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Effect of Incorporation of Green Synthesized ZnO Nanofillers on Mechanical and Anti-fungal Activity of PMMA Denture base Resin

Green Synthesized ZnO Nanofillers for Denture Base Resin

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Abstract— *ZnO nanoparticles that are green synthesized using co-precipitation method and using Piper betel aqueous extract (PZnO) as bioreductant were incorporated into the powder matrix of commercially available heat cure acrylic denture base resin (PMMA) in 0.25%, 0.5% and 1% w/v ratio. PMMA specimen with/without the incorporation of PZnO nanofillers was prepared according ADA specification #12 and ISO 1567:1998 standards and tested for flexural strength, surface roughness and fungal adhesion inhibition. Mean flexural strength of PMMA standard (without PZnO nanofiller addition) was 71.285 MPa, while those incorporated with 0.25%, 0.5% and 1% PZnO showed 73. 278, 75. 886 and 72.037 MPa, respectively. Surface roughness assessment among test and control groups was statistically significant (p<0.05) with PMMA+ 1% PZnO nanofiller showing more roughness than other two groups, but less than control group. There was no statistically significant inhibition of fungal adhesion to PMMA sample surface both by test and control groups. The study concludes that PMMA with incorporation of 0.5% PZnO as nanofiller showed optimum results by the virtue of improved flexural strength and enhanced surface smoothness. Hence addition of green synthesized PZnO into PMMA matrix can be considered to augment its mechanical properties.*

Keywords- Polymethyl methacrylate;Green synthesized ZnO; Flexural Strength; Surface roughness

1. INTRODUCTION

Conventional complete dentures still remain the treatment of choice in providing prostheses to edentulous patients and the most feasible option among rural Indian population [1]. The prime requisite of denture base material includes adequate physical and mechanical properties, esthetics and biocompatibility with oral tissues. Polymethyl methacrylate (PMMA) is one such polymeric material, which even today remains the material of choice in making dentures due to economic, medical, technical and esthetic reasons [2]. However, it is not considered an ideal material because of its inferior physical and mechanical properties and poor microbial resistance [3].

Yet another issue that is commonly faced by denture wearers is colonization of oral microbiota, especially the *Candida albicans* on denture surface leading to denture induced stomatitis, an inflammatory mucosal condition [4]. Earlier, studies were conducted to improve physico-mechanical properties of PMMA material, by adding macro-fillers, fibres, nanofillers and hybrid reinforcements. Metal fibres and glass fibres of different sizes were incorporated to PMMA matrix to improve transverse strength and modulus of elasticity with varying results [5].

Uniformly sized metallic nanoparticles have attracted researchers all over the world because of their excellent physical and mechanical properties, good thermal stability with added advantage of inherent antimicrobial activity

[6]. Earlier, TiO_2, ZrO_2 and CNTs have been used to improve the mechanical properties of PMMA denture base resin and addition of very small quantity of NPs was sufficient to achieve the desired results [7-9].

The tissue surface and undercuts of dentures can act as host of microorganisms like *C albicans* and can cause conditions like denture induced stomatitis. Metal oxide NPs like Ag, ZnO, CaO and TiO_2 have been incorporated into the matrix of both heat and self polymerized acrylic resin with varying results on antifungal properties [10, 11]. ZnO NPs synthesized using plant extracts are known to have potent antifungal activity against fungi causing plant diseases [12]. ZnO NPs synthesized using *C majus* aqueous extract has earlier shown potent antifungal activity against *C albicans* [13]. Taking these researches into account, in the present study ZnO NPs that are synthesized using aqueous extract of *Piper betel* leaves using co-precipitation method were incorporated in powder of heat polymerizing PMMA denture base resin. Effect of addition of green synthesized ZnO NPs over the physical/mechanical properties and fungal adhesion inhibition properties of acrylic resin was also studied.

2. MATERIALS AND METHODS

A. *Synthesis of PZnO Nanoparticles*

Synthesis and Characterization of PZnO NPs was carried out using our previous method. Synthesized NPs were 69nm in diameter and short rod shaped flakes in morphology [14].

B. *Determination of antifungal activity of PZnO nanoparticles*

Antifungal activity of PZnO NPs was determined using our previous protocol [15]. PZnO NPs were serially diluted from 20µg/mL to 2.5 µ g/mL, in ultrapure water and sonicated for 45 min to get complete dispersion. The cell density of *C albicans* pure sample was adjusted to 10^6 CFU/mL using 0.5 McFarland standard and using sterile potato dextrose broth for dilution. The antifungal activity was performed using agar well diffusion method in triplicates.

Testing effect of incorporation of PZnO nanoparticles in PMMA resin

In total, 35 specimens were prepared for flexural strength measurement and antifungal activity determination with dimensions of 65x10x2.5 mm and 10x10x1 mm according to the ADA specification no 12 and ISO/DIS 1567:1998 standards respectively. Finely ground PZnO powder as nanofiller, in different concentrations was mixed to powder matrix of DPI heat cure acrylic PMMA resin. For uniform distribution, the PZnO + PMMA mixture was ground thoroughly using agate mortar for 30-45 min. Further, this mixture and monomer liquid were mixed according to manufacturer's instructions and specimens were cured in water bath at 100^o C for 45 min and bench-cooled before de-flasking. Specimens were wet ground with silicone carbide grinding papers of 200- 600-grit sizes and preserved in distilled water at room temperature.

C. *Determination of Flexural Strength*

Test and control specimens were subjected to flexural strength measurement using 3-point bend test on Lloyd Universal Testing Machine at cross-head speed of 2.5mm/min. The specimens were loaded until they fractured and the peak load was recorded. Flexural strength was calculated using the formula

$$F=3WL/3bd^2$$

Where (F= Flexural strength of **PMMA groups**; W=Peak load at which specimen fractured; L=Distance between the supporting points in mm b=Width of the specimen in mm; d=Specimen thickness in mm. Fig 1 shows the preparation and flexural strength measurement PMMA samples of different groups).

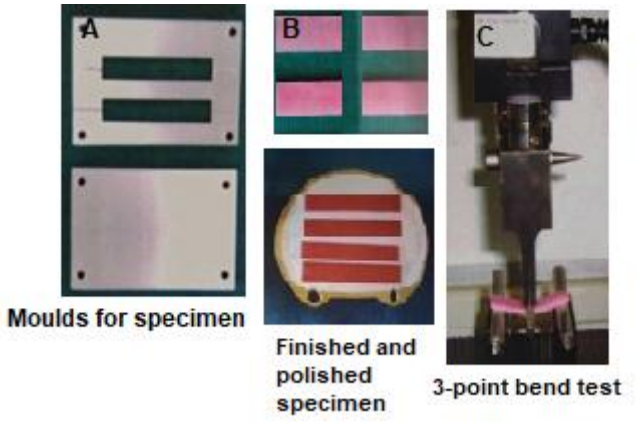

Fig 1-Preparation and flexural strength measurement of PMMA specimen of different groups

D. Determination of surface roughness using atomic force microscopy

The average surface roughness of finished and polished resin specimen with/without PZnO incorporation was determined using atomic force microscopy using AFM studies (APER, Italy). Surface of samples were scanned using intermittent contact mode at a scan rate of 0.5 Hz and the images were obtained at 1000 X resolution. The experiment was performed in triplicates on area of size 5x5 µm.

E. Determination of fungal adhesion inhibition to PMMA specimen

The samples that are subjected to fungal adhension inhibition studies were sterilized using ethylene oxide vapors. The experiments were performed according to the method followed in our previous work [15].

3. RESULTS AND DISCUSSION

F. Determination of antifungal activity of PZnO NPs

Minimum Inhibitory concentration of PZnO NPs was determined using agar well diffusion method, in serially diluted samples in concentration range of 20-2.2 µg/mL. The experiment was carried out in triplicates and the average of all three readings was taken. Table 1 shows the details of antifungal activity shown by different concentrations of PZnO NPs. As per the data, the MIC was 5µg/mL, and at lower concentration it did not cause any inhibitory activity.

Table-1-Determination of antifungal activity and Minimum inhibitory concentration of PZnO

Sl No	Zone of inhibition (in mm)			
	20 µg/mL	10 µg/mL	5µg/mL	2.2µg/mL
PZnO	24 ± 0.02	19 ± 0.04	12.6 ± 0. 02	-
Std (Pen-Stryp)	30 ± 0.04	21± 0.02	17± 0.02	9± 0.04

Several studies were conducted earlier to assay antimicrobial activity of ZnO NPs, which have explained the mechanism of action that is attributed to formation of Reactive Oxygen Species and killing of microorganisms due to electrostatic forces when NPs get adsorbed to surface of microbes [16, 17]. The antibacterial /antifungal activity of ZnO NPs was attributed to their size, shape and concentration. With increase in concentration and decrease in size, the antimicrobial properties also get increased [18]. In one of the earlier studies, the ZnO nanoparticles in whiskers form were found to have better antifungal activity against *C albicans* [19]. In our study, the NPs measured an average size of 69 nm and the nano-flakes were short rod like in appearance, which caused significant antifungal activity only at higher concentrations.

G. Determination of Flexural Strength

Flexural strength of PMMA specimens incorporated with 0.25% 0.5% and 1% (w/v) ZnO NPs was determined using 3-point bend test using UTM at cross head speed of 2.5 mm / min. The experiment was performed in triplicates and the mean flexural strength was expressed in MPa. Results of test are given below in table 2. From the results it could be inferred that the significant increase in flexural strength was achieved at lower concentrations. At the concentration of 0.5% w/v, maximum flexural strength (at selected concentrations) of 75.886 MPa could be achieved. Even at 0.25% concentration, the flexural strength achieved was more than standard PMMA. But at higher concentration of 1%, there was reduction in flexural strength. The reason could be that, at lower concentration, the NPs cross-link polymeric molecules leading to pseudocrystalline condition and forming an ordered system. However, increase in concentration of NPs in polymer reduces chemisorptions interaction of PZnO nanofiller with polymer, which leads to disordered structure of nanocomposite and reduces the mechanical strength [20].

Table-2-Determination of flexural strength using 3-point bend test

Group Name	Mean Flexural Strength (MPa)	Standard Deviation
PMMA control	71.285	71.19 ± 0.2
PMMA+ 0.25%	73.278	73.26 ± 0.2
PMMA+ 0.5%	75.886	74.29± 0.2
PMMA+ 1%	72.037	72.78± 0.4

H. Determination of average surface roughness

Surface roughness of finished and polished denture base resin is one of the major determinants of hygiene of denture bases as it directly affects colonization and multiplication of oral microbes on denture surface. Surface roughness of PMMA specimen incorporated with 0.25%, 0.5% and 1% PZnO NPs and control was measured using AFM (APER, Italy).

Results of AFM studies showed that among test and control group specimens, the control specimen had more surface roughness. Incorporation of PZnO NPs at 1% ratio did increase roughness, while addition of 0.5% and 0.25% PZnO as nanofiller caused better surface with more smoothness (as represented in Fig 2). A one way ANOVA study was performed to determine statistical significance. The study showed that there exists a statistically significant difference ($p<0.05$) among test and control group and also among test group specimen.

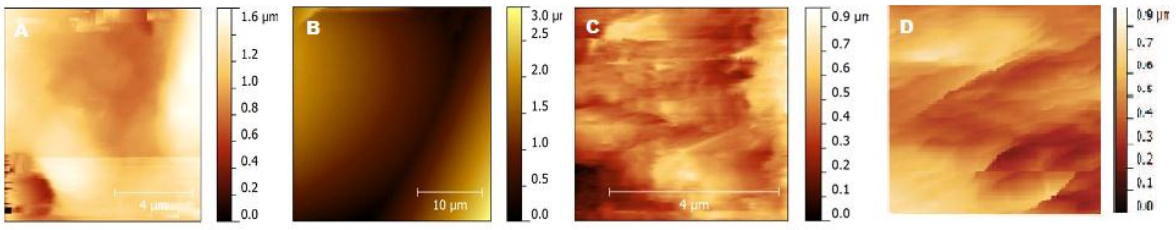

Fig 2-Determination of surface roughness using Atomic Force Microscope 2A-control, 2B-0.25 ZnO+PMMA, 2C-1% ZnO+PMMA, 2D-0.5% ZnO+ PMMA

The roughness value was expressed as Ra (Average surface roughness). Ra value is the average of set of individual measurements of surface's peaks or valleys [21]. Figure 3 shows average surface roughness of PMMA specimens with/without PZnO NPs incorporation in different concentrations.

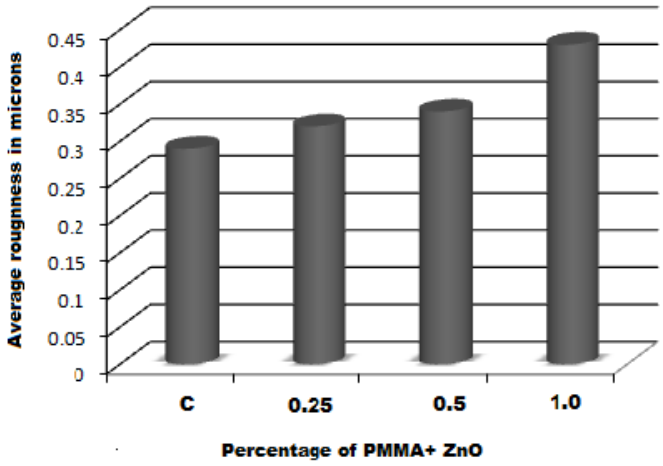

Fig 3-Average surface roughness caused by different concentrations of PZnO with PMMA

4. Determination of fungal adhesion inhibition

Results of fungal adhesion inhibition showed that there was no statistically significant difference in inhibition of fungal adhesion among test and control group specimens which was confirmed by 2-way analysis ofvariance. There was only about 2-3% fungal adhesion inhibition in those specimens that are incorporated with 1% PZnO NPs that might be probably due to release of very small amount of ZnO NPs from the specimen. PMMA in denture base resin is basically a hydrophobic polymer. Earlier studies have suggested that antimicrobial activity of nanoparticles incorporated polymers depends on diffusion of water molecules into polymer and release of nanoparticles to the aqueous medium [22, 23]. Since PMMA is a hydrophobic material it might not have allowed water to diffuse into intricacies of bulk PMMA resin specimen to facilitate the release of Zn^{2+} ions. So results of adhesion study could be well correlated with innate lack of antifungal activity of resin specimen that have been attempted to be developed into indigenously antifungal by incorporation of other nanoparticles [24].

5. CONCLUSIONS

Present study investigates effect of incorporation of green hydrothermally synthesized ZnO (PZnO) NPs on flexural strength, surface roughness and fungal adhesion inhibition of heat cure acrylic denture base resin (PMMA). The PZnO NPs were ~69 nm and rod shaped flakes. The NPs had an antifungal activity against *C albians* and showed an MFC of 5µg/ml. This PZnO was incorporated at 0.25, 0.5 and 1% w/v ratio in powder matrix of PMMA resin and the specimens were prepared. Specimens that were incorporated with 0.5% PZnO showed noticeable increase in flexural strength as compared to control. While increase in PZnO concentration proved detrimental to flexural strength of cured resin samples. Incorporation of 0.5% PZnO caused significant betterment in surface smoothness (Decrease Ra value in AFM studies). Though PZnO NPs showed fairly good antifungal activity, it did not facilitate inhibition of fungal adhesion to the PMMA specimen (which received their addition) in a statistically significant manner. Hence from this preliminary study, the authors conclude that green hydrothermally synthesized PZnO nanoparticles noticeably improved the physico-mechanical properties of heat cure acrylic denture base resin, while they did not cause noticeable fungal adhesion inhibition in the concentrations considered in the study.

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