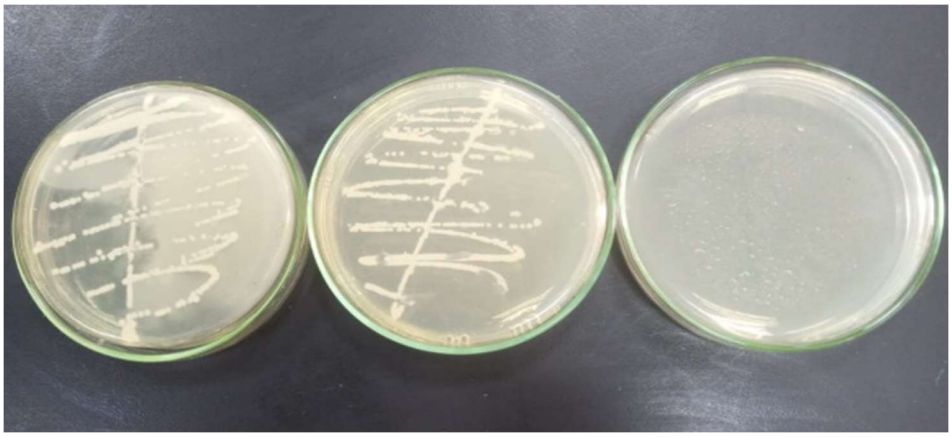

Figure 2 Reduction of bacterial growth on Muller Hinton agar plates with increased concentration of (CuO)-containing PGF (culture from broth containing test specimens group B on the left, group C on the middle and group E on the right, inoculated with *S. aureus*)

The agar diffusion test assessed the release of used antimicrobial material. The specimens were placed on Muller Hinton agar plates inoculated with Staph aureus and *K. pneumonia* (1.0×106 CFU). The plates had incubated for 18 to 24 hours at 37C, then the zone of inhibition for bacterial growth was determined visually (Figure 3).

Statistical analysis

Differences between groups were analyzed using one-way variance analysis, followed by the Student-Newman-Keuls-Multiple-Comparisons test. Differences were considered significant when $p < 0.05$.

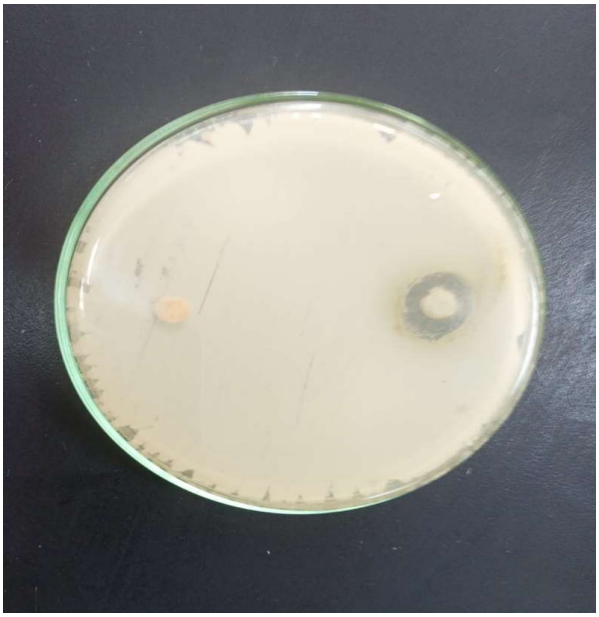

Figure 3 The agar diffusion test showed a zone of inhibition around the specimen disc containing 30% (CuO)-containing PGF (group E on the right) versus control (group A on the left)

3. RESULT

The result showed a substantial antimicrobial effect of CuO toward *S. aureus*, creating an inverse relationship as the concentration of CuO increased until it completely inhibited the microbial growth at 30% CuO per weight concentration in both diluted and undiluted groups. For *K. pneumonia*, the antimicrobial effect was boosted as the percentage weight of CuO increased, especially in the diluted suspension group that reached zero bacteria with 30% CuO per weight. On the other hand, the mean number of *K. pneumonia* found in the undiluted group was 2000. Finally, the lowest antimicrobial effect was observed on the undiluted *Candida albicans*, showing a mean of 30000 bacteria with 30% CuO per weight. A summary of the influence of incorporating different ratios of (CuO)-containing PGF into PMMA resin material on the viability of *K. pneumonia, S. aureus* and *C. albicans* biofilm cells are presented in (Table 1) (Figure 4).

Table 1 Effects of different percentages of (CuO)-containing PGF incorporated into PMMA specimens on the activity of *K. pneumonia, S. aureus* and *C. albicans* biofilm cells (P< 0.05)

	K. pneumonia initial suspension (160,000)		*K. pneumonia* diluted suspension (60.000)		*S. aureus* initial suspension (160,000)		*S. aureus* diluted suspension (60,000)		*Candida albicans* initial suspension (160,000)		*Candida albicans* diluted suspension (60,000)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	10000000	149909	994700	6395	10000000	77028	1000000	52281	10000000	365148	8000000	666667
5% per weight concentration	170000	1247	100000	943	130000	943	60000	1247	130000	816	100000	3712
10% per weight concentration	80000	1054	1000	62	4000	156	1000	66	120000	667	10000	816
20% per weight concentration	40000	1247	1000	62	1000	77	0	0	80000	816	5000	816
30% per weight concentration	2000	163	0	0	0	0	0	0	30000	943	3000	816

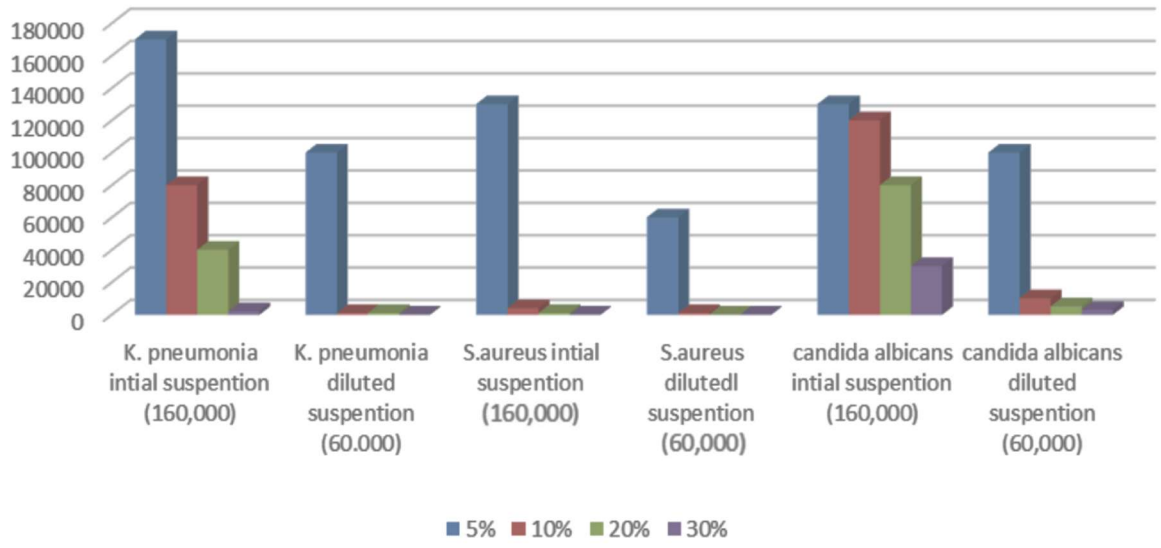

Figure 4 Effects of different percentages of (CuO)-containing PGF incorporated into PMMA specimens on the activity of *K. pneumonia*, *S. aureus* and *C. albicans* biofilm cells (P< 0.05)

The antimicrobial activity of (CuO)-containing PGF was evaluated on gram-negative bacteria (*K. pneumonia*) and gram-positive bacteria (*S. aureus*) and (*C. albicans*). The outcomes of the test suggest bactericidal activity against all tested microorganisms. A significant drop in all tested microorganisms was observed compared to the control groups (P< 0.05). For all microbial species, the highest effect of the antimicrobial material was observed in the 30 % ratio group. There was a highly significant difference between group E and other groups. According to the agar diffusion test, a significant zone of inhibition was observed around the specimen disc containing 30 % (CuO)-containing PGF to the *S. aureus* (group E).

4. DISCUSSION

In the present study, CuO containing PGF was added to conventional heat-cured PMMA to evaluate their antibacterial and antifungal effects, especially *Candida albicans*, which contribute to oral diseases. The CuO-containing PGF-modified specimens significantly reduced colony counts for all the tested microorganisms. The antibacterial activity of the filler was performed using a "wet" inoculation technique by applying initial and diluted suspensions of the selected organism, a common evaluation method (Grass et al., 2011). In addition, it represents the clinical situation and simulates the condition of the oral cavity.

The antimicrobial effect increased proportionally with the increased ratio of loaded filler, with the highest inhibition observed at 30% concentration; this can be attributed to the ability of copper ions and phosphate-based glasses to form a localized antibacterial delivery system (Neel et al., 2005). Although most of the antibacterial processes of copper surfaces are well understood, a few things need clarification. On the other hand, copper's antibacterial action appears to be directly tied to its oxidative behavior and the solubility qualities of copper oxides (Hans et al., 2016).

This study reveals the inhibitory effect in the following order: *S. aureus* > *K. pneumonia* > *C. albicans*. Gram-negative bacteria are more resistant to antiseptics and disinfectants than Gram-positive bacteria; this may be due to the outer membrane's structure, which reduces cell penetration by antibacterial chemicals (Franklin and Snow, 2005). A previous study by Mirizadeh et al., (2018) investigated the effect of adding quaternized N, N-dimethylaminoethyl methacrylate monomer into a dental resin material; they found similar results to gram-positive and gram-negative bacteria, while their results showed more inhibition of *C. albicans* than Gram-negative bacteria (Mirizadeh et al., 2018).

Our results supported the previous studies on the antifungal activity of copper oxide against *C. albicans* (Kruk et al., 2015; Usman et al., 2013). As of the clinical implantation perspective of the restorative dentist, the main application of an antibacterial agent "Copper oxide" is to control existing caries or as a prophylactic against new lesions. The mechanism of action of copper oxide is to inhibit the glycolysis that decreases the acid production, having stronger action among the aerobic bacteria *Streptococcus mutans* than the glass ionomer material (Thneibat et al., 2008).

On the other hand, the Prosthodontist take the advantage of incorporating low wt% of copper oxide into the restorative materials to enhance the antimicrobial effect without affecting the mechanical and physical properties (Abuelenain, 2018), which help the Periodontist to reduce the gingival and periodontal disease by affecting the sanguis bacteria in dental plaque (Mulligan et al., 2003).

5. CONCLUSION

Within the limitations of this study, we can conclude that: Incorporating copper oxide with phosphate-based glass fibers into a PMMA resin material reduces gram-positive/ gram-negative bacteria and fungi. The inhibitory effect was more substantial against *S. aureus* than against *K. pneumonia* and *C. albicans*. Further study is recommended to investigate the physicomechanical properties of different concentrations of copper oxide containing phosphate glass fiber.

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Author Contributions

The authors confirm contribution to the paper as follows: Study conception and design Ahmed B EI Okl, Samar H Abuzinadah, Mohamed Aboshama; data collection: Osamah Alsulimani, Salem K Aloubathani; analysis and interpretation of results: Gehan A El Olimy, Samar H Abuzinadah Samah M Awad; Mohamed Aboshama; draft manuscript preparation: Rayyan A Kayal, Abdulrahman J Alhaddad. All authors reviewed the results and approved the final version of the manuscript.

Ethical Approval

The IRB issued by the Faculty of Dentistry (Assuit branch), Al-Azahr University: AUAREC20220010-02.

Informed consent

Not applicable.

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Conflict of interest

The authors declare that there is no conflict of interests.

Data and materials availability

All data sets collected during this study are available upon reasonable request from the corresponding author.

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