

Effect of Addition of Titanium Oxide and Zirconium Oxide Nanoparticles on the Surface Roughness of Heat Cured Denture Base Resins: An In-Vitro study

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Abstract:

Background: With the recent advancements in nano-technology, titanium dioxide nanoparticles (TiO_2 NP) and zirconium oxide nanoparticles (ZrO_2 NP) are being used as filler to improve the mechanical and biological properties of heat cured denture base resins. Surface roughness of denture base resins is an important physical property as roughened denture surfaces tend to accumulate plaque, leading to oral candidiasis. The aim of the study is to compare and evaluate the effect of ZrO_2 NP and TiO_2 NP on the surface roughness of heat cured poly-methyl methacrylate (PMMA).

Materials and Methods: Previously silane TiO_2 NP was mixed with heat cure PMMA polymer with the help of Twin-Screw Extruder. A homogenous mixture of PMMA polymer reinforced with 2% TiO_2 NP was made. Similar procedure was done using ZrO_2 NP. 90 wax patterns of dimension 10mm diameter & 2mm thickness were prepared using a metal die for surface roughness measurement which was further divided into three groups: GROUP 1-PMMA; GROUP 2- TiO_2 NP and PMMA; GROUP 3- ZrO_2 NP and PMMA. Excess material was trimmed with acrylic bur, and the surface of the test specimens was standardized by wet grinding with silicon carbide papers and polished with pumice polishing disc, rinsed with tap water, and air dried. Followed by measurement of surface roughness of the samples using a Surface roughness tester 3200.

Results: The highest mean surface roughness was found to be of Group 2 (TiO_2 NP + PMMA), $0.4095 \mu\text{m}$ with the standard deviation of 0.02871 while the lowest mean surface roughness was found to be of Group 1 PMMA group ($0.2278 \mu\text{m}$ with standard deviation of $.07769$), while the mean surface roughness of ZrO_2 NP + PMMA group was found to be $0.3503 \mu\text{m}$ with standard deviation of $.04627$. Statistically significant difference in surface roughness was found between all three groups.

Conclusion: Within the limitations of the study, it can be concluded that addition of TiO_2 NP and ZrO_2 NP brings about significant changes in surface roughness of PMMA. Addition of TiO_2 NP causes greater change in surface roughness in PMMA when compared to ZrO_2 NP.

Keywords: ZrO_2 , TiO_2 , PMMA, Nanoparticles, Surface Roughness, surface properties, denture base resins

Introduction

Poly-methyl methacrylate (PMMA), a common biomaterial, has been used for several decades as denture base resin, because of its biocompatibility, relative lack of toxicity, and excellent esthetic appearance. However, polymeric products do not show optimum mechanical strength and biologic properties. Thus, there is a need to improve the mechanical properties of PMMA for better clinical performance. Nanotechnology has recently been used in the prosthodontic field for material enhancement purposes. Nanofillers in the form of Nanoparticles (NP) are added in order to improve the properties of PMMA. Recently, zirconium oxide NP (ZrO_2 NP) and titanium dioxide NP (TiO_2 NP) have been added in PMMA and studied. Incorporating ZrO_2 NP and TiO_2 NP into PMMA has shown to improve PMMA properties.^[1,2] Incorporating ZrO_2 NP in PMMA has shown to increase the impact strength, flexural strength, compressive strength, fatigue strength, fracture toughness and hardness of PMMA.^[3,4] Similarly, it has been found that on adding TiO_2 NP, the flexure strength, fracture toughness, hardness, as well as thermal conductivity of PMMA increases significantly. In addition to this, a significant increase in impact strength and a significant decrease in water sorption and solubility was found.^[5,6]

Amongst the various biomechanical properties surface roughness is a property of clinical significance. A smooth and highly polished surface is desired to prevent microbial and plaque accumulation. Previous studies have shown the accumulation of plaque and microbial adhesion in roughened acrylic surfaces. The surface roughness of 0.2 μm for acrylic resins has been shown to be clinically acceptable.

Even though several studies have reported the effect of addition of TiO_2 NP and ZrO_2 NP on surface roughness of PMMA individually, a comparative evaluation with ZrO_2 NP or TiO_2 NP enforcements has not yet been reported. Thus, the need of the study was felt. The null hypothesis of the current study was that no significant difference in the surface roughness of PMMA would be found on addition of TiO_2 NP and ZrO_2 NP. The aim of the current study was to compare and evaluate the effect of ZrO_2 NP (2%wt of 30-50 nm) and TiO_2 NP (2%wt of 30-50 nm) on surface roughness of heat cured PMMA, using a Surface roughness tester 3200.

Material and Methods

Even coating of the surface of the TiO_2 NP (Nano Research Lab, Gopalpur, Jharkhand) was done with silane coupling agent (MONOBOND-S, Ivoclar Vivadent) following which TiO_2 NP was dissolved in acetone in the jar for the purpose of wetting (Fig 1). The silaned NP were then incorporated into the PMMA polymer. 10 grams of the silaned TiO_2 NP of 30 - 50 nm diameter (Nano Research Lab, Gopalpur, Jharkhand) was mixed with 500 grams heat cure PMMA polymer [DPI-Dental Product of India] with the help of Twin-Screw Extruder (Fig 2). Thus, a homogenous mixture of 510 grams of PMMA polymer reinforced with 2% TiO_2 NP was made. Similar procedure of silanization and incorporation into the PMMA polymer was done using ZrO_2 NP. Similar procedure was done for ZrO_2 NP of 30 -50 nm diameter.

To fabricate the samples, wax patterns of dimension 10mm diameter & 2mm thickness were prepared using a metal die. 90 such wax patterns were prepared for surface roughness measurement. All the wax patterns were invested in dental flask using dental plaster in two stage pour technique, where 3 wax patterns were invested in each flask (Fig 4). This was followed by dewaxing and packing. The packing was done according to the groups. 90 dewaxed flasks were equally divided in 3 groups where 30 samples were packed with PMMA for Group 1 (Fig 8), 30 samples were packed with TiO_2 NP reinforced PMMA for Group 2 (Fig 9) and 30 samples were packed with ZrO_2 NP reinforced PMMA for Group 3 (Fig 10). For each of the groups, the respective polymer was mixed with the monomer in a ratio as per the manufacturer's instructions in a clean and dry porcelain mixing jar for 30 seconds. When it reached dough stage, it was packed into the mold space and the flasks were closed and kept under pressure of 2000 psi for bench curing for 30 minutes. Once the bench curing was done, the flasks were clamped and polymerized using short curing cycle of 74°C for 90 minutes and then boiled for 1 hour. The flasks were then allowed to bench cool to room temperature and the specimens were retrieved. Excess material was trimmed with acrylic bur, and the surface of the test specimens was standardized by wet grinding with silicon carbide papers (400, 600, and 800 and 1200 grit) and polished with pumice polishing disc, rinsed with tap water, and air dried. All procedures were done by one investigator. All the samples were visually inspected by one researcher. Samples which were damaged/fractured/ incompletely fabricated, dimensionally incorrect, with voids or any other surface defects were eliminated. All the selected specimens were stored in distilled water at room temperature for 1 week.

The sample size was calculated using Openepi software based previous study. The sample size for each group was calculated at 95% confidence interval at 80% power using the sample size formula. Thus, a total of 30 samples was made from each group for measuring surface roughness with the help of surface analyzer instrument, Surface Roughness Tester 3200 at (ITS engineering college, Greater Noida, Uttar Pradesh). Three readings for each sample were taken and a mean of three readings for each sample was recorded (Fig 3). One sample from each group was randomly selected for SEM. For this purpose, it was painted with a gold/palladium film layer using 10 -1 hPa/Pa combustion room pressure and a current of 10 mA for 180 s in the gold/palladium coating unit (Sputter Coater SC7620, Polaron, VG Microtech, Uckfield, UK). SEM (Nova Nanosem 450, American Company) images were obtained with magnifications of 10000x, 20000x and 50000x. The specimens were evaluated with SEM not only to examine the surface irregularities and other defects but also to observe the incorporation of fillers in polymer matrix. Data obtained was tabulated in Excel sheet and the mean and standard deviation was calculated for all three parameters separately and analyzed by ANOVA test ($p=0.001$). The data was coded and entered into Microsoft Excel spreadsheet. Analysis was done using SPSS version 20 (IBM SPSS Statistics Inc, Chicago, Illinois, USA) Windows software program. Descriptive statistics included computation of means and standard deviations. The Kruskal Wallis test (ANOVA) [for quantitative data within three groups] was used for quantitative data comparison of all parameters followed by post hoc test (Turkey HSD test). Level of significance was set at $P \leq 0.05$.



Fig 1: TiO_2 NP and ZrO_2 NP



Fig 2: Twin screw extruder



Fig 3: Surface Roughness Tester 3200



Fig 4: Wax patten invested in in flask



Fig 5: Thirty wax pattens of control group for fabricating surface roughness specimens.

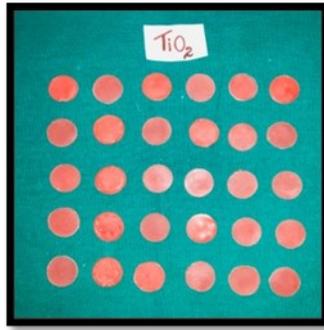


Fig 6: Thirty wax pattens of titanium oxide group for fabricating surface roughness specimens.

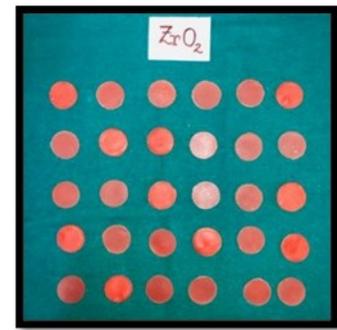


Fig 7: Thirty wax pattens of zirconium oxide group for fabricating surface roughness specimens.

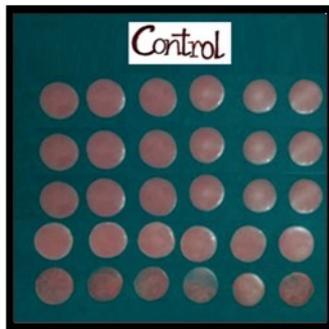


Fig 8: Thirty samples of control group for fabricating surface roughness specimens.

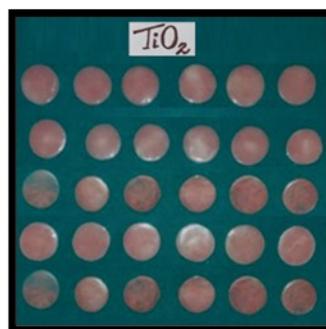


Fig 9: Thirty samples of titanium oxide group for fabricating surface roughness specimens.

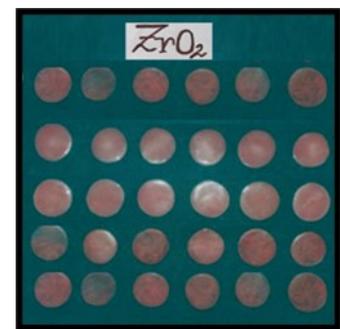


Fig 10 : Thirty samples of zirconium oxide group for fabricating surface roughness specimens.

Results:

The highest mean surface roughness was found to be of Group 2 (TiO₂ NP + PMMA), 0.4095 μm with the standard deviation of 0.02871 while the lowest mean surface roughness was found to be of Group 1 PMMA group i.e., 0.2278 μm with standard deviation of .07769, while the mean surface roughness of ZrO₂ NP + PMMA group was found to be 0.3503μm with standard deviation of .04627(Fig11). Statistically significant difference in surface roughness was found between all three groups (Table:1).

SEM Analysis

The surface morphology of PMMA group showed even distribution of PMMA particles, spherical shape and narrow size distribution (Fig12).The surface morphology of sample containing TiO₂ NP PMMA showed even distribution of TiO₂ NPs in the PMMA matrix and absence of any agglomeration of TiO₂ NP (Fig13). Similarly, the surface morphology of the sample containing ZrO₂ NP PMMA group showed even distribution of ZrO₂ NP in the PMMA matrix, and absence of any agglomeration of ZrO₂ NP (Fig14).

Descriptive analysis of Surface Roughness values of the samples.

Groups	N	Mean	Std. Deviation
Control	30	.2278	.07769
ZIRCONIUM OXIDE+PMMA	30	.3503	.04627
TITANIUM OXIDE+PMMA	30	.4095	.02871
Total	90	.3292	.09339

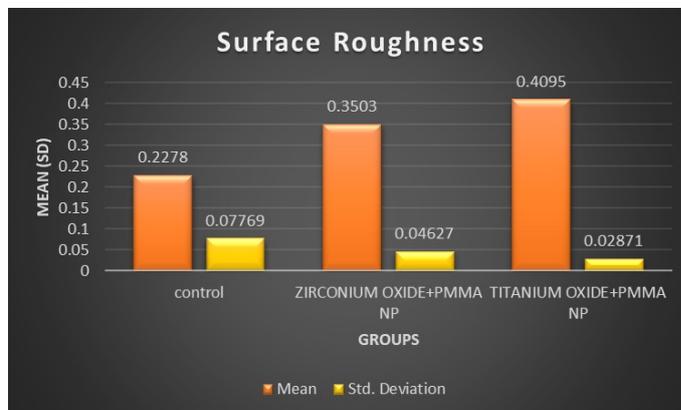


Fig 11: Mean value and standard deviation of the surface roughness values of Control group ZrO₂ NANOPARTICLES+ PMMA group and TiO₂ NANOPARTICLES+ PMMA group.

Table: 1

Multiple Comparisons of mean surface roughness between different groups (Post hoc Turkey's HSD test)									
Dependent Variable: Surface Roughness									
Tukey HSD									
(I) Groups	(J) Groups	Mean Difference (I -J)	Std. Error	P value	95% Confidence Interval for mean		Minimum	Maximum	ANOVA F value
					Lower Bound	Upper Bound			
Control	ZrO ₂ NANOPARTICLES+PMMA	-.12250*	.01414	.000	-.1562	-.0888	.2672	.4272	85.836
	TiO ₂ Nanoparticles+PMMA	-.18167*	.01414	.000	-.2154	-.1479	.3555	.4559	
ZrO ₂ NANOPARTICLES+PMMA	Control	.12250*	.01414	.000	.0888	.1562	.0947	.3541	
	TiO ₂ Nanoparticles+PMMA	-.05917*	.01414	.000	-.0929	-.0254	.3555	.4559	
TiO ₂ Nanoparticles+PMMA	Control	.18167*	.01414	.000	.1479	.2154	.0947	.3541	
	ZrO ₂ Nanoparticles+PMMA	-.05917*	.01414	.000	-.0254	-.0929	.2672	.4272	

*. The mean difference is significant at the 0.05 level.

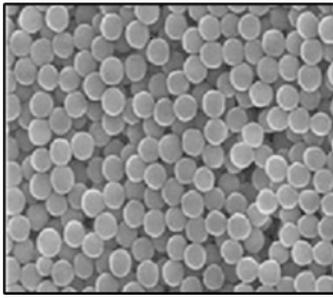


Fig: 12(A)

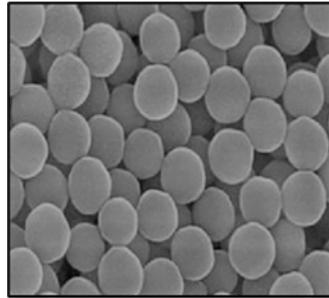


Fig: 12(B)

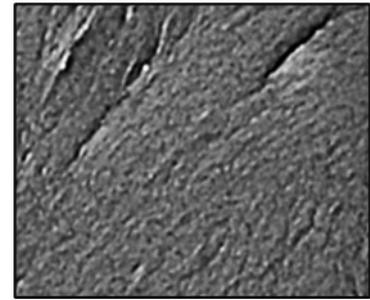


Fig: 12 (C)

Fig 12 A: Surface morphology of PMMA :Even distribution of PMMA particles,spherical shape and narrow size distribution. Image under 20000x A magnifications, HV = 2000 kv, Width 10mm

Fig 12 B: Surface morphology of PMMA : Even distribution of PMMA particles,spherical shape and narrow size distribution. Image under 50000x A magnifications, HV = 2000 kv, Width 10mm

Fig 12 C: Surface morphology of PMMA :Image under 10000x A magnifications, HV = 2000 kv, Width 10mm

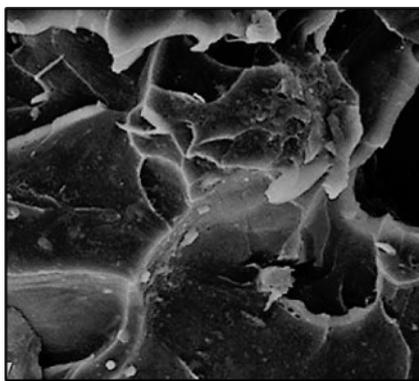


Fig:13(A)

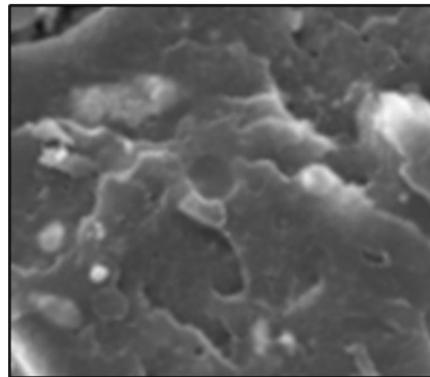


Fig: 13 (B)

Fig 13 A: Surface morphology of PMMA + ZrO₂ nanoparticles: Surface changes ascribable to the presence of 2% ZrO₂ nanoparticles in heat polymerized acrylic resin. Image under 2000x A magnifications, HV = 2000 kv, Width 10mm

Fig 13 B: Surface morphology of PMMA + ZrO₂ nanoparticles: Surface changes ascribable to the presence of 2% ZrO₂ Nanoparticles in heat polymerized acrylic resin. Image under 5000x A magnifications, HV = 2000 kv, Width 10mm

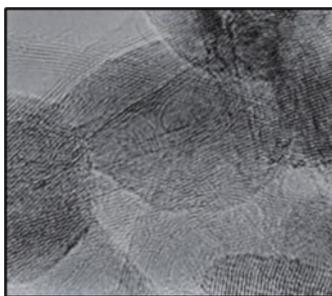


Fig: 14(A)

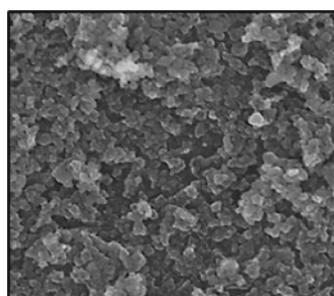


Fig: 14(B)

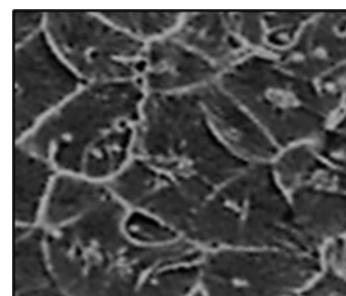


Fig: 14(C)

Fig 14 A: TiO₂ nanoparticles in seen in PMMA resin. Image under 2000000x A magnifications, HV = 2000 kv, Width 10mm

Fig 14 B: Even distribution of 2% TiO₂ nanoparticles with PMMA particles, absence of any agglomeration of TiO₂ Nanoparticles Image under 50000x A magnifications, HV = 2000 kv, Width 10mm

Fig 14 C: Surface morphology of PMMA: Surface changes ascribable to the presence of 2% TiO₂ Nanoparticles in heat polymerized acrylic resin. . Image under 2000x A magnifications, HV = 2000 kv, Width 10mm.

Discussion

Denture base surface properties are of clinical significance, as they directly affect the longevity of the denture. Surface roughness as one of the surface properties of denture base materials has been investigated utilizing different in-vitro methods. According to ISO 20795-1, the critical Ra of PMMA denture base resin that is clinically acceptable should not be above 0.2 μm . An increase of roughness above this value leads to accumulation of bacteria.^[7] However, the aforementioned studies were performed on artificial materials such as cellulose acetate. Thus, direct comparisons between surface roughness data of different studies have to be thoughtfully considered due to differences in methodology and settings of surface analysis along with tested surfaces. Furthermore, comparison of roughness values obtained using a contact profilometer cannot be compared to values obtained with the non-contact optical interferometers. The current study used a contact type of profilometer, which uses a selected micro area with high spacial resolution and does not create any marks or distortion on the samples. Surface Roughness Tester 3200 has a diamond stylus which determines the surface roughness of the samples. With this contact-type surface roughness instruments, a stylus tip makes direct contact with the surface of a sample. The detector tip is equipped with a stylus tip, which traces the surface of the sample and electrically detects the vertical motion of the stylus. It electrically measures the Ra in microns.

ZrO₂ NP and TiO₂ NP when added to denture base resins have shown to significantly improve certain properties like flexural strength, impact strength of PMMA, however, on addition of these particles the comparative effect on surface roughness is not known. The current study was undertaken to evaluate the effect on the surface roughness on addition of two different types of NP in PMMA. Several studies have shown that on incorporating ZrO₂ NP in PMMA, significant improvement in mechanical properties such as impact strength, fracture toughness, flexural strength, compressive strength, fatigue strength, hardness and surface roughness.^[8,9] Various studies have been previously done with different percentages of ZrO₂ NP (i.e. 1%, 2%, 3.5%, 4%, 5% etc.) and it has been shown that as the concentration of the NP increased, the mechanical properties improved. However, percentages between 1 – 5 % by weight has been stated to be most appropriate as percentages above 7 % leads to massive changes in color of the acrylic. Thus, in the current study, 2% of ZrO₂ NP was used^[10-14]. Similarly, addition of TiO₂ NP into acrylic resin, has showing to improve the physical and mechanical properties of the resulting hybrid material. TiO₂ NP remains one of the preferred alternatives because of their ease of availability, low toxicity, chemical stability, robust physical properties, antibacterial activity, and cost-effectiveness. Reinforcements with TiO₂ NP has shown to improve mechanical as well as physiochemical properties of PMMA when added at very low concentrations.^[15,12,13] Thus, the second NP selected in the current study was TiO₂ NP.

To improve the bond strength ZrO₂ NP and TiO₂ NP with PMMA, the use of silane coupling agent has been recommended^[5,10,16]. Moreover, properties like hardness increases and surface roughness increases when silaned ZrO₂ NP and TiO₂ NP were incorporated in acrylic resin, while apparent porosity, water sorption, and solubility decreased^[8,9]. Thus, in the current study, silaned ZrO₂ NP and TiO₂ NP was incorporated in PMMA polymer.

In the current study the Ra value of PMMA was found to be within acceptable value with mean being 0.2278 μm , however, the mean Ra of both the groups (ZrO₂ NP+ PMMA=0.3503 μm and TiO₂ NP+ PMMA=0.4095 μm) was higher. The Ra values of Group 2 and 3 being higher than acceptable limits. The results of our study are similar to previous studies. Ergun G et al showed an increase in the Ra on addition of ZrO₂ NP in different percentages. Aljafery and Mah showed the surface roughness of 2% ZrO₂ – Al₂O₃ NP was significantly increased when compared to the control group.^[10] Moreover, many have shown increase in the Ra values is proportional to the concentration of NP.^[5] In agreement with the previous studies, the observed difference in Ra in the different groups may be due to different microstructural characteristics of materials and the form of the particles added.^[5]

As the surface gets rougher the Ra values increase resulting in more sites for microbial adhesions and colonization's like *candida albicans*^[5,6,10] Thus, even though on addition of ZrO₂ NP and TiO₂ improvement in certain mechanical properties is observed, the surface roughness of PMMA also increases. Thus, additional methods to improve the surface roughness of PMMA must be researched and recommended.

The ratio of the NP used should be at a rate that can be well distributed within the resin matrix and does not disrupt the continuity of the resin matrix.^[5] Use of higher percentages of addition of NP has shown to form agglomerates affecting the properties. The SEM micrographs in the current study showed excellent surface characteristic of the three samples. Moreover, no big agglomeration of these NP was observed, indicating a uniform particle distribution. The SEM analysis showed the ZrO₂ NP and TiO₂ NP in the polymer matrix are uniformly filled with NP as desired for improving the mechanical properties.^[17-20]

While interpreting the results of this investigation, one should take the limitations of the study into consideration, which includes it in vitro setting that does not simulate the oral cavity.^[21] Thus, the direct implications of the results must be exercised with caution.^[22] However, in this experimental study, the use of standardized experimental conditions was as advantage. Long-term studies are recommended for further evaluation of correlating the surface roughness and microbial adhesion in ZrO₂ NP and TiO₂ NP.

Conclusion

Within limitations of the current study, it can be concluded that:

- The mean surface roughness TiO₂ nanoparticles+ PMMA group was the highest (0.4095 μm) when compared to the other groups, while the control group showed the lowest surface roughness of 0.2278 μm.
- Statistically significant difference in the surface roughness of heat cured PMMA was observed on addition of ZrO₂ NP and TiO₂ NP.

Conflict of Interest

There is no conflict of interest in the present study.

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