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The Medical Study of Denture Base Resin Poly(Methyl Methacrylate) Reinforced by ZnO and TCP Nanoparticles

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Abstract

Denture base poly (methyl methacrylate (PMMA) resin is one of the most frequently used materials in denture base synthesis, but due to its poor mechanical properties, PMMA can be considered a medium for the attachment and growth of a variety of pathogenic bacteria and fungi, particularly due to PMMA's pores and rough surface. The porosity percentage and surface roughness of the PMMA resin sample was lowered in this study, which resulted in a reduction in microorganisms' surface adhesion by varying the ratios of additives such as zinc oxide (ZnO) and tri-calcium phosphate (TCP) nanoparticles with (1, 2, 3, and 10% wt percent) for each additive separately, and 3% as a combination of ZnO and TCP nanoparticles in an equal ratio. Additionally, mechanical features such as surface hardness are developed, which is a critical attribute for polishing and easy finishing, as well as offering great scratch resistance during denture base cleaning. These results indicated that when compared to the other groups, PMMA (ZnO wt. 1%) and TCP-wt. 1%) reinforced composite resins demonstrated the best optimum properties. Additionally, it was discovered that adding 1% of NPs improved the mechanical qualities, which benefited the biological properties by reducing bacterial adherence to the PMMA composite resin.

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1. Introduction

Nanotechnology is the next generation of wealth creation since it has a positive influence on the economy. Nanotechnology has recently been integrated into a variety of aspects of life, including hydrogen fuel cells, medical, solar cells, electronics, food, energy, and the environment [1], [2]. The materials employed in nanotechnology have been subjected to a variety of physical and chemical techniques to achieve desirable features such as increased efficacy, small size [3], and extensive surface area [4]. As a result of its unique physicochemical features in biological applications, metallic NPs have been active in extensive research studies, methodologies, and advanced micro-and nanotechnology applications [5]. Since 1937, acrylic resins have been the most frequently utilized material in the fabrication of dentures [6]. This material has several advantages: it is simple to polish, has an acceptable appearance, is stable in the mouth, is reasonably priced, is easy to fabricate, and is lightweight [7] [8]. Increased material hardness results in reduced abrasion which offers strong resistance to scratching during prosthodontics cleaning and hence a longer service life for components exposed to severe frictional stress [9]. The

early causes of oral disorders including tooth decay and gingivitis are the adherence of certain microbes to certain surfaces in the mouth and the production of dental plaque on teeth and restorative materials [10], [11]. Many factors influence the quality and the amount of bacterial colonization on a given surface. As an example, bacterial adhesion is increased by rough surfaces, high free surface energy [12], and surface porosity. The surface porosity of the biomaterial surface seems to enhance adhesion and shield bacteria from antibiotics and the host's immune systems [13]. The interaction of nanoparticles with certain materials results in nanoparticle aggregation on the membrane's surface and blocking of the membrane's pores [14]. The primary objective of this study is to determine a correlation between surface porosity and bacterial adhesion. Additionally, to determine the optimal nanoparticle-to-biocompatible nanoparticle ratio for reducing surface porosity and thus microbial proliferation while remaining biocompatible, and determine the optimal nanoparticle-to-biocompatible nanoparticle ratio for minimizing microbial growth while remaining biocompatible. The hardness of the composite samples should be determined. Determine the composite materials' antibacterial and biocompatibility properties. By adding TCP-NP to the biocompatible acrylic resin, you can make the biomaterial more bioactive.

2. Theoretical Part

2.1. Preparation of the Dental Flask

The PMMA resin sample was made in two halves of a dental flask: the first half was filled with stone and the second half was filled with polymer clay (40403) mm in dimension. After coating the polymer clay with vaseline before pouring the stone, the second half was filled with stone and allowed overnight to dry as shown in Figure 1. The approach is based on W. A. Hussain et al., 2020) [15].

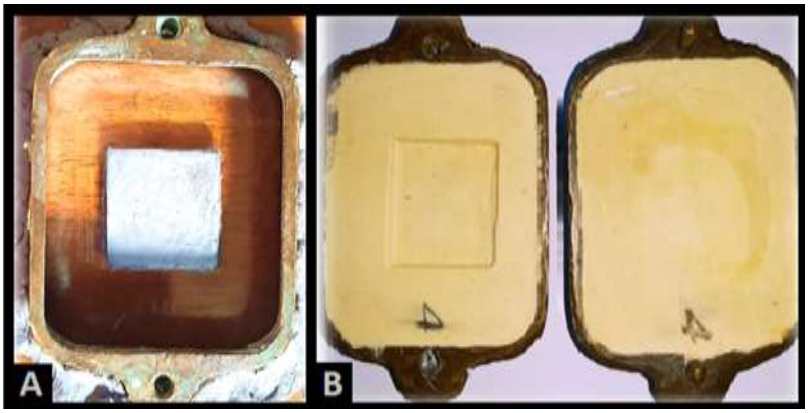

Figure 1: Preparation of the dental flask for the samples

2.2 Preparation of the Samples

According to the manufacturer's guidelines, the denture base acrylic resin PMMA (DURACRYL PLUS, produced by Spofa Dental Business in Czechia) was created. By combining the PMMA powder with the monomer in a ratio of 6 g to 2.5 mL per specimen for each TCP-NPs (FLUIDINOVA, Porto, Portugal) and ZnO-NPs (Hongwu International Group Ltd., China), we used the following ratios (1, 2, 3, and 10% wt%) for each type of nanoparticle added to the samples. Furthermore, some samples included 3 wt% nanoparticles in the form of an equal mix of TCP-NPs and ZnO-NPs. Control specimens, as stated in Table 1, were prepared without the addition of nanoparticles. Before initiating the polymerization process, hydraulic pressure was gradually increased to 1 ton using a hydraulic press (manual hydraulic press 10-ton shop press), then polymerized in a normal electric heater by immersing them in 25C water and raising the temperature until they boiled within 1 hour. After cooling, the specimens were removed from the flask [16]. Finally, all samples were polished and sliced into 16 samples (10 mm×10 mm) as shown in Figures 2 and 3.

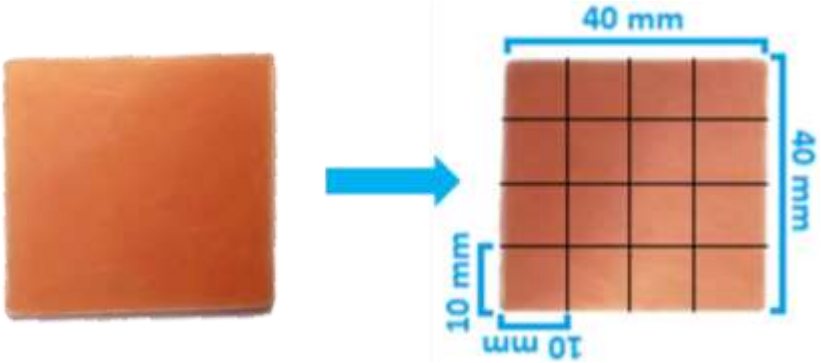

Figure 2: Each of the samples was cut the same, into 16 pieces with (10 × 10 × 3) mm dimensions.

Table 1: Quantification of the acrylic resin species.

Samples	ZnO wt%	TCP wt%	PMMA wt%
Pure PMMA	0	0	100
TCP-1	0	1	99
TCP-2	0	2	98
TCP-3	0	3	97
TCP-10	0	10	90
TZ-3	1.5	1.5	97
ZnO-1	1	0	99
ZnO-2	2	0	98
ZnO-3	3	0	97
ZnO-10	10	0	90

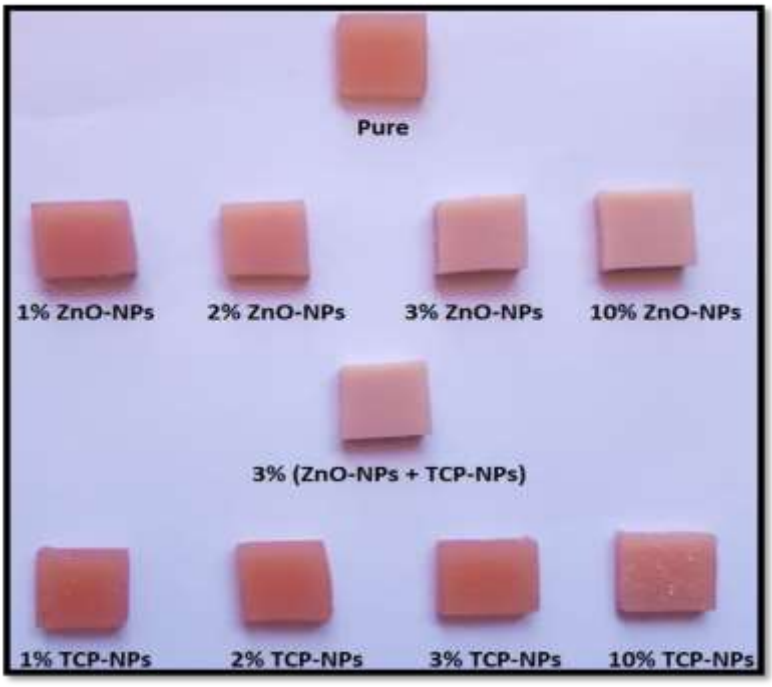

Figure 3: The form and color of the samples, which we notice in order to maintain the aesthetics of the sample after it has been reinforced with nanoparticles in various ratios.

2.3 Hardness Shore D

To evaluate the hardness of acrylic resin samples, the Shore D scale was employed. The Shore D scale is used to test hard polymers, semi-rigid plastics, and hard rubber. The test was carried out in the laboratory using the

durometer D-XD (Shore hardness tester TH210). The results were achieved after measurements of five different points in each sample.

2.4 Blood Compatibility

2.4.1 Hemolysis Assay

Our initial step is washing the samples in 70% alcohol and immersing them in 2 ml of 0.9 sodium chloride for 2 days at 25C before starting the experiment. A healthy donor donated blood, which was centrifuged to separate the red blood cells (RBC) from the plasma and then washed five times to make sure there would be no plasma remaining on the RBC. This was followed by gently mixing 1ml of RBC with 4ml of phosphate-buffered (PBS) saline, creating a 20 % v/v of RBC suspension. 0.5 mL of the sample solution was transferred to a 200 µl suspension and kept in the water bath for 90 minutes at 37 ^{o}C, then centrifuged for 4 minutes at 1000 RPM, additionally, the optical density of the filtrate solution was determined at 541 nm with the use of a spectrophotometer. In the positive control, 200 µl of the RBC suspension was added to 1 mL of water, whereas the negative control was 200 µl of the RBC suspension added to 1 mL of PBS. In parallel, three tests were conducted on each sample group. The following equation was used to determine the hemolysis ratio:

$$\boldsymbol{Hemolysis}\ (\%) = (\boldsymbol{As} - \boldsymbol{An})/(\boldsymbol{Ap} - \boldsymbol{An}) \times \mathbf{100}\% \quad (1)$$

As, An, and Ap are the absorbance of the sample, the negative control, and the positive control, respectively. To create a blood film, the sediment (RBC) was stained with Lishman stain for 2 minutes, dried, and washed with distilled water. The blood film was studied under a 40x magnification light microscope [17][18].

2.4.2 Prothrombin Time (PT) and The International Normalized Ratio (INR) Assay

After the blood was taken from a healthy volunteer, it was placed into tubes with sodium citrate and gently mixed, centrifuged, and then 180 µl of plasma was mixed with 20 µl of the samples' solution. Then 50 µl of this mixture was applied to a semi-automated device (from Human Company, Germany) until the temperature reached 37 ^{o}C, then 100 µl of thromboplastin liquid was applied, and finally, the results of PT and INR were recorded. As a control group in the PT and INR analyses, 0.9 sodium chloride was used. Each sample group was examined three times in rapid succession [18].

2.5 The apparent porosity ratio

Microbe adherence in PMMA is directly affected by porosity; therefore, increasing the apparent porosity will enhance microorganism adhesion [19]. Archimedes' concept was used to determine the apparent porosity. This approach is based on the weight change of the samples when they are wet and dry, as well as the weight of the samples when they are submerged in water. The apparent porosity of the samples was determined using the following equation:

$$\boldsymbol{A.P.} = (\boldsymbol{S} - \boldsymbol{D})/(\boldsymbol{S} - \boldsymbol{I}) \times \mathbf{100}\% \quad (2)$$

Where D is the dray sample weight, S is the saturated sample weight and I is the immersed sample weight [20].

2.6 Microorganism Adhesion to PMMA-NPs' Surface

Microorganisms adhering to certain surfaces in the human oral cavity and the creation of dental plaque on these surfaces are the fundamental causes of a variety of oral disorders, which may lead to other health issues [21]. Klebsiella pneumoniae, gram-negative bacteria, and Staphylococcus aureus, gram-positive bacteria, were taken from Yarmouk Teaching Hospital patients, cultivated on MacConkey agar, isolated, and stained with gram stain, and identified using VITEK® 2. To begin the experiment, stock solutions of 1.48×10^{8} cells/ml of bacteria were prepared in normal saline. For 15 minutes, all of the samples were sterilized in an autoclave. Under sterilizing conditions, 10 µl of the stock was applied to the surface of each sample and incubated for 4 hours. Following three washes with 0.9 percent sodium chloride to remove non-adherent bacteria, the samples were centrifuged for 20 minutes at 4000 RPM with 1ml of 0.9 percent sodium chloride to release adherent bacteria from the sample surface into the solution. 50 µl of the solution was then cultured using the spread plate method on MacConkey Agar for *Klebsiella pneumoniae* and nutrient agar for *Staphylococcus aureus*. As a control, we utilized a 100% PMMA sample.

3. Results and Discussion

3.1 Hardness Shore D

Because of the enhanced dispersion and interaction with PMMA, the acrylic resin reinforced with nanoparticles exhibited a much better Shore-D hardness value than the nano particle-free sample. In conclusion, TCP-NPs and ZnO-NPs bonded with PMMA resulted in acrylic nanocomposites with outstanding mechanical characteristics. As a result, it was noticed that the hardness progressively rises as the proportion of nanoparticles increases and that the hardness of acrylic resin reinforced by TCP-NPs is greater than that of acrylic resin reinforced by ZnO-NPs at the same ratios. Furthermore, TCP-NPs have a greater capacity than ZnO-NPs to enhance the hardness of the resin alloy, because synthetic TCP possesses hardness comparable to that of real teeth, its insertion into dental base composites increases the composite's hardness. However, as compared to pure acrylic resin, ZnO-NPs still contribute significant hardness characteristics, which grow progressively as the ZnO-NPs ratio in the acrylic resin samples increases. As seen in Figure 4, NPs had a considerable influence on increasing the hardness value, which resulted in the highest hardness (90.9 N/mm2) in the 10% TCP-NPs sample, compared to the other samples.

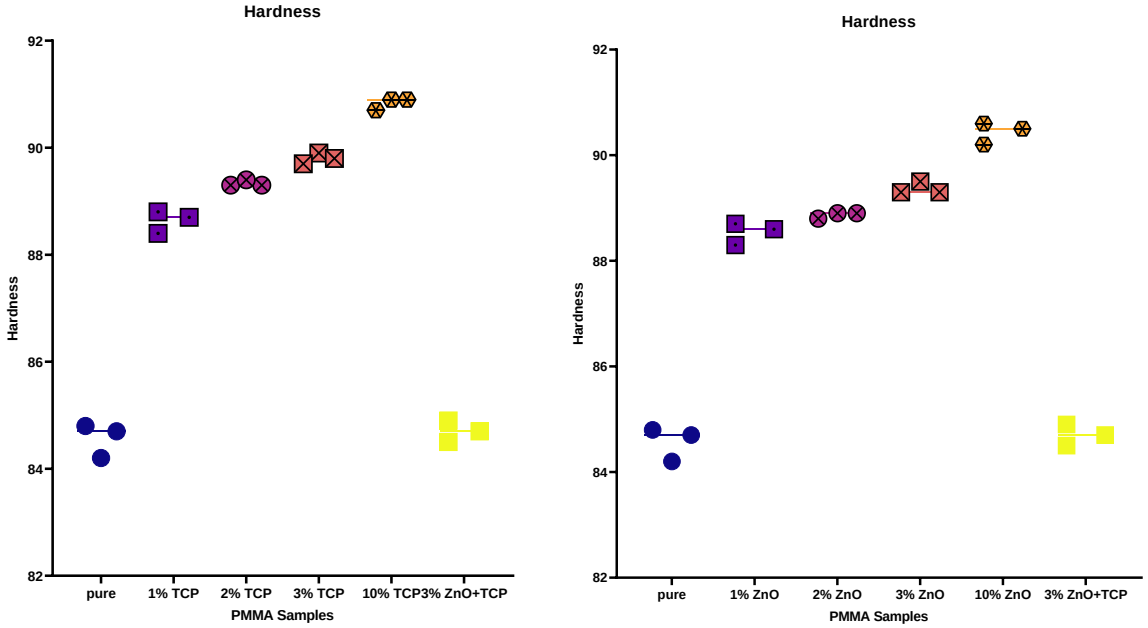

Figure 4: The hardness of the samples increases linearly with the rise in the nanoparticles ratios.

3.2 Blood Compatibility Assays (In vitro)

3.2.1. Hemolysis Assay

This study aimed to create viable PMMA composite resins for clinical applications that would come into direct contact with human blood and tissue. If the blood compatibility performance criteria of the composite materials are not met, it not only impacts their clinical use in the oral area but also poses significant hazards to the patient's health. A variety of PMMA resin composite samples were tested for blood compatibility, including hemolysis, prothrombin time (PT), and the International Normalized Ratio (INR). Compared to a positive control that induced 100 percent hemolysis of RBCs, the lower concentration ranges of PMMA-NPs composites had no detrimental effect on RBC morphology. Most hemolysis occurred in a composite sample containing 10 wt% ZnO NPs, although even then it was less than 5%. This study shows that nanoparticle composites are very compatible with the blood when used at low concentrations (Figure 5), and according to this study, they might be used in a variety of applications, including toxicology.

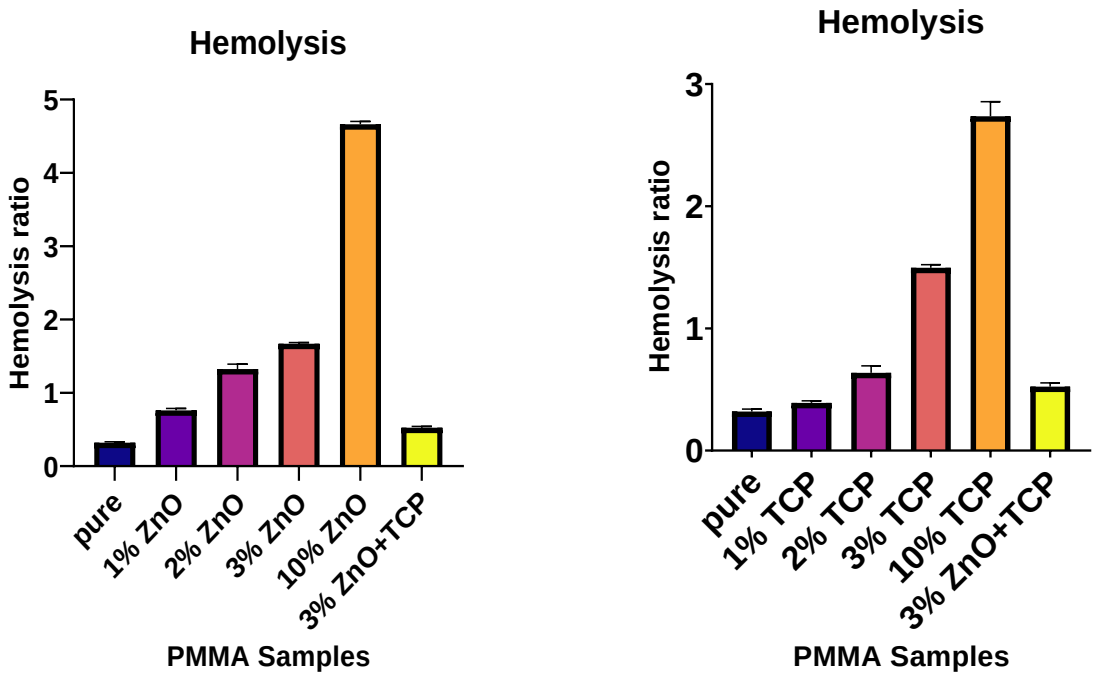

Figure 5: Images of the proportion of blood hemolysis in human RBCs after incubation with various amounts of sample suspension (A) PMMA-ZnO-NPs composite samples (B) PMMA-TCP-NPs composite samples.

3.2.2. Prothrombin Time (PT) and International Normalized Ratio (INR) Assay

PT and INR are typical plasma coagulation test measures [20]. Under the experimental conditions, PT and INR readings are adversely affected by high ratios of nanoparticles in PMMA-TCP and ZnO composites. They are not recommended for use in clinical applications; samples with lower ratios are preferred, as shown in Figures 6 and 7. Those results are comparable to the ones of (S. Chen et al., 2019) [18] who investigated the influence of a TiO2-NPs composite on PT values.

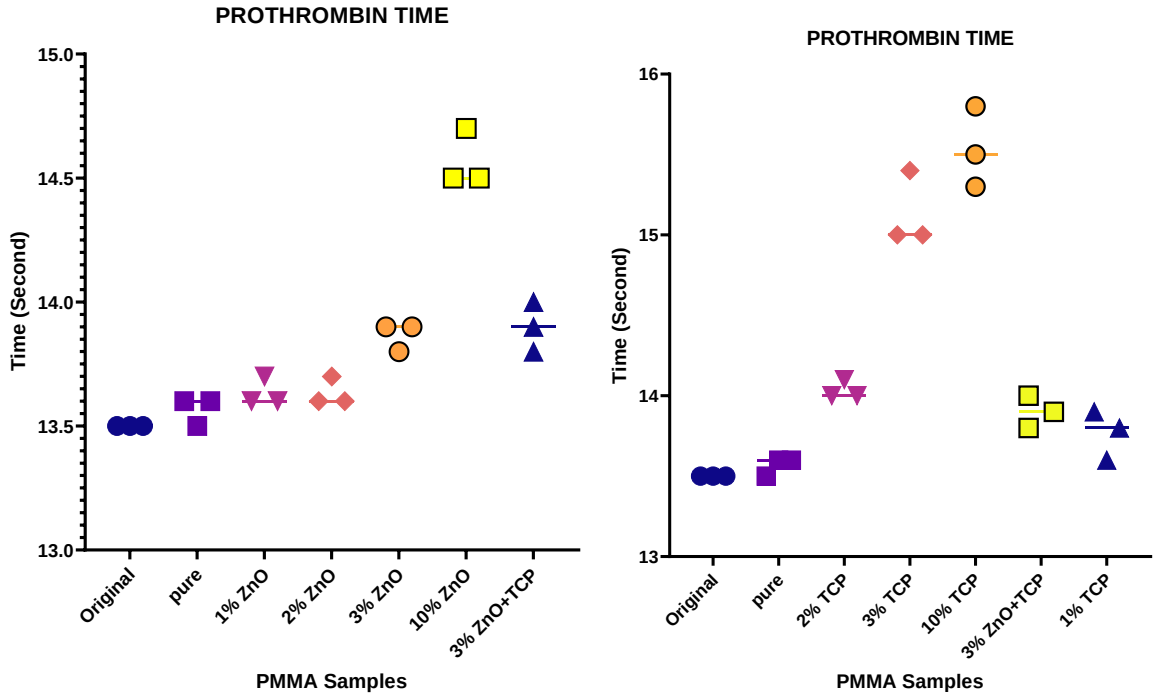

Figure 6: When compared to samples with a low concentration, samples with a high concentration have a significant influence on the analytical findings.

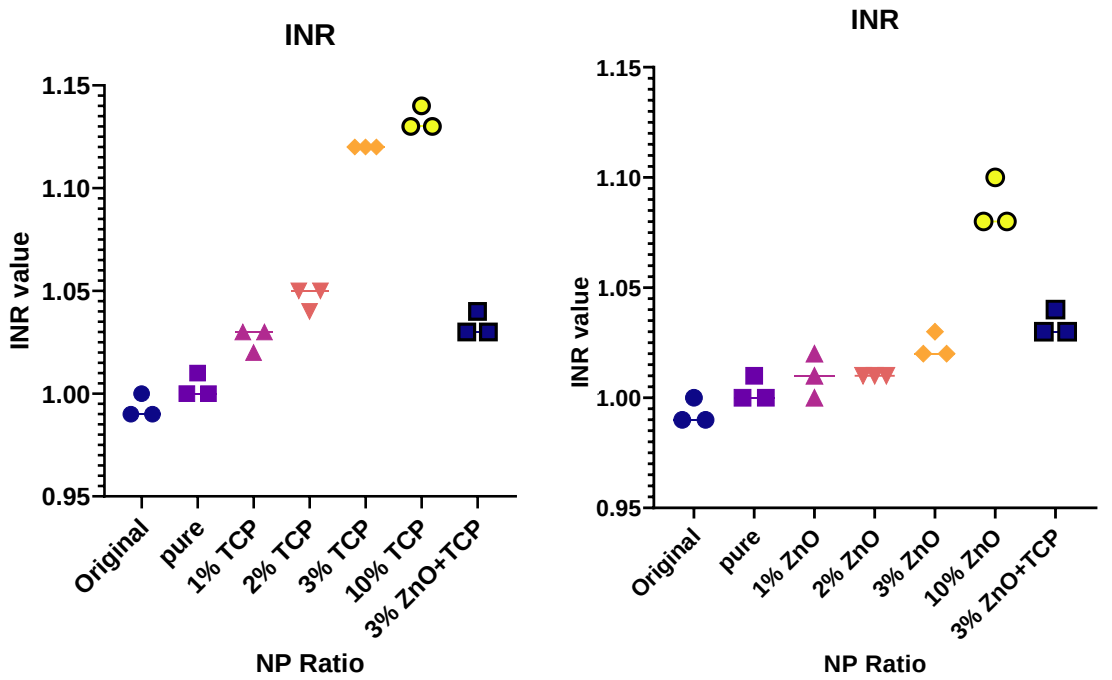

Figure 7: In comparison to low-concentration samples, samples with a high concentration have a considerable effect on the analytical results.

3.3 Apparent Porosity Ratio

Reduced porosity and surface roughness are critical variables in limiting bacteria's adherence to the surface. As shown in this research, the continual addition of nanoparticles to PMMA results in a steady reduction in surface porosity, as compared to the nano particle-free PMMA utilized as a control. (Figure 8). The lowest percentage of porosity occurs in 10% (nanoparticles wt%) samples, particularly 10% TCP samples.

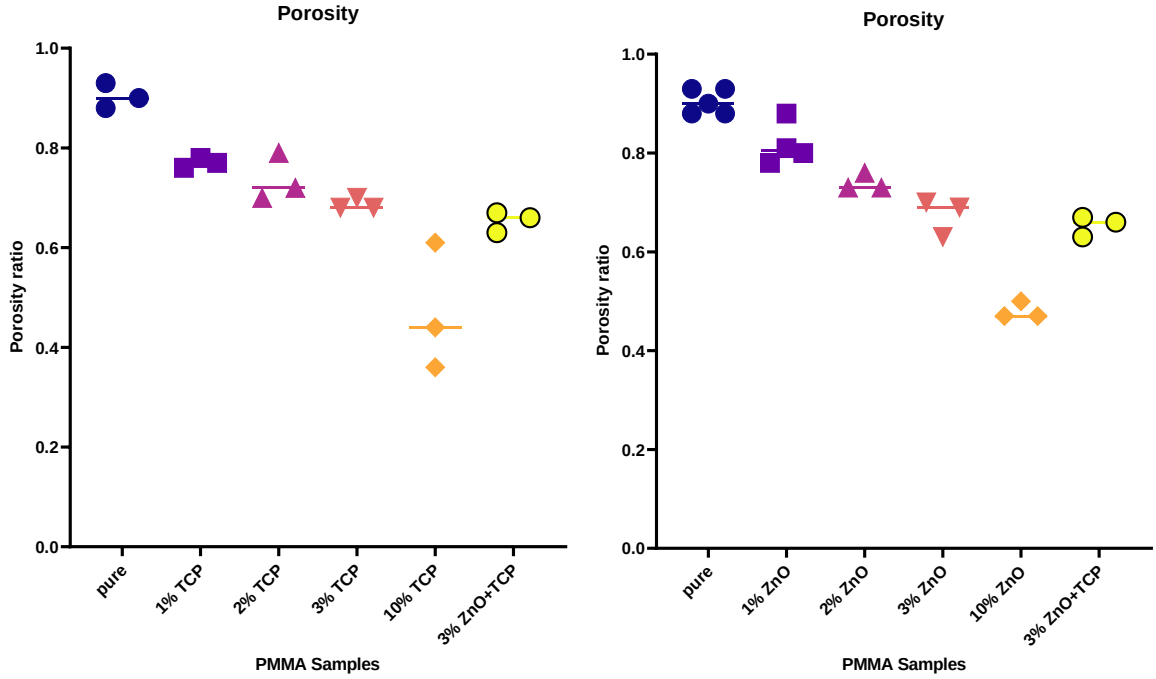

Figure 8: In this diagram, you can see how the apparent porosity of the samples changes as more TCP-NPs and ZnO-NPs are added to them.

3.4 Microorganism Adhesion to PMMA-NPs' Surface

Next, bacterial adherence to samples' surfaces was examined to detect the potential of bacteria to reduce biofilm development. The area available for adhesion is greatly increased when the surface is roughened (Quirynen & Bollen 1995) [22]. The addition of TCP-NPs and ZnO-NPs to PMMA samples resulted in a substantial decrease in apparent porosity on the surface of the acrylic resin PMMA, which considerably reduced bacterial adhesion on the membrane.

Figure 9: This graph illustrates a reduction in the number of S. aureus adhering colonies to the surfaces of samples reinforced with certain ratios of nanoparticles in comparison to a PMMA-free nanoparticles sample that has been used as a control.

Figure 10: The graph depicts a decrease in the number of klebsiella pneumoniae colonies on the surfaces of samples reinforced with various ratios of nanoparticles in comparison to a PMMA-free nanoparticles control sample.

4. Conclusions

The following findings might be derived within the restrictions of the current study: (1) The hardness value of the PMMA specimens was increased by increasing the nanoparticles ratio. (2) PMMA-TCP and ZnO nanoparticles composite have a slight effect on blood hemolysis, however, all samples were within normal range. (3) In clinical

applications, it is not recommended to utilize PMMA-TCP and ZnO composites with large ratios of nanoparticles since they have a significant impact on PT and INR values; samples with 1 and 2 wt% of nanoparticles ratios are more recommended for usage. (4) Increasing the nanoparticles ratio lowered the apparent porosity of the samples. (5) A decrease in apparent porosity was associated with a reduction in bacterial adhesion.

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Conflict of Interest
There are no potential conflicts of interest with this manuscript's publishing.

References
[1] R. Wennersten, J. Fidler, and A. Spitsyna, "Nanotechnology: A New Technological Revolution in the 21st Century," in *Handbook of Performability Engineering*, Springer, London, 2008, pp. 943–952.
[2] N. Ali, A. Taha, and D. Ahmed, "Characterization of Treated Multi-Walled Carbon Nanotubes and Antibacterial Properties," *J. Appl. Sci. Nanotechnol.*, vol. 1, no. 2, pp. 1–9, 2021, doi: 10.53293/jasn.2021.11636.
[3] Z. Khedaer, D. Ahmed, and S. Al-Jawad, "Investigation of Morphological, Optical, and Antibacterial Properties of Hybrid ZnO-MWCNT Prepared by Sol-gel," *J. Appl. Sci. Nanotechnol.*, vol. 1, no. 2, pp. 66–77, 2021, doi: 10.53293/jasn.2021.11634.
[4] R. Saleh, O. Salman, and M. Dawood, "Physical Investigations of Titanium Dioxide Nanorods Film Prepared by Hydrothermal Technique," *J. Appl. Sci. Nanotechnol.*, vol. 1, no. 3, pp. 32–41, 2021, doi: 10.53293/jasn.2021.3513.1024.
[5] A. Jawad, Q. Thewaini, and S. Al-Musawi, "Cytotoxicity Effect and Antibacterial Activity of Al2O3 Nanoparticles Activity against Streptococcus Pyogenes and Proteus Vulgaris," *J. Appl. Sci. Nanotechnol.*, vol. 1, no. 3, pp. 42–50, 2021, doi: 10.53293/jasn.2021.3944.1061.
[6] H. Sharhan, Z. Rasheed, and J. Oleiwi, "Synthesis and Physical Characterization of PMMA/PP and PMMA/PAN Composites for Denture Applications," *J. Appl. Sci. Nanotechnol.*, vol. 1, no. 3, pp. 13–23, 2021, doi: 10.53293/jasn.2021.3759.1040.
[7] S. A. Ibrahim, S. H. Lafta, and W. A. Hussain, "Impact strength of surface treated SS316L wires reinforced PMMA **," vol. 30, pp. 272–278, 2021.
[8] W. A. Hussain, S. M. H. Al-Jawad, and S. A. Hannon, "Effect of carbon fibre layer with alumina and tri calcium phosphate on mechanical properties of denture base," *Int. J. Nano Biomater.*, vol. 10, no. 1, pp. 22–33, 2021, doi: 10.1504/IJNBM.2021.114691.
[9] A. O. Alhareb, H. M. Akil, and Z. A. Ahmad, "Impact strength, fracture toughness and hardness improvement of PMMA denture base through addition of nitrile rubber/ceramic fillers," *Saudi J. Dent. Res.*, vol. 8, no. 1–2, pp. 26–34, 2017, doi: 10.1016/j.sjdr.2016.04.004.
[10] S. A. Mosaddad *et al.*, "Oral microbial biofilms: an update," *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 38, no. 11. Springer, pp. 2005–2019, Aug. 01, 2019, doi: 10.1007/s10096-019-03641-9.
[11] W. H. Bowen, R. A. Burne, H. Wu, and H. Koo, "Oral Biofilms: Pathogens, Matrix, and Polymicrobial Interactions in Microenvironments," *Trends Microbiol.*, vol. 26, no. 3, pp. 229–242, Mar. 2018, doi: 10.1016/J.TIM.2017.09.008.
[12] M. Aziznezhad *et al.*, "Comparison of the effect of resin infiltrant, fluoride varnish, and nano-hydroxy apatite paste on surface hardness and streptococcus mutans adhesion to artificial enamel lesions," *Electron. physician*, vol. 9, no. 3, pp. 3934–3942, 2017, doi: 10.19082/3934.
[13] L. Montanaro, C. R. Arciola, A. B. Adhesion, and P. Infection, "to Irregular or Porous Surfaces," in *Handbook 0/ Bacterial Adhesion: Principles, Methods, and Applications*, R. J. An Y. H. and Friedman, Ed. Bologna:

Humana Press, Totowa, NJ, 2000, pp. 331–332.

[14] S. Bandehali, F. Parvizian, A. R. Moghadassi, S. M. Hosseini, and J. N. Shen, "Fabrication of thin film-PEI nanofiltration membrane with promoted separation performances: Cr, Pb and Cu ions removal from water," *J. Polym. Res.*, vol. 27, no. 4, 2020, doi: 10.1007/s10965-020-02056-x.

[15] W. A. Hussain, B. A. M. Bader, M. Y. Slewa, and L. H. Alwan, "Impact and flexural strength of kaolinite / glass fibers reinforced heat-cured acrylic denture," *Mater. Sci. Forum*, vol. 1002, no. July, pp. 340–349, 2020, doi: 10.4028/www.scientific.net/MSF.1002.340.

[16] S. Y. Taher and W. A. Hussain, "The effect of acidic treatment of carbon fiber on denture mechanical properties," *J. Phys. Conf. Ser.*, vol. 1879, no. 3, pp. 1–10, 2021, doi: 10.1088/1742-6596/1879/3/032082.

[17] M. H. Nematollahi, M. Torkzadeh-Mahanai, A. Pardakhty, H. A. Ebrahimi Meimand, and G. Asadikaram, "Ternary complex of plasmid DNA with NLS-Mu-Mu protein and cationic niosome for biocompatible and efficient gene delivery: a comparative study with protamine and lipofectamine," *Artif. Cells, Nanomedicine Biotechnol.*, vol. 46, no. 8, pp. 1781–1791, 2018, doi: 10.1080/21691401.2017.1392316.

[18] S. Chen, J. Yang, Y. Jia, and B. Lu, "TiO 2 and PEEK Reinforced 3D Printing PMMA Composite Resin for Dental Denture Base Applications," *nanomaterials*, vol. 9, no. 7, pp. 1–18, 2019.

[19] R. Giti, K. Zomorodian, M. Firouzmandi, Z. Zareshahrabadi, and S. Rahmannasab, "Antimicrobial Activity of Thermocycled Polymethyl Methacrylate Resin Reinforced with Titanium Dioxide and Copper Oxide Nanoparticles," *Int. J. Dent.*, vol. 2021, 2021, doi: 10.1155/2021/6690806.

[20] A. A. Jock, F. A. Ayeni, A. S. Ahmed, and U. A. Sullayman, "Evaluation of the Refractory Properties of Nigerian Ozanagogo Clay Deposit," *J. Miner. Mater. Charact. Eng.*, vol. 01, no. 06, pp. 321–325, 2013, doi: 10.4236/jmmce.2013.16048.

[21] L. Costa de Medeiros Dantas, J. Paulo da Silva-Neto, T. Souza Dantas, L. Zago Naves, F. Domingues das Neves, and A. Soares da Mota, "Bacterial adhesion and surface roughness for different clinical techniques for acrylic polymethyl methacrylate," *Int. J. Dent.*, vol. 2016, pp. 1–6, 2016.

[22] M. Quirynen and C. M. L. Bollen, "The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man," *J. Clin. Periodontol.*, vol. 22, no. 1, pp. 1–14, Jan. 1995, doi: 10.1111/J.1600-051X.1995.TB01765.X.