Aya A. Shaher [1]
Rand B. Lutfi [2]
Shaimaa A. Naser [1]
Haneen A. Basim [1]
Ghufran F. Hasan [1]

[1] Nanotechnology and Advanced Materials Research Center, University of Technology, Baghdad, IRAQ
[2] Department of Physics, College of Education, Al-Iraqia University, Baghdad, IRAQ

Impact of Al_2O_3-PSA on Biological and Mechanical Properties of Soft Acrylic used in Medical Applications

Among orthopedic surgery materials, poly(methylmethacrylate) (PMMA) is most commonly used for its excellent mechanical properties and rapid self-setting time. However, PMMA has been a major hurdle, namely, their poor antimicrobial (i.e., adhesion) properties, can accelerate infections and cause bacterial colonization and to have poor bioactivity. The powders of Aluminum oxide (Al_2O_3), peach kernal seed (PKS) with particles size in a range of (73 nm) and (9.5 nm), respectivly, were added to PMMA cement. Their influence on FTIR, compression strength, hardness, wettability, antibacterial and cytotoxicity properties of PMMA was assessed. The result showed a considerably improvement in the values of these properties for both groups of bio composite specimens comparing with neat PMMA. All bio composite specimens reinforced with peach kernal seeds PKS powder in nanometre size showed the highest properties as compared with the bio composite specimens strengthened by nano Aluminium oxide Al_2O_3.

Keywords: Nanofillers; Soft acrylic resin; Biocomposites; Biomaterials
Received: 21 January 2025; **Revised:** 18 March 2025; **Accepted:** 25 March 2025

1. Introdication

The biomaterial has not been around for a very long time. Nonbiological compounds were first introduced into the human body thousands of years ago [1]. The majority of biomaterials are already accessible for use in the development of these materials, either individually or in combination. these materials have various atomic congurations, resulting in a wide range of structural, physical, chemical, and mechanical properties, as well as a wide range of potential applications in the body [2,3]. Poly(methylmethacrylate) (PMMA) is a synthetic amorphous linear polymer commenly used as biomaterials, derived from methyl methacrylate (MMA) ($C_5H_8O_2$) by a polymerization process, and it is often used as a biomedical material. It is a transparent and thermoplastic. PMMA is regarded as a preferable material for a number of biomedical applications because of its high processability, handling characteristics, and biocompatibility on the consideration of its satisfactory mechanical properties, ease of manufacturing process, good appearance, biocompatibility, low toxicity, and low cost[4,5] . A major limitation It lacks antimicrobial properties and is prone to microbial infection [6,7]. Biofilms easily colonize PMMA due to the absence of ionic charges, and the presence of electrostatic and hydrophobic interactions [8], while microbes with hydrophobic cell surface have been found to favor biofilm formation in hydrophobic material surfaces like PMMA [9][10]. Various works have been carried out in order to enhance the properties of PMMA by integrating particles into the host matrix. A number of studies have reached the conclusion that the biological and mechanical properties of PMMA material can be improved by using several types of particles introduced into the resin matrices. This is a common method used to enhance composite mechanical characteristics [4]. The various component elements retain some of their original identities, but they combine and provide their greatest qualities to the finished material's properties. These composite materials are stronger and more durable. In relation to this work, composites contain polymer matrices reinforced with natural and synthitic materials that are of the particulate variety. Composites made of natural materials include cotton, flax, jute, sisal, and hemp, whereas those made of synthetic materials include carbon, glass, etc. The agricultural wastes are utilised as polymer reinforcement instead of synthetics reinforcement, which ha expanded since natural fibers are sustainably beneficial, non-toxc, and have a low cost with reduced weight [11,12]. Nanomaterials of various types and compositions have been exploited for different applications (e.g., antibecteriall, hemostatic and wound healing) owing to their ability to provide an enhanced surface area [5].

Among the particles that have been widely used for this strengthening function is Al_2O_3. Al_2O_3 particles have been widely applied in the field of biomaterials. These nanoparticles belong to the family of metal oxide nanomaterials and from the structural point-of-view, they are assembled as corundum-like structure in which six oxygen atoms surround one aluminium atom. Similar to the other metal oxide nanoparticles, Al_2O_3 nanoparticles can be readily handled and are easily accessible. Also, these cost-effective nanomaterials possess high surface area as

well as mechanical strength; and they have exceptional chemical stability towards high temperatures and harsh conditions such as abrasive environment. Further, they possess a low electrical conductivity [13]. Moreover, the exceptional optical properties of Al_2O_3 nanoparticles are used as a model for investigation of the properties as well as structural and electronic variations of nanomaterials. In addition, the bioinertness and easy surface functionalisation allows their use in the biological environment [14].

There are several peach kernel shell (PKS) biomass applications. In particular, data is given on the use of PKS as a concrete filler, as a substrate for growing mushrooms, and as a component of a composite material with polypropylene. Moisture, carbohydrates, ash, proteins, and fatty acids content in PKS amounts, in particular, to 27.3 ± 0.15%, 68.2 ± 0.04%, 0.46 ± 0.01%, 1.84 ± 0.01%, 2.20± 0.02% [15] . One of the ways to use PKS is the application as a bio-material to use in medical application.

2. Materials and Methods

PMMA-Al_2O_3-PKS (PTP) hybrid nanocomposite were prepared via an ultrasonic mixing method were the average size of Al_2O_3 was 73.1 nm and PKS was 9.5 nm as shown in figures (1a) and (1b). Al_2O_3 ceramic material supplied by Zhoushan Mingri Nanomaterials Co. Ltd. (China) with purity of 99% was used. The PKS natural material utilized that taken from peach fruit. These natural materials were crushed and ground by the porcelain ball mill to reach these values of the particle size, and then a ball mill was utilized to achieve nano size.

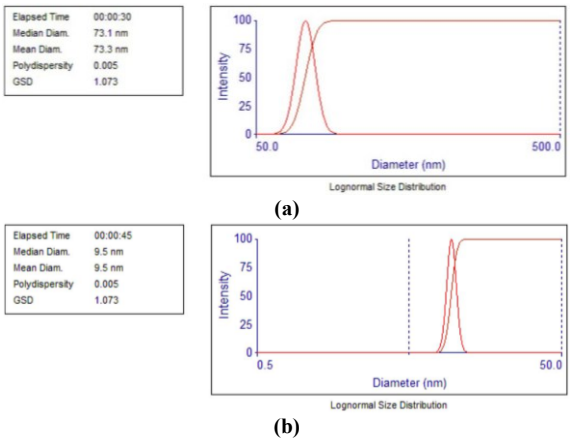


Fig. (1) Particle size analysis for (a) Al_2O_3 and, (a) PKS

The matrix material used in this study is soft acrylic which self-curing PMMA provided by MOONSAR company. This type of material characterized by many properties compared to other kind of PMMA polymer with density of 1.175 g/cm^3. This matrix composed of liquid (monomer) and powder (hardener) is blended in proportion to the percentage of a combination, which equals to 100/2 according to the manufacturer's specifications.

The natural nanoparticles treated with solution of 5% alkali (sodium hydroxide) to remove lignin, pectin, waxy substances, and natural oils covering the external surface of the material cell wall. Then, the particles washed several times with distilled water to remove excess alkali sticking on their surface, and finally washed with distilled water, after that the treated particles were dried at room temperature for 5 days and finally kept in hot air oven at 50-60 °C until dry the sieving to determine particle size.

The cast PMMA samples classified into six types (P, PA1, PA2, PA3, PP1, PP2, PP3, PAP) based on the ratio of nano-powders in PMMA matrix (table 1). The soft acrylic resin was prepared by ultrasonic mixing method at various attempt were prepared by mixing PMMA as liquid monomer with different weight percentage of each type of nano-powders (Al_2O_3 and PKS) at room temperature for 15-20 min to get homogenous mixture. After that, the PMMA powder then added to the mixture and gradually mixed. At last, the resultant solution was poured in the silicon mold and left to dry at room temperature for 7 days to ensure dry of samples. The sample then expelled from the silicon form, with an extremely smooth upper and lower surface. Afterward, they were exposed to complete the procedure to be prepared for the resulting tests. Soft acrylic resin without Al_2O_3 and PKS particles was utilized as the control cement for comparative purposes.

Table (1) Classification of prepared polymeric samples

No.	Symbol	Sample concentration
1	P	100% PMMA
2	PA1	98% PMMA- 2% Al_2O_3
3	PA2	97% PMMA -3% Al_2O_3
4	PA3	96% PMMA- 4% Al_2O_3
5	PP1	98% PMMA- 2% PKS
6	PP2	97%PMMA- 3%PKS
7	PP3	96%PMMA- 4%PKS
8	PAP	94%PMMA- 2% Al_2O_3-4%PKS

3. Characterization and Test Methods

Compressive strength of all prepared samples having dimensions of 12.5 mm in diameter and 25 mm in height were tested using a microcomputer-controlled electronic universal testing machine (WDW-5E) with 50 kN load, a test speed of 0.5 mm/min and with an average of three samples for each value by employing the following relation:

$$\sigma = F/A \quad (2)$$

Hardness is the resistance that a material exhibits to deformation that results from corrosive forces or the deepening of the surface. Five points of a sample were examined and the average of recorded data was reported as Shore A has been used to measure the hardness of the samples at room temperature based on

ASTM D2240 standard test method for shore hardness. It shows how we measure the depth of penetration of a specified type of indenter into soft materials like rubber or plastic [16].

Hydrophilicity of the reinforced specimens was conducted to determine the tangent angle of a distilled water droplet that had dropped on the surface specimen using a high-resolution camera. The test was performed with a CAM110 (Germany) device at the department of chemical engineering, University of Technology – Iraq.

The *in vitro* antibacterial activity of the reinforced specimens against *Staphylococcus aureus* was tested using agar well diffusion. This test was designed to identify antibacterial (biological) activity by measuring the inhibition zone of the reinforced specimens against varieties of bacteria. We cultured each bacterial isolate in nutritional broth at 37 °C for 18-24 hours. Placing 0.1 cc of each bacterial solution on nutritional agar at 37 °C for 24 hours after incubation. One colony was introduced to a test tube with 5 mL of normal saline to create a bacterial suspension with moderate turbidity, similar to 1.5x108 CFU/ml. Using a sterile cotton swab, bacterial suspension was carefully dispersed over Mueller-Hinton agar medium for 10 min. Agar layer has three 5-mm wells each plate. Remove the agar discs, add of prepared specimens with a diameter (6mm) to each well using a micropipette. Then incubated plates at 37 °C for 18 hours and measured the inhibitory zone diameter.

Cytotoxicity was assessed using an MTT assay at culture times ranging from 1 to 3 days. The cells were cultured and sustained in DMEM supplemented with 10% FBS "Fetal Bovine Serum, France," and 1% PSF "Antibiotic Antimycotic Solution; Sigma, USA," in a humidified incubator with an atmosphere of 5% carbon dioxide in air at 37°C. At 37°C, cells were separated utilizing 0.25% trypsin (USA) and 0.1% ethylenediaminetetraacetic acid (Germany) in phosphate-buffered saline (PBS). The specimens were placed in 24 well culture plates. Five drops (40 µl) of cultured cells were then spread to the samples with a concentration of 10000 cells/well. The microplate was then incubated for four hours after adding the MTT solution. The MTT solution was eliminated, and each well received a dose of DMSO solvent to dissolve the formazan salts. At a wavelength of 545 nm, the absorbance was measured using an ELISA reader (Stat Fax-2100, Miami, FL, USA). The outcomes were derived from the mean values of three separate, triplicate-conducted experiments. On the third day, cell viability was assessed by measuring the ratio of the sample's absorbance to that of the control. DAPI nuclear staining was conducted to investigate chondrocytes cellular activity using fluorescence microscopy.

4. Results and Discussion

The compression strength values results obtained from compression test that carried out on PMMA composite materials and hybrid reinforced PMMA composite materials for all groups specimens are discussed in Fig. (2). The relationship between the weight ratios (2, 3, and 4%) adding of natural and ceramic nanoparticles content and the compression strength of the specimens. It can be noticed that the values of compression strength increased with increasing the weight fraction of both types of particles for both groups of composite materials. This is owing to the nature of bonding strength and strengthening mechanism, which have high compression strength. Furthermore, it could be attributed to these nanoparticles ability to strengthen the resin and prevent crack propagation [17].

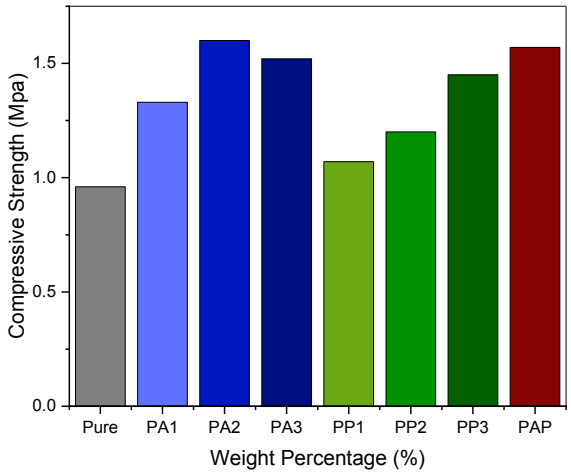


Fig. (2) Compressive strength of prepared polymeric samples

Also can be noticed in this Figure, that the values of compression strength for first group ceramic composite samples, are higher than the values of compressive strength, for second group natural composite samples. This is due to the improvement of the mechanical properties that is associated with the addition of Al_2O_3 particles, which is related to the nature of Al_2O_3 particles which are have high compression strength, comparing with PSK particles. Because the nanomaterials are consistently immersed with substantial bonding physically cross-linking (supra-molecular), inhibiting the cracks propagation within the matrix [18]. It is also worth noting that the inclusion of Al_2O_3 and PKS improved compression strength with higher results found when reinforcing with Al_2O_3 than with PKS powders. This is because Al_2O_3 and PKS powders have higher compressive strengths than PMMA polymer matrix, subsequent in the effective hybrid samples' compressive strength [19].

It is assumedthat differences in filler content, type, size, and dispersion in the polymer matrix will influence the mechanical properties of the composite

groups [20]. Figure (3) show the hardness values of bio nanocomposite specimens with an additional weight fraction of nanoparticles (2, 3 and 4%) content in the polymer matrix. The addition of nanofillers resulted in a grandul increase in hardness values as the weight fraction of these powders increased, the maximum results obtained were at (3 wt.%) for natural powders and (4 wt.%) for ceramic powders. The hardness of the modified soft acrylic resin nanocomposite groups was raised could be related to the reduction in interparticle spacing. As the particle loading in the polymer matrix increases, leading to an increase in surface resistance.

These results indicate that a higher concentration of reinforcement makes the composite surface structure more compact than lower concentration of reinforcements content, letting the surface withstand a more significant load [21]. The last behavior was due to using stiff and hard reinforcing powders and may be associated with these powders that work as obstacle to the PMMA chains motion.

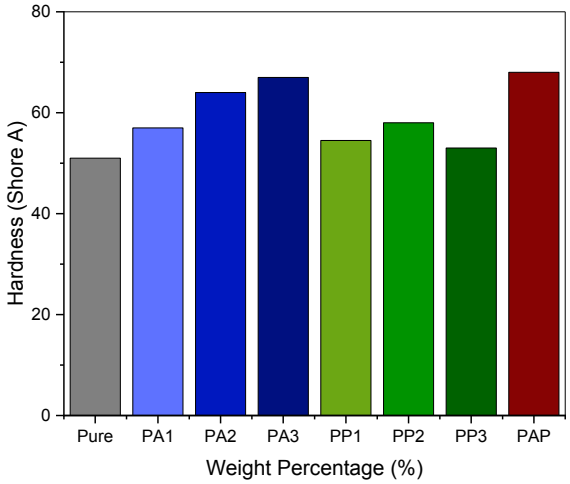
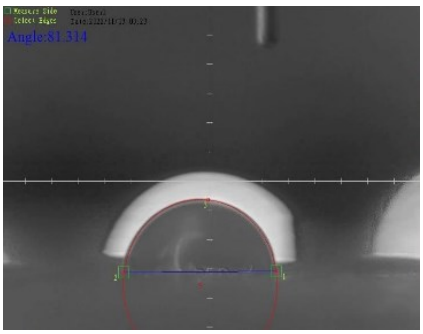
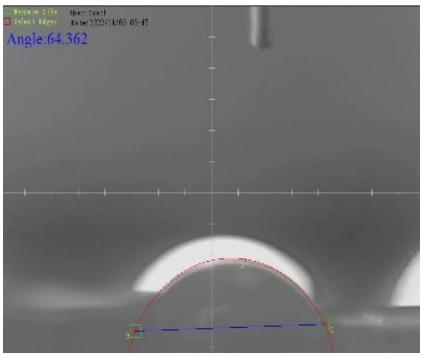


Fig. (3) Hardness (shore A) of prepared polymeric samples

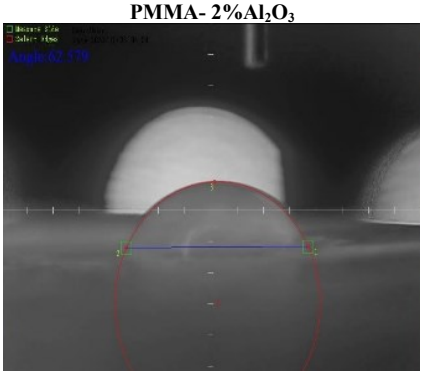
It is a most common technique to assess the surface hydrophilic and hydrophobic ability of the materials which is important for various cellular responses. Figure (4) show the contact angle measurements taken on all of the reinforced specimens (pure, composite and hybrid) after 30 seconds of a drop of water being dropped on the surface of the specimen. When the powder weight ratios in the matrix increased, the value of contact angle for the samples diminished. This behavior can be related to the strong affinity of the nanocomposites demonstrates that the inclusion of natural and ceramic powders into the matrix materials increases the specimens hydrophilic character, particularly at high concentrations. This finding shows that the presence of these additive changes the surface of the specimen and minimizes surface tension in comparison with pure PMMA specimen [22]. We discovered that the contact angle is often less than 90° and that the drop has been almost dispersed and absorbed by reinforced samples, showing that the reinforcement samples have good wettability. However, with the specimens that included PKS, the absorption was better, allowing for the drop to be dispersed more effectively [23].



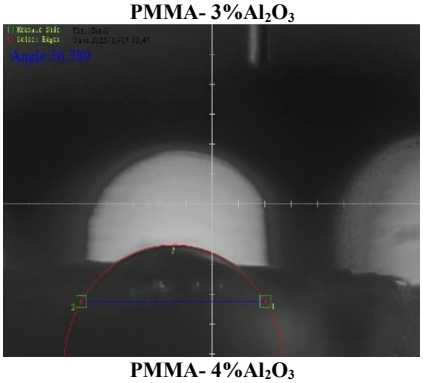
Pure



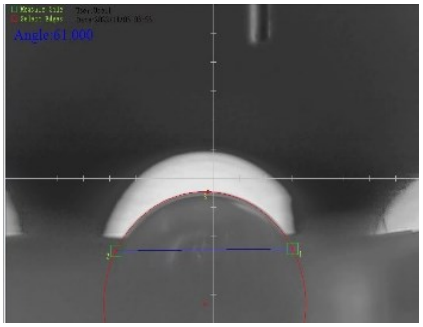
PMMA- 2%Al_2O_3



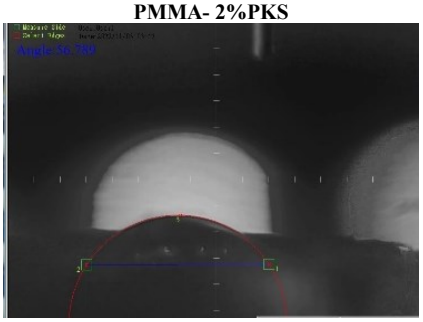
PMMA- 3%Al_2O_3



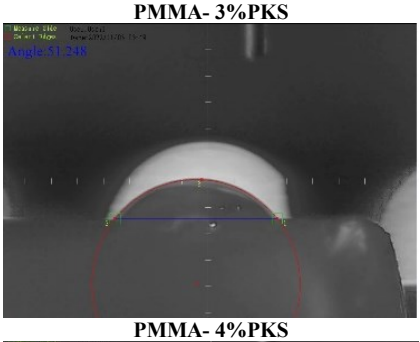
PMMA- 4%Al_2O_3



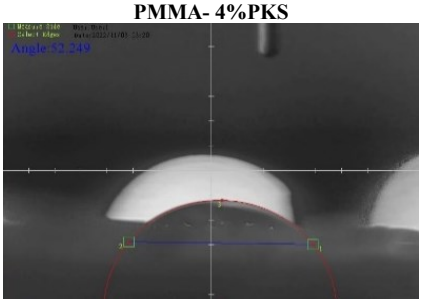
PMMA- 2%PKS



PMMA- 3%PKS



PMMA- 4%PKS

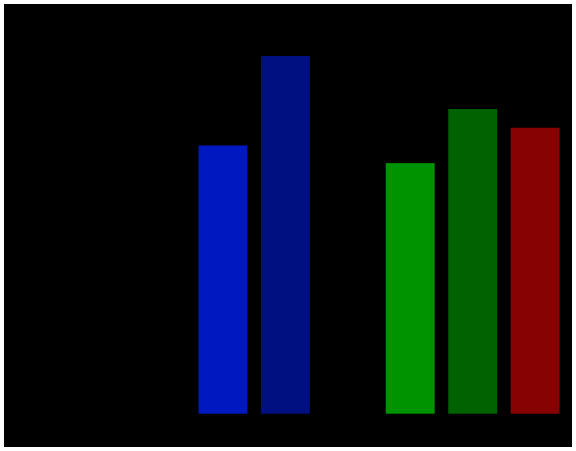


PMMA-4%Al_2O_3-4%PKS

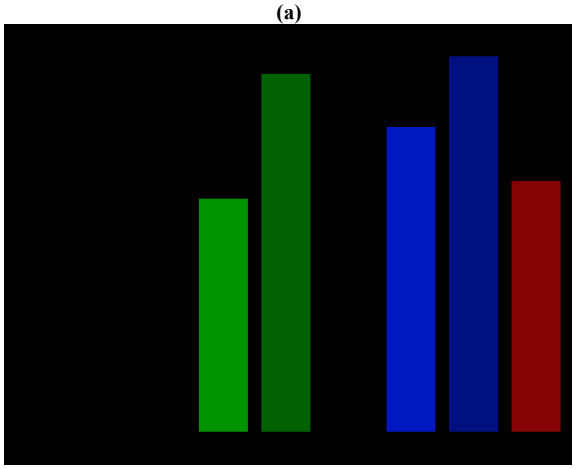
Fig. (4) Images of contact angle measurements of polymeric samples

Results of the antibacterial activity of PMMA specimens strengthened with Al_2O_3 and PKS against *Streptococcus mutans* (*S. mutans*) and *Staphylococcus aureus* (*S. aureus*) bacteria after finishing the specific incubation periods were assessed by observing the change in the inhibition zone surrounding each of the test samples in figures (5a) and (5b), respectively. In general, the introduction of nanopowders conferred a marked antibacterial activity. By comparing the viability of the biofilm unstrengthen (control) and strengthened with Al_2O_3 and PKS.

The results show that no inhibition zone was observed around pure polymer matrix and reinforced with less weight percentage of nano-fillers added. Including nanoparticles at high percentage within the polymer matrix led to the formation of an inhibition zone around each test sample towards *S. mutans* and *S. aureus* compared to pure polymer matrix. The greater inhibition zone appeared to indicate the existence of antimicrobial activities [24,25]. The antibacterial activity increased as the concentration was increased. The antibacterial activity of polymer matrix reinforced with ceramic nanoparticles higher than polymer matrix reinforced with natural nanoparticles [26].



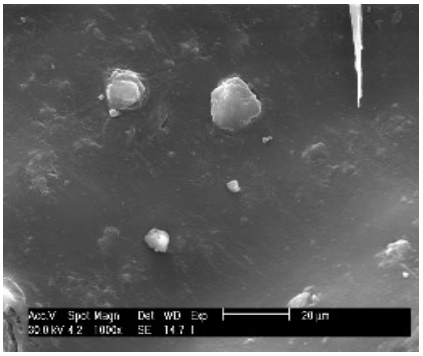
(a)



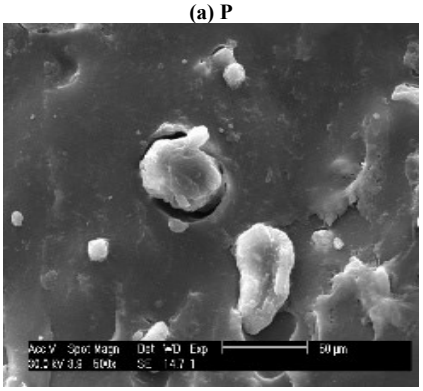
(b)

Fig. (5) Images of polymeric samples after 24 hrs against (a) *Staphylococcus aureus* and (b) *Streptococcus mutans*, respectively

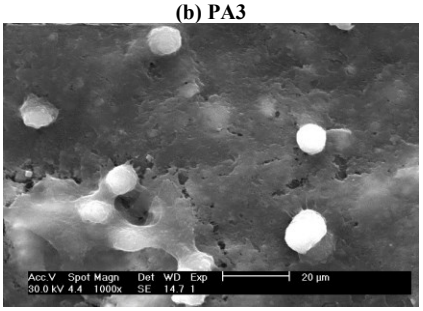
MTT analysis showed that all grafted specimens (neat sample, PA3, PP3 and PAP) supported cell proliferation after culture time. The results of SEM images, as shown in Fig. (6), demonstrate that cell viability in the grafted sample containing reinforcement materials are much higher than in the pure specimens due to the release of ions in the culture media during the dissolution of the cement [27]. Incorporation of natural and ceramic into resin matrix leads to further adhesion of the cells on the cement surface [28].



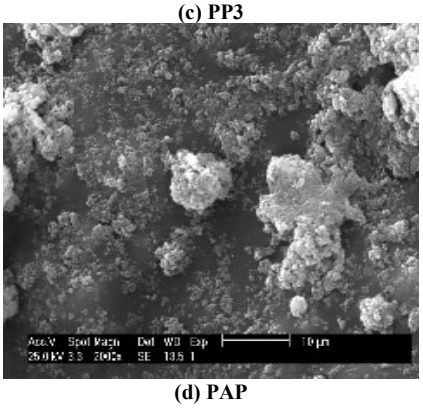
(a) P



(b) PA3



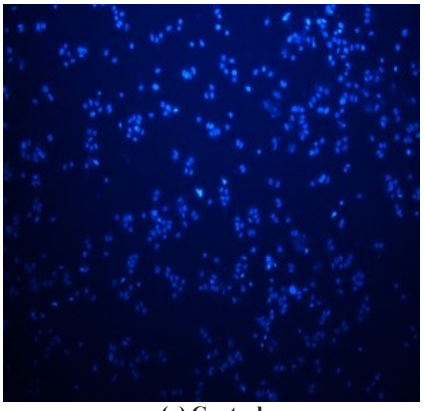
(c) PP3



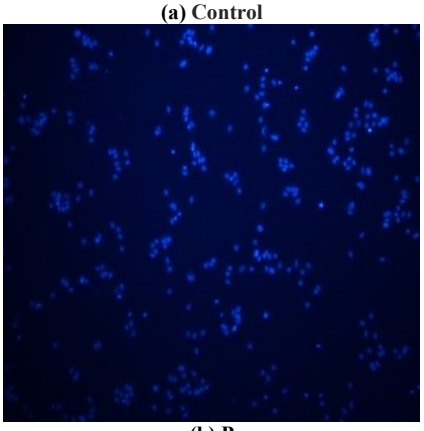
(d) PAP

Fig. (6) SEM images of chondrocytes cells cultured for 3 days on polymeric samples

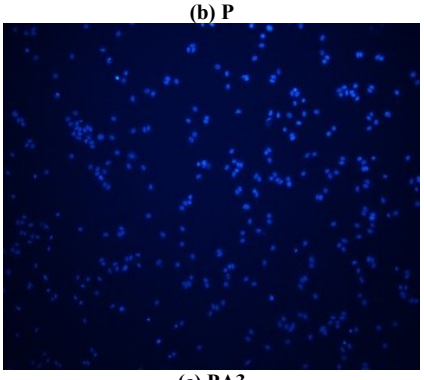
Faction and nuclei (DAPI) staining of cells was performed to examine the cellular spreading and morphology. After being seeded, DAPI nuclei staining of chondrocytes cells further confirms that a larger number of cells spread and attach on PP3 vs. pure sample in Fig. (7). Similarly, PMMA matrix containing PKS, Al_2O_3 appear able to induce cell function and accelerate cellular activity. Overall, the embedded of natural and ceramic nano-powders as the reinforcement significantly enhances bioactivity and biocompatibility.



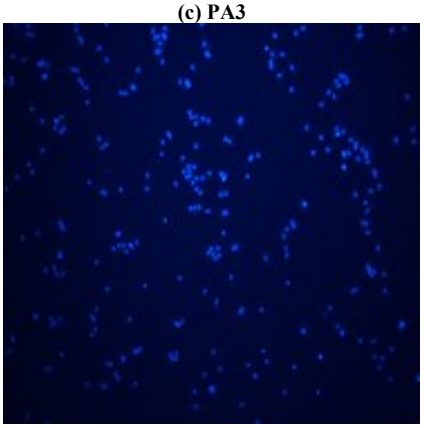
(a) Control



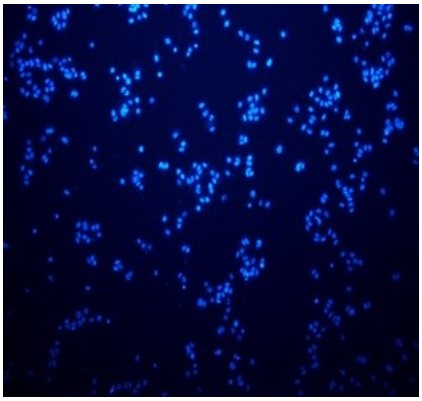
(b) P



(c) PA3



(d) PP3



(e) PAP

Fig. (7) DAPI nuclei staining of chondrocytes cells for 3 days on polymeric samples

5. Conclusion

The results of the production polymer nanocomposites with peach kernel shell (PKS) nanoparticles, and Al_2O_3 nanoparticles, it was concluded that the mechanical properties for the strengthened polymer matrix increased with increasing expansion of (PKS, Al_2O_3) nanoparticles in matrix. The most elevated estimation of compressive strength of reinforced polymer matrix from PAP samples. The hydrophobicity of strengthened polymer matrix decreased with adding the weight percentage of nanofillers (PKS, Al_2O_3). So, the PMMA transforms to hydrophilic with adding natural and ceramic fillers. The biological properties including antibacterial and MTT assay showed that the adding strengthened nano-powders into polymer matrix led to improve antibacterial activity and cytocompatibility. The outcomes show that the advancement of properties of acrylic resin (PMMA) with expansion (PKS, Al_2O_3) can be utilized in the uses of medical applications. On the basis of these results, it can be conclusion that the addition of nano fillings nanoparticles to bio PMMA material, is one of the promising materials in biomedical application.

References

[1] J.D. Bronzino, "Biomaterials", Ch. V in **"Biomedical Engineering Fundamentals"**, Taylor & Francis (FL, 2006).

[2] Q.A. Hamad, F.J. Al-Hasani and N.K. Faheed, "Comparative Study of Biotin and Hydroxyapatite on Biological Properties of Composite Coating", *Int. J. Biomater.*, 2022 (2022) 11.

[3] R.I.M. Sarx et al., "Corrosion and surface modification on biocompatible metals: A review", *Mater. Sci. Eng.*, 77 (2017) 1261–1274.

[4] M.E.P. Purnomo et al., "An Overview of Titanium Dioxide Effect on Mechanical Properties of PMMA-TiO_2 Nanocomposites", *J. Int. Dent. Med. Res.*, (2023) 1797–1803.

[5] E.B. Abdelazim et al., "*in vitro* and *in vivo* studies of Syzygium cumini- loaded electrospun PLGA/PMMA/collagen nanofibers for accelerating topical wound healing", *RSC Adv.*, 14 (101) (2024) 101–117.

[6] M. Topouzi et al., "Reinforcement of a PMMA resin for interim fixed prostheses with silica nanoparticles", *J. Mech. Behav. Biomed. Mater.*, 69 (2017) 213–222.

[7] A.N. Arf et al., "Assessment of the Antifungal Activity of PMMA-MgO and PMMA-Ag Nanocomposite", *Kurdistan J. Appl. Res.*, 9(1) (2024) 66–76.

[8] P. Protopapa et al., "Reinforcement of a PMMA resin for fixed interim prostheses with nanodiamonds", *Dent. Mater. J.*, 30(2) (2011) 222–231.

[9] S. Puri et al., "Antifungal effect of piezoelectric charges on pmma dentures", *ACS Biomater. Sci. Eng.*, 7(10) (2021) 4838–4846.

[10] A. Shahir, A. Al.Zubaidi and W. Salih, "The Influence of the Inclusion of Nano Ceramic Particles on the PMMA Composite Properties for Biomaterials Applications", *Eng. Technol. J.*, 41(12) (2023) 1–14.

[11] R.B. Lutfi and W.H. Jassim, "Fabrication natural gelcoats of (epoxy/coffee fibers) and (epoxy/cantaloupe fibers) composites with high wear and thermal resistance", *AIP Conf. Proc.*, 2769 (2023).

[12] J. Lade et al., "Characterization of Polymethyl Methacrylate (PMMA) Composites with Graphite", *E3S Web Conf.*, 430 (2023).

[13] A. Sezavar, S. Zebarjad and S. Sajjadi, "A Study on the Effect of Nano Alumina Particles on Fracture Behavior of PMMA", *Technologies*, 3(2) (2015) 94-102.

[14] P. Hassanpour et al., "Biomedical applications of aluminium oxide nanoparticles", *Micro Nano Lett.*, 13(9) (2018) 1227–1231.

[15] I.G. Shaikhiev, N.V. Kraysman and S.V. Sverguzova, "Review of Peach (Prúnus pérsica) Shell Use to Remove Pollutants from Aquatic Environments", *Biointerface Res. Appl. Chem.*, 13(5) (2023) 1–14.

[16] S. Mosalman, S. Rashahmadi and R. Hasanzadeh, "The effect of TiO_2 nanoparticles on mechanical properties of poly methyl methacrylate nanocomposites", *Int. J. Eng.*, 30(5) (2017) 807-817.

[17] S.K. Alsaedi, "Preparation and Characterization of Polymer Blend and Nano Composite Materials Based on PMMA Used for Bone Tissue Regeneration", MSc thesis, University of Technology - Iraq (2017).

[18] M. Hashem et al., "Influence of titanium oxide nanoparticles on the physical and thermomechanical behavior of polymethylmethacrylate (PMMA): A denture

base resin", *Sci. Adv. Mater.*, 9(6) (2017) 938–944.
[19] S.H. Ahmed and W.M. Salih, "Enhancement of the Characteristics for Polymeric Blend (PMMA/UP) Used in Socket Prosthetic", *AIP Conf. Proc.*, 2437 (2022).
[20] A. Alrahlah et al., "Influence of the Physical Inclusion of ZrO_2/TiO_2 Nanoparticles on Physical, Mechanical, and Morphological Characteristics of PMMA-Based Interim Restorative Material", *Biomed Res. Int.*, 2022(1) (2022) 1-11.
[21] H. Sosiati et al., "The mechanical and physical properties of microcrystalline cellulose (MCC)/sisal/PMMA hybrid composites for dental applications", *Mater. Res. Exp.*, 10(3) (2023) 035301.
[22] A.I. Martínez-Pérez et al., "Characterization and sliding wear performance of PMMA reinforced with SiO_2 nanoparticles", *J. Thermoplast. Compos. Mater.*, 33(7) (2020) 867-881.
[23] N.A.S. Aljazy, A.E.H.J. Al-mossawi and A.K. Al-rikabi, "Basrah Journal of Agricultural Sciences Study of Antibacterial Activity of some Date Seed Extracts", *Basrah J. Agricul. Sci.*, 32 (2019) 247-257.
[24] A.H. Phakatkar et al., "Novel PMMA bone cement nanocomposites containing magnesium phosphate nanosheets and hydroxyapatite nanofibers", *Mater. Sci. Eng. C*,109 (2020) 11049.
[25] T. Russo et al., "Preliminary focus on the mechanical and antibacterial activity of a PMMA-based bone cement loaded with gold nanoparticles", *Bioact. Mater.*, 2(3) (2017) 156–161.
[26] S.T. Alzayyat et al., "Antifungal Efficacy and Physical Properties of Poly(methylmethacrylate) Denture Base Material Reinforced with SiO_2 Nanoparticles", *J. Prosthodont.*, 30(6) (2021) 500–508.
[27] F. Pahlevanzadeh et al., "Development of PMMA-Mon-CNT bone cement with superior mechanical properties and favorable biological properties for use in bone-defect treatment", *Mater. Lett.*, 240 (2019) 9–12.
[28] M.M. Aljumaily et al., "Superhydrophobic nanocarbon-based membrane with antibacterial characteristics", *Biotechnol. Prog.*, 36(3) (2020).

