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ORIGINAL ARTICLE

Anti-Inflammation Effects of Silver Nanoparticles-Zinc Polycarboxylate Cement (AGNPS-ZPCCEM)

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ABSTRACT

Though, zinc polycarboxylate cement(ZPCCEM) is a good root filling block of cement, But its efficiency against microbes was low or non-existent.ThE purpose Of our studying is Evaluation of the effects of adding silver nanoparticles to the (S-NP) to (ZPCCEM), on antimicrobial effectiveness towards the three Microorganisms appear in human infection, including, Escherichia-coli (E.-coli) are a Gram-negative, Staphylococcus-aureus (S.aureus) is a Gram-positive round-shaped bacterium, a member of the Bacillota, and Candida albicans It appears to be used as an organism for fungal pathogens, these microorganisms are of the most prevalent taxa, that caused Chronic periodontitis and pulpitis Combined 4g of powder zinc polycarboxylate cement with 3 drops of liquid zinc polycarboxylate cement to give zinc polycarboxylate cement(ZPCCEM) and mixed with various amounts of Silver nanoparticles powder to give (AgNPs-ZPCCEM) composites. The diagnosis of AgNPs, (ZPCCEM) and (AgNPs-ZPCCEM) were carried out with FTIR and FESEM analysis. To evaluate the antimicrobial effectiveness, agar diffusion and broth dilution methods were used. In the agar diffusion test, (ZPCCEM) And (AgNPs-ZPCCEM), showed zones of inhibition against three microorganisms. The (AgNPs-ZPCCEM), showed the most superior synergy with (AgNPs-ZPCCEM). The activity of the (AgNPs-ZPCCEM) and cement alone was increased up to 28 and 35 mm against E-coil respectively, against Staphylococcus aureus. Similarly, the antimicrobial activity of (AgNPs-ZPCCEM) and cement alone was shown at 23 and 31 mm respectively, while the antimicrobial activity of (AgNPs-ZPCCEM) and cement alone was 15 and 12 mm respectively, against Candida albicans.

Keywords: silver nanoparticle, zinc polycarboxylate cement, AgNPs-ZPCCEM, antimicrobial activity.

INTRODUCTION

Many dental treatments require restorations and indirect devices to be attached to cement. Dental types of cement currently in use include (zinc phosphate–silicophosphate–polycarboxylate–glass ionomer–zinc oxide, eugenol, and resin-based blocks of cement (1). The results in a salt matrix similar to a cross-linked polycarboxylic zinc gel containing the Residual zinc oxide particles (2)(4) This type of cement also belongs to a class of materials known as base-acid reaction blocks of cement, which are Suitable at body temperature, thus kill any associated Problems.

ocean thermal erosion tissues is a common problem with PMMA types of cement (5). As a result of the configuration of salt, Porosity is also produced. However, achieving porosity always comes as a compromise to the mechanical properties of the final batch product

Zinc polycarboxylate cement is Moreover characterized by the Bone adhesion ability (5,6). It can also be observed by eye and proven as a chemical and mechanical bond (6). However, the factors that allowed the use of cement in the base or restoration are its compressive strength and rapid action (7-13). Although there are no biological similarities between polycarboxylic acids, they have been discovered to form molecular and mechanical bonds with native dental materials. (14-16). Peters and others. showed that polycarboxylic zinc cement was Biocompatible in transplant studies in such a way fibrous capsule layer of Finally, collagen is formed next to a cement-like acrylic bone cement, a behavior that classes cement as a biomaterial (17). In ordinary acrylic joint arthroplasties, The main contributor of the capsule to dilution, sterilization and killing is this fibrous layer (18).

The addition of metal oxides to root-end–filling materials can improve the physicochemical and antimicrobial properties of these materials (19,4).

More efforts were done to develop the anti biological effectiveness of types of cement and the other root-end–filling materials, by using new counted materials like silver nanoparticle AgNPs and plant extracts which take a lot of interestAlthough it has been proven that AgNPs are Good antimicrobial agent, reports of use AgNPs with MTA and CEM and Comparing the inhibitory effects of rare bacteria and fungi (20,17-21,19).

Silver nanoparticles AgNPs are widely used particles, Nanoparticles, in particular, serve as an antimicrobial factor for medical purposes [14, 15]. AgNPs can inhibit the growth of microorganisms intake on silver ions with the same amounts of silver [22,16, 17].

Particle size has also been associated with antimicrobials; Smaller molecules give more bactericidal activity compared to larger molecules [23,18-24,20].

Therefore, in this work, the sol-gel method has been used to synthesize silver nanoparticles (AgNPs). Therefore, this research aimed to study the effects AgNPs with different amounts On the antimicrobial activity of CEM and CEM/ AgNPs nanocomposite toward most three Important types of microorganisms.

Escherichia coli (E-coli) is a Gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms [21]. Most E. coli strains are harmless, but some serotypes (EPEC, ETEC, etc.) can cause serious food poisoning in their hosts and are occasionally responsible for food contamination incidents that prompt product recalls [22]. The harmless strains are part of the normal microbiota of the gut and can benefit their hosts by producing vitamin K2,[23] and preventing colonization of the intestine with pathogenic bacteria, having a mutualistic relationship [24]. E. coli is expelled into the environment within fecal matter. The bacterium grows massively in the fresh fecal matter under aerobic conditions for three days, but its numbers decline slowly afterward [25]. The bacterium can be grown and cultured easily and inexpensively in a laboratory setting. E. coli is a chemoheterotroph whose chemically defined medium must include a source of carbon and energy.[26] E. coli is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA. Under favorable conditions, it takes as little as 20 minutes to reproduce [27].

Staphylococcus aureus is a Gram-positive round-shaped bacterium, a member of the Bacillota, and is a usual member of the microbiota of the body, it's a major bacterial human pathogen that causes a wide variety of clinical manifestations [28]. Infections are common both in community-acquired as well as hospital-acquired settings and treatment remains challenging to manage due to the emergence of multi-drug resistant strains such as MRSA (Methicillin-Resistant Staphylococcus aureus) [29]. S. aureus is found in the environment and is also found in normal human flora, located on the skin and mucous membranes (most often the nasal area) of most healthy individuals [28]. S. aureus

does not normally cause infection on healthy skin; however, if it is allowed to enter the bloodstream or internal tissues, these bacteria may cause a variety of potentially serious infections [28]. Transmission is typically from direct contact. However, some infections involve other transmission methods [30].

Candida albicans (C. Albicans) is commonly used as a model organism for fungal pathogens. It is generally referred to as a dimorphic fungus since it grows both as yeast and filamentous cells. However, it has several different morphological phenotypes including the opaque, GUT, and pseudohyphal forms. C. Albicans was for a long time considered an obligate diploid organism without a haploid stage. albicans is easily cultured in the lab and can be studied both in vivo and in vitro. Depending on the media different studies can be done as the media influences the morphological state of C. Albicans. A special type of medium is CHROMagar™ Candida, which can be used to identify different species of candida. Table 1 showed the scientific classification of microorganism species used in our study

Table 1: Scientific classification of microorganism species .

Scientific classification	E. coli	S. aureus	C. albicans
Domain	Bacteria	Bacteria	Fungi
Phylum	Pseudomonadota	Bacillota	Ascomycota
Class	Gammaproteobacteria	Bacilli	Saccharomycetes
Order	Enterobacterales	Bacillales	Saccharomycetales
Family	Enterobacteriaceae	Staphylococcaceae	Saccharomycetaceae
Genus	Escherichia	Staphylococcus	Candida
Species	E. coli	S. aureus	C. albicans

MATERIALS AND METHODS

Preparation of Silver Nanoparticles: Silver nanoparticles powder was prepared by the sol-gel method successfully by dissolving 2.5 g silver nitrate (AgNO3) in 800ml of distilled water, heating the solution to 95-100 °C for boiling after that adding drops by drops Slowly (125ml) from 0.01M of trisodium citrate (C6H5O7Na3) as a reducing agent and regularly by using magnetic stirrers to mix and heat the solutions 40°C [34,20] following reaction equation is calculated;

$$4Ag+ + C6H5Na3 + 2H2O \rightarrow 4Ag\downarrow + C6H5O7H3 + 3Na+ + H+ + O2\uparrow$$

during preparation begin mirror sliver appearance in-wall container . after losing time of adding solution let without heat only move for 15 min. The solution is kept in a dark place to precipitate, after which it is washed with distilled water twice, filtered with filter paper, and dried at a temperature of 40°C for 15 minutes.

2.2 (AgNPs-ZPCCEM) Composite preparation

Mix 4g of powder zinc polycarboxylate cement (Aghesor, America) with 3 drops of liquid zinc polycarboxylate cement successfully and mix that mixture with 1.4g of Silver nanoparticles powder. The diagnosis of AgNPs, (ZPCCEM) and (AgNPs-ZPCCEM) were carried out with FTIR and FESEM analysis[35].

2.3 Antimicrobial Activity

Antimicrobial activity of AgNPs, (ZPCCEM), and (AgNPs-ZPCCEM) were checked against 3 different bacteria as described by TM Media, Titan Biotech Ltd. Dissolving 1 g of sheep Blood Agar (BA) powder (Infusion Agar)(from TM Media, Titan Biotech Ltd, Delhi, India) in 23.75 ml of distilled water, then heating the solution employing a hot plate for 15 minutes to the boiling point, and upon completion of the heating, blood is added to the solution, then the solution is stored in the incubator at 37 °C for 24 hours, Microbial Colonies were harvested and suspended and turbidity was adjusted by adding either of bacteria suspended. A sterile cotton-tipped swab was used to inoculate 0.1mL of the suspension onto the surface of a (BA) plate to achieve a lawn of bacterial growth, bacteria and fungi were cultured Stores in the incubator, and stored upside down [36].

RESULTS AND DISCUSSION

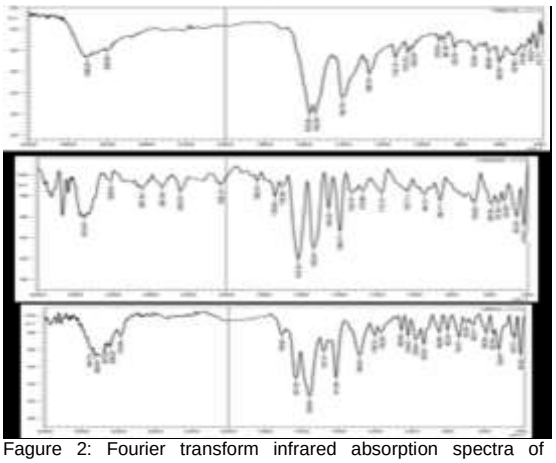
Diagnosis: The first evidence of the formation of silver particles is the appearance of a silver mirror on the walls of the reaction Becker during the reaction as shown in Figure (1).

Figure 1: Silver Mirror of Silver Nanoparticles.

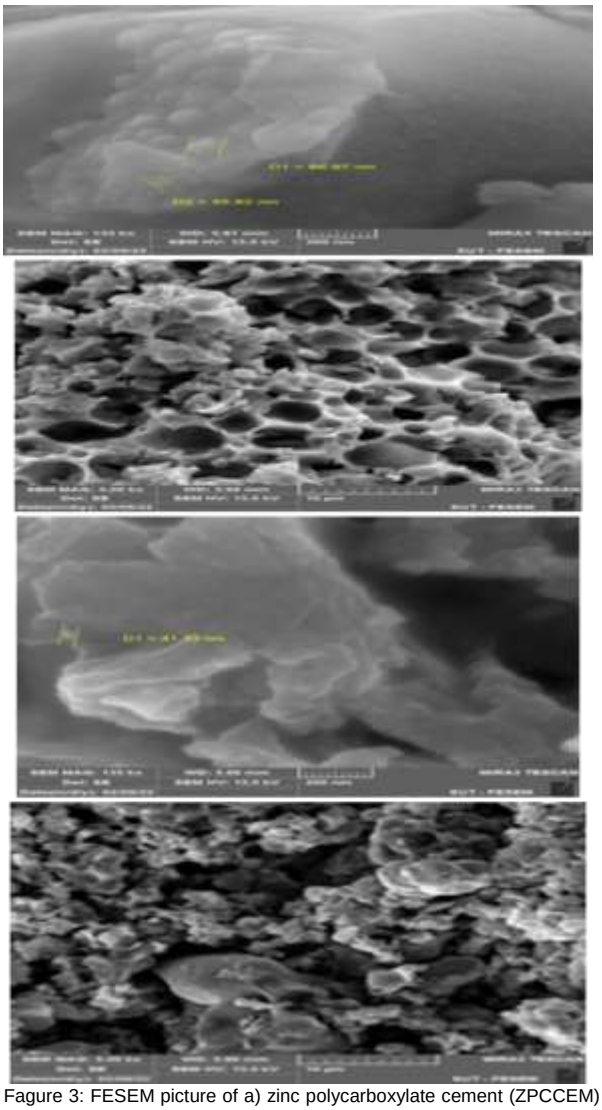
To detect the possible functional groups of the samples were investigated by FTIR spectra recorded by Spectrum FTIR (SHIMADZU). figure 1a showed the FTIR spectrum of AgNPs, which appeared in bands at (605.65), (524.64), and (412.77) indicating silver particles formation. The very strong absorption bands at 1570.06, 1543.18, and the strong absorption band at 1404,18 represent the presence of NO2 which may be from AgNO3 Solution, the metal precursor involved in the Ag nanoparticles synthesis process. The strong interaction of water with the surface of Silver could be the reason for the O-H stretching mode peaks at 3405.29, and 3106.69, and O-H in-plane bending mode peaks at 1269.16 and 1134,14 [37, 38].

Figure 1b showed the FTIR spectrum of zinc polycarboxylate cement (ZPCCEM), which appeared in the main band at 1558.48 cm1, which is attributed to the stretching mode of absorption of C=O of the carboxyl group in the zinc polycarboxylate salt[39]

, while figure 1c showed the FTIR spectrum of (AgNPs-ZPCCEM), which appeared at (455.30) and (416.77), as well as the peaks of (ZPCCEM), indicating the formation (AgNPs-ZPCCEM) Composite



Fagure 2: Fourier transform infrared absorption spectra of a) Silver Nanoparticles , b) zinc polycarboxylate cement (ZPCCEM) and c) (AgNPs-ZPCCEM) composite.



Fagure 3: FESEM picture of a) zinc polycarboxylate cement (ZPCCEM) and b) (AgNPs-ZPCCEM) composite.

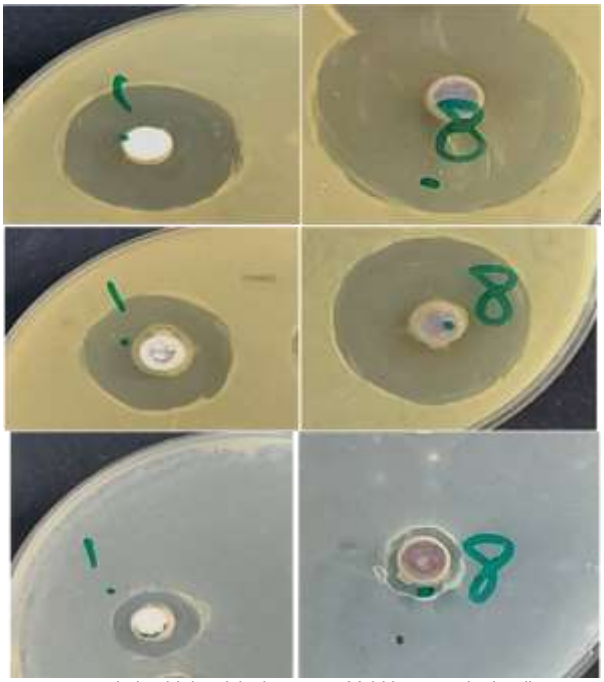
Unlike other dental blocks of cement, the setting reaction of the polycarboxylate cement produces little heat. The setting rate is affected by the potency of zinc oxide, the ratio of powder to liquid, the presence of additives, particle size, as well as the concentration and molecular weight of polyacrylic acid. Keeping these factors constant and introducing silver nanoparticles as an antibacterial reagent, the setting times of the produced cement were affected, as reported in Table 3. All types of cement were set at approximately 4.5 min. Adding different kinds of silver nanoparticles changes the setting reactions of the produced cement compounds, and the best addition of silver nanoparticles is 0.31g, which reduces the setting time for cement compound production to about 3.15 minutes. The decrease in preparation time for the addition of silver nanoparticles could be attributed to the behavior of silver nanoparticles as a catalyst to activate the reaction of ZnO with PAA which causes the observed setup reaction. In addition, this criterion is explained in terms of surface area, with small crystals being relatively more reactive than large crystals due to the higher surface area of the former when compared to the latter. It was also found that the addition of silver nanoparticles increases the density of cement formed. This result makes a good space for the designer for cost considerations[]

Antimicrobial activity: The effect of the antimicrobial activity of zinc polycarboxylate cement (ZPCCEM) and NanoSilver- zinc polycarboxylate cement (AgNPs-ZPCCEM) were evaluated against a clinical isolate of three important microorganisms used in this study, which include E-coil, Staphylococcus aureus, and Candida albicans.

The (AgNPs-ZPCCEM) showed strong antimicrobial activity(Table 1 and figure 4), this synergistic increase in activity was estimated by evaluating a fold increase in the diameter zone of the (AgNPs-ZPCCEM) compared to cement alone, and showed the most superior synergy with (AgNPs-ZPCCEM). The activity of the (AgNPs-ZPCCEM) and cement alone was increased up to 28 and 35 mm against E-coil respectively, against Staphylococcus aureus. Similarly, the antimicrobial activity of (AgNPs-ZPCCEM) and cement alone was shown at 23 and 31 mm respectively, while the antimicrobial activity of (AgNPs-ZPCCEM) and cement alone was 15 and 12 mm respectively, against Candida albicans. The enhancement of the area diameter as a function of antimicrobial activity indicates that the addition of silver nanoparticles to the cement, leads to an increase in the antimicrobial activity against Staphylocuas aureus and E-coil, while it decreases against Candida albicans.

Table 1: Antimicrobial activity in terms of fold increase in the diameter zone for zinc polycarboxylate cement (ZPCCEM) and (AgNPs-ZPCCEM) composite.

Microbial species	Inhibition zone diameter (mm)		
	Bacteria Variety	ZPCCEM	AgNPs- ZPCCEM
E-coil	Gram (-)	35	28
S. aureus	Gram (+)	31	23
C. albicans	Gram (+ & -)	15	12



Fagure 4: Antimicrobial activity in terms of fold increase in the diameter zone for zinc polycarboxylate cement (ZPCCEM) and (AgNPs-ZPCCEM) composite.

CONCLUSION

Data collected from agar diffusion assays, show that adding low amounts of silver nanoparticles to zinc polycarboxylate cement (ZPCCEM) to give (AgNPs-ZPCCEM) composite, can be a valuable alternative to increase the antimicrobial effects of this substance against a clinical isolate of three important microorganisms used in this study, which including E-coil, S.

aureus, and C. Albicans. on the antimicrobial effectiveness of endodontic cement.

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