Original Research Article

~~AN IN VITRO STUDY TO EVALUATE~~ EVALUATION OF THE TRANSVERSE STRENGTH OF HEAT-~~-~~CURE~~D~~ PMMA RESIN REINFORCED WITH ~~BY~~ VARIOUS ~~DIFFERENT~~ CONCENTRATIONS OF TWO DIFFERENT NANOPARTICLES: AN *IN VITRO* STUDY

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ABSTRACT

Purpose: The purpose of the study was to evaluate the effect of various concentrations of titanium dioxide and zirconia nanoparticles on the transverse strength of heat-cure PMMA resin ~~reinforced with various nanoparticles of different concentrations~~.

Method: One hundred samples of PMMA resin were made and divided into five groups (20 samples for each group). The test specimens were divided into five groups depending on the concentration of reinforcing nanoparticles as Group 1,2,3,4 and 5; Group 1: PMMA unreinforced (control group), Group 2: PMMA reinforced with 2.5% nanozirconia, Group 3: PMMA reinforced with 5% nanozirconia, Group 4: PMMA reinforced with 2.5% titanium dioxide nanoparticles, and Group 5: PMMA reinforced with 5 % titanium dioxide nanoparticles. Universal testing machine was used to conduct a three-point bending test and evaluate the transverse strength of samples. Comparison of mean transverse strength for various groups was carried out was done employing one way analysis of variance and Bonferroni *post hoc* tests.

Comment [RKA1]: Incomplete sentence

Results: The highest and lowest mean transverse strength were observed in ~~of~~ Group 3 and 1, respectively. Bonferroni *post hoc* honestly significant difference multiple comparison for mean transverse strength increase in strength to be statistically significant between all the groups ($P < 0.05$) except between the samples of group G1 and G5 and G2 and G3.

Comment [RKA2]: Consider revising

Conclusion: Addition of nanoparticles in all concentrations significantly increased transverse strength of heat cure PMMA resin as compared to control group. The best result was obtained after adding 5% of nanozirconia particles to the conventional heat polymerized acrylic resin.

KEYWORDS

PMMA, nanozirconia, titanium dioxide nanoparticles, transverse strength

1. INTRODUCTION

Edentulism has been a matter of great concern to a majority of people, be it partial or complete. Replacement of teeth by artificial substitutes plays a vital role in leading a normal life.[1]

Since 1930, acrylic resin polymethyl methacrylate (PMMA) has been the most popular material for denture fabrication. Not only it provides an accurate fit, good esthetics, stability in the oral environment but also is easy to handle in laboratory and clinics. Despite many advantages this material exhibits certain limitations which render failure to fulfill mechanical requirements for dental applications. These include low fracture resistance and plaque accumulation[2], high coefficient of thermal expansion and relatively low modulus of elasticity. Fracture of maxillary dentures is twice more common than that of mandibular dentures. Fractures caused outside the mouth are usually a result of heavy impact forces or a high stress rate while those caused within the mouth are usually caused in function and can be attributed to a fatigue phenomenon i.e. low and repetitive stress rate which commonly occurs over a period of time. This type of fracture is typically seen in midline of maxillary dentures than in mandibular dentures.[3]

Release of PMMA from the dentures has reported to cause irritation to mucosa.[5] Also being a radiolucent material it cannot be imaged using standard radiographic techniques hence in cases of accidental ingestion of prosthesis, aspiration or traumatic impaction of dental appliance, their detection can become painstaking and invasive procedures may need to be carried out.[4]

There have been several attempts to improve the mechanical properties of acrylic resins such as chemical modification or reinforcement with glass fibers, metal oxides and nanoparticles.

Recently, incorporation of nanofillers has been suggested to improve mechanical properties of PMMA. Fine particle size enables the homogenous distribution of nanofillers in PMMA matrix and has reportedly improved the thermal properties of PMMA by increasing its thermal stability compared with ~~pure~~ PMMA. However, size, shape, type and concentration of nanoparticles added affect the properties of resin.[5]

The few studies conducted on the effect of nanoparticles on the transverse strength have been more or less conclusive and unclear.[6]

Comment [RKA3]: Concise the sentence and rephrase.

Comment [RKA4]: Release of residual MMA.

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Titanium dioxide and zirconium oxide nanoparticles have become popular as reinforcement nanofillers recently. Titanium is used since it increases the surface hydrophobicity , reduces the adherence of biomolecules, aids in colouring, has antimicrobial properties and improves mechanical properties of PMMA resins. Spherical particles of titanium dioxide have been used to improve the flexural strength as spherical particles increase the polishability and mechanical properties. Other structures such as nanotubes and fibers which have been recently discovered show much better properties.[7]

Zirconia has exhibited excellent biocompatibility and being white in colour it is less likely to interfere with esthetics.[4]

Zirconium oxide nanoparticles mechanically reinforce the polymers and allows for high impact strength, fracture toughness, hardness and density of the reinforced PMMA matrix.[8]

Modifying nanozirconia powder by coating with a layer of trimethoxysilypropylmethacrylate (TMSPM) renders more radiopacity as it decreases the radiographic density and allows more absorption of radiation.[4]

Hence, the purpose of this study was to evaluate and compare transverse strength of heat cured PMMA resin after its reinforcement with zirconium oxide and titanium dioxide nanoparticles in concentrations 2.5 wt % and 5wt % each.

2. MATERIAL AND METHODS

2.1 The study proceeded as follows:-

i. Fabrication of metal dies-

   Three stainless steel metal dies of dimensions 65mm x 10mm x 3 mm were fabricated. The selection for dimensions of the dies was based on ADA specification no. 12.

ii. Fabrication of test samples-

   Preparation of molds for fabrication of wax pattern:

a. The stainless steel metal dies were impressed upon putty material (Affinis, New Delhi, India) so as to create a mold space. Molten wax (No.2, Rolex, Ashoosons Dental Care Pvt. Limited, Delhi, India) was then poured onto the mold spaces so created and left to cool. The wax patterns of dimension 65 x 10 x 3mm as per ISO 1567 standardization were obtained after cooling. These patterns were lubricated with a thin layer of petroleum jelly (Loba Chemie, India) and invested in Type II dental plaster (Dentex, India) . After the investing materials had set, the flasks were placed for dewaxing in a conventional water bath. The molds so created were thoroughly flushed with hot water. The flasks were left to cool followed by application of a layer of separating media (DPI Cold Mould Seal, Bombay Burmah Trading Corporation Ltd., Mumbai , India) to prepare the flasks for packing. The appropriate amount of heat cure acrylic resin required was prepared from a mixture of polymer and monomer in the ratio of 3:1 by volume i.e. 3 parts of polymer and one part of monomer with the help of electronic weighing machine (Jeejex Digital Electronics SF-400, Jiya Sales, India).

Comment [RKA6]: Consider reframing the entire paragraph.

iii. Division of samples into various groups (Fig.1):

G1 - Control (DPI Heat Cure, Bombay Burmah Trading Corporation Ltd., Mumbai , India)

G2- DPI Reinforced with 2.5 wt% nanozirconia particles (ZrO_2, Purity 99.9%, Average particle size: 30-50nm, NanoResearch Lab, Jamshedpur, Jharkhand, India)

G3 - DPI Heat Cure with 5 wt% nanozirconia particles

G4 - DPI Heat Cure with 2.5% titanium dioxide nanoparticles (TiO_2, Anatase, Purity: 99.9 %, Average particle size : 10-20nm, NanoResearch Lab, Jamshedpur, Jharkhand, India)

G5 - DPI Heat Cure with 5 wt% titanium dioxide nanoparticles

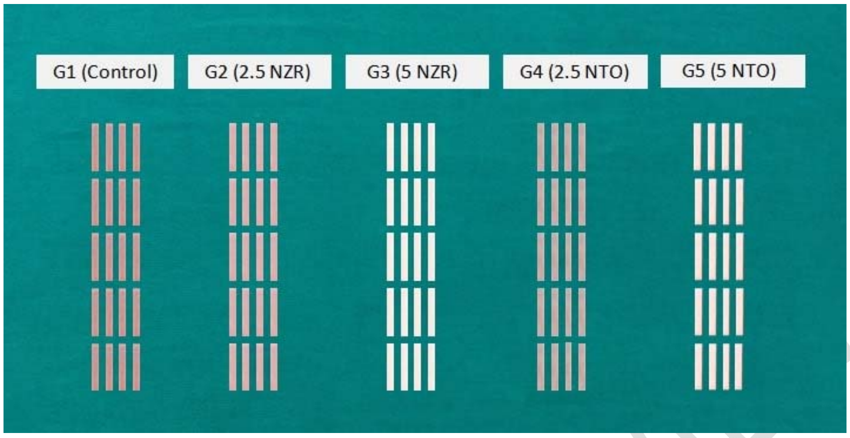

Fig. 1 - Specimens for each group

a. Preweighed nanoparticle powder was separately added to the acrylic resin powder to form the desired formulation and thoroughly mixed using a mortar and pestle (Uniteck Scientific & Electronic Industry, Chandigarh, India).

b. Proportionate amount of the monomer and the polymer was taken in the mixing jar and thoroughly mixed until dough stage was attained. The acrylic dough was kneaded and packed in to the ~~T~~the flask (Varsity Flask, No-7, S.S. Products, India.). ~~was closed~~ ~~and~~ Trial closures were~~as~~ carried out using hydraulic press (Unident Instruments India Private Limited, India) to remove the excess flash and provide uniform distribution of acrylic dough throughout the mold cavity. The flasks were then secured tightly to maintain the pressure and bench cured ~~. After removal of flash, flask was then clamped and pressure maintained~~ for 30 minutes to allow proper penetration of monomer into polymer. The flask was then immersed in a~~n~~ thermostatically controlled water bath such as acrylizer (Unident Instruments India Private Limited, India) at room temperature. The temperature of water bath was raised to 74°C, held for 8 hours, then raised to 100°C for an hour. After the completion of the curing cycle, the flask was removed from the water bath and bench cooled for 30 minutes, immersed in cool tap water for 15 minutes prior to deflasking. Samples were then contoured using carbide bur, finished with sandpaper and polished using slurry of coarse pumice. The width and thickness of each samples was measured using a digital vernier calliper (PRECISE, Sudershan Pvt Ltd, Delhi, India) with a resolution of 0.01mm. Since width and thickness were factors assessed for determining transverse strength, only the resin samples with a slight variation in size up to + 0.3mm were included in the study.

In this manner, a total of 100 acrylic samples divided into five groups each containing 20 samples of compression moulded heat cure acrylic denture base resin were fabricated.

All the specimens were stored in distilled water at a temperature of 37° C for 48 hours prior to transverse strength testing.[9]

2.2 Calculation of Transverse Strength:

To determine transverse strength, fracture load was measured using the three-point bending test according to ISO 178 on a universal testing machine (ASIAN Test Equipments, Micronix Intruments, India) . The~~n~~ specimens were placed on a 3-point flexure apparatus with the ~~and the~~ support span of ~~was~~ 50 mm. Load was applied at the midpoint of the sample with a crosshead speed of 5mm/min until the specimen fractured and fracture load was recorded (Fig. 2).[7] The transverse strength values of each specimen were derived using formula:

$$TS = \frac{3WL}{2bd^2}$$

where TS is the transverse strength (in MPa), W is the fracture load (N), L is the distance between the two supports, b is the specimen width, and d is the specimen thickness. The data thus obtained was subjected to statistical analysis.

Comment [RKA7]: Resolution or least count?

Comment [RKA8]: Is it total in total dimension (LxBxH)of the specimen?

Comment [RKA9]: repeated

Comment [RKA10]: Mention the details of descriptive statistics used along with the statistical package details in this section.

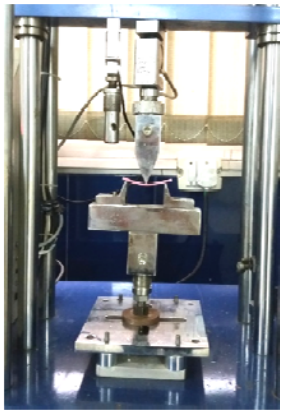

Fig. 2 - Testing the specimen on Universal Testing Machine

2.3 SEM ANALYSIS:

Comment [RKA11]: Full form

SEM (FEI Nova NanoSEM 450, USA) was used to examine the surface of fractured cross-section of the specimens. The acceleration voltage, used to perform SEM evaluation, was set at 10 kV and the working distance was 5.3 mm with a 3 spot size. ~~A 10 nm gold-palladium coating was applied~~ The specimens were gold sputtered to provide conductivity to the material. Images were recorded at different magnifications to study distribution of particles (Fig. 3a-e and Fig. 4a-e).

Comment [RKA12]: Are all these SEM images for studying the distribution of nanoparticles?

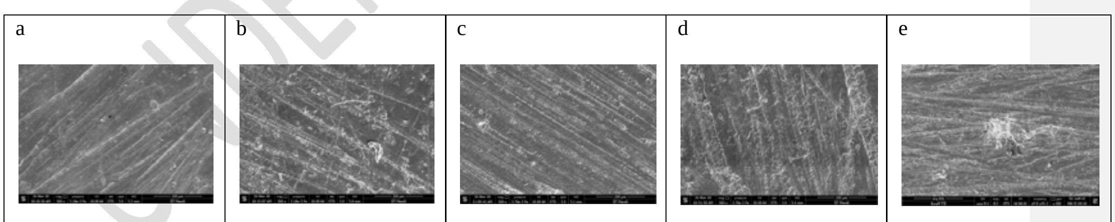

Fig. 3 - SEM images at 500x

Comment [RKA13]: Reframe the legend

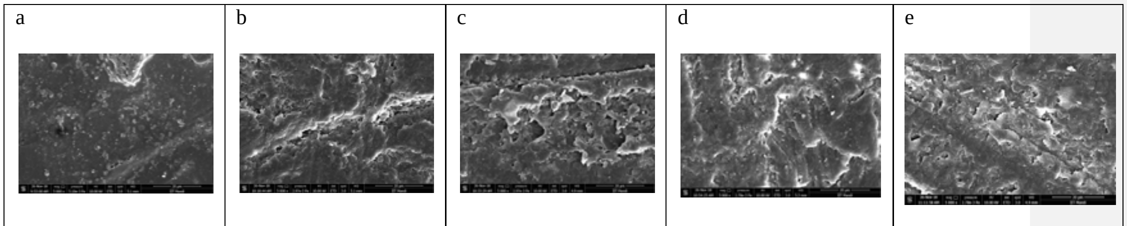

Fig. 4 - SEM images at 5000x

3. RESULTS

In the present study, all reinforced ~~experimental~~ groups ~~other than unreinforced PMMA have shown~~ showed a definite increase in mean transverse strength compared to control groups.~~with reinforcements~~. Data was analysed using computer statistical software STATA and SPSS-20.0, IBM software, Chicago.

The mean transverse strength (**MPa**) obtained for control and reinforce groups were ~~different categories of nanoparticles as well as with control have been~~ summarized in **Table 1.** Group 3 specimens showed Maximum transverse strength ~~values were obtained with **G3**,~~ followed by **G2**, **G4**, **G5** and group 1 exhibited less strength.~~minimum strength was obtained for **G1**.~~

In the present study all experimental groups other than unreinforced PMMA have shown a definite increase in mean transverse strength with reinforcements.

One-way ANOVA (Table 2) showed significant differences among the groups (P<0.001). ~~A multiple comparison test by one - way ANOVA as shown in **Table 2** revealed a significant difference among the means obtained for all the groups (*P* <0.001)~~.

For comparison within the group i.e. multiple group comparison: **Posthoc Bonferroni** test was applied which shows that the increase in transverse strength was statistically significant for all experimental groups in comparison with samples of unreinforced PMMA group except between G1 and G5 (*P*= 1.000) and G2 and G3 (*P*= .74).

Comment [RKA14]: 1.Reframe the legend 2.Don't have to provide different SEM images with different resolutions, If the SEM images are showing the dispersion of nanoparticles. Choose the images with the best resolution and provide them.

Comment [RKA15]: Mention this at the end of methodology.

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Comment [RKA17]: Consider rephrasing the paragraph and also provide the Post-hoc analysis details in a table.

Table 1- Descriptive statistics of transverse strength values (MPa) obtained for control and two types of nanoparticles:

Groups	N	Mean (MPa)	StandardDeviation	Standard Error Mean
G1	20	94.800	9.83483	2.19913
G2	20	134.82	12.66516	2.83202
G3	20	148.96	28.65139	6.40664
G4	20	119.26	6.91676	1.54663
G5	20	101.98	14.43716	3.22825

Table 2: Intergroup comparison of mean of transverse strength (MPa) among various studied groups:

Comment [RKA18]: Anova/Post-hoc?

Transverse strength (MPa)	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	40373.034	4	10093.258	37.822	**0.000***
Within Groups	25351.795	95	266.861		
Total	65724.828	99			

4. DISCUSSION

Polymethylmethacrylate (PMMA) is the most popular material used widely in various fields of Prosthodontics. Amongst various mechanical properties it is the impact strength and fatigue strength of this material that are of utmost importance and in denture base polymer they are not found to be entirely satisfactory. The ultimate flexural strength (also called transverse strength or modulus of rupture) of a material reflects the potential of material to resist fatal failure under a flexural load.[7] Hence, high flexural strength can be regarded as an important determinant for the success of dentures. Compressive, tensile and shear strengths collectively form the flexural strength of a material. As the flexural strength increases, the force required to fracture the material also increases.[10]

Comment [RKA19]: Consider rephrasing the sentence.

Transverse strength represents the type of loading borne by a denture in the mouth. A high value of transverse strength of denture base acrylic is desirable as it provides a superior clinical performance by the dentures.[1] Fatigue phenomenon is the low and repetitive stress rate which commonly occurs over a period of time. This fatigue failure is not dependent on strong biting forces. Relatively small stresses caused by mastication over a period of time can contribute to formation of a small crack , which propagates through the denture thereby resulting in a fracture. The maximum bite forces in a patient wearing complete dentures have been noted up to 700 N, but these values are reduced drastically (100 - 150 N) with the removal of dentures. Fractures of denture occur essentially because of concentration of stresses and increased flexing.[2]

Comment [RKA20]: This sentence is not necessary as it was mentioned in the previous sentence that the biting force does not contribute to the fatigue failure.

Recently, incorporation of nanofillers has been suggested to improve PMMA properties. The structure of material that has particles of a nanometer size possesses special properties. It can be rendered to the high ratio of surface area to volume. Amongst a variety of nanoparticles available like silver, copper, zinc, silicon, titanium and their oxides, titanium dioxide has gained importance recently because of its higher photocatalytic activity, high stability, low cost and safety towards both humans and the environment. On the other hand, some studies found that titanium dioxide nanoparticles did not improve the transverse strength of PMMA. This could be attributed to clustering of the particles within the resin matrix that ~~which~~ weakened the denture prosthesis~~resin~~. It was found that TiO_2 nanoparticles has an effect on thermal stability of PMMA resin and it caused a decrease in the thermal expansion coefficient and contraction. A decrease in elastic modulus, transverse strength and toughness was reported. Addition of nanozirconia was suggested to improve the mechanical properties of PMMA. This helped in increasing the impact strength, transverse strength, compressive strength, fatigue strength, as well as its fracture toughness and hardness. An antifungal effect has also been reported which may play a preventive role in patients susceptible to fungal infections.[11]

Use of nanoparticles is based on the principle that reduction of filler size increases the mechanical properties of resins. Spherical particles of titanium dioxide have been reported to improve the transverse strength as they increase polishability and mechanical properties. It also increased the surface hydrophobicity, reduced the adherence of biomolecules, aided in colouring and exhibited antimicrobial properties.[7]

The quest has been on for the most suitable concentration of different nanoparticles which can be added to the acrylic resin so that transverse strength is improved manifolds. However, it was found that concentrations above 5% have led to massive changes in the colour of acrylic. Therefore, two concentrations 2.5% and 5% were selected.[12]

The increase in transverse strength at 5% concentration of nanozirconia can be attributed to the high interfacial shear strength between nanofiller and matrix due to formation of cross-links or supra molecular bonding which covers or shields the nanofillers. This in turn prevents propagation of crack. Also the crack propagation can be changed by improving the bonding between nanofiller and resin matrix. An increase in transverse strength that occurred with addition of 2.5wt% zirconium dioxide nanoparticles can be attributed to the uniform distribution of the very fine size of nanoparticles that allows them to enter between linear macromolecular chains of the polymer.[4]

The SEM micrograph studies have shown good surface characteristics with different nanozirconia concentrations. Moreover, a uniform distribution of particles was assumed as no big agglomeration was found. The SEM analytical studies have revealed that as the concentration increased, the polymer matrix got filled with nanoparticles that stopped crack propagation, resulting in stronger material. Uniform dispersion of the nanoparticles into the resin matrix filled the inter-polymeric chain spaces, which shows the importance of the additive content of the nanoparticles.[13]

Comment [RKA21]: With???

Comment [RKA22]: Transverse strength is one of the mechanical property.

Comment [RKA23]: Not all the titania nanoparticles exhibit antimicrobial properties.

Comment [RKA24]: Why only these two concentrations? Please provide specific reasons

Increase in transverse strength on addition of titanium dioxide nanoparticles in concentration 2.5% in PMMA matrix can be attributed to uniform dispersion of the small sized filler particles. This is responsible for the improved fracture resistance of PMMA. Addition of titanium dioxide nanoparticles up to 2.5 % increased the strength, above which the strength decreased This can be explained by adversely affected degree of conversion which in turn leads to increase in the level of residual unreacted monomer that acts as plasticizer. Incorporation of nanoparticles causes these particles to agglomerate and aggregate. The agglomerated compounds can act as stress concentrating center in the matrix and adversely affect mechanical properties of the polymerized material. It is easily noted that the content of nano additives is of critical importance.[14]

Comment [RKA25]: Please justify

By using scanning electron microscopy it has been observed that, at a concentration of 2.5%, nanoparticles of titanium dioxide were well distributed in specimen. The particles maintained their original size and had an active role in improving the mechanical properties. However, when the concentration was increased to 5.0%, the SEM images have demonstrated that the nano-oxides had agglomerated to a different extent, which resulted in a decrease in the mechanical properties of the resin.

Comment [RKA26]: Compare this study with other similar studies.

In the *in vitro* present study only one mechanical property i.e. transverse strength was taken into consideration. So, further studies may be carried out *in vivo* conditions to understand its effect in oral environment.

5. CONCLUSION

Within the limitations of this study, following conclusions were drawn:

- Addition of titanium dioxide nanoparticles beyond the concentration of 2.5 % decreased the transverse strength of conventional heat polymerized acrylic resin.
- The best result was obtained ~~after adding~~with incorporation of 5% ~~of~~ nanozirconia particles to the conventional heat polymerized acrylic resin.

According to the results of this *in vitro* study, it could be concluded that nanoparticles may be considered as a new approach for denture base reinforcement. The reinforcement resulted in significantly higher transverse strength as compared to unreinforced resin.

However, only one mechanical property i.e., transverse strength was taken into consideration in this *in vitro* study. Further studies considering other mechanical, esthetic and biological properties can be carried out.

Comment [RKA27]: Consider rephrasing the sentences.

COMPETING INTEREST

Authors have no competing interest

REFERENCES

Comment [RKA28]: Maintain uniformity in all the references which includes; Authors. Title of the manuscript. Name of the journal. Year; Volume(Issue):page numbers.

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Comment [RKA29]: Maintain the uniformity in citing the journal titles.
Use journal abbreviations

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