SYNTHESIS AND CHARACTERIZATION OF METALLIC AND POLYMERIC NANOPARTICLES AND THEIR EFFECT ON THE ANTIBACTERIAL PROPERTIES OF MICROHYBRID COMPOSITE RESIN

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ABSTRACT

INTRODUCTION: During the past decade, composites have become the most commonly used restorative materials. The rate of dental caries following treatment with composite resin is high. Therefore, one of the most applicable methods for preventing enamel demineralization around the restorations is using dental materials resistant to the bacterial accumulation

OBJECTIVES: To synthesis and characterize antibacterial nanoparticles and to evaluate the effect of blending microhybrid composite with zinc oxide nanoparticles (ZnO), Chitosan (Cs) and combination of both Chitosan/Zinc oxide nanoparticles (Cs/ZnO) and properties of these nanoparticles on the composite resin.

MATERIALS AND METHODS: Three antibacterial nanoparticles were prepared and characterized in terms of particle size, zeta potential, shape, morphology and functional group determination. Minimum inhibitory concentration of the nanoparticles was determined. The nanoparticles were incorporated into commercial microhybrid composite resin. The antibacterial properties against *Streptococcus mutans* were evaluated by disc diffusion test and direct contact test. The results were analyzed using ANOVA test at $p \leq .05$ significance level.

RESULTS: For agar diffusion disc, incorporation of ZnO nanoparticles into the composite resin results in an antibacterial effect which lasted for up to 12 weeks, while for the Cs and Cs/ZnO nanoparticles the antibacterial effect lasted for up to 2 weeks. The direct contact test visualized under SEM also showed that incorporation of ZnO nanoparticles into composite resin to be the most inhibitory in all the 4 groups, denoting that ZnO-NPs has a far better inhibitory effect than Cs-NPs and Cs/ ZnO-NPs.

CONCLUSIONS Antibacterial nanoparticles could be synthesized and characterized by Zetasizer NanoZS, scanning electron, transmission microscopy and Fourier transform infrared spectroscopy. Incorporation of ZnO, Cs and Cs/ZnO nanoparticles into the composite resin could significantly inhibit the *S. mutans*. The antimicrobial efficacy of the ZnO nanoparticles blended with microhybrid composite resin was confirmed for a duration up to 12 weeks.

KEYWORDS: Composite resin, ZnO, Chitosan nanoparticles.

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INTRODUCTION

Composite resin is the most widely used dental restorative material in practice today due to its superior esthetics and ease of handling (1). However, a number of clinical studies have reported shorter longevity and higher failure rates for composite restorations compared to amalgams. One of the main reasons of failure was secondary caries or recurrent caries (2).

Furthermore, as various laboratory and clinical researches have demonstrated, comparing to other restorative materials or dental hard tissues, more plaque accumulation occurs on resin composites which results in a higher prevalence of secondary caries around composite resin restorations (3). The more biofilm formation on resin composites is related to its surface roughness and free energy, that is the outcome of resin type, filler size, and percentage of fillers (4).

In light of these findings, recent studies pay growing attention to the antibacterial activity of composite resins in order to reduce the risk of recurrent decay around esthetic direct restorative materials. Several methods have been used to inhibit biofilm growth that contributes to dental caries (4).

Some metal oxides, such as magnesium, zinc, silver and calcium are proven to have antimicrobial characteristics. This property is enhanced when the oxides are in nano scale level (5).

Nanotechnology is the science of evaluating and producing materials in nano-dimensions by resizing of atoms to prepare materials with better properties. The presence of very small particles leads to superior properties of the material. These unique properties, which are the subject of quantum mechanics, have attracted a great deal of interest (6).

Currently, the metallic and polymeric nanoparticles are thoroughly being explored and extensively investigated as potential antimicrobials. The antimicrobial activity of the nanoparticles is known to be a function of the surface area in contact with the microorganisms. The small size and the high surface to volume ratio, i.e., large surface area of the nanoparticles enhances their interaction with the microbes to carry out a broad range of probable antimicrobial activities.(7)

Streptococcus mutans is one of the major species of bacteria responsible for dental caries. Several researches argued that among metallic agents, the silver nanoparticles are the most effective metal for inhibiting the growth of *S. mutans* (8). However, the major drawback of silver in restorative dental materials is the cosmetic changes of tooth colored materials (3). Hence, insoluble, white, tooth-colored or colorless antibacterial polymer such as Chitosan or metal oxide powders such as zinc oxide (ZnO), may be more interesting in dental composites (4).

Zinc oxide has shown anti *S. mutans* properties which will be enhanced with higher concentrations. Chitosan is a widely used material due to its biodegradable, nontoxic, non-antigenic, and biocompatible properties. It is a biopolymer isolated from shellfish, crab and shrimp. Chitosan is reported to exhibit numerous health-related beneficial effects, including strong

antimicrobial and anti-oxidative activities in food. Chitosan is one of the most abundant substances in nature (5,9).

Because nanoparticles are expected to be more effective in penetrating and disrupting bacterial cell membranes, that is why we choose ZnO and chitosan nanoparticles in our study. Nano-Chitosans are effective against a variety of organisms, and if antimicrobial agent combined to other such as ZnO nanoparticles, the antimicrobial effect could be exaggerated (5), for that reason Chitosan/ ZnO nanoparticles was also studied.

The null hypothesis to be tested in this study is that incorporation of metallic and polymeric nanoparticles will have an antibacterial effect and reduce bacterial adhesion on commercial microhybrid composite resin material in the short and long term.

MATERIALS AND METHODS

Preparation of the nanoparticles

A commercially available zinc oxide dispersion nanoparticles (50 wt % in water) was supplied from Sigma Aldrich chemical company (Batch # MKBL 4351). The nano-suspension freeze dried by vacuum Freeze Drying Machine (CHRIST - BETA, Zeichnug Nr. GT-3635, Germany) work at 220 V, 50 Hz, 1000W. The nano-solutions were placed in 15 ml petri dish, frozen in liquid nitrogen and freeze dried by vacuum Freeze Drying Machine at a pressure of 26.5 pa and 5% saccharose which was used as cytoprotectant. The lyopholized particles were then characterized. Chitosan nanoparticles (Cs-NPs) were prepared by ionic gelation method (10), in brief, 0.5% Chitosan solution was prepared by dissolving chitosan (Sigma–Aldrich Chemical Co. Ltd.) with sonication in 1% (w/v) acetic acid solution until the solution was transparent. Sodium tripolyphosphate (TPP) (Sigma–Aldrich Chemical Co. Ltd.) was dissolved in deionized water at the concentration of 0.25%. The TPP solution was added drop by drop using a 22-gauge micro-infusion syringe pump at rate of 1 mL/min, the formation of chitosan-TPP nanoparticles started spontaneously via the TPP initiated. The ratio of chitosan: TPP was 3:1. The nanoparticle suspensions were gently stirred for 48hrs at room temperature at 3000 rpm. Nanoparticles precipitated by centrifugation at 10000 rpm for 1 hr. The supernatants were discarded, and the chitosan nanoparticles were washed three times with deionized water. Then the nanoparticles were lyophilized using vacuum Freeze Drying Machine before further use or analysis. then the nanoparticles' dry powder was stored in desiccator at room temperature.

To prepare Chitosan/ZnO nanoparticles (Cs/ZnO -NPs), 0.5% Chitosan solution was prepared by dissolving chitosan with sonication in 2% (w/v) acetic acid solution until the solution was transparent. To this solution 0.5 gm ZnO nano powder was added. The nanoparticle suspensions were gently stirred for 72 hrs at room temperature at 3000 rpm. After magnetic stirring, 1M NaOH was added drop by drop until the solution attained pH 10. Nanoparticles were precipitated by centrifugation at 10000 rpm for 1 hr. The supernatants were discarded, and the Chitosan/ZnO nanoparticles were washed three times with deionized water to remove any sodium hydroxide. Then the nanoparticles were lyophilized using vacuum Freeze Drying Machine before further use or analysis. Finally the nanoparticles' dry powder was stored in desiccator at room temperature (11).

Characterization of the nanoparticles

The three types of nanoparticles were characterized by Zetasizer NanoZS (Malvern Instruments Ltd, Malvern, UK.) to determine particle size, polydispersion index (PDI) for homogeneity and zeta potential for stability of the nanoparticle in the solution. Transmission Electron Microscope (TEM) (JEOL-100 CX, Japan.) and scanning Electron Microscopy (JSM-5300, JEOL, Japan) were used to determine shape and morphology. Functional groups were determined by Fourier transform infrared (FTIR) spectrometer (Shimadzu, Tokyo, Japan).

The nanoparticles were dissolved in double distilled water and I ml was placed in the quartz glass cuvette, this cuvette was placed in the Zetasizer NanoZS. With the help of the software, a curve with the particle size distribution, zeta potential and PDI number was obtained.

Determination of the nanoparticles ratio to be blended with composite resin

The ratio of nanoparticles to be incorporated were determined according to the minimum inhibitory concentration (MIC).

Determination of the MIC of ZnO NPs was done using the two-fold dilution method (1-4096 mg/L) according to Clinical and Laboratory Standards Institute (CLSI) guidelines.(12) MICs were performed in 96-well microplates (Greiner, Wemmel, Belgium). Serial two fold dilutions of 2x strength ZnO NPs were performed in sterile distilled water. An overnight culture of the tested isolate in Brain Heart Infusion broth (BHI) (Oxoid, Cambridge, UK) was diluted in fresh double strength BHI till it reached 10^5 CFU/ml. A 0.05 ml suspension of the *S. mutans* (ATCC#25175) in double strength BHI was added to the well with 0.05 ml of the antibiotic solution, to obtain a final concentration of $5x10^5$cfu/mL. Inoculated and uninoculated chitosan free broth were included and the microtiter plates were incubated aerobically at 35ºC for 18 hours (12). The MIC was then defined as the lowest concentration of the antibiotic in which there was no visible growth after overnight incubation. The same was applied to determine the MIC for Chitosan nanoparticles and Chitosan/ZnO nanoparticles and were triplicated, on three different days.

Blending of nanoparticles with microhybrid composite resin

Based on the results obtained from the MIC, several nanoparticles percentage by weight were tested for the antimicrobial activity done in the pilot study, then 1.5% by wt ZnO-NPs, 1.7% by wt chitosan nanoparticles and 1.7 % by wt chitosan/ZnO-NPs were selected to be incorporated into the microhybrid composite for further testing.

The nanoparticles were dispersed mechanically to the microhybrid composite resin (Filtek Z250 universal restorative) and homogeneously mixed in a dark room (x-ray film processing room), these modified composites were stored in completely opaque bottles until each test was performed (5,4,13).

SEM-EDX (Scanning electron microscopy with an energy dispersive X-ray analytical system) (JSM 5300 JEOL, Japan- Link ISIS) analysis was performed to confirm the homogeneity of the distribution of the nanoparticles in the composite resins and provide chemical microanalysis, where elements with relative values expressed in weight percentage was determined (14,15).

Grouping of the test specimens

Group I: Control group, microhybrid composite resin (Filtek Z250 universal restorative) (3M ESPE, St. Paul, MN, USA.).

Group II: Microhybrid composite resin blended with ZnO-NPs.

Group III: Microhybrid composite resin blended with Cs-NPs.

Group IV: Microhybrid composite resin blended with Cs/ZnO-NPs.

The antibacterial effect of each material was evaluated against *S. mutans* (ATCC#25175) using the agar diffusion test and direct contact inhibition. A custom made Teflon disk-shaped mold (2mm thickness x 6 mm diameter) was used to prepare the specimens. Initially the mold was slightly overfilled with the material, and pressed flat with a microscopic glass slide to extrude excess composite material then the composite was light polymerized following the manufacturers' instructions with a visible light polymerization unit (TULIP 100 B, Being Foshan Medical Equipment Co. Ltd) for 40 seconds, excess composite resin was removed with sterilized instruments.

The specimens for the 2 tests were divided according to the aging period at the time intervals into 4 subgroups (Table 1).

All specimens were stored immediately in tightly covered sterile test tubes containing pyrogen free water. During the storage period, the samples were stored in an incubator at 37 °C (16).

Disc diffusion test

A total of eighty discs, twenty for each group were divided according to the test aging period as shown in (Table 1) mainly 24hrs, 2 weeks, 6 weeks and 12 weeks. The modified Kirby Bauer disc diffusion

method was carried out according to CLSI recommendations for disc diffusion susceptibility testing (12). Composite resin discs were laid on BHI. Agar plate was incubated at 35°C for 24 hrs and the zone of inhibition was recorded in millimeters.

Direct contact inhibition test (DCT) of composites evaluated by scanning electron microscopy:

A total of forty eight discs, twelve for each group and divided according to the test aging period in (table 1) Bacterial attachment was demonstrated by direct contact inhibition studies. Composite discs were inoculated with 10 µL (~10^7 CFU) bacterial solution without additional nutrients, incubated for 1 hr at 37 °C, then wells of the 96-well plate were filled with 750 µL BHI broth without sucrose and allowed to culture for an additional 23 hrs (3).

Twenty four hours fixation was performed in glutaraldehyde then dehydration was completed in a gradient ethanol series (30%, 50%, 70%, 80% then absolute), in each concentration the specimen was placed for 5 min 3 times before transferring to the higher concentration. Samples were coated with gold in vacuum evaporator sputter coater and visualized with Scanning Electronic Microscopy at 25 keV beam energies.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Statistical comparison was carried utilizing AVOVA test with *P value* set at 5% level

Table 1: Showing grouping and subgrouping of the Disc diffusion test and Direct contact test.

Subgroup / Group	A (24hs)		B (2ws)		C (6ws)		D (12ws)	
	a	b	a	b	a	b	a	b
I	5	3	5	3	5	3	5	3
II	5	3	5	3	5	3	5	3
III	5	3	5	3	5	3	5	3
IV	5	3	5	3	5	3	5	3
TOTAL (n)	128							

a: disc diffusion test. (n=80)

b: direct contact inhibition of composites evaluated by SEM. (n=48)

RESULTS

Preparation and characterization of the nanoparticles

Particle size distribution by Zetasizer NanoSZ, the mean particle sizes and polydispersion index (PDI) were 38.19 nm and 0.5 (i.e. less than 1) for Zinc oxide nanoparticles (fig. 1a), 82.87 nm and 0.598 (i.e. less than 1) for chitosan nanoparticles (fig. 1b), and 185.7 nm and 0.3 (i.e. less than 1) for Cs/ZnO-NPs respectively (fig. 1c), which indicates homogeneous nature of the formulation.

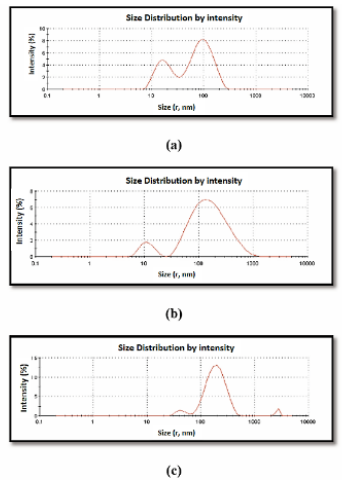

Figure 1: particle size distribution for the nanoparticles.

ZnO nanoparticles, b) Cs nanoparticles, c) Cs/ZnO nanoparticles

Zeta potential was performed to estimate the surface charge of nanoparticles in solution showing (+ 30.9 mv ± 4.8) for ZnO NPs (fig. 2a), (+ 23.9 mv ± 6.87) for Cs NPs (fig. 2b), and (-7.87 mv ± 5.8) for Cs/ZnO NPs respectively (fig. 2c).

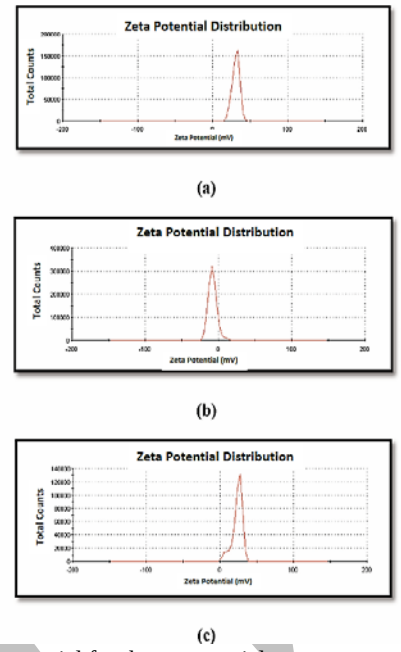

Figure 2: zeta potential for the nanoparticles.
a)ZnO nanoparticles, b) Cs nanoparticles, c) Cs/ZnO nanoparticles

The shape of the nanoparticles was determined by TEM and revealed completely spherical particles with low level of agglomeration for ZnO NPs and CsNPs. However, Cs/ ZnO NPs showed high level of agglomeration. (Fig. 3)

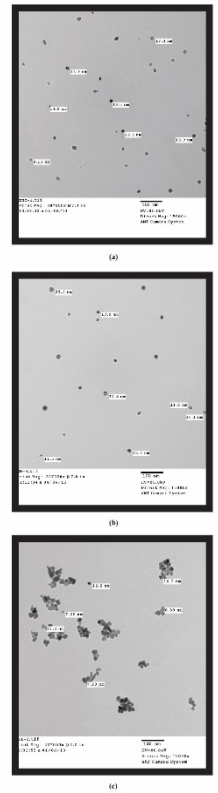

Figure 3:TEM images for the nanoparticles**.**
a)ZnO nanoparticles, b) Cs nanoparticles, c) Cs/ZnO nanoparticles

The surface morphology of the prepared nanoparticles was determined by SEM and revealed completely spherical particles with smooth surface for ZnO NPs and Cs NPs. (Fig. 4)

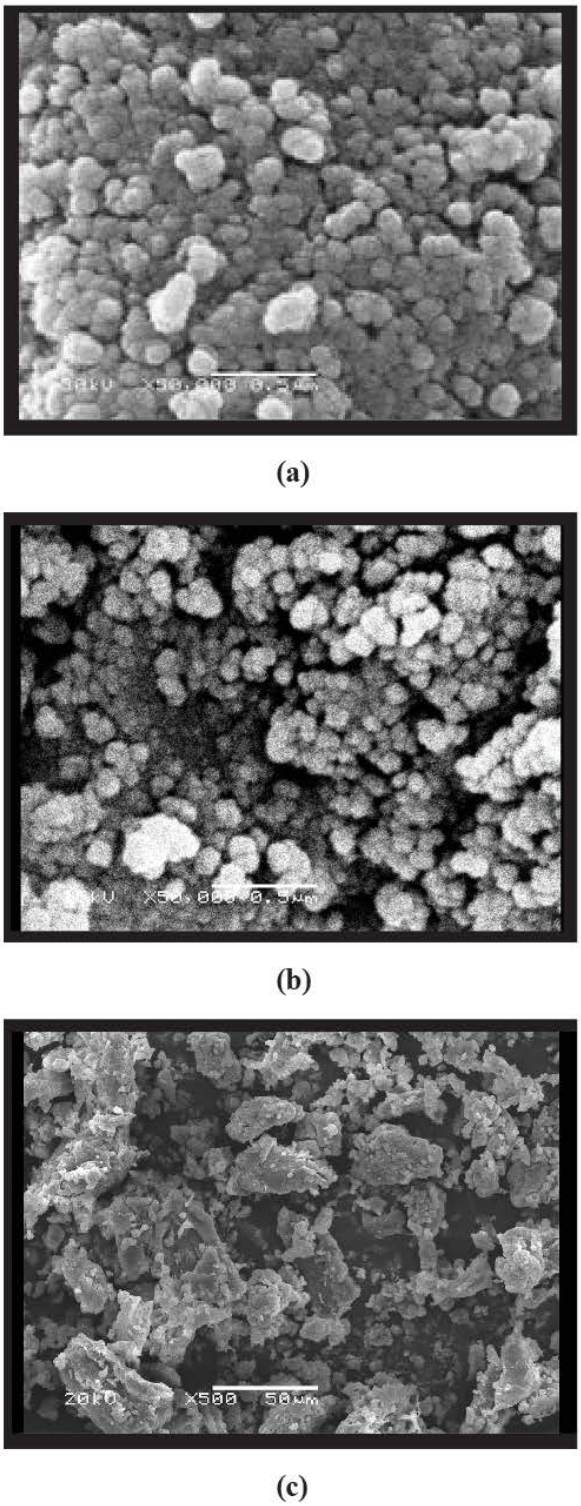

Figure 4:SEM for the nanoparticles.
ZnO nanoparticles, b) Cs nanoparticles, c) Cs/ZnO nanoparticles

For the FTIR spectra of the nanoparticles (fig. 5),

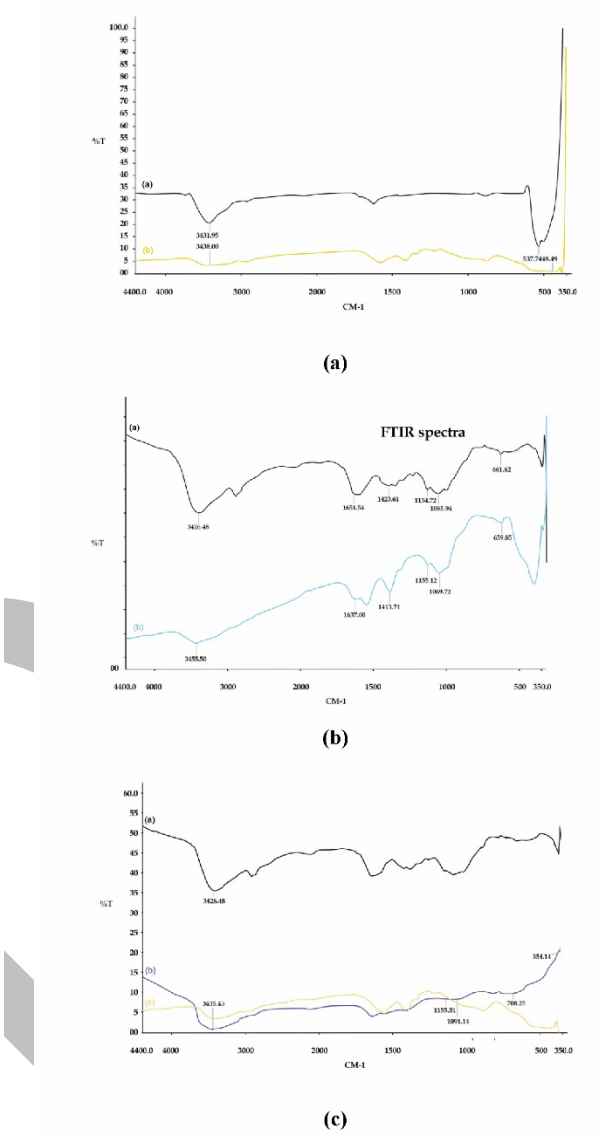

Figure 5:FTIR spectra of the nanoparticles
a) ZnO powder b) ZnO nanoparticles
a) Cs powder b) Cs nanoparticles
a) Cs powder b) Cs/ZnO nanoparticles c) ZnO nanoparticles

the IR of ZnO powder showed an IR band of (Zn-O) at 537 Cm^{-1} which is shifted in the nanoparticles form to 448 Cm^{-1}. The broad band at 3431 Cm^{-1} for ZnO powder which is at 3438 Cm^{-1} for Zno NPs suggest the presence of hydroxyl group. For Chitosan nanoparticles, the conserved characteristic absorption bands between Chitosan powder and Chitosan nanoparticles appeared at 3426 cm-1, 1651 cm^{-1}, 1423 Cm^{-1},1154 Cm^{-1}, 1085 Cm^{-1}, 661 Cm^{-1} with small shifting compared with chitosan powder with the formation of new characteristic absorption band at 1565 (NH2 cross linking). For Cs/ZnO NPs IR spectrum, the characteristic absorption bands appeared at wave number 3435 Cm^{-1} which shifted compared with 3426 Cm^{-1} for chitosan powder, this shifting related to the formation of H2 bond between Zn and chitosan and the other wave number at 1155, 1091, 708 with the formation of new bond at wave number at 354 Cm^{-1} which correspond to vibration of O-Zn-O group.

Nanoparticles' MIC by broth microdilution method

The MIC for ZnO-NPs, Cs NPs, and Cs/ ZnO-NPs was 32 µg/ml, 2048 µg/ml, and 1024 µg/ml respectively.

Disc diffusion test

The results shown in tables 2 and 3, indicating that the highest mean width of inhibition zone was observed for group II (microhybrid composite resin blended with ZnO nanoparticles) which lasted for a period up to 12 weeks and the lowest mean width of inhibition zone was observed for group IV (microhybrid composite resin blended with Cs/ZnO nanoparticles) which lasted for a period up to 2 weeks and these results were statistically significant at P≤0.05.

Table 2: Comparison between the different studied groups according to Width of inhibition zone (mm) in each storage period

	Width of inhibition zone (mm) Mean ± SD			
	Subgroup A **(24hrs)**	Subgroup B **(2 weeks)**	Subgroup C **(6 weeks)**	Subgroup D **(12 weeks)**
Group I	$6.0^{a} \pm 0.0$	$6.0^{a} \pm 0.0$	$6.0^{a} \pm 0.0$	$6.0^{a} \pm 0.0$
Group II	$18.0^{b} \pm 0.71$	$16.0^{b} \pm 1.58$	$14.0^{b} \pm 0.71$	$8.0^{b} \pm 0.71$
Group III	$18.0^{b} \pm 1.87$	$10.0^{c} \pm 1.0$	$6.0^{ac} \pm 0.0$	$6.0^{ac} \pm 0.0$
Group IV	$15.80^{b} \pm 1.48$	$10.0^{c} \pm 0.71$	$6.0^{ac} \pm 0.0$	$6.0^{ac} \pm 0.0$
p	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$

p: p value for F test (ANOVA) for comparing between the different studied group
Different superscript in each Colum are significant
*: Statistically significant at $p \leq 0.05$

Table 3: Mean zone of inhibition of the 4 tested materials for the 4 testing conditions.

	Width of inhibition zone (mm) Mean ± SD				**p**
	Subgroup A **(24hrs)**	Subgroup B **(2 weeks)**	Subgroup C **(6 weeks)**	Subgroup D **(12 weeks)**	
Group I	$6.0^{a} \pm 0.0$	$6.0^{a} \pm 0.0$	$6.0^{a} \pm 0.0$	$6.0^{a} \pm 0.0$	-
Group II	$18.0^{a} \pm 0.71$	$16.0^{ab} \pm 1.58$	$14.0^{b} \pm 0.71$	$8.0^{c} \pm 0.71$	$<0.001^{*}$
Group III	$18.0^{a} \pm 1.87$	$10.0^{b} \pm 1.0$	$6.0^{c} \pm 0.0$	$6.0^{c} \pm 0.0$	$<0.001^{*}$
Group IV	$15.80^{a} \pm 1.48$	$10.0^{b} \pm 0.71$	$6.0^{c} \pm 0.0$	$6.0^{c} \pm 0.0$	$<0.001^{*}$

p: *p value* for F test (ANOVA) with repeated measures for comparing between different period
Different superscript in each raw are significant
*: Statistically significant at $p \leq 0.05$

Direct contact inhibition test

Samples were visualized with scanning electron microscopy for the results of the direct contact inhibition test (DCT) to visualize the amount of bacteria that adhere on the surface of the composite resin. (Fig 6). The largest number of adherent bacteria was observed for group I, III and IV (12 weeks), while group II showed the least number of the adherent bacteria that was observed up till 12 weeks.

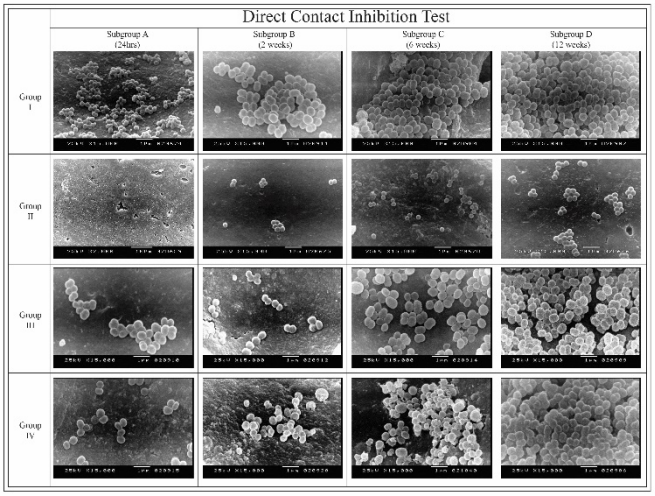

Figure 6: SEM pictures of the direct contact test

DISCUSSION

The integration of a countless number of nanoparticles into dental materials leads to an advancement by producers to enhance the chemical and physical properties of these materials (17).

Zinc has been used in dentistry for a long time as the leading filler constituent of dental cements. ZnO-NPs have been applied as a coating material along with nano-hydroxyapatite (18) and incorporated into dental resins (14). Past studies were done in effect exhibiting antibacterial action and biofilm growth inhibition by ZnO-NPs in composite (3,4).

The antibacterial effect of ZnO-NPs and Cs-NPs as fillers in microhybrid composite was evaluated in this study. Although their antimicrobial properties were previously described, studies focusing on a combination of both have been very scarce. Microhybrid composite incorporated with 1.5% by wt ZnO-NPs, 1.7% by wt chitosan nanoparticles and 1.7 % by wt Cs/ZnO-NPs were utilized.

The ZnO nanoparticles samples showed completely spherical particles with mean particle size of 38.19 nm and polydispersition index was 0.5 (i.e. less than 1) ensuring sample homogeneity and surface charge of nanoparticles in solution of + 30.9 mv ± 4.8. The infra-red (IR) spectra of zinc oxide showed an IR band of (Zn-O) at 537 cm^{-1} which is shifted in the nanoparticles form to 448 cm^{-1}. The broad band at 3431 cm-1 for ZnO powder which is at 3438 cm^{-1} for Zno NPs suggest the presence of the hydroxyl group indicating that the composition of the prepared nanoparticles did not change from the original particle size at the macroscale level.

Perfectly spherical Cs NPs with discrete distribution, mean particle size to be around 82.87 nm and polydispersition index was 0.598 (i.e. less than 1) and surface charge of + 23.9 mv ± 6.87 were obtained by ionic gelation method. FTIR spectra of the Cs NPs showed a new formed absorption peak at 1565 cm^{-1} which represents the cross linking of amino group of Chitosan with polyphosphoric group of tripolyphosphate (TPP) ensuring that the formed chitosan is in a nano form.

Cs-ZnO NPs was effectively prepared by means of a simple and cost effective chemical precipitation method. The prepared composite revealed spherical particles with mean particle size of 185.7 nm and polydispersition index was 0.3 (i.e. less than 1), which indicates homogeneous nature of the

formulation and the surface charge of nanoparticles in solution -7.87 mv ± 5.8. FTIR showd the formation of new bond at wave number 354 cm^{-1} which correspond to vibration of O-Zn-O group indicating that the composition of the prepared nanoparticles did not change from the original particle size at the macroscale level.

Our results demonstrated that the MIC for ZnO-NPs, CsNPs, and Cs/ ZnO-NPs was 32 µg/ml, 2048 µg/ml, and 1024 µg/ml respectively. Microhybrid composites incorporated with ZnO-NP demonstrated the most elevated mean zone of inhibition followed by chitosan nanoparticles and Cs/ ZnO-NPs. The mean zone of inhibition diminished as the composite aged, yet group II (ZnO-NP) remained the highest for subgroup A and B (24 hrs and 2 weeks with no statistical significance between the two) and remained so till 12 weeks. The results were statistically significant.

In this study, the mean width of inhibition zone was 18.0 mm for group (II and group III) and 15mm for group IV. The range of the zone of inhibition values obtained from this study (6mm - 18.0mm) were in accordance with Mahapoka et al. (15) and Mirhashemi et al.(5). Our results were in disagreement with Hojati et al (4) and Sevinc and Hanley (3) who showed that there were no inhibition zones around the test specimens and they explained their results that due to the insolubility of the ZnO-NPs, an adequate amount of Zn^{2+} could not leach to the surrounding environment to establish an antibiotic efficacy.

The results of the current study were in agreement with Mirhashemi et al (5) found that the zone of inhibition with modified composite resin with Cs/ ZnO-NPs and explained this as the combination of the two nanoparticles made an enhancement of the antibacterial effect of each nanoparticles.

In order to estimate the sustainability of the ZnO-NPs efficacy in the resin matrix the aging test was performed by DCT. We preferred water aging as most of the studies reported have evaluated the initial inhibition only, and it is imperative to determine the effect over a longer time interval. The DCT visualized under SEM also showed Group II to be the most inhibitory among the 4 groups, denoting that ZnO-NPs has a far better inhibitory effect than Cs-NPs and Cs/ ZnO-NPs combination

These results show that incorporation of ZnO-NPs into the resin composites could significantly inhibit the *S. mutans* strains. This was in agreement with other studies (14,19). One mechanism that is considered to explain the antimicrobial properties of ZnO-NPs is that they generate active oxygen species such as H_2O_2 which inhibit growth of pathogens (20). It may also be attributed to their ability to interact with the cell membrane of several bacterial species (21). Toxicological mechanisms of zinc ions play a vital role in biofilm inhibition by hindering the active transport and metabolism of sugars along with disrupting enzyme systems of dental biofilms by displacing magnesium ions essential for enzymatic activity of the plaque (3). Zinc can also reduce acid production by *S. mutans* biofilms, which includes both *S. mutans* and *S. sobrinus*, and also has an ability to inhibit glucosyltransferase activity, thus impeding decalcification in vitro and in vivo (22).

On the other hand, chitosan binds to the membrane of bacterial cells causing a boost in membrane permeability with an associated increase in the outward flow of ions and proteins from the microbial cell; and inhibition of mRNA transcription and change of protein translation owing to binding of chitosan to the DNA of several micro-organisms (23).

Our results showed that ZnO-NPs have induced an antibacterial activity in resin composite; which was significantly higher than other groups. Thus, the incorporation of ZnO-NPs provides beneficial antibacterial properties. More studies are required however, to determine the mechanical modifications of adding NPs as filler to dental composite.

CONCLUSION

From the results of this study, the following could be concluded:

Chitosan and Chitosan/ZnO nanoparticles could be synthesized by ionic gelation method and simple precipitation method respectively which is considered simple and cost effective. They were also characterized by Zetasizer NanoZS, SEM, TEM and FTIR.

The MIC for these nanoparticles were determined to be 2048 µg/ml and 1024 µg/ml respectively.

Incorporation of ZnO, Cs and Cs/ZnO nanoparticles into the composite resin could significantly inhibit the *S. mutans*.

The antimicrobial efficacy of the ZnO nanoparticles blended with microhybrid composite resin was confirmed for a duration up to 12 weeks.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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