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Original Article

Evaluation of Flexural Strength of Heat Cure PMMA Denture Base Material Reinforced with Various Concentrations of Zirconium Oxide Nanoparticle: An In-vitro Study

Lazyan L. Raouf¹, Souza A. Faraj¹, Bruska A. Azhdar^{2*}

Abstract

Objective: The aim of this study was to investigate the effect of zirconium oxide (ZrO₂) nanoparticle dispersion on the flexural property of heat-cure polymethylmethacrylate denture base material.

Methods: Eighty specimens were divided into 10 groups of eight specimens; Group 1 unreinforced acrylic, group 2: treated with ethanol alcohol, Groups 3,4,5 and 6 reinforced acrylic specimens with 1, 3, 5, and 7 wt% of untreated zirconium oxide nanoparticle (u-ZrO₂) and Groups 7,8,9 and 10 reinforced acrylic specimens with treated and sonicated zirconium oxide nanoparticle in ethanol alcohol (t-ZrO₂). X-ray diffraction test (XRD) was used to study the dispersion quality of the tested specimens, and universal testing machine was used to evaluate flexural strength and flexural modulus. SPSS software program used to compare groups results statistically.

Results: The results revealed significant decrease in flexural strength with u-ZrO₂ nanoparticle addition in different concentration groups (p<0.05), while with t-ZrO₂ nanoparticle addition, above 3 wt% statistically significant decrease was noted as compared to the conventional group. However, the flexural modulus increased with increasing the both of u-ZrO₂ and t-ZrO₂ nanoparticles addition. XRD results showed better dispersion quality of the t-ZrO₂ than u-ZrO₂ throughout the acrylic resin denture base material.

Conclusions: Flexural strength decreased inversely with increasing u-ZrO₂ concentration, while it decreases significantly above 3 wt% of t-ZrO₂ nanoparticle concentration. The flexural modulus increased with increasing both of u-ZrO₂ and t-ZrO₂ nanoparticles. Reduced nanoparticle's cluster size of t-ZrO₂ allows more nanofiller to be incorporated to denture base materials with less adverse effect on the mechanical properties.

Keywords: Denture base, PMMA, Zirconium oxide nanoparticles, Mechanical properties.

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1. Department of Prosthodontics, College of Dentistry, University of Sulaimani, Sulaimani, Iraq.
2. Nanotechnology Research Laboratory, Department of Physics, College of Science, University of Sulaimani, Sulaimani, Iraq.

* Corresponding author: bruska.azhdar@univsul.edu.iq.

Introduction

Polymethylmethacrylate (PMMA) denture base material is the most common used material in the construction of dental prostheses, because it has the advantages of being biocompatible, aesthetically acceptable, relatively cheap, easily manipulated and good in terms of optical properties⁽¹⁻³⁾, but it has deficiency in some of its mechanical properties. The acrylic resin reinforcement with nanofillers including metal oxide nanoparticles which is one of the methods to improve the mechanical properties of heat-cured acrylic resin⁽⁴⁾.

Zirconia (ZrO_2), is one of the most widely used metal oxide nanomaterials to improve PMMA, has good mechanical strength and surface properties, and it is biocompatible^(5,6). These properties make it useful for improving the mechanical properties of acrylic resin denture base materials^(7,8). Zirconia nanofillers have been used to improve mechanical properties, predominantly impact and flexural strength. Maximum impact and flexural properties of acrylic denture base material were observed using reinforced with 5 wt% ZrO_2 nanofillers⁽⁸⁾. Homogenous dispersion of nanoparticles within the acrylic resin is crucial to improve the acrylic based nanocomposites' properties⁽⁹⁻¹³⁾. To maintain a uniform dispersion of nanoparticles within the acrylic resin matrix, previous studies have focused on silane coupling agents^(8,14,15). Almost all described the benefits of these coupling agents on the acrylic denture base's mechanical properties.

To date, in both in vivo and in vitro studies, less effort has been made to improve the dispersion of nanoparticles within the acrylic resin matrix of the heat cure acrylic resin without using silane, and coupling agents. Complicated extra procedures and sensitive techniques and materials were required for silanization, such as aromatic hydrocarbons (toluene) or alcohol (acetone), which are known for deleterious effects on skin, or eye contact or inhalation. Previous studies^(8,16,17) have not discussed the shelf life, environmental effects, or possible hazards of these agents in the chemical treatment of nanoparticles. However, this sonication method aimed to get nanoparticle dispersion in acrylic resin, without the need for the third material addition and with less possibility of the denture base materials being adversely affected mechanically.

The aim of this study is to study the effect of addition of different weight concentrations of both of untreated and treated and sonicated ZrO_2 nanoparticles on the flexural strength, and flexural modulus of heat-cure PMMA

denture base material. The null hypothesis is that the flexural strength and flexural modulus of heat-cure PMMA denture base material will not be affected by the addition of ZrO_2 nanoparticles.

Materials and methods

ZrO_2 nanoparticles of diameter ranging from 20–30 nm was purchased from (Sky Spring Nanomaterials Inc.) Absolute ethanol alcohol (99.5% absolute) (EMPARTA® ACS) was purchased from Merck (Merck-KGaA.), and both powder and monomer of heat-cured PMMA (SR Triplex Hot ®) were purchased from Ivoclar Vivadent Inc.

Nanoparticle dispersion process

Nanoparticle concentrations of 0.5, 1.0, 1.5, 2.5, and 3.5 grams (g) corresponding to 1 wt%, 3 wt%, 5 wt%, and 7 wt% of 50 grams of heat-cure PMMA were mixed with the complementary weight ratio of the PMMA powder, respectively. The powders mixture was shaken on a custom made roller mixer to provide a uniform dispersion of the ZrO_2 nanoparticle throughout the PMMA powder⁽¹⁸⁾. While in the second method of ZrO_2 nanoparticle dispersion, the same ratios of ZrO_2 nanoparticles (1 wt%, 3 wt%, 5 wt% and 7 wt% were mixed with 50 mL of the absolute ethanol alcohol and kept under sonication (Qsonica-700 sonicator) on ice at 400 watts for 30 minutes to form a suspension. Then the suspension was added to the remaining complementary weight of 50 g of PMMA powder in a glass jar for 30 minutes, with a continuous shake on a two-dimensional roller mixer of 150 rpm. The mixture was dried on a magnetic stirrer (Daihan Labtech Co., Ltd.) and vacuumed at -0.75 bar by a rotary vacuum pump (Eurovac, Thompson-CSF) for 120 minutes. The damp mixture was warmed with a thermally controlled hot-plate on the stirrer at 40°C for 30 minutes, and the mixture was then totally dried by placing in a dry oven (Memmert GmbH Co.) at the same temperature for an extra 60 minutes⁽¹⁹⁾.

Preparation of acrylic resin matrix

Purchased and untreated ZrO_2 nanoparticle was nominated as u- ZrO_2 , while treated and sonicated ZrO_2 nanoparticle with absolute ethanol alcohol was described as t- ZrO_2 .

The acrylic specimens' preparation method for the (group 1) conventional group is as follow, and for the rest of the other groups are the same but with different (u- ZrO_2 /t- ZrO_2) concentration and dispersion methods:

A volume of heat-cured PMMA monomer liquid was added to the mixture of PMMA/ZrO₂ nanoparticle (whether t-ZrO₂ or u-ZrO₂), and they were blended as directed by the manufacturer (1 mL: 2.34 g) in a vacuum mixer (Wehmer Ltd., Inc.) for two minutes. The mixed acrylic was left in situ for an extra three minutes to allow the acrylic resin dough to mature.

The acrylic resin dough was applied in a trail closure, a metallic plate at the center holding the specimen design, in accordance with the International Organization for Standardization (ISO) standards (ISO 1567; 1999)⁽²⁰⁾ for testing the flexural strength and flexural modulus. On both sides of the design holding plate, 6 mm thick metallic plates were used as covers. After a trial closure and checking for any voids, they were placed under hydraulic pressure of 49 kPa (5 tons/m²) for five minutes. Then, six metallic clamps were used to hold the plates tightly together. They were placed in a water bath (Julabo, Gerhard-Juchheim-Strasse Co., Ltd.) starting from cold water (10–15°C) as recommended by the acrylic resin manufacturer, with the temperature increased for boiling temperature and kept for 45 minutes, then the set acrylic left in situ overnight in the water bath. After demolding, the set acrylic-nanocomposite was cut into individual samples using a laser cutting machine (GH-E1390 Co., Ltd.) used for cutting the specimens according to the test standards⁽²¹⁾. The specimens were highly polished with silicon carbide paper (Al-alamain Inc. Ltd.) with 240, 400, 1000, and 1200 grit sizes, respectively, to remove any remaining flashes and make the acrylic samples smooth and shiny in appearance.

Characterization

u-ZrO₂ nanoparticle, t- ZrO₂ nanoparticle, purchased PMMA powder, treated PMMA with ethanol alcohol, dispersed u-ZrO₂ nanoparticle in PMMA powder, and dispersed of t-ZrO₂ nanoparticle in PMMA powder were characterized using X-ray diffractometer (XRD) to study the dispersion quality of the ZrO₂ nanoparticles (Panalytical Xpert Pro ®, ul. Radzikowskiego, Kraków, Poland).

Mechanical properties analysis

The ten groups of samples were defined as conventional, ethanol control, and (1wt%, 3 wt%, 5 wt% and 7 wt%) for both u-ZrO₂ and t-ZrO₂ addition. Each group was represented by eight specimens. Tests were carried out after conditioning the samples for 48 hours in distilled water at 37°C for the flexural strength and flexural modulus⁽²⁰⁾.

Rectangular-shaped specimens were prepared to carry out the flexural strength and flexural modulus tests. The

specimens were prepared according to ISO 1567:1999⁽²⁰⁾, having dimensions of 64*10*3.3 mm. Each sample was placed on two circular supporting beams of a universal testing machine (Cussons Technology Co. Ltd. Manchester, UK) with a space of 50 mm between the supporting points. A gradual loading was applied by the upper beam of the universal testing machine in the middle of the supported sample, at a cross head speed of 5mm/min. till the failure of the sample under flexural force occur, the data was collected, calculated and read automatically by use of a computer software program supported by the testing machine.

$$\text{Flexural strength (Fs)} = 3Pl / 2bd^2$$

$$\text{Flexural modulus} = Pl^3/4bh^3d$$

Where **F_s** is the flexural strength in MPa, **P** is peak force at the bending fracture in Newtons, **l** is the length of the specimen in mm, **b** is the width of the specimen in mm, **h** is the distance subjected to the flexural loading and **d** is the thickness of the specimen in mm. Three-point flexural test used in this study to evaluate the bending modulus and the amount of force needed to fracture a specimen under a three-point bending force. The stiffness of materials under bending forces are measured by measuring their flexural modulus⁽¹⁴⁾.

Statistical analysis

SPSS (ver. 22.0) software (SPSS Inc.) was used for statistical analysis. One-way ANOVA testing was applied for determining the difference between the means of the groups, where the significance alpha level was set at p<0.05, followed by a post-hoc Tuckey's test whenever the results were significant.

Results

X-ray diffraction test

The XRD patterns of tested u-ZrO₂, t-ZrO₂ nanoparticle are illustrated in Figure 1, XRD results of untreated PMMA powder and treated PMMA powder in absolute ethanol alcohol are shown in Figure 2(A). A random selection technique is used to study one of the weight percent groups that was 5 wt% for both of the prepared powders of the PMMA/ZrO₂ in both conventional mechanically shaking technique on a roller mixer, and the study's technique of dispersing nanoparticle in absolute ethanol alcohol using ultrasonic homogenizer. Studying ZrO₂ phases from diffraction peaks showed that both of ZrO₂ phases were the same before and ethanol treatment and none of ethanol alcohol peaks was seen in the XRD results. On the other hand, both of XRD tests for the treated PMMA/t-ZrO₂ and untreated PMMA/u-ZrO₂ were studied, the former group showed

Table 1: Mean \pm SD and 95% CI of flexural strength of specimens with different u-ZrO₂ nanoparticle concentrations as compared to conventional group.

Groups	Mean values (MPa) \pm SD	95% CI	One-way ANOVA p-value
Conventional	160.22 \pm 12.73	149.58 -170.87	–
Ethanol control	158.47 \pm 12.88	147.70 - 169.23	p>0.05
1 wt% U-ZrO ₂	115.97 \pm 26.20	94.06 - 137.87	p<0.05
3 wt% U-ZrO ₂	113.85 \pm 26.19	91.95 - 135.75	p<0.05
5 wt% U-ZrO ₂	108.73 \pm 18.57	93.21 - 124.26	p<0.05
7 wt% U-ZrO ₂	114.27 \pm 18.09	99.15 - 129.40	p<0.05

Table 2: Mean \pm SD and 95% CI of flexural strength of specimens with different t-ZrO₂ nanoparticle concentrations as compared to conventional group.

Group	Mean values (MPa) \pm SD	95% CI	One-way ANOVA p-value	independent sample t-test
Conventional	160.22 \pm 12.73	149.58 -170.87	–	–
Ethanol control	158.47 \pm 12.88	147.70 - 169.23	p>0.05	–
1 wt% t-ZrO ₂	150.67 \pm 21.97	132.30 - 169.04	p>0.05	vs. 1 wt% u-ZrO ₂ p<0.05
3 wt% t-ZrO ₂	103.91 \pm 6.37	98.59 - 109.23	p<0.05	vs. 3 wt% u-ZrO ₂ p>0.05
5 wt% t-ZrO ₂	109.34 \pm 7.61	102.98 - 115.70	p<0.05	vs. 5 wt% u-ZrO ₂ p>0.05
7 wt% t-ZrO ₂	96.27 \pm 18.21	81.04 - 111.50	p<0.05	vs. 7 wt% u-ZrO ₂ p>0.05

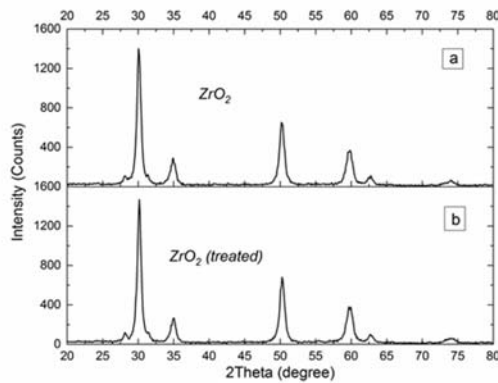


Figure 1: XRD results of ZrO₂ nanoparticles: (a) untreated ZrO₂ nanoparticles, and (b) treated and sonicated ZrO₂ nanoparticles.

Table 3: Mean \pm SD and 95% CI of flexural modulus of specimens with different u-ZrO₂ nanoparticle concentrations as compared to conventional group.

Group	Mean values (MPa) \pm SD	95% CI	One-way ANOVA p-value
Conventional	7543.75 \pm 238.82	7344.10 - 7743.41	–
Ethanol Control	7475.17 \pm 74.66	7412.75 - 7537.59	–
1 wt% U-ZrO ₂	14203.80 \pm 331.02	13927.06 - 14480.54	p<0.05
3 wt% U-ZrO ₂	14144.33 \pm 474.35	13747.77 - 14540.90	p<0.05
5 wt% U-ZrO ₂	14639.85 \pm 728.07	14031.16 - 15248.53	p<0.05
7 wt% U-ZrO ₂	14352.10 \pm 524.32	13913.76 - 14790.43	p<0.05

Table 4: Mean \pm SD and 95% CI of flexural modulus of specimens with different t-ZrO₂ nanoparticle concentrations as compared to conventional group.

Group	Mean values (MPa) \pm SD	95% CI	One-way ANOVA p-value	independent sample t-test p-value
Conventional	7543.75 \pm 238.82	7344.10 - 7743.41	–	–
Ethanol control	7475.17 \pm 74.66	7412.75 - 7537.59	–	–
1 wt% t-ZrO ₂	7608.87 \pm 101.56	7523.96 - 7693.78	p>0.05	vs. 1 wt% u-ZrO ₂ p<0.05
3 wt% t-ZrO ₂	7611.20 \pm 143.06	7491.60 - 7730.80	p>0.05	vs. 3 wt% u-ZrO ₂ p<0.05
5 wt% t-ZrO ₂	7697.26 \pm 130.89	7587.83 - 7806.68	p>0.05	vs. 5 wt% u-ZrO ₂ p<0.05
7 wt% t-ZrO ₂	7791.21 \pm 168.20	7650.59 - 7931.83	p<0.05	vs. 7 wt% u-ZrO ₂ p<0.05

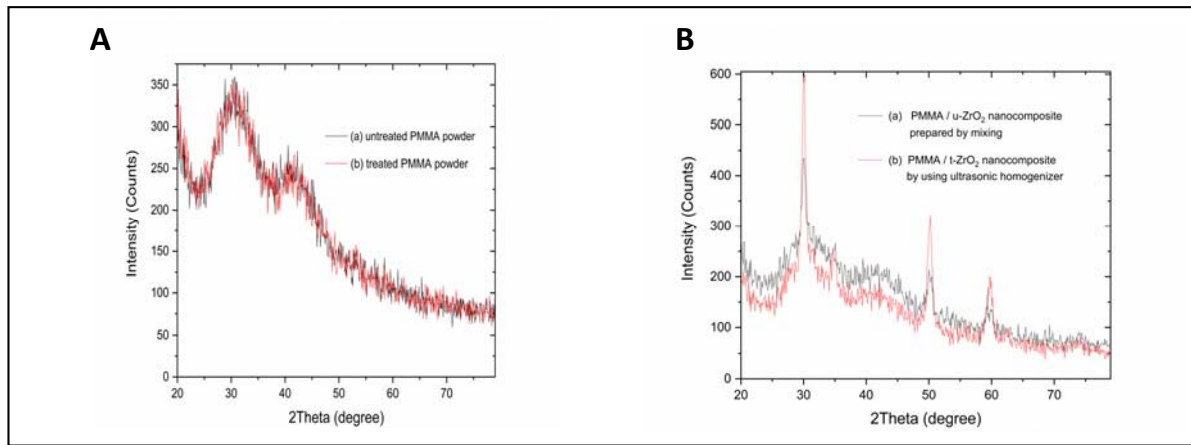


Figure 2: (A) XRD of untreated PMMA powder and treated PMMA powder in absolute ethanol alcohol, (B) XRD of nanocomposite mixed conventionally and by homogenizer.

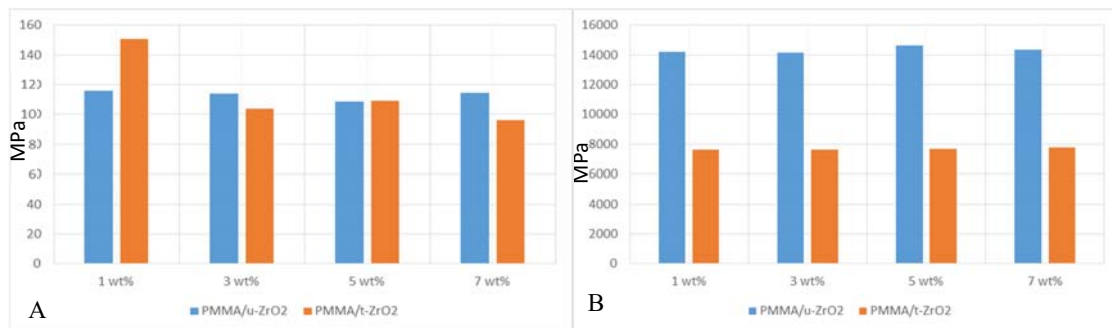


Figure 3: (A) Flexural strength (MPa) of reinforced acrylic denture base material with ZrO_2 nanoparticle prepared by two different dispersion methods. (B) Flexural modulus (MPa) of reinforced acrylic denture base material with ZrO_2 nanoparticle prepared by two different dispersion methods.

higher dispersion quality than the latter group of the shake dispersion technique as shown in Figure 2(B).

Flexural strength test

The flexural strength for the specimens prepared by mechanically shaking dispersion technique decreased significantly with increasing u- ZrO_2 nanoparticle, and it was directly proportional to the u- ZrO_2 nanoparticle concentration, Table 1. Also, the flexural strength of the specimens prepared by ZrO_2 nanoparticle treatment and sonication in ethanol alcohol using ultrasonic homogenizer (t- ZrO_2) resulted in decreased flexural strength and this decrease is directly proportional with increasing the nanoparticle's concentration, Table 2. Different concentration of nanoparticles groups means of flexural strength are compared in Figure 3. One-way ANOVA showed that decreased flexural strength is statistically significant for the groups above 3 wt%

group ($p < 0.05$). The flexural modulus was opposite to the flexural strength in both of the dispersion techniques, Table 3 and Table 4, for the u- ZrO_2 addition the flexural modulus increased with increasing the ZrO_2 nanoparticle concentrations, and they were statistically significant when compared to the conventional group. While for the t- ZrO_2 addition only the 7 wt% group was statistically significant. Different concentration groups' means of flexural modulus are compared in Figure 3(B).

Discussion

In dentistry, ZrO_2 nanoparticle has got a great attention for reinforcement of dental materials because of high fracture toughness, radiopacity, and high biocompatibility⁽²²⁾.

An important factor of the nanofillers to reinforce the mechanical properties of the reinforced heat-cured

acrylic denture base material is its size. Larger nanoclusters result in stress concentration areas, crack formation and propagation throughout the matrix of the polymer, and finally failure of the polymer⁽²³⁾. In this study, a new method was used to disperse ZrO₂ nanoparticles uniformly and to fill the interstitial free volume within the acrylic matrix.

The XRD results as shown in Figure 2 (A) “the red line”, showed a uniform t-ZrO₂ nanoparticle distribution throughout the acrylic resin matrix when compared to the u-ZrO₂ as shown in Figure 2 (B) “the black line”. The XRD analysis showed sharp steep 2 θ curves of treated and sonicated PMMA/t-ZrO₂ mixture, that indicate better crystalline structure configuration and less amorphous phase of t-ZrO₂ nanoparticle as compared to PMMA/u-ZrO₂ mixture.

The flexural strength evaluation of the acrylic denture base material based on three-point bending system, since it reflects the loading arrangement in the clinical situation. The flexural strength results of Table 1 and Table 2 showed that flexural strength decreased with increasing the nanoparticles concentration in both dispersion process. However, the sonication process reduced the size of the nanoclusters according to XRD findings, but the numbers of clusters were increased, as well, and each of this clusters act as inclusion bodies and stress concentration areas, thus increased micro-cracks formation and propagation. The finding of this study was in agreement with previous studies, which reported that using low concentrations of TiO₂-SiO₂ nanoparticles (0.5 wt% and 1 wt%) adversely affect the flexural strength of the acrylic denture base material.

Similarly addition of different types of nanoparticles in different concentrations resulted in decrease of flexural strength^(15,22,24,25). While the other studies which used salinization and coupling agents to provide an organic-inorganic bonding which provides a good bonding of the metallic nanoparticles to the acrylic as they are used as the third materials to provide a uniform distribution of the nanoparticles and forming an organic-inorganic bonding. Consequently, this distribution and bonding are responsible for improving mechanical properties^(8,16,26-28). Based on these bonding properties of the coupling agents, researchers reported that flexural strength has increased with increasing the nanoparticles ratio, however at a certain weight percentage, the flexural strength declined again due to nanoparticles agglomeration and forming stress concentration areas^(23,29,30).

The value of the flexural modulus is an indicator for the resistance of the material against deformation⁽³⁰⁾, in addition to the findings of the current study which showed that the flexural strength decreased with

increasing the nanoparticles ratio, but the case is reverse for the flexural modulus. Thus, the flexural modulus increased with increasing the nanoparticles ratio, Table 3 and Table 4, and as a result, the stiffness of the acrylic-based nanocomposite increased as well as more nanoparticles can fill the resin matrix interstitially. This finding is in agreement with the previous studies^(22,28,29).

In current study, the t-ZrO₂ nanoparticles were making smaller clusters. Purchased nanoparticles u-ZrO₂ has large nanoparticle clusters, this tendency restricted mechanical properties improvement of the denture base material. The major tendency is to reduce the size of filler particles from agglomerated micro-nanometer scales to smaller aggregated particles in a nanometer scale⁽³¹⁾, and this has been the main goal of many other researchers to improve the mechanical properties of heat-cured acrylic denture base material⁽³²⁾. The clusters of nanoparticle within the polymer matrix form areas of stress concentration which result in crack formation and propagation. The size of clusters is very important in improving the mechanical properties of the reinforced heat-cured acrylic denture base material. The large size of clusters of nanoparticles results in stress concentration areas, crack formation and propagation throughout the matrix of the polymer, and finally the failure of the polymer⁽³³⁾.

Conclusions

Adding both of u-ZrO₂ and t-ZrO₂ nanoparticles to heat cured acrylic resin denture base material decreases flexural strength. While, flexural modulus (bending modulus) increases with increasing both of u-ZrO₂ and t-ZrO₂ nanoparticles' concentration. u-ZrO₂ nanoparticle increases flexural modulus and stiffness of the acrylic resin significantly when compared to t-ZrO₂ nanoparticle's in different concentration groups. Using an ultrasonic homogenizer for nanoparticles dispersion in absolute ethanol alcohol result in smaller ZrO₂ nanoparticles' clusters.

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