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RESEARCH PAPER

The Effect of ZrO_2 Nanoparticles Addition on Candida Adherence and Tensile Strength of 3D Printed Denture Base Resin

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ABSTRACT

To enhance 3D printed denture base resin performance; ZrO_2 nanoparticles were added to improve the biological and mechanical behavior. (110) specimens (50 dumbbell- shaped and 60 discs) were 3D printed and divided into five groups per test (n=10). The control group for each test included unreinforced 3Dprinted denture base resin, while the test groups reinforced with (1, 2, 3, and 4 %) nanoZrO_2; with positive control of nystatin 1.4% for candida adherence test. Tensile strength was evaluated using universal testing machine while candida test was evaluated by spectrophotometer device through optical density verification. The study showed significant increase in antifungal activity of the 3Dprinted denture base resin after adding nano ZrO_2 .The tensile strength mean was significantly higher than the control group; although the mean was decreasing with increasing the ZrO_2 NPs. The addition of ZrO_2 nanoparticles increasing the antifungal activity of the 3D denture base resin, the increasing was proportional to the nanoparticles concentration. The tensile strength of the 3D denture base resin was significantly improved with 1% of ZrO_2 NPs concentration among 2, 3 and 4%.

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INTRODUCTION

PMMA still the most friendly denture base material for many practitioners, in spite of its limitations of low mechanical and physical properties with the long process of fabrication [1]. Digital technology in dentistry as a whole and prosthodontics in particular has shown to have many benefits in terms of precision results of fabrication and speed of manufacturing [2-8]. However, there are still some issues that must be resolved, such as the poor mechanical properties of the base materials used in 3D printing dentures [2-5].

Despite being close to the ISO-accepted value of 65 MPa for flexural strength, 3D printed denture base materials have the lowest flexural strength and surface hardness compared to conventional and milled denture base materials. Thus, its clinical applications are constrained [5,6,8,9].

Numerous studies looked into many ways to overcome the aforementioned restrictions, modification of post-polymerization time, layer thickness, printing orientation, and the addition of nanoparticle fillers like TiO_2, Al_2O_3, and SiO_2 as metal oxide nanoparticles that appear to improve some mechanical properties of the 3D-printed denture base resin [10-13]. According to Gad et al. (2022) adding SiO_2 NPs to 3D printed denture base resin improves flexural strength and impact strength without significantly affecting surface roughness [13]. Additionally, Alshaikh et al. (2022), stated that the 3D printed denture base resin was significantly increased in flexural strength, impact strength, and hardness with no appreciable

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changes in surface roughness after addition of ZrO_2 NPs [14]. ZrO_2 mimics the appearance of natural teeth and reduces peri-implant inflammatory reaction, which makes it more biocompatible than other ceramic materials like alumina. Having high strength, fracture toughness, and surface hardness, it is a biocompatible metal oxide [14-17]. Additionally, it exhibits thermal stability, corrosion resistance, antifungal and antibacterial activity against Candida albicans and Asergillus niger [17-19].E.coli and S. aureus were used as model strains of gram-negative and gram-positive bacteria, respectively, in antibacterial activity tests of ZrO_2 NPs. capable of effectively inhibiting the growth of bacterial cultures ,it was discovered that ZrO_2 NPs were significantly more effective against S. aureus than E. coli, with S. aureus bacterial growth being inhibited by ZrO_2 NPs to a greater than 90% degree. Scanning electron microscopy (SEM) analysis of the morphology of bacterial cells revealed that nanoparticles and nanocomposite permanently damaged the cell membrane [17,18].

ZrO_2 NPs may work well as a 3D printing material reinforcement technique .To the best of the authors' knowledge, no studies have previously examined the impact of adding ZrO_2 NPs on the ability of 3D-printed resins on candida adherence and increase tensile strength [19].

Therefore, the aim of this study was to evaluate the effect of adding ZrO_2 NPs to 3D-printed denture-base resins on candida adherence and on tensile strength.

MATERIALS AND METHODS

110 specimens were designed according to specification for each test, (60) disks of 2x10 mmfor candida test and (50) dumbbell shape specimen for tensile strength test with dimension given by (ASTM specification D-638M, 1986) (20), divided into 5 groups (n=10) according to the ZrO_2 nanoparticles concentration (1, 2, 3, and4 %) by weight.

Optiprint laviva (dentona , Germany) 3D printed denture base resin of light pink color was used with DLP open system microlay versus 385 dental printer by exporting the STL file from microform computer software program .Pure resin was placed on mechanical mixer machine before adding the nanoparticles for 120 min; then addition of nanoparticles in mentioned concentrations and distributed into several bottles with continuous stirring in magnetic stirrer for 30 minutes at 60°C to decrease the resin viscosity, then stirred at room temperature for 8h to obtain homogenous nanocomposite for printing procedure [20]. Each layer was printed with a 50 µm layer thickness in (1.61) sec/slice in vertical Z axis following manufacturing instructions. Cleaning with isopropyl alcohol 99.9% before immersion in glycerol and placing in UV light polymerization unit for 10minutes to complete the polymerization prior to finishing the samples by removing the supports and base with low speed rotary instrument and polishing with polishing machine and cloth in a wet condition [21,22]. The whole procedure was done by one operator to insure applying same preparation conditions .The specimens immersed in distilled water 48hs at 37°C prior to testing [23].

Testing procedure

Candida test: sterile disks were incubated with a candida culture for 24 hours before being removed, washing with normal saline to remove any remaining candida, staining with crystal violet for 20 minutes, rewashing with normal saline, and

Table 1. The mean values and standard deviation of Candida albicans adherence test.

	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Control	10	0.13510	0.031631	0.11247	0.15773	0.099	0.186
Nystayin	10	0.03670	0.004373	0.03357	0.03983	0.030	0.042
1%	10	0.08120	0.001317	0.08026	0.08214	0.079	0.083
2%	10	0.03800	0.001155	0.03717	0.03883	0.036	0.039
3%	10	0.03180	0.001317	0.03086	0.03274	0.030	0.034
4%	10	0.02470	0.001947	0.02331	0.02609	0.023	0.028

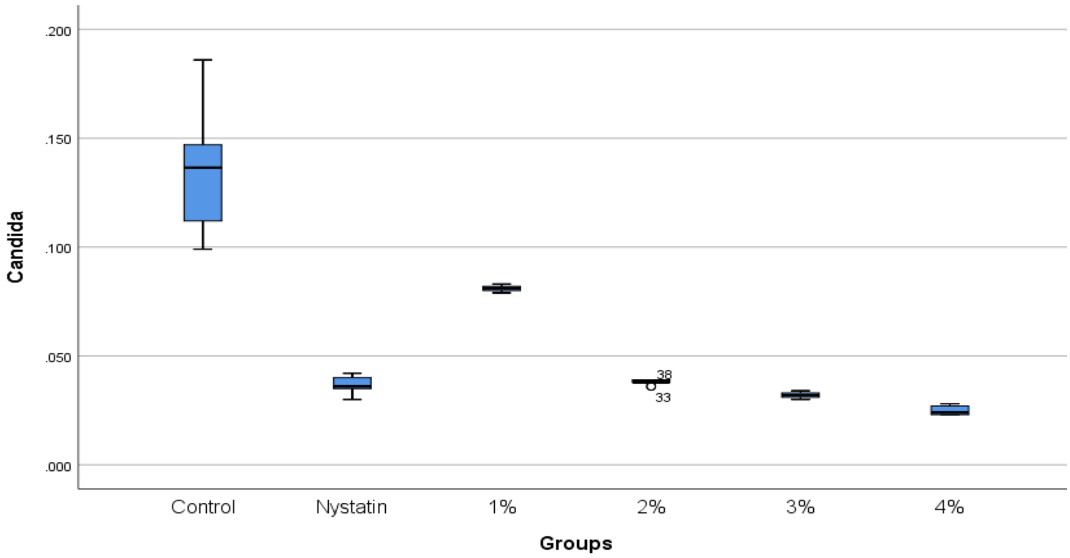


Fig. 2. Boxplot for standard deviation and median of tensile test.

then immersing in 3 ml of ethanol alcohol (96%) for three minutes [24-27]. The optical density was then confirmed. The tensile strength: Each specimen's tensile strength had been evaluated using a universal testing machine. The ends of the material specimen are typically clamped on two jigs spaced apart by a specific amount, stretching the specimen as the two jigs separate until there is damage to the specimen.

Tensile strength was calculated by formula: T.S.MPa=Maximum force (N.)\ Area (mm)

RESULTS AND DISCUSSION

Evaluating the adherence ability of Candida albicans to 3D printed denture base resin after ZrO_2 addition by OD verification, mean and standard deviation with confidence interval in Table 1, as shown; the minimum antifungal activity of 3D printed denture base resin after adding 1% nano ZrO_2, and maximum value was with 4% nano ZrO_2 at 95% confidence interval. Boxplot to describe the SD and median between minimum and maximum range of candida adherence test Fig. 1.

According to test of homogeneity of variance (Levene test) Table 2 and test of ANOVA Table (3) a highly significant differences (p ≤0.01) demonstrated between study groups and control group at a significant level of (0.01%).

According to the significant results, comparison between each 2 groups was decided to be evaluated by Games-Howell test. Post hoc test (Games–Howell) was selected for multiple comparisons of incorporation to compare the mean values among all study groups in Table 4.

The same for tensile test as mean and standard deviation was conducted with confidence interval at 95% in Table (5) and demonstrated in Fig. 2. The variances of tested groups for tensile strength were analyzed by Levene's test of homogeneity in Table 6 to decide the test of multiple comparisons of the results. Comparison of means for tensile test results of the experimental groups using ANOVA in Table 7 and the result was highly significant.

With this significant result, Games-Howell test

Table 2. Test of homogeneity (Levene test).

Test of Homogeneity of Variances					
		Levene Statistic	df1	df2	Sig.
Candida	Based on Mean	12.785	5	54	0.000

Table 3. ANOVA test.

ANOVA					
Candida					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	0.091	5	0.018	106.568	0.000
Within Groups	0.009	54	0.000		
Total	0.101	59			

Table 4. Games-Howell multiple comparisons test.

(I) GroupsC		Mean Difference (I-J)	Sig.	
Control	Nystayin	.098400*	0.000	Sig.
	1%	.053900*	0.004	Sig.
	2%	.097100*	0.000	Sig.
	3%	.103300*	0.000	Sig.
	4%	.110400*	0.000	Sig.
Nystatin	1%	-.044500*	0.000	Sig.
	2%	-0.001300	0.936	Non sig.
	3%	0.004900	0.053	Non sig.
	4%	.012000*	0.000	Sig.
1%	2%	.043200*	0.000	Sig.
	3%	.049400*	0.000	Sig.
	4%	.056500*	0.000	Sig.
2%	3%	.006200*	0.000	Sig.
	4%	.013300*	0.000	Sig.
3%	4%	.007100*	0.000	Sig.

*The mean difference is significant at the 0.05 level.

Significant results revealed that the data were normally distributed.

(Table 8) was selected to compare between the mean values among all study groups. SEM images of the samples surfaces at 100,000× magnification force revealed significant difference between the pure 3Dprinted resin(A) (with no addition) that appears to have broad scattered pores with irregularity compared to the 2% (C) and 3% (D) nano ZrO_2 ;while the images of the 3D resin with 2% and 3% shows the dispersion of nanoparticles within the material to give more compact and regular surface with more diminished pores and particle size of less than 50 µm of ZrO_2 NPs in (C) than (D) and this explains the ductility of the group (D) which gives the result of tensile strength Fig. 3.

Fig. 4 also shows significant differences in the surface of the pure 3Dprinted denture base resin (A) and the 2% (B),3% (C) ZrO_2 NPs at 4000 magnification force of SEM to prove the chemical

Table 5. The mean values and standard deviation for tensile strength test.

	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Control	10	14.4513	1.14383	13.6331	15.2695	12.77	16.79
1%	10	29.2360	5.60986	25.2229	33.2491	22.77	37.59
2%	10	24.5021	9.79926	17.4921	31.5121	13.32	36.26
3%	10	23.4326	0.64503	22.9712	23.8940	22.45	24.35
4%	10	22.3438	0.78028	21.7856	22.9020	21.18	23.43

reaction between the resin and the nanoparticles which was supported by the FTIR readings in Fig. 5, both (B) and (C) showed homogenous and good distribution of nanoparticles within the resin matrix with some clusters may be shown at 3% nano ZrO_2.

The FTIR results showing significant difference between the pure 3D printed resin (0%), 2% nano ZrO_2 3D resin and 3% nano ZrO_2 3D resin especially between 806- 636 cm1 range of spectra which indicate the presence of ZrO_2 within the polymer of the 3D printed denture base resin ,differences between peaks of 2% and 3% ZrO_2 resin as appeared at 752 cm^{-1} suggests the chemical reaction between the polymer resin and the nanoparticles, as the most intense peak of band for 2% NPs at 690 cm1, while for 3% NPs at 694 cm 1, with similarity to some extent between the spectra of the pure 3D resin and the reinforced resin attributed to the vibration and stretching of CH_3 and CH_2 groups at 1716-1381 cm1 bands with vibration of ester group C=O at 1180-1149 cm1, and this confirm the homogenous dispersion of the nanoparticles within the 3D printed resin material.

The effect of ZrO_2 NPs addition on the properties of 3D printed denture base resin was testing in this study regarding antifungal activity and tensile strength; according to the results, the null hypothesis was rejected because the addition of ZrO_2 NPs significantly affect the Candida albicans adherence and tensile strength. The present study showed an increase in antifungal activity of 3D printed denture base resin when ZrO_2 NPs were added. DS is a condition linked to Candida albicans that frequently returns in people who wear complete dentures. An important step in the colonization and pathogenesis that results

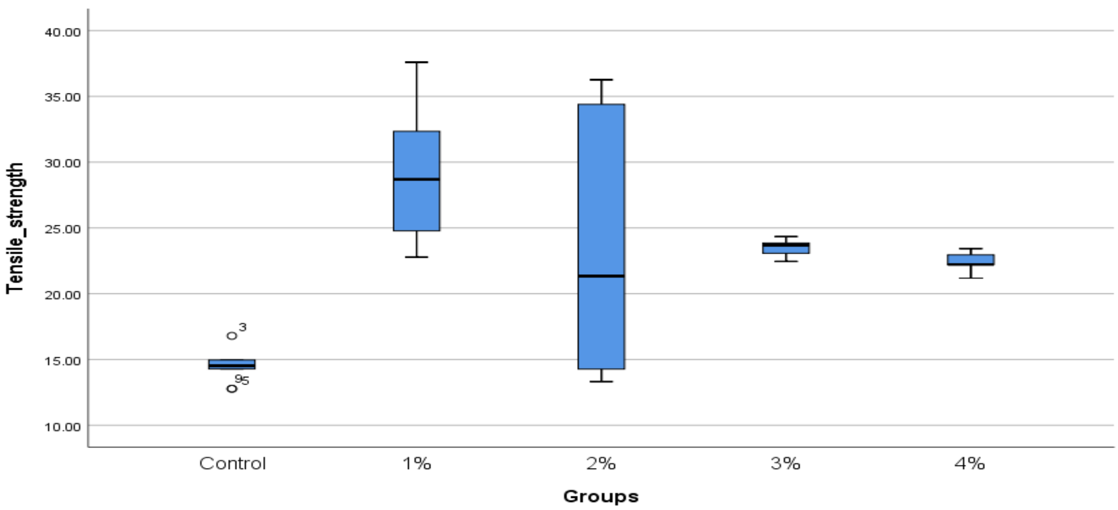


Fig. 2. Boxplot for standard deviation and median of tensile test.

Table 6. levene's test for tensile strength.

Test of Homogeneity of Variances					
		Levene Statistic	df1	df2	Sig.
Tensile_strength	Based on Mean	29.507	4	45	0.000

Table 7. ANOVA test for tensile strength.

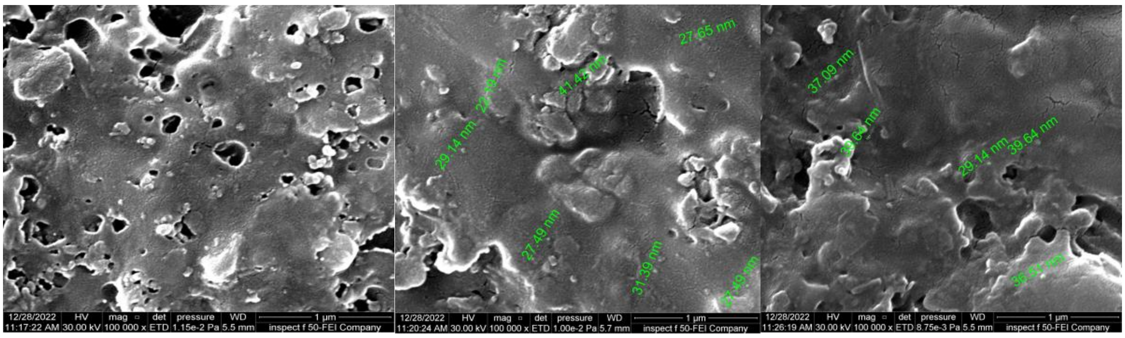
ANOVA Tensile_strength	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1146.281	4	286.570	11.036	0.000
Within Groups	1168.464	45	25.966		
Total	2314.745	49			

Table 8. Games-Howell multiple comparisons test.

(I) GroupsT		Mean Difference (I-J)	Sig.	
Control	1%	-14.78470*	0.000	Sig.
	2%	-10.05080	0.060	Non sig.
	3%	-8.98130*	0.000	Sig.
	4%	-7.89248*	0.000	Sig.
1%	2%	4.73390	0.681	Non sig.
	3%	5.80340	0.057	Non sig.
	4%	6.89222*	0.023	Sig.
2%	3%	1.06950	0.996	Non sig.
	4%	2.15832	0.953	Non sig.
3%	4%	1.08882*	0.024	Sig.

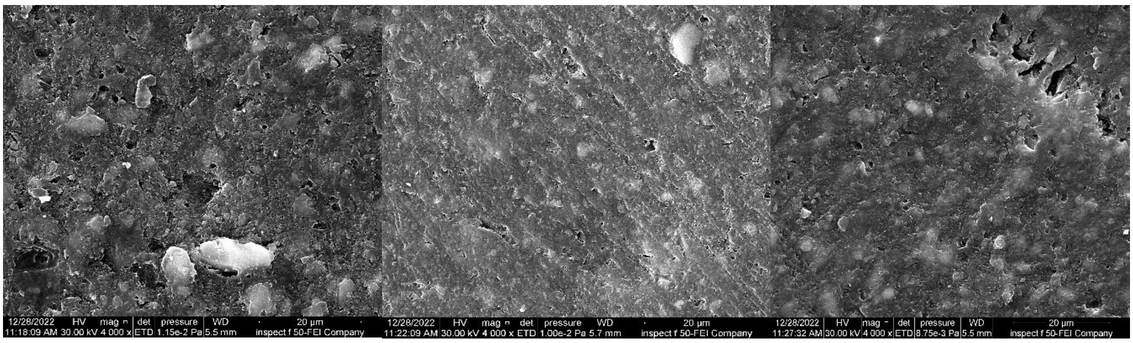
* The mean difference is significant at the 0.05 level.

in DS is C. albicans' adherence to the intaglio surface of a denture base [29]. It was claimed that mechanical cleaning techniques fall short of completely eliminating bacteria from denture surfaces, as a result, numerous attempts have been made to use a range of antifungal drugs to minimize C. albicans adherence and subsequent colonization on the denture base, but these treatments have shown to be ineffective and for short term [31]. Additionally, a variety of methods have been used to prevent fungal attachment to denture bases, including surface modification using various coatings or adding an antifungal component to a PMMA denture base [32]. Due to their outstanding scientific, technological, and medicinal characteristics, ZrO_2 NPs have drawn a lot of attention. ZrO_2 NPs were discovered to have super antibacterial and antifungal properties.



(A) (B) (C)

Fig. 3. SEM images (100,000x),(A) 3D printed resin with no addition, (B) 3D resin with 2% ZrO_2 NPs, (C) 3D resin with 3% ZrO_2 NPs.



(A) (B) (C)

Fig. 4. SEM images (4000X),(A) 3Dprinted resin with no addition , (B) 3D printed resin with 2% ZrO_2 NPs , (C) 3D printed resin with 3% ZrO_2 NPs.

Numerous studies have documented the beneficial effects of ZrO_2 NPs on Aspergillus niger and Candida albicans [29,30].

In this study, results indicate significant reduction in candida adherence after addition of ZrO_2 NPs to the 3D printed resin. The association between antifungal activity and ZrO_2 NPs concentration is consistent with previous studies involved modification of PMMA with ZrO_2 NPs [31,32]. Zirconium oxide nanoparticles shows outstanding antibacterial efficacy against Candida albicans and bacterial infections by interfering with cell function and deform fungal hyphae, drastically inhibited the growth of fungus strains [33], Scanning electron microscopy (SEM) analysis of the morphology of bacterial cells revealed that ZrO_2 nanoparticles and nanocomposite permanently damaged the cell membrane of bacteria [17,18].

Regarding tensile strength; addition of ZrO_2 NPs in different concentrations (1,2,3 and 4%) result in significant increase of tensile strength of 3Dprinted denture base resin in regard to control group, and this coincide with previous studies that proved the significant increase in mechanical properties with the addition of ZrO_2 NPs [13,15].The improvement in tensile strength may be related to the nano- ZrO_2 fillers' effective dispersion, which increases strength due to their nano size and aids in internally filling the matrix [30]; although the increase in NPs concentration result in decreasing of the tensile strength and this could be explained due to the agglomeration of the nanoparticles incorporated within the 3D resin which act as stress concentration spots in the matrix and this lead to decreasing the mechanical properties, and this result match the finding of Chladek et al (2013) who found that the mechanical properties of nanocomposites reinforced by silver NPs decreased as NPs concentration increased

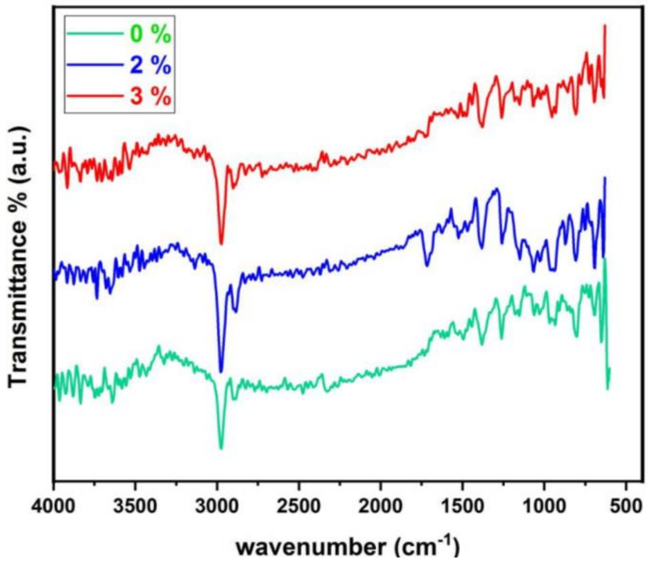


Fig. 5. FTIR spectra of 3D printed denture base resin with 0%,2%&3% ZrO_2 NPs addition.

[15,33-35]. Similary in 2010 Chatterjee showed that increasing in titanium oxide nanoparticles decreased the tensile strength [35]. Additionally, the tensile strength is decreased by the presence of agglomerated fillers that form loosely bounded clusters and alter the mechanism of crack propagation [33-35]. Based on these results, the outstanding act of ZrO_2 NPs as antifungal fillers cannot be ignored, with many other properties due to their specific characteristics making them suitable for denture base reinforcement material. Still further investigations are recommended with more concentrations of ZrO_2 NPs on other physical and mechanical properties of 3D printed denture base resin.

The limitations of this study were using one type of 3Dprinted denture base resin, with only 4 concentrations of ZrO_2 NPs. More concentration will give better idea about the behavior of ZrO_2 NPs within the 3D printed resin for denture base, moreover the conditions of testing did not simulate oral environment. Therefore, in vivo and clinical investigations are required.

CONCLUSION

Within the limitation of this study, it was concluded that the addition of ZrO_2 NPs to 3D printed denture base resin increases its antifungal activity, and this increase is directly proportional to the nanoparticles concentration .The tensile strength also increased significantly when 1% ZrO_2 NPs were added , but it was decreased as increasing the NPs concentration. Caution must be taken to properly select the appropriate concentration of ZrO_2 NPs in order not to affect other properties adversely.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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