Improvement anti biological effect of Dycal® Calcium Hydroxide Liner cement by addition of ZnO NPs

Mohammed Q. Mohammed[1], Entisar Alabodi[2]
1,2College of Education Ibn Al-Haitham, Chemistry Department, University of Baghdad, Baghdad, Iraq
Correspondence to: Entisar Alabodi, Email: entisaree2000@gmail.com

Abstract

Successful root canal therapy depends on preventing bacterial development during treatment sessions. The most frequent method for doing this is by using Dycal® Calcium Hydroxide, but some researchers have indicated that Dycal is ineffective in this area. This study aimed to evaluate the inhibitory effects of Dycal® Calcium Hydroxide based on Staphylococcus, E. coli, and Candida with those of zinc oxide nanoparticles generated by chemical and green synthesis. Characterization of the prepared samples utilized field emission scanning electron microscopy (FE-SEM), energy dispersive X-ray (EDX), Fourier- transforms infrared spectroscopy (FTIR), and ultraviolet-visible spectroscopy (UV-Vis.). The amorphous form and particle sizes of the ZnO NPs ch. and gr. were revealed by the SEM images to be 24.87 nm and 33.58 nm, respectively. FTIR data analysis centered on peaks between 4000 and 450 cm-1. The ZnO NPs (ch. and gr) absorption spectra had a constant peak at a wavelength of 300 nm. The Mann-Whitney test was used to examine the data. The findings show that ZnO NPs gr. synthesis at (25, 50, and 75) % concentrations could considerably produce an inhibitory zone that was greater than dycal alone (P value 0.5). The growth inhibition zone was substantially larger in the calcium hydroxide composition that contained 75% zinc oxide nanoparticles gr than in other ratios.

Keywords: Zinc oxide nanoparticles, Green synthesis, Inhibition area, Antibacterial, Dycal®alcium hydroxide.

1- Introduction

Bacteria in the root canal have a big impact on the stability, development, and extension of root lesions during root canal treatment [1-3]. Due to the complex architecture of the root canal and the challenges of physically preparing all root sites to eradicate bacterial infections, the use of antibacterial medications is indicated during root canal clearing [4]. Researcher Bystrom confirmed despite the adoption of these methods, some bacterial agents remain in the canal [5], and that if the canal is left empty in between treatments, the bacteria grow and eventually overpopulate the treatment area. This problem has usually been resolved by filling the canal with calcium hydroxide in between treatments [6, 7]. The mineral calcium hydroxide is antimicrobial. Although calcium hydroxide is frequently utilized, specific research has questioned its use. According to some studies, germs grow more after calcium hydroxide treatment and dentin inactivates calcium hydroxide's antibacterial effect [8, 9]. Considering calcium hydroxide's shortcomings, efforts have been made to enhance the substance's antibacterial capabilities. Numerous investigations have demonstrated that adding Silver or Copper to calcium hydroxide enhanced its antibacterial effects [10, 11]. Metal oxides have been utilized as antibacterial for a long time. Zinc oxide and magnesium oxide, among other types of metal oxides, have antibacterial properties [12, 13]. Keep in mind that the efficacy of these elements depends on their contact surface. Technical advancement, the capacity to lower metal particle sizes from micro to nanoscale, and an increase in the contact area of metal particles are all necessary to achieve increased antibacterial properties [14–16]. Additionally, a typical method to boost their antibacterial properties is to use multiple antibacterial chemicals. The synergistic effects of antibacterial agents reduce the need for higher treatment dosages to maintain side effects to a minimal [17, 18].

According to the aforementioned data, root canal treatment is not entirely avoided when calcium hydroxide is used alone. The current study sought to examine the antibacterial properties of calcium hydroxide in combination with zinc oxide nanoparticles prepared by green and chemical synthesis because there hasn't been a thorough study comparing the antibacterial properties of these particles in combination with calcium hydroxide and because metallic nanoparticles have antibacterial properties and can increase the antibacterial properties of calcium hydroxide.

In this paper, ZnO nanoparticles were chemically and biologically synthesized utilizing composites to examine the effects of this addition on the inhibitory of Dycal® Calcium Hydroxide based on Staphylococcus, E. coli, and Candida. The composite is characterized by field emission scanning electron microscopy, X-ray diffraction, Fourier transforms infrared, and ultraviolet-visible (UV-Vis) spectroscopy. The major dental caries bacteria were also examined for resistance to the chemically and biologically generated ZnO NPs using the broth microdilution technique advised by CLSI M7A8 [19-27].

Materials and experimental

2-1 materials used

The base paste was formed of 3-butylene glycol, zinc oxide, calcium tungstate, iron oxide, and calcium phosphate. The starter materials were calcium hydroxide, also known as Dycal (produced in the USA). And Zn $(NO_3)_2.6H_2O$ for environmentally friendly ZnNP production.

2-2 Green synthesis of ZnO NPs:

ZnO NPs were produced using the same technique as the researcher Bela [17]. While stirring continuously (1200 rpm) for two hours at 60 ^oC, the 50 mL solution of Zn $(NO_3)_2.6H_2O$ (0.5 M) was added dropwise to the aqueous solution of the 10 grams of Murraya paniculata leaves. The precipitate was rinsed three times with ethanol and double-distilled water before being centrifuged at 6000 rpm for 15 minutes. Before being calcined at 400 ^oC for an hour, the dark-colored precipitate was held at 100 ^oC in a hot air oven for 24 hours [28].

2-3 Antibacterial testing

The effectiveness of ligands and their metal complexes against pathogenic bacteria under aerobic conditions was examined using the good diffusion method. Mueller-Hinton agar was used to test the inhibitory activity against all pathogenic bacteria. Each indicator microorganism (Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Bacillus subtitles, Candida albicans, and Microsporum spp.) was expanded in nutrient broth before being infused with 1.5 x 108 colony-forming unit (CFU) of bacteria and (1.5 x 106) CFU of mold and yeast per milliliter of agar plates. Each of the six millimeter-deep wells on the Mueller-Hinton agar plate received 100 micro L of ligands and their metal complexes. To find bacteria, plates were incubated for 24 hours at 37°C. For the types of fungus, plates were incubated for 72 hours at 28°C. The inhibitory zone diameter (mm) was used to calculate the activity [29]. The samples were examined at the Environmental Laboratory Center of the University of Baghdad.

2-3 Device of characterization

Field emission scanning electron microscopy (FE-SEM) (Make: JEOL India Pvt. Ltd., Delhi, India). Fourier transforms infrared (FTIR) spectrometer (Perkin Elmer, Buckinghamshire, UK) spectrometer (for stability). Spectrophotometer (UV-Vis) (Jenway-USA) at range used (200-1200) nm and three hundred-seven hundred nm

3- Results and discussion

FESEM images of green, chemically generated dycal and dycal containing ZnO NPs are shown in Figs. 1a, b, and c. The FESEM pictures (Figure 1a) show the crystal and amorphous morphologies of the dycal. Figure 1b, c shows the chemical and environmentally friendly synthesis of ZnO NPs, which have a variable and amorphous appearance. The FESEM image in Figure 1 shows how the "image J" software determined the particle size. Dycal produced particles that were 59.09 nm in size, while chemical synthesis and green synthesis produced particles that were (24.87, and 33.58) nm in size, respectively.

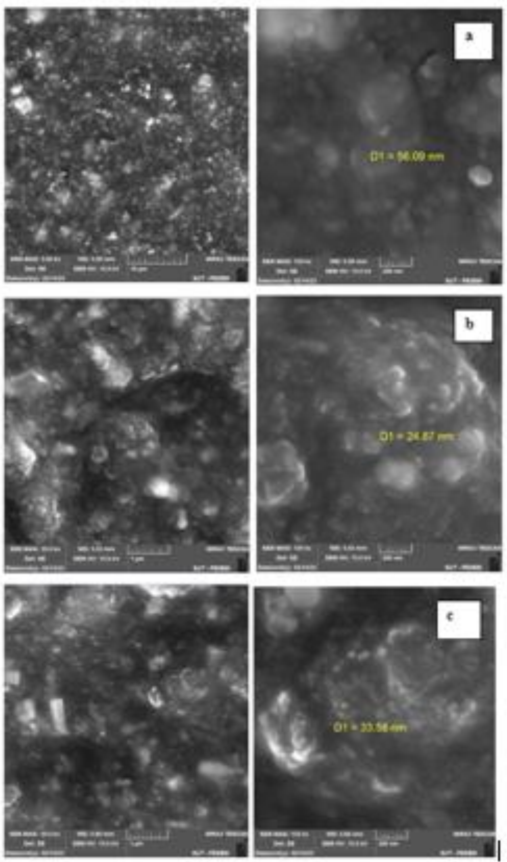

Figure 1. The FE-SEM images of the dycal with ZnO NPs, (**a**) pure dycal, (b) dycal: ZnO NPs ch., and (**c**) dycal: ZnO NPs gr.

The EDX spectra of the dycal, dycal with ZnO NPs ch., and dycal with ZnO NPs gr. samples are shown in Fig. 2a, b, and c. The names and proportions of the elements are shown on the labeling for the prepared samples. There is no question that the samples contain Zn and O, and no impurities were found that were larger than the EDX detection limit. Additionally, fig.2a displayed the percentage of dycal's constituent elements, and fig.3b displayed the findings of dycal with ZnO NPs ch. It is evident from fig.2c that the percentage of ZnO (Zn, O) appeared in addition to the percentage of dycal's constituent components in all three samples. We observe that there are no contaminants, which supports the correctness with which the research materials were prepared

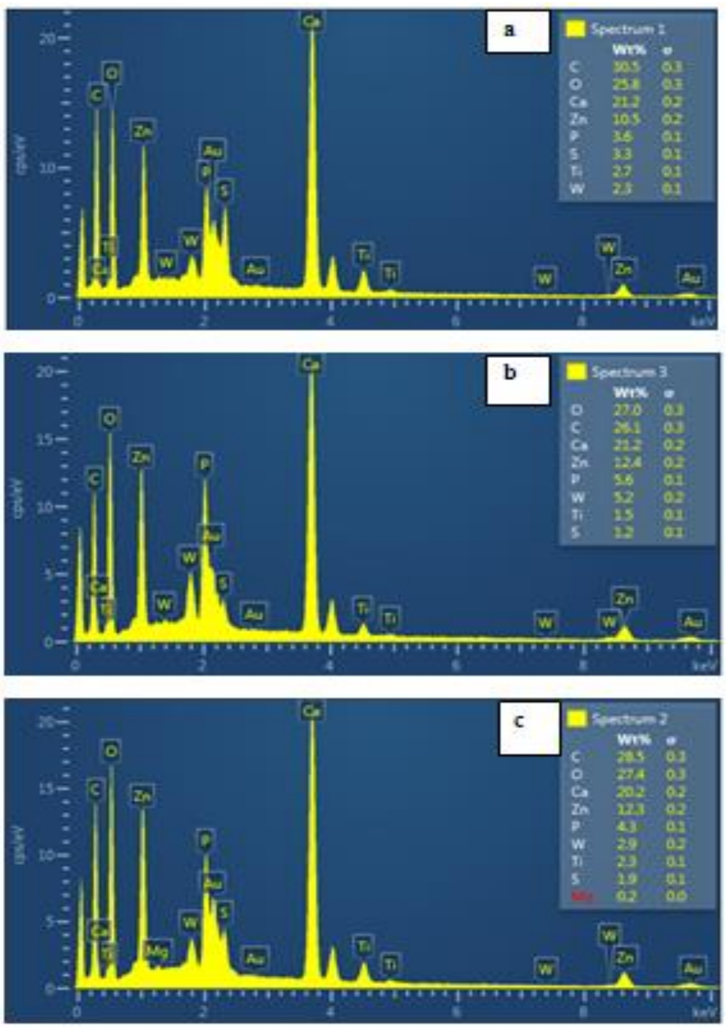
a
Spectrum 1
Wt% σ
C 30.5 0.3
O 25.8 0.3
Ca 21.2 0.2
Zn 10.5 0.2
P 3.6 0.1
S 3.3 0.1
Ti 2.7 0.1
W 2.3 0.1
b
Spectrum 3
Wt% σ
O 27.0 0.3
C 26.1 0.3
Ca 21.2 0.2
Zn 12.4 0.2
P 5.6 0.1
W 5.2 0.2
Ti 1.5 0.1
S 1.2 0.1
c
Spectrum 2
Wt% σ
C 28.5 0.3
O 27.4 0.3
Ca 20.2 0.2
Zn 12.3 0.2
P 4.3 0.1
W 2.9 0.2
Ti 2.3 0.1
S 1.9 0.1
cps/eV
keV

Figure 2: EDX diagrams of dycal with ZnO NPs, (a) dycal, (b) dycal: ZnO NPS ch., (c) dycal: ZnO NPS gr.

Figures 3a, b, and c show the FTIR spectra of dycal, dycal with ZnO NPs chemical synthesis, and dycal with ZnO NPs green synthesis. Using a spectroscopic type and grade of KBr in a wave number range of (4000 to 450) cm^{-1} at the diffuse reflectance mode, the data of FTIR spectra were measured as pellets. Bands of absorption of the strong broad in the higher region contained 3641.60 cm-1 of dycal, 2924.09 cm-1 of dycal with ZnO NPs ch., and ZnO NPs gr., as shown in fig. 3a, b, and c. These powerful broads are brought on by the stretching and vibration of a hydroxyl group (OH). The three samples' respective peaks at (3298.28, 2924.09, 2854.65, 2298.65, 2298.28, and 2864.65 cm-1 are caused by the vibration of stretching of (CH) groups. The absorption bands (1730.08, 1666.50, 1600.92, 1534.05, 1597.09, and 1597.06) cm-1 were used to represent the (C=O) amine groups. The peaks at (1469.75, 1391.31, 1257.59, 1219.01, and 1469.67) cm-1, which correspond to three samples, respectively, are caused by the (-C-H-) groups' bending vibration band. A (C-O) stretching vibration is present in the band display at (1152.29, 1068.56, and 1033.85) cm-1. The (=C-H) banding vibration groups are seen at (860.25, 806.25, and 813.96) cm-1, although this band was not visible in the ZnO NPs ch. experiment. The peaks (759.95, 709.80, 798.53, 756.10, 706.95, and 601.79) cm-1 and (799.95, and 601.80) cm-1 are not far from the banded vibration of (O-H) groups. The peaks (563.21, 416.62, and 567.07 cm-1) are due to the stretching vibration of the (Zn-O) band [30-33]. These findings showed that the presence of C-O, -C-H-, and -OH, which may be connected to the reaction's processing, is indicated by the peaks at (4000-450)cm-1

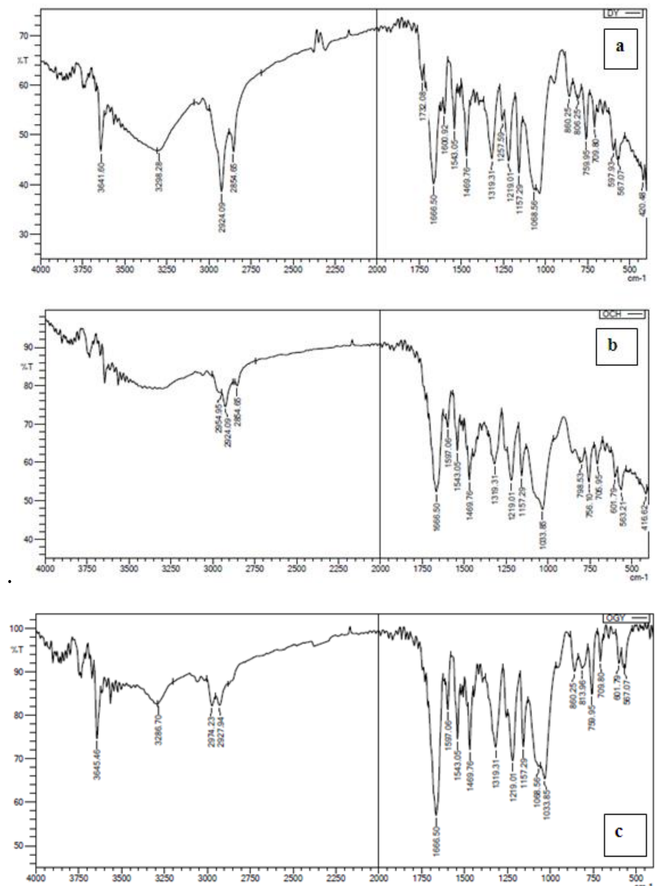

Figure 3. FTIR spectra of (a) pure Dycal, (b) Dycal: ZnO NPs ch., (c) Dycal: ZnO NPs gr.

UV-visible spectroscopy is the most effective method for examining the optical characteristics of the dycal and dycal with ZnO NPs. The absorption spectra of pure dycal, pure dycal with ZnO NPs ch., and pure dycal with ZnO NPs gr. are shown in Fig. 4. The 240 nm high and constant peak is associated with the dycal, while the 210 nm and 220 nm peaks are associated with the dycal with ZnO NPs ch. and gr., respectively. The absorbance peak for the chemically and environmentally produced ZnO NPs is visible at a wavelength of around 300 nm, which is consistent with the formation of smaller particles [34]. The SEM image further supports the aforementioned claim by displaying the smaller-sized particles. Additionally, it provides proof that the mono-disappearing nature of the NPs distribution is characterized by the higher absorption of sharp dycal and ZnO NPs [35].

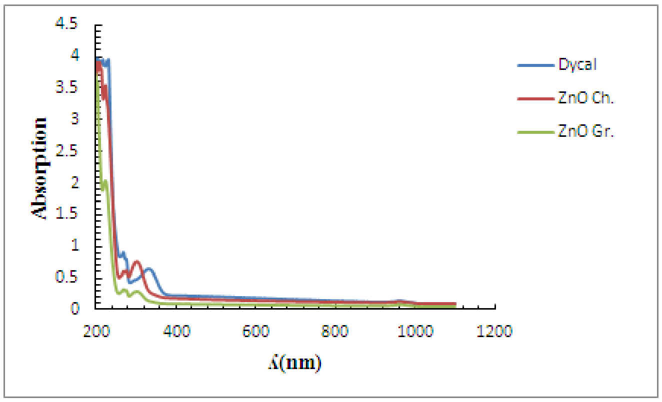

Figure 4. UV-visible spectra of dycal with ZnO NPS gr. and ch.

In Fig. 5 and Table 1, the average diameter of the inhibitory zone for the dycal alone and the composite with ZnO NPs (gr., ch.) synthesis is displayed. However, overall, the addition of zinc oxide is favorable or increases from the inhibition zone. The results show that there are relative changes in the inhibition zone across the groups. The statistical findings revealed a strange data distribution. Thus, the Mann-Whitney test was used to examine the data. According to the statistical findings, the inhibitory zone diameter for the selected kind of bacteria was substantially different at (25, 50, and 75) % concentrations of dycal for 75% dycal (P value 0.05). According to the statistical analysis of the dycal composite with ZnO NPs green synthesis, the inhibition zone of dycal + (25, 50, and 75)% ZnO NPS gr. differed significantly from that of dycal ®calcium hydroxide alone (P value 0.05). The results showed that the inhibition zone was larger than that of dycal alone for all bacteria chosen, as shown in Table 1. we use the independent sample t-test to check whether there are significant differences between groups or not using 3 different measures (0.02 g / 0.05 g / 0.07 g). There are significant differences between dycal, ZnO-NPs gr and ZnO-NPs ch in Candiad with (0.07) gm. The diameter of the inhibitory zone for each of the selected bacteria was significantly different when dycal and ZnO NPs were combined (P 0.05). (Table 1). The inhibition zoon was equivalent to or less than dycal alone, according to the results in those cases. According to the findings of the current investigation, adding ZnO gr NPS to other composites increases bacterial resistance and increases the diameter of the inhibitory zone for dycal with ZnO gr synthesis at 75% concentration. However, the addition of ZnO ch NPs to the dycal demonstrated the limited impact of bacterial resistance. These results agree with the results of the researchers Samiei [36], and Alann [37].

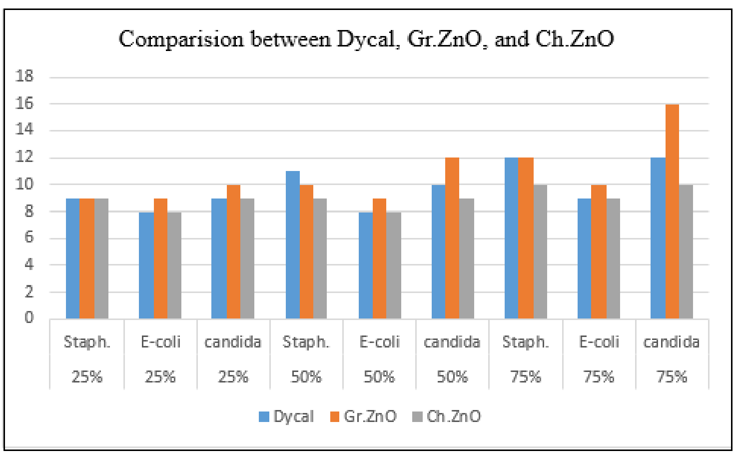

Figure 5: The histogram of the inhibition zone in different concentrations of Dycal and dycal: ZnO (Gr, CH) synthesis.

Table 1 Comparison between the inhibition zone of ZnO-NPs (Gr. and Ch.) synthesis and dycal

Bacterial	0.07 gm			0.05 gm			0.02 gm		
	E.coli	Staph	Candid	E.coli	Staph	Candiad	E.coli	Staph	Candiad
Dycal	9	12	12	8	11	10	8	9	9
ZnO Gr. Sy.	10	12	16	9	10	12	9	9	10
ZnO Ch.Sy.	9	10	10	8	9	9	8	9	9
p-value	0.06999	0.07423	0.00009**	0.05598	0.07999	0.07014	0.07113	1	0.06997

** The difference is considered to be highly statistically significant under 0.01.

4- Conclusion

It is clear from this research that using dycal and dycal with ZnO NPs (gr, ch) synthesize enhances antibacterial activity against E. coli, Staph, and candida. However, the mixture is taken into consideration because different therapeutic components each have benefits and drawbacks. To prevent the adverse effects of drugs that reduce antibacterial activity, it is thought to be preferable to utilize different antibacterial agents separately. To develop an effective drug composition, inhibit bacterial recolonization, boost antimicrobial activity, and provide successful root canal therapy, more research is required. Within the confines of the current investigation, it can be concluded that the addition of nano zinc oxides prepared by green synthesis for dycal®calcium hydroxide nanoparticles has improved the antibacterial of E. coli, Staph, and Candid while adding the prepared nano zinc oxide in chemical synthesis, it had a limited or equal effect of the dycal in the antibacterial to the selected bacteria.

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