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Original research article

The influence of nanoparticles on the mechanical and biological properties of temporary acrylics

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Abstract

Objective: **The aim of this study was to evaluate the flexural strength (FS) and antifungal properties of autopolymerized polymethylmethacrylate (PMMA) reinforced with zirconium oxide (ZrO_2) and hydroxyapatite (HA) nanoparticles (NPs).**

Materials and Methods: ZrO_2 and HA-NPs were incorporated into cold cured PMMA at a rate of 1%. 65×10×3 mm specimens prepared for FS and 2×10 mm disc specimens for *Candida Albicans* (*C. Albicans*) adhesion test (n=10). Surface roughness was recorded for each specimen via a profilometer. Flexural strength test and *Candida* adhesion tests were performed. Statistical analysis was done using one-way analysis of variance and post-hoc Bonferroni tests. ($p<0.05$)

Results: Based on the findings, the addition of NPs resulted in a decrease in FS. In comparison to other groups, ZrO_2 (55.47 ± 9.40) showed a significant decrease in FS ($p<0.05$). In addition, the adhesion of *C. albicans* was significantly reduced by ZrO_2 (16.5 ± 5.8) in comparison to the control group ($p<0.05$).

Conclusion: ZrO_2-NPs incorporated into temporary acrylic reduced FS and prevented *Candida* adhesion.

Keywords: Candida Albicans; Flexural Strength; Nanoparticles; Polymethyl Methacrylate.

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[Abstract in Turkish is at the end of the manuscript]

Introduction

Polymethylmethacrylate is a commonly used material in prosthetic dentistry for the production of artificial teeth, denture bases and dentures, obturators, and temporary crowns. Providing both functional and aesthetic benefits, temporary dental acrylics, also known as provisional or interim materials, are essential for patient satisfaction during the interim period between tooth preparation and final restoration placement.[1] Well-constructed provisional restorations play a crucial role in safeguarding the well-being of oral support tissues. They serve as a protective barrier that effectively shields against tooth sensitivity and the infiltration of microbes. With these considerations in mind, enhancing the temporary acrylic resins' physical, mechanical, and chemical attributes becomes imperative.

Flexural strength (FS), a mechanical property, measures the ability of a material to resist bending or deformation under applied loads. In the context of provisional dental restorations, FS is a critical determinant of their structural integrity and clinical performance.[2,3] However, the potential antibacterial properties of interim restorations hold promise for improving oral health outcomes. By limiting bacterial colonization and biofilm formation, interim restorations could help reduce the risk of secondary infections such as peri-implantitis, marginal gingivitis, and halitosis.[1]

Recent advances in nanotechnology have led to the incorporation of nanoparticles (NPs) into PMMA, offering enhanced mechanical, anti-microbial, and aesthetic benefits. PMMA enhanced with NPs finds application in the fabrication of denture base materials. Reinforcing NPs, such as silica, hydroxyapatite, titanium dioxide, or zirconia are incorporated into PMMA matrices to improve the mechanical properties of dentures.[4-7] Also, NPs with inherent antimicrobial and antifungal properties, such as silver NPs, can be incorporated.[8]

The aim of this study is to evaluate the flexural strength and antifungal effects by adding %1 hydroxyapatite and zirconium dioxide NPs to temporary acrylic resin. The null hypothesis of the study is that the addition of nanoparticles will have no effect on the flexural strength and antifungal properties of PMMA.

Materials and Methods

Specimen preparation

In accordance with the American Dental Association Specification no. 12, 30, rectangular shaped specimens (n=10) with dimensions of 65×10×3 mm were prepared for the flexural strength test.[9] For the *Candida* adhesion test, 30 disc specimens, 10 mm wide and 2 mm thick (n=10) were manufactured using a stainless steel mould.[10,11] (Figure 1 A-B) The samples were divided into three groups as control, 1% ZrO_2-NPs (99.9% purity, 30 nm particle size, Nanografi, Ankara, Türkiye) 1% HA-NPs (99.9% purity, 50 nm particle size, Nanografi, Ankara, Türkiye). The NPs were weighed with an electronic precision balance and added to auto polymerized acrylic resin polymer powder (Integra, Birlesik Group Dental Ankara, Türkiye) at a concentration of 1% by weight. The mixture was prepared following the manufacturer's instructions to obtain a homogeneous distribution of the particles and then poured into a metal mould for polymerization. After polymerization, the specimens were immersed in distilled water at 37°C for 24 hours to remove any remaining residual monomers. The samples were polished on both sides with 500, 1000, 1500, and 2000 grit abrasive paper.

Surface Roughness Measurement

The arithmetic mean roughness, recorded in micrometers, was determined by the probe of the profilometer (Perthometer M2, Germany) straight across the test sample surface. The process was performed twice on various surfaces, and the means of the two measurements were analyzed. The experiment was performed with the following parameters: diamond stylus tip radius 5μ, stylus speed 0.25mm/s, and cut-off length 0.8mm.[12] Roughness values between 0.30 and 0.38 μm were accepted as the reference.

Flexural Strength Test

A universal testing machine (Instron 5581, Norwood, USA) was used for flexural strength tests. A load of 5 kN was applied to failure at a crosshead speed of 5 mm/min.[9] The load at failure was recorded and the FS was calculated using $S=3FL/2bd^2$.

S= Flexural Strength (MPa), F= Load at Failure (N), L= Distance between two supports (50 mm), b = Specimen width (mm), d= Specimen thickness (mm). (Figure 1. C)

Candida Albicans Culture Conditions and Determination of Surface Adherence

The *C. albicans* V6 strain was used in this study. A single colony from an agar plate was inoculated into 10 mL of Brain Heart Infusion (BHI) broth and incubated

Table 1. *Normality (Shapiro-Wilk) test results according to groups.*

Tests of Normality		
Shapiro-Wilk		p
Flexural Strength	Control	0.589
	HA	0.915
	ZrO_2	0.628
C. Albicans	Control	0.243
	HA	0.499
	ZrO_2	0.708

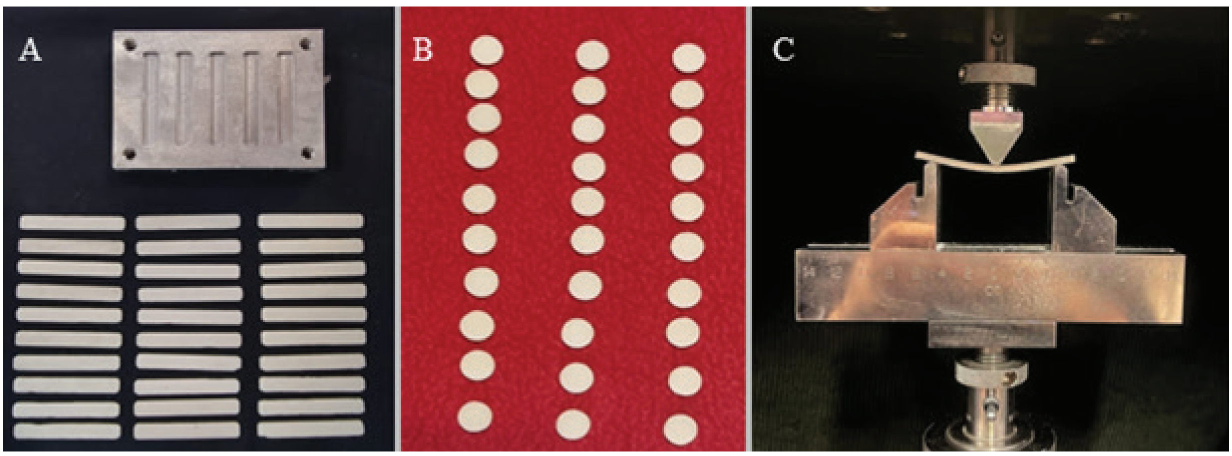

Figure 1. A. *Metal mold and flexural strength test samples of autopolymerizing acrylic resin (65×10×3 mm)* ***B.*** *C. albicans adhesion test discs (2×10 mm)* ***C.*** *flexural strength test application on universal testing machine.*

***Table 2.** C. Albicans and Flexural Strength values (Mean+SD) of nanoparticle-reinforced groups.*
[a-b]There is no difference between groups with the same letter for each row.

	Control	ZrO_2	HA	p
Flexural Strength (MPa)	67.65±2.09[a]	55.47 ± 9.40[b]	62.75 ± 5.70[ab]	<0.05
***C. Albicans* (CFU)**	28 ± 8.2[a]	16.5 ± 5.8[b]	34.2 ± 9.2[a]	<0.05

overnight at 37°C. Then, 1.5 mL of the overnight culture was used to inoculate 30 mL of fresh BHI broth, which was then incubated overnight at 37°C to obtain the main culture.

Before use, the discs were sterilized by immersing them in 70% ethanol (v/v) and then drying them under sterile conditions in a laminar airflow hood. Following this, they were exposed to UV light for one hour to ensure complete sterilization.

The yeast cells of the main culture were collected by centrifugation at 3220g for 10 minutes at 5 °C (Eppendorf 5810R, with an Eppendorf Swing-bucket rotor A-4-62, Hamburg, Germany) and washed three times with 10 mM potassium phosphate buffer solution (pH 7). The resulting cell pellet was resuspended in potassium phosphate buffer to a McFarland 2 turbidity. Then, 2 mL of this *Candida* cell suspension was inoculated with all the discs inside 24-well plates and incubated for 2 hours at 37°C. After 2 hours, the discs were immersed in 2 mL of potassium phosphate buffer and gently lifted to wash off any non-adherent yeast cells. Subsequently, the discs were placed in 10 mL of saline solution supplemented with 0.04% Tween 80. The saline solutions containing the discs were vortexed for 2 minutes each to detach the yeast cells that had adhered to the discs. The detached fungal cell suspensions were then diluted 10-2 and 10-3. Finally, 100 µL of each dilution was inoculated onto BHI agar plates. The plates were incubated at 37°C for 48 hours, and all resulting colonies were carefully counted and recorded.

Statistical Analysis

The statistical analysis was performed using IBM SPSS Statistics 22 (Chicago, USA). Descriptive statistics (mean, standard deviation) were employed. The normality of the data was determined using the Shapiro-Wilk test. The one-way ANOVA and post-hoc Bonferroni test were used to analyse the groups, with a significance level of $p<0.05$.

Results

The results of the normality tests for the groups are presented in Table 1. The results of the descriptive statistics test and the differences between the groups are presented in Table 2. FS mean values differed according to the groups ($p<0.05$). The mean value was 67.65 MPa in the control group, 55.47 MPa in the ZrO_2, and 62.75 MPa in the HA group. FS value in the ZrO_2 group ($p=0.023$) was statistically significantly lower than the Control. The mean value in the HA group was not different from both the control and ZrO_2 groups.

The mean values of *C. albicans* cells attached differ according to the groups ($p<0.05$). There was no difference between the control and HA groups, and the mean values were 28.0 and 34.2 CFU, respectively (Figure 2). The mean *C. albicans* value in the ZrO_2 group was 16.5 CFU, which was statistically lower than all other groups ($p=0.009$, $p=0.000$). Specifically, the amount of yeast cells attached to ZrO_2 was significantly less than the amount attached to the control and HA groups (Figure 3).

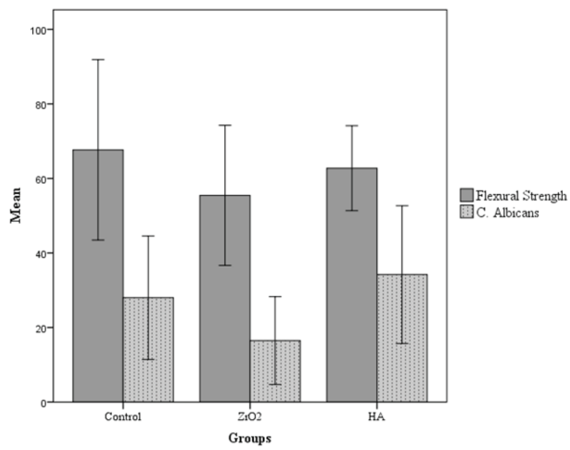

***Figure 2**. Flexural strength values (MPa) of biomaterials. Microbial attachment of C. albicans on different types of biomaterials measured by colony forming units (CFU).*

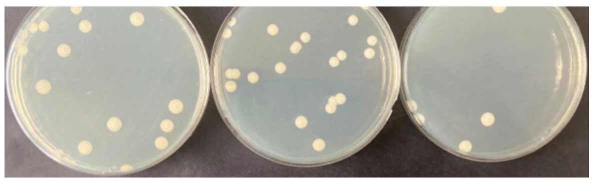

***Figure 3.** Images of C. albicans colony forming units (CFUs) attached to three different types of biomaterials: **A)** control, **B)** hydroxyapatite, and **C)** zirconia. The CFUs were measured following a 10-4 dilution, and the images demonstrate the level of attachment to acrylic resin.*

Discussion

Temporary restorations are designed to maintain the structure, function, and aesthetics of damaged or missing teeth until permanent restorations, such as crowns or bridges. The success of these restorations depends on their ability to withstand the functional demands of mastication, occlusion, and oral hygiene.

ZrO_2-NPs exhibit exceptional mechanical strength, fracture, and corrosion resistance. This makes them especially suitable for dental implants, crowns, and bridges, where longevity and stability are critical.

Furthermore, ZrO_2-NPs demonstrate outstanding biocompatibility with oral tissues. Hydroxyapatite, a mineral found in bone and teeth, is becoming increasingly popular as a versatile material in dentistry due to its biocompatibility and ability to bond with natural tissues. Incorporating HA-NPs into dental treatments provides a variety of benefits, including bioactive properties indicating osseointegration, bone regeneration, and assistance in the repair of bone tissue or periodontal defects, reducing the risk of caries.[12-15] When all of these properties were evaluated, and since there are no studies in the literature that compare ZrO_2 with HA, these two materials were selected. In addition, a review of previous studies showed that increasing the NP concentration had a negative effect on the physical, mechanical, and biological properties of PMMA.[16,17] Therefore, a concentration of 1% was investigated.

Examining the results of our study, it can be observed that the groups with added NPs exhibited reduced FS. However, it was found that the addition of ZrO_2-NPs to the group resulted in an increased antifungal effect, whereas HA-NPs reduced it. When all these results are evaluated, the null hypothesis of the study is rejected.

According to the International Organization for Standardization (ISO 4049) and the American National Standards Institute (ANSI)/American Dental Association (ADA) Specifications no. 27, interim fixed prosthesis materials must exhibit a minimum flexural strength of 50 MPa when subjected to a flexural strength test.18 In this study, all tested specimens demonstrated FS values exceeding 50 MPa, indicating that different NPs with %1 concentration are suitable for use in fabricating provisional restorations.

When previous studies were examined, Ergun *et al.*,[6] used 5-10-20% ZrO_2 NPs and the FS decreased, Kul *et al.*,[7] used 10% ZrO_2-NPs and the FS decreased but was not statistically significant. The results of our study are in agreement with these studies. However, there are many studies where ZrO_2 NPs at lower (%1-2.5-3-5-7.5) concentrations increase the FS of PMMA.[2,3,19,20] Also, Aldabib and Ishak[5] found that the FS increased up to 5% in their study in which HA-NPs were added to PMMA at various concentrations.[5] However, Zebarjad *et al.*[21] reported that the addition of increasing concentrations of HA nanocomposite similarly had no significant effect on the FS in their study evaluating the mechanical properties of PMMA. We attribute this difference to the agglomeration of the NPs. Polymers or other molecules bound to the surface of nanoparticles can prevent or promote the agglomeration of NPs. Furthermore, the addition of suitable stabilizers, use of coupling (silane) agent, and physical methods such as ultrasonication and controlled synthesis conditions can be provided to prevent agglomeration.[22-24]

Several studies have assessed the impact of ZnO, TiO_2 and Ag-NPs on biofilm formation on PMMA.[8,25-27] Nevertheless, the assessment of ZrO_2 and HA-NPs is restricted. Gad *et al.*[28] reported that the addition of ZrO_2-NPs to cold-cured acrylic resin was an effective method of reducing *Candida* adhesion to PMMA. Abualsaud *et al.*[29] found that the incorporation of 2.5-5% and 7.5% ZrO_2-NPs in PMMA inhibited the formation of *C. albicans* biofilms. Our study aligns with these studies. Few studies have been conducted on HA-NPs to evaluate their antibacterial efficacy. Nonetheless, a study by Elboraey *et al.*[30] which utilized HA-NPs as a drug delivery mechanism for metronidazole, suggested that the antibacterial effect was greater in the HA-metronidazole group. In their study evaluating the antibacterial activity of HA-NPs, Ragab *et al.*[31] concluded that the HA-NPs are effective against common gram-negative and gram-positive bacteria, depending on the highly reactive oxygen species. Nevertheless, the effectiveness of HA-NPs synthesized in this study when combined with PMMA remains uncertain as they were not evaluated together. Furthermore, the role of free oxygen radicals in this combination is unclear.

Considering these discoveries, the inclusion of ZrO_2-NPs to enhance the antifungal attributes holds promise for enhancing oral hygiene and curbing the incidence of gingivitis and halitosis linked to temporary restorations. Despite the potential reduction in the FS, this approach could present a viable strategy for diminishing bacterial accumulation in short-term clinically utilized temporary restorations. Nevertheless, it is crucial to acknowledge the constraints of not investigating various nanoparticle concentrations and the absence of assessments of the impact of aging, intraoral dynamics, and different materials.

Conclusion

ZrO_2-NPs are effective in preventing bacterial adhesion in temporary acrylics. However, its clinical application is limited due to the reduced flexural strength of PMMA by incorporating ZrO_2-NPs. HA does not display a significant effect on either aspect. To further understand the efficacy of nanoparticles, *in vitro* and clinical studies are necessary.

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Nanopartiküllerin geçici akriliklerin mekanik ve biyolojik özellikleri üzerine etkisi

Özet

Amaç: Bu çalışmada, zirkonyum oksit (ZrO_2) ve hidroksi-apatit (HA) nanopartikülleri (NP) ile güçlendirilen otopolimerize polimetilmetakrilatın (PMMA) bükülme dayanımı (BD) ve antifungal özellikleri değerlendirilmiştir.

Gereç ve Yöntem: ZrO_2 ve HA-NP'ler otopolimerizan PMMA içerisine %1 oranında ilave edilmiştir. BD için 65×10×3 mm'lik ve *Candida* albicans (C. Albicans) adhezyon testi için 2×10 mm'lik disk örnekler hazırlanmıştır (n=10). Yüzey pürüzlülüğü her numune için profilometre kullanılarak ölçülmüştür. Bükülme dayanımı ve *Candida* adhezyon testleri uygulanmıştır. Elde edilen veriler ANOVA ve Post-Hoc Bonferroni testleri kullanılarak istatistiksel olarak analiz edilmiştir. Anlamlılık düzeyi $p<0.05$ olarak kabul edilmiştir.

Bulgular: Bulgulara göre, NP'lerin eklenmesi BD'de bir azalmaya neden olmuştur. Diğer gruplarla karşılaştırıldığında, ZrO_2 ilavesi (55.47 ± 9.40) BD'de anlamlı bir azalmaya sebep olmuştur. ($p<0.05$) Ayrıca, ZrO_2 ilavesi (16.5 ± 5.8) kontrol grubuna kıyasla C. Albicans adhezyonunu önemli ölçüde azaltmıştır.($p<0.05$)

Sonuç: Geçici otopolimerizan akriliğe ilave edilen ZrO_2-NP'ler bükülme dayanımını azaltmakta ve *Candida* adhezyonunu önlemektedir.

Anahtar Kelimeler: Bükülme Dayanımı; *Candida* Albicans; Nanopartikül; Polimetilmetakrilat