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RESEARCH PAPER

In Vivo and In Vitro Biological, Histopathological and Mechanical Investigations of Modified PMMA with Different Nano-additives in Rabbit Model

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ABSTRACT

This study examined the effects of reinforcing poly methyl methacrylate (PMMA) acrylic resin with silicon oxide (SiO_2) and apricot seed shell (ASS) nanoparticles using the ultrasonic mixing technique for cartilaginous joint applications. The microstructure, functioning groups, tensile strength, elastic modulus, compressive strength, hardness, hydrophilicity, in vitro cytotoxicity, cell culture, antibacterial effect, and in vivo performance of the sample with and without (SiO_2) and (ASS) reinforcements were evaluated. The experimental investigation of samples after reinforcement with one type of filler or a combination of both fillers reveals that the mechanical and biological properties were enhanced. The highest improving rates of tensile strength, modulus of elasticity, compressive strength, and hardness were (66.52%, 261.5%, 49.5%, and 41.66%) for the (PMMA-1.5% SiO_2NP) composite specimen and (97.82%, 469.23%, 86%, and 60.42%) for the (PMMA-1.5% SiO_2NP and 7.5% ASSNP) hybrid composite sample. The PMMA samples were better at attracting water after being reinforced. The antibacterial activity results against Streptococcus mutans (S. mutans) and Staphylococcus aureus (S. aureus) show that neat PMMA did not exhibit any inhibition. But by mixing nanoparticles within the matrix, bacteria growth reduces with the increased loading of nanoparticles in the composites. The experimental results of the in vivo study using a rabbit model demonstrated that the (PMMA-1.5%SiO_2-7.5%ASS) sample showed a significant response without any signs of inflammation around the surgical site compared to those obtained for a neat PMMA sample. The synthesized hybrid nanocomposite possesses excellent bioactivity and mechanical properties, promoting the attachment of chondrocyte cells compared to the PMMA neat sample.

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INTRODUCTION

Several researchers from other fields now find the biomaterials domain to be an exciting topic. The vast majority of biomaterials have been made accessible for use in the creation of these materials, either individually or in combination. Each of these materials has different atomic configurations, leading to a variety of structural, mechanical, chemical, and physical characteristics, as well as a variety of possible uses in the human body [1]. Since the early 1900s, acrylic, or polymethyl methacrylate (PMMA), has been clinically

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used in biomedical applications. Because of its biocompatible properties, PMMA was employed for hard contact lenses found by accident. PMMA is also used in dentistry as a significant material in the production of dentures. Then, "dental acrylic" is used in total hip arthroplasty surgery to cement an orthopedic prosthesis[2]. PMMA is a linear thermoplastic polymer with a backbone carbon chain, and its long chains are smooth and thin, allowing them to slide more readily together, making the material softer [3]. PMMA has several advantages, including ease of process-ability, light weightless, low fabrication cost, lack of toxicity, and high bio-stability in the human body. Nevertheless, the initial clinical outcomes were inappropriate due to insufficient biological and mechanical factors. For instance, lack of antibacterial activity, poor level of bioactivity (bio-inert material), and mechanical performance are considered drawbacks of the PMMA, which limit its clinical applications [2,4].

Numerous studies have been conducted to enhance the mechanical and biological capabilities of PMMA. Most of them involve introducing additives as reinforcing elements due to the unique characteristics of nanoscale materials that cannot be achieved with non-nano materials [5]. Researchers in many medical domains have turned to nanomaterials for their physicochemical attributes, such as ultra-small forms, high ratios of surface area to weight, and improved chemical reactivity. Because of their advanced advantages, they have been used as antibacterial agents. Because of their small size, such nanoparticles may quickly enter microbial cells and induce inhibiting mechanisms. Nanoparticles can be bactericidal by limiting their food supply or disrupting cellular membranes [6]. In addition, several recent research studies have turned to incorporating different nanomaterials to produce hybrid nanocomposites as potential approaches to enhancing PMMA's mechanical performance. Hybrid composites are made up of two or more inorganic or organic parts that work together to make a new material that is better than the originals. These parts can be inorganic-inorganic (TiO_2-Ag), organic-organic (starch-cellulose), or inorganic-organic (TiO_2-starch) [7].

Das and Biswas [8] evaluated the physical and mechanical behavior of coir fiber of various lengths and concentrations strengthened by the epoxy composite loaded with Al_2O_3 powder. The experimental investigation reveals that including 15 wt.% and 12-mm-long coir fiber enhances composite materials' mechanical strength and physical properties. Phakatkar et al. [9] prepared a nanocomposite material specimen of the PMMA resin material, including hydroxyapatite (HA) nanofibers and magnesium phosphate (MgP) nanosheets. The results demonstrated that expanding these filler mixes into the PMMA matrix increases the compressive strength, antibacterial properties, and bioactivity. 2.5% PMMA and 7.5% HA nanofibers and MgP nanosheet composite samples exhibited the best mechanical and biological performance. Barua et al. [10] researched the influence of different zinc oxide (ZnO) nanoparticle contents on the attitude of a hydroxyapatite-PMMA-based composite bone scaffold. The outcomes illustrated the best optimized biological, physical, and mechanical properties by incorporating 5% ZnO within PMMA-HA composite materials. Alsaedi et al. [11] explored upgrading the mechanical performance of a blend of acrylic bone cement with 15% PMMA strengthened by a mixture of Zr_2O and MgO nanopowders with distinctive concentrations by weight. The results reveal that the incorporation of 1.5 wt.% ZrO_2 and 1 wt.% MgO leads to improving the young modulus and tensile strength of nanocomposite specimens. Hamdi [12] studied the mechanical, physical, biocompatibility, and morphological performance of PMMA acrylic resin by adding various contents of multi-walled carbon nanotubes (MWCNT) and alumina (AL_2O_3). The results revealed that the 99% PMMA+1% Al_2O_3 specimen significantly improved the mechanical and several physical characteristics of PMMA composite samples. Ahmed and Salih [13] analysed the effect of the PMMA acrylic resin on its physical and mechanical performance when loaded with 0.3 wt.% nanoparticles walnut shell nanoparticles (WSP) and hemp fibers (HF) with a 0.5mm length at a different weight percentage. The results indicated that the hybrid polymer nanocomposite specimens made from PMMA and 0.3% WSP nanoparticles loaded with 0.9% HF had better physical and mechanical characteristics. Barapatre et al. [14] studied the effect of inserting different sorts of powder (polyetheretherketone (PEEK), zirconium oxide (ZrO_2), and hybrid) at nano-scale as support on the mechanical performance of the PMMA matrix. The study revealed the flexural strength of hybrid nanocomposite utilizing PMMA

matrix supplemented with 1.5 wt.% PEEK and 1.5 wt.% ZrO_2 nanoparticles was better than the PMMA control, PMMA/PEEK, and PMMA/ZrO_2 composite samples. Fadil and Hashim [15] investigated the effect of including nanomaterials (silicon oxide (SiO_2)- cerium oxide (CeO_2)) in ratios of 1.4, 2.8, 2.8, 4.2, and 5.6 wt.% on the antibacterial application. The results showed that the PMMA/SiO_2/CeO_2 samples have a good homogeneity distribution of SiO_2/CeO_2 nanoparticles within the polymeric mixture with good antibacterial activity. The inhibition zone diameter was observed to increase with an increase in the SiO_2/CeO_2 nanoparticle concentrations.

Based on the previous studies mentioned above, we would like to clarify that most of the researchers' work was applied to orthopedic applications, and there were no previous studies on the manufacture of articular cartilage. In this study, natural (apricot seed shell nanoparticles (ASSNP)) and ceramic (silicon dioxide nanoparticles (SiO_2NP)) were utilized to support a polymeric acrylic resin to examine its biological, morphological, and mechanical properties owing to their excellent biocompatibility, low toxicity, and capacity to be functionalized with a range of molecules and polymers.

MATERIALS AND METHODS

Soft Acrylic Resin Poly methyl methacrylate

PMMA soft acrylic resin was used as a polymer matrix material with a density of 1.18 g/cm^3 to prepare hybrid nanocomposite specimens. It was equipped by the Ortotek company in Turkey. The PMMA matrix was reinforced with two types of nanomaterials.

Particles Reinforcement Materials

Two types of nanoparticles were used as reinforcement materials. The first type was ceramic particles with selected weight fractions of 0.5, 1, and 1.5%, and the second was natural particles with selected weight fractions of 2.5, 5, and 7.5%. These particles were added to the soft

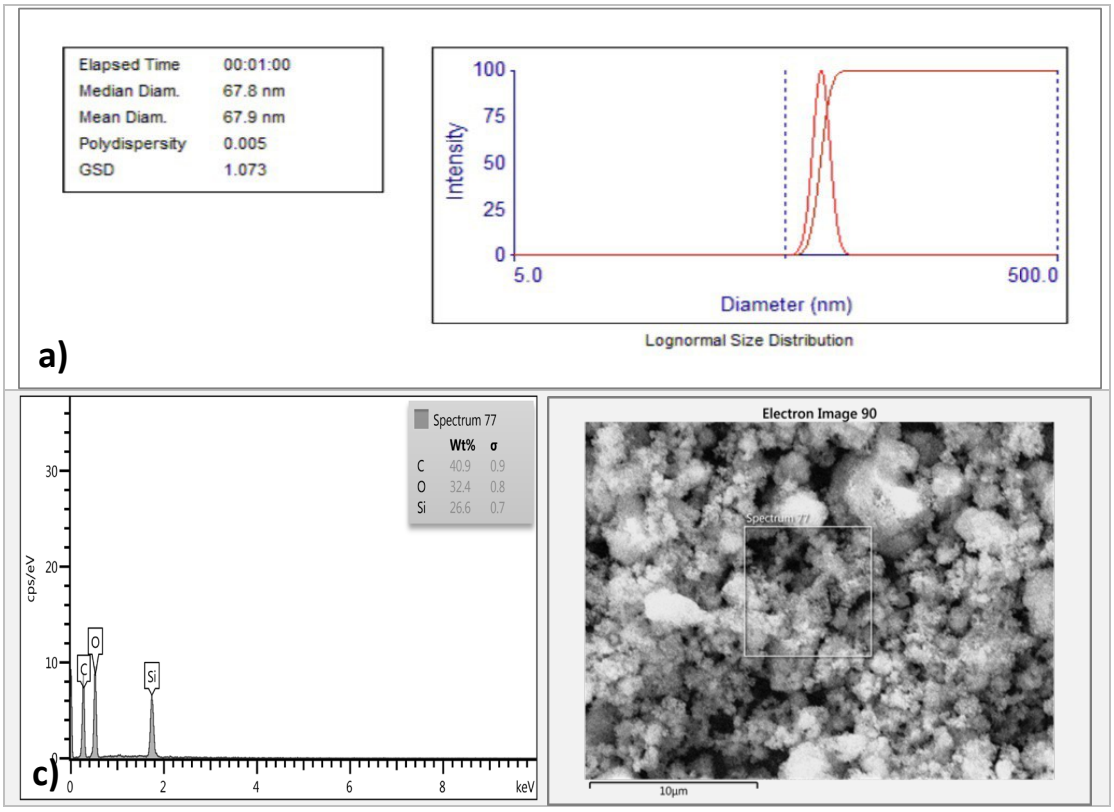

Fig. 1. Characteristics of Silicon Oxide (SiO_2) Powder Used, a(Particle Size Distribution, and b(EDS spectrum and SEM images.

acrylic resin.

Silicon dioxide Nanoparticles

The first reinforcement type was ceramic material (silicon dioxide nanoparticles (SiO_2NP)), which is a popular name for silica. It represents one of the most complicated and available classes of ceramic substances, with a density of 2.3-2.65 g/cm^3. Strong covalent bonds generate each unit of silicon dioxide. Two atoms of oxygen and one atom of silicon are given as nanopowders. Silica was purchased from Skyspring Nanomaterials, Inc., USA, with a high purity of 99.9%, outstanding resistance to chemical and thermal shock, exceptional strength, transparency, and electrical insulation. Fig. 1a presents the particle size analysis (PSA) utilized to determine the SiO_2 particles' average size and distribution. The SEM-EDS "scanning electron microscopy-energy dispersive spectroscopy" test for the SiO_2NP is shown in Fig. 1b.

Apricot seed shells nanoparticles (ASSNP)

An apricot seed shell is a very hard shell covering the kernel (seed) of an apricot, rough, reddish, or purplish brown, provided from Iraq. Currently, the utilization of apricot seed shells (ASS), which make up around 10% of the entire fruit mass, is viewed as waste material. The 0.896 cm^3/g density of the finely powdered apricot seed shell suggests that ASS can serve as a resource for producing antioxidant supplements, stabilizers and preservatives. [16][17]. The average size and distribution and the SEM-EDS test for the (ASS) powder are shown in Figs. 2a and b.

Alkali Treatment of Natural Nano-powders

The nano-powders of apricot seed shells (ASS) were rinsed many times using distilled water to eliminate any dirt. It was immersed in a 5% (weight/volume) alkaline solution (NaOH) at 25°C for 2 hours. The nanoparticles treated with an alkaline treatment were extensively rinsed with distilled

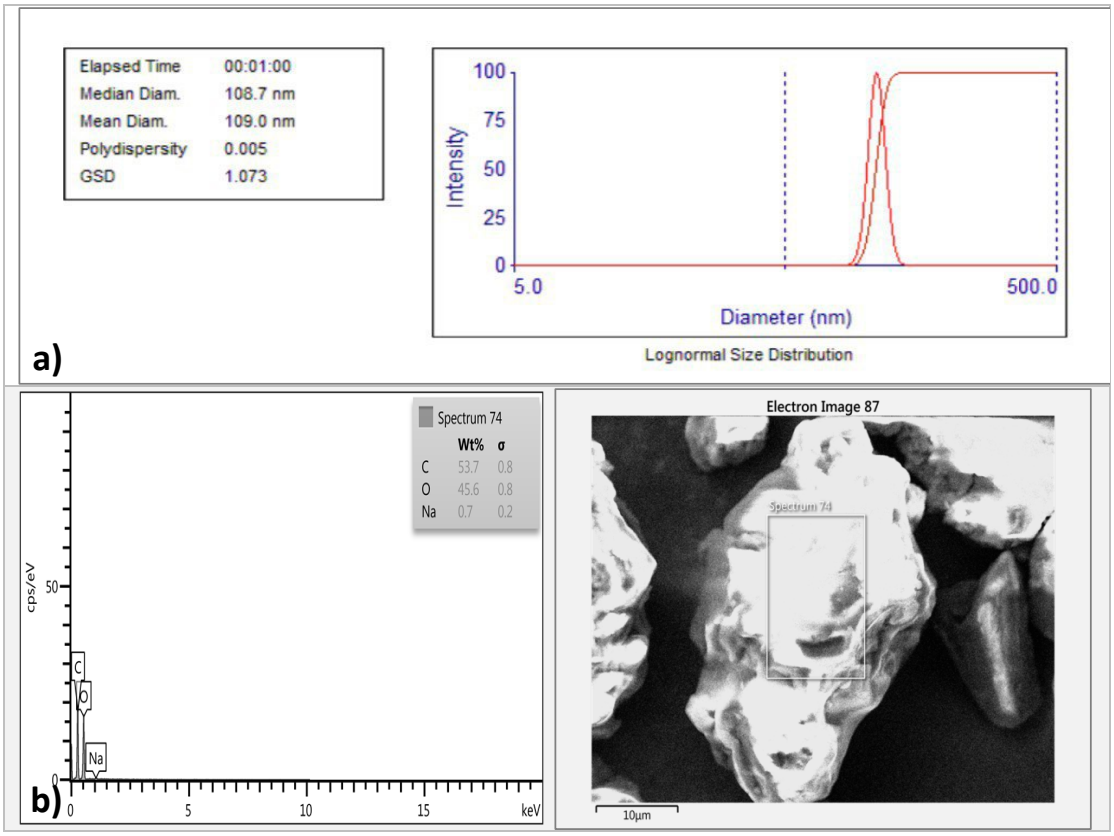

Fig. 2. Characteristics of Apricot Seed Shell (ASS) Powder Used, a(Particle Size Distribution, and b) EDS spectrum and SEM images.

water to remove any residual sodium hydroxide solution clinging to their surface. Subsequently, distilled water was used to raise the pH of the solution to 7. The untreated nanoparticles were exposed to room temperature air drying for five days before being put into an oven at 50 to 60 degrees Celsius to finish drying.

Preparation of Composite and Hybrid Samples

In this study, PMMA was prepared in two forms, the first one as liquid resin materials and the second part as powder hardener materials according to the manufacturer's recommended ratio of 100:2. To prepare composite samples, the liquid monomer (MMA) and one type of nanoparticle (SiO_2 and ASS) should be homogeneously and continuously mixed utilizing ultrasonic mixing at room temperature for 20-30 min. The reinforced PMMA samples were classified into eight types based on the ratio of SiO2 nanoparticles and ASS nanoparticles in PMMA matrix (Table 1). The PMMA matrix was reinforced with 1.5% SiO_2NP and 7.5% ASSNP to prepare hybrid nanocomposite specimens. The ultrasonic mixing method was utilized to set the distribution up at room temperature for 20-30 minutes. After that, the specified ratio of hardener powder was added to the nanocomposite and hybrid nanocomposite mixtures and mixed for 5-10 minutes. The prepared mixture was poured into a silicon mold and kept at ambient temperature for approximately 24 hours to complete polymerization. Afterward, the prepared specimens with smooth surfaces were expelled from the mold to prepare for the subsequent tests.

RESULTS AND DISCUSSION

The FTIR technique was employed in the 400-4000 cm^{-1} range to identify compounds and define mixture components following ASTM E1252-98 international standards. The FTIR spectroscopy test was performed by the test instrument TENSOR-27, manufactured by "Bruker Company, Germany". The principle of the FTIR test is that the infrared radiation (IR) passes through a material, part of the IR radiation is absorbed, and the passed radiation is measured.

The contact angle experiment 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.

A SEM device of model "Tescan VEGA-SB" was utilized to analyze the PMMA samples' surface morphology reinforced with nanomaterials. All specimens were sputtered with a thin layer of gold to ensure high image quality.

The Tensile test was done using a universal tensile device with a 10 mm/min crosshead speed. The tensile properties of the specimens were determined depending on ASTM D638. The tensile properties were determined following the ASTM-D638 international standard [18].

The compressive strength of the samples was completed by the same instrument used during the tensile test as indicated by the standard ASTM D-695. The applied compression load on the sample progressively rose until it fractured [19].

The hardness (Shore A) test is employed to evaluate the nanocomposite hardness based on ASTM D-2240. The prepared specimens with a 40 mm diameter and 4 mm thickness were used to perform the hardness test [20].

The samples with a 6 mm diameter were incubated for 24 hrs. at 37ºC, and then agar was uniformly covered with several microlitres of bacteria solution (106 bacteria). This test was designed to identify antibacterial (biological) activity by measuring the inhibition zone of the prepared hybrid nanocomposite specimens against varieties of bacteria.

The *in vitro* test (MTT) assay technique was

Table 1. Specimens' classification.

Samples	Percentage(%)		
	PMMA	SiO_2	ASS
Pure	100	0	0
PMMA-0.5%SiO_2	99.5	0.5	0
PMMA-1%SiO_2	99	1	0
PMMA-1.5%SiO_2	98.5	1.5	0
PMMA-2.5%ASS	97.5	0	2.5
PMMA-5%ASS	95	0	5
PMMA-7.5%ASS	92.5	0	7.5
PMMA-1.5%SiO_2-7.5%ASS	91	1.5	7.5

utilized to assess cytotoxicity. The Tehran Pasteur Institute in Iran provided the chondrocyte cell line. The cells were cultured and sustained in DMEM "Dulbecco's Modified Eagle Medium; Gibco, Life Technologies, Waltham, MA, USA," supplemented with 10% FBS "Fetal Bovine Serum; BioWest SAS, Nuaille, France," and 1% PSF "Antibiotic Antimycotic Solution; Sigma-Aldrich®, St. Louis, MO, 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 (Gibco, Invitrogen, Waltham, MA, USA) and 0.1% ethylenediaminetetraacetic acid (Merck, Darmstadt, Germany) in phosphate-

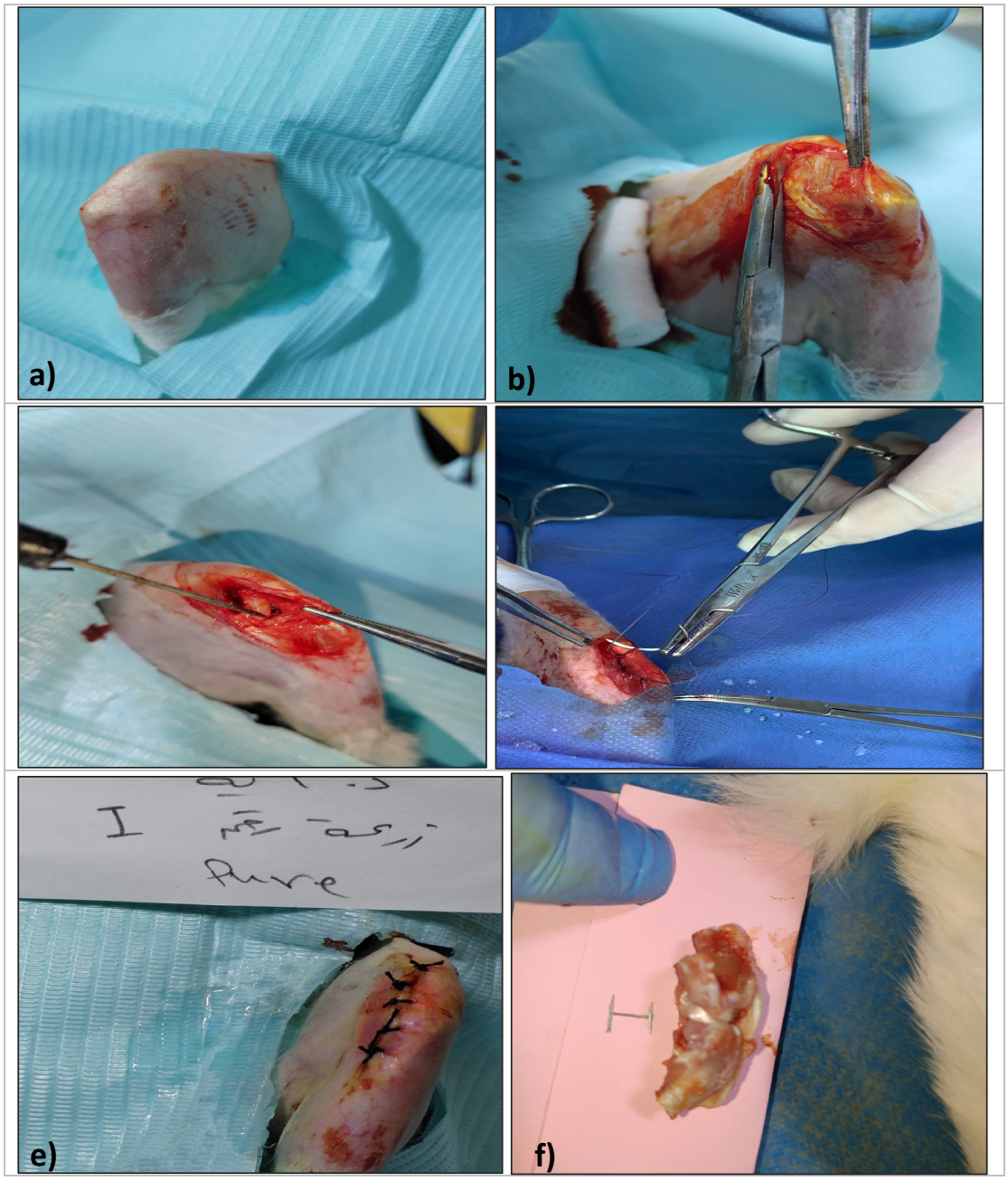

Fig. 3. (a) Region shaving and skin sterilization, (b) skin refecting, (c) insertion of sample between bones of knee joints, (d)& (e) suturing and (f) bones block of knee joint for histological analysis.

buffered saline (PBS). The control, pure, PMMA-1.5%SiO_2, PMMA-7.5%ASS, and PMMA-1.5%SiO_2-7.5%ASS 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 10,000 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.

Firstly, histological analysis had been applied only to reinforced samples that showed the optimum antibacterial activity and less toxicity. The ethical assessments of the use of animals (rabbits) in this research were done using laboratory animals (males with 2.25–3 kg as weight) at the department of pathology animal diseases of the Veterinary Medicine at the University of Baghdad. All animals were housed individually in a temperature-, light-, and humidity-controlled environment. Anesthesia was induced through injected intramuscularly of each animal with a combination of ketamine hydrochloride and xylazine. Continuity of incision the skin of the knee joint until it reached to cartilage. The implant was

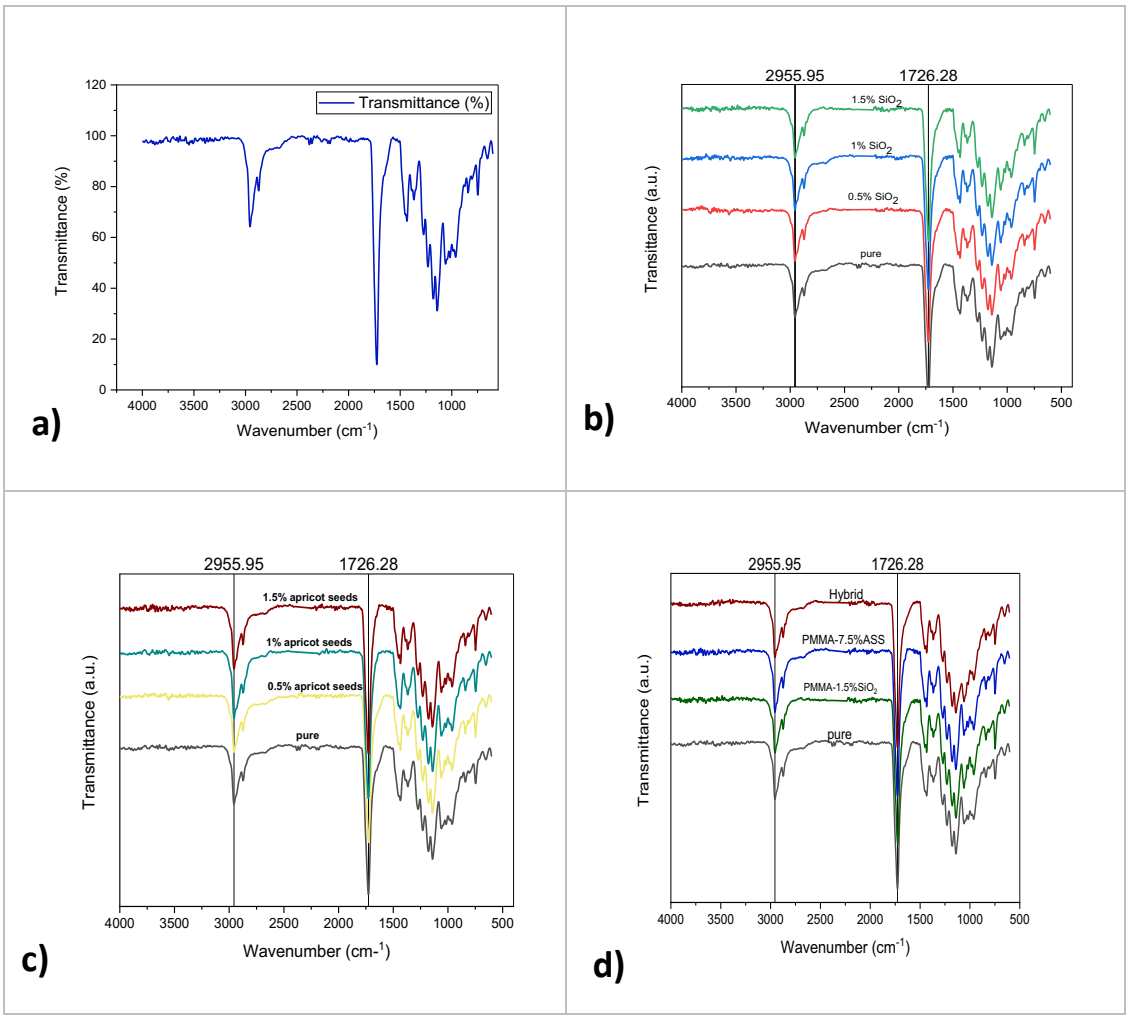

Fig. 4. FTIR Spectrum for: a) Pure PMMA, b) PMMA-X% SiO_2 composite samples, c) PMMA-X% ASS composite samples and d) PMMA Nanocomposite and Hybrid sample.

put in place between the bones of joint instead of cartilage, as shown in Fig. 3. The test was carried out by inserting the implants (specimens) between the joint bones of knee of rabbits for 45 days. After routine closure of the wound, resorb-able sutures for subcutaneous tissues, silk non-absorb-able for the skin incision. The surgical site was cleansed with iodine before being bandaged.

After the time required was completed for implantation, the rabbits were sacrificed. Then extracted the sample (reinforced implant and part of joints) and washed in distilled water to eliminate hair and blood, as illustrated in Fig. 3. The sample remained in a 10% formalin solution for fixation, the formalin is changed after 24, 72, and 120 hours until it is ready for histological analysis which include slide preparation (Dehydration) by immersing in an alcohol (70%, 80%, 90%, 95%, 100%) each concentration for 2 hours. The aim of this step is to remove the water from the cells. After that, clearance with xylene was used to remove alcohol twice, which every time took 2 hours. The embedding in paraffin was then used to penetrate tissue components with paraffin wax, by placing it in an oven at a melting temperature of the wax between (58-60 ºC). After that, (Blocking step) was done by putting the specimen in the mold. Microtome instrument was used to Microtome for the slice in thickness of 5-6 µm in the water bath at (37 °C). Finally, the images of histological were captured using a digital camera attached with the light microscope that used to analyze the section of implantation. An images analysis program (Image J, version 1.8.0) was used to import and analyses the images. The chemical affinity of the PMMA matrix towards the nanoparticles surface was investigated via Fourier transform infrared spectroscopy (FTIR). From FTIR analysis spectra results, pure PMMA, composite, and hybrid FTIR spectra detected no noticeable difference or shift in the positions of the peaks. The major features of the pure PMMA spectra are the α-methyl (1748 and 1373 cm^{-1}), ester-methyl, and methylene C-H stretching (3100–2800 cm^{-1}) and bending (1500-1350 cm^{-1}) modes. The PMMA FTIR spectrum in Fig. 4a demonstrates the functioning groups contained in the prepared PMMA. A sharp peak is observed at 1733.25 cm^{-1} because of the stretching vibration of C=O ester carbonyl group. The C-O-C (ester bond) stretching vibration was observed with a broad

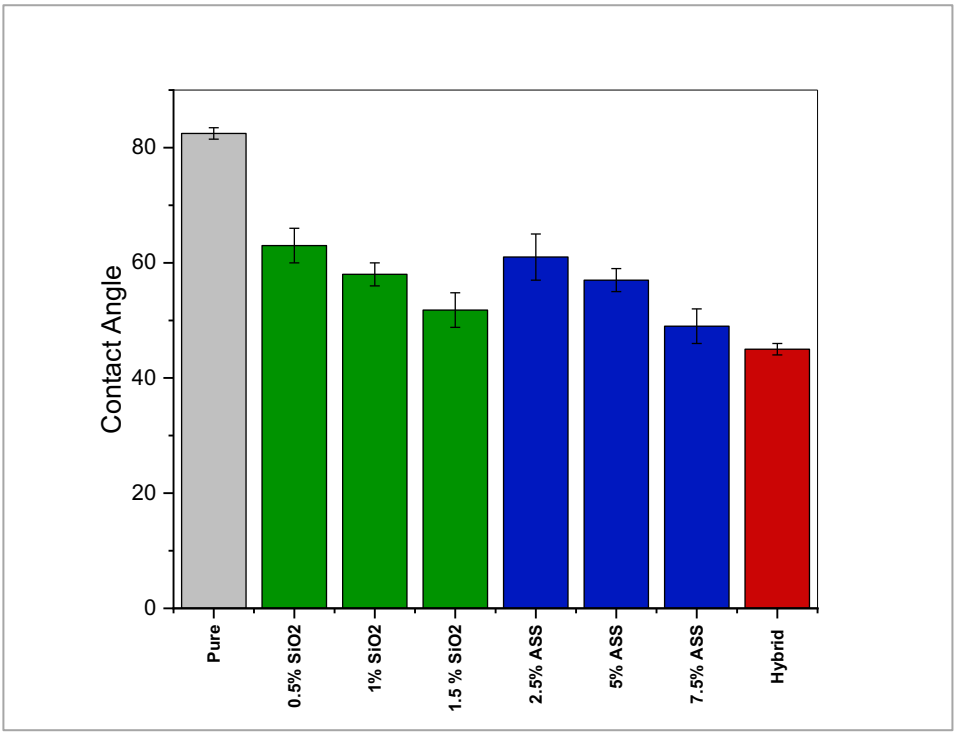

Fig. 5. Contact Angle of the Nano-composite and Hybrid PMMA Polymeric Specimens.

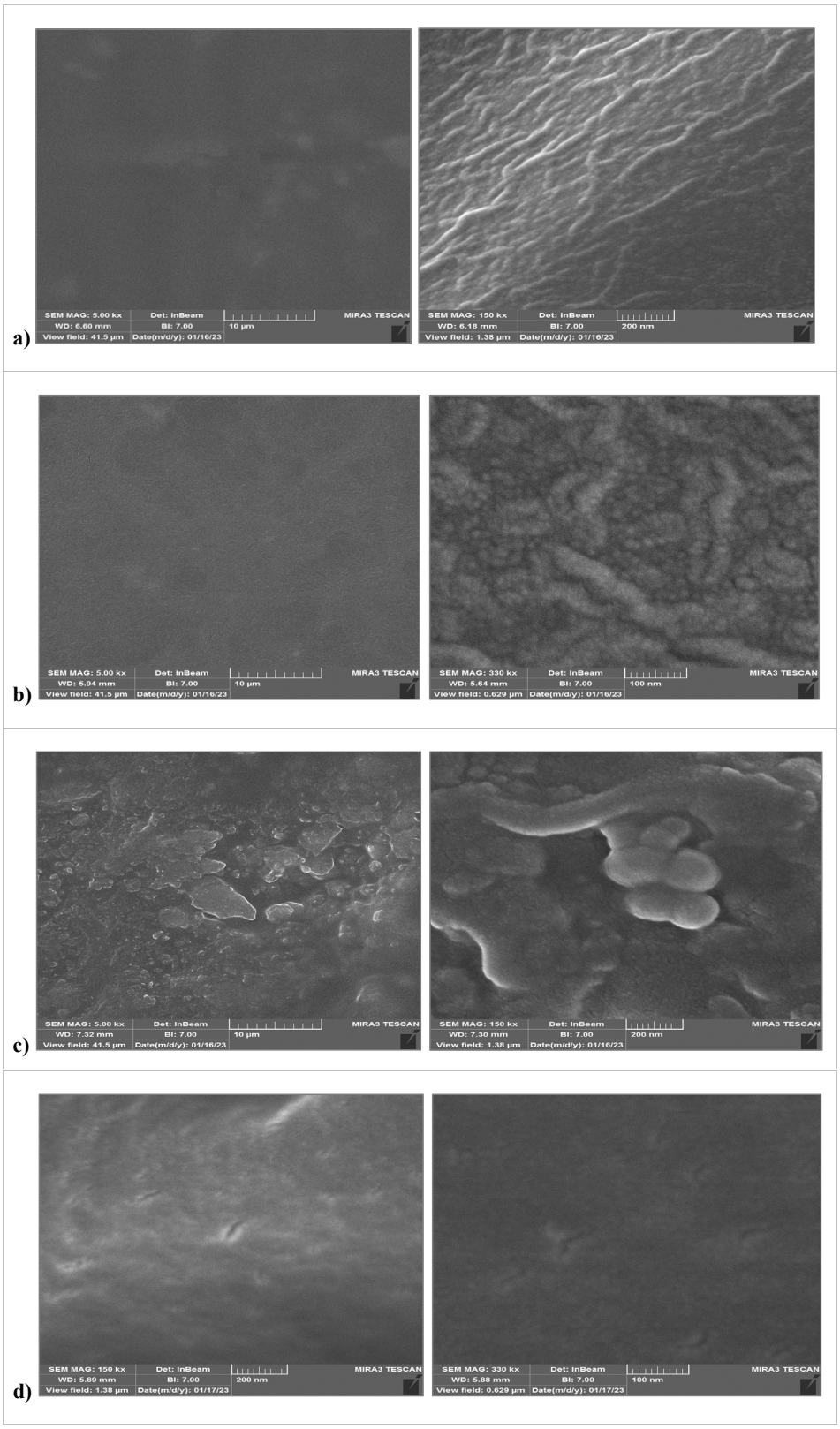

Fig. 6. SEM Image of a) neat PMMA sample, b) PMMA-1.5%SiO_2 Nanocomposite Sample Surface, c) PMMA-7.5%ASS Nanocomposite Sample Surface and d) PMMA-1.5%SiO_2-7.5%ASS Hybrid Nanocomposite Sample Surface.

peak of 1260-1000 cm^{-1}. The characteristic band appears at 1450.42, 1434.13 cm^{-1}, and 1368.83 cm^{-1}, related to the methyl group (C-H) bending. The peak at 1275.66-1230.80 cm^{-1} is associated with C-O bond stretching vibrations in the ester group. At 1180.40 cm^{-1}, the band appears to relate to CH3 wagging, and two bands were observed attributed to CH3 twisting with a range of 1162.90 and 1059. 52 cm^{-1}. The vibration modes resulting from C-C stretching show at 962.21 cm^{-1}, while the vibration bands at 984.87 and 840.76 cm^{-1} are caused by the C-C bond stretching vibration. To fully characterize the PMMA composite samples, the band was examined before and after including nanoparticles at different weight fractions. The infrared spectra of PMMA composites reinforced with 0, 0.5, 1, and 1.5wt.% of SiO_2Np, samples reinforced with 0, 2.5, 5, and 7.5wt.% of ASSNP, and sample reinforced with a combination (1.5% SiO_2NP+7.5% ASSNP) are shown in Figs.4-d, respectively. The infrared spectrum of these hybrid and composite specimens shows that no new shifts in the peaks were noticed for the prepared specimens, including SiO_2 or/and ASS nanoparticles. This could be due to the physical bond, lack of chemical reaction, and cross-linking between the matrix constituents. This indicates that the state of miscibility of the prepared composite constituents is improving. Thus, no new production takes place in these polymer composite specimens.

The evaluation of contact angles makes it easier to determine how hydrophilic samples of PMMA polymer matrix with reinforcement are. Contact angle measurements were performed on all reinforced specimens (both composite and hybrid specimens), and the findings are shown in Fig. 5. A water droplet was left on the specimen's surface for 30 seconds to collect the measurements. Contact angles under 90 degrees denote the solid material's hydrophilic nature, signifying strong wettability as the liquid spreads across the surface. On the other hand, higher contact angles above 90 degrees indicate a hydrophobic solid, where the liquid is isolated on the surface and does not spread [6]. The results demonstrated that the addition of one or both components of nano-powders within a polymethyl methacrylate matrix reduced the water contact angle, increasing the hydrophilicity of the hybrid nanocomposite samples compared to control samples. Compared to the composite samples, the hybrid had a reduced contact angle. Size, quantity, distribution, and interfacial bonding of the reinforcing phase are only a few examples of the variables that directly effect the physical and mechanical characteristics. These characteristics also affect the morphology and microstructure of composites. SEM micrograph investigations were carried out on the sample surfaces to establish a correlation between the mechanical properties of the polymer matrix (unreinforced sample) and the polymer nanocomposites of (PMMA-1.5% SiO_2 and PMMA-7.5% ASS) samples and their microstructural characteristics. The images in Fig. 6a show that the surface of the unreinforced sample has a homogeneous, uniform morphology. Conversely, Figs. 6b-d show a substantial amount of embedded nanoparticles within the matrix material. This incorporation creates a strong interfacial link between the composite material and improves compatibility between the acrylic resin (PMMA) and the reinforcing nanoparticles. It is an essential component of the acrylic resin structure. This enhances the mechanical characteristics. Adding SiO_2 and ASS nanoparticles into the polymer material acrylic resin resulted in changes in the surface morphology, which were relatively good and evenly distributed over the entire matrix. This may indicate the formation of a homogeneous growth mechanism of SiO_2 and ASS without aggregates.

Figs. 7a-c show how adding different amounts of SiO_2NP and ASSNP affects the tensile properties of polymer composite and hybrid groups of PMMA acrylic resin specimens, such as their ultimate tensile strength, elastic modulus, and percentage of elongation. Figs. 7a, and b shows that the tensile strength and elastic modulus values of PMMA composite and hybrid material samples were improved. In contrast, the elongation value in Fig. 7c is reduced by combining silicon oxide SiO_2 or/and apricot seed shell nano-fillers compared to the polymeric matrix. This behavior might be attributed to high interfacial bonding between the PMMA resin matrix with these nano-fillers and the random and regular allocation of powder material inside the matrix, reducing reinforcement materials' agglomeration (grouping). Also, the PMMA chain slippage was decreased by occupying the spaces inside the PMMA matrix, generating a positively enhanced modulus of elasticity and ultimate tensile strength [21][22]. The polymeric nanocomposites (PMMA-1.5% SiO_2) were discovered to have greater estimates of elastic modulus and tensile strength individually at a

rate of 261.5% and 66.52%, respectively, when compared with PMMA-pure samples, but a low rate of elongation. The robust interconnection between the PMMA matrix and these particles and the inherent characteristics of SiO_2 particles may be attributed to the improvement in tensile characteristics. These particles can impede crack propagation inside the PMMA matrix via the reinforcement mechanism [23].

Tensile strength of the (PMMA-1.5% SiO_2-7.5% ASS) hybrid nanocomposite material was increased compared to the PMMA composite due to the incorporation of SiO_2 and ASS nano-powder into the PMMA resin matrix. This can be attributed to the better tensile strength and elastic modulus of ASSNP and SiO_2NP in contrast to the PMMA matrix and each type of filler's role in bearing the load applied to the hybrid composite matrix. The heightened bond strength between PMMA and the reinforcing material is another factor that may have contributed to this improvement. These factors collectively enhanced the flexural strength values of the hybrid laminated composite specimens [24]. The results of the composite and hybrid materials show that the nano-hybrid content, consisting of 1.5% SiO_2 + 7.5% ASS, produced improvements in tensile strength and modulus of elasticity of 97.82% and 469.23%, respectively.

In this investigation, the compressive strength of the specimens was evaluated using a uniaxial compression test, and the outcomes are shown using a uniaxial bar chart, as displayed in Fig. 7d. The figure shows how adding natural and ceramic powders to the PMMA matrix affects compressive strength values. The outcomes exhibited that the compressive strength of all specimens improved, and the resulting reduction in movement of the particles of the formed nanocomposite and hybrid samples of PMMA combined with nano-powders caused the polymeric chains to stiffen by inhibiting crack migration, demonstrating compression resistance against vertical stress applied. When comparing (PMMA-SiO_2NP) with (PMMA-ASSNP) results, polymeric nanocomposites PMMA-1.5% SiO_2 were revealed to have higher estimations of compression resistance, reaching a rate of 49.5% [25].

The hybrid nanocomposite samples might absorb, transmit, and distribute force uniformly over their cross-section. Thus, including SiO_2 and ASS nano-powder in the PMMA resin matrix to form a hybrid composite material improved the compressive properties by 86% compared with the PMMA particulate composite [26].

PMMA composite and hybrid composite hardness measurements from this investigation are presented in Fig. 7e. This illustration portrays the effects of adding two different reinforcing agents (SiO_2 and ASS nanoparticles) on the PMMA matrix's hardness properties. Generally, all the results of hardness composite samples increased with the inclusion of SiO_2 or ASS nanoparticles in their matrix. This increase may be due to the fact that hardness is generally considered a property of the surface; therefore, this behavior of hardness is expected. It might also be due to the stiff and strong nature of the constituents, which relates to strong matrix/nanoparticle compatibility, resulting in improvements in mechanical qualities such as hardness [27]. The hardness value for the PMMA+SiO_2 sample has a maximum enhancement rate of 41.66% compared to other samples.

Presenting SiO_2 and ASS together in the sample yields a considerable increase in hardness (60.4%), where a drop in the homogeneity and conglomerates of SiO_2 and ASS in matrix PMMA contact occurs. As well, that is related to the good coherence extent resulting from the wettability of surfaces of SiO_2 and ASS nanoparticles with the liquid of the polymer resin matrix, which leads to making the surface harder by inhibiting the motion of the polymeric chains along the stress direction [28, 12, 29].

After finishing the specific incubation periods, the results of the influence of the addition of nanoparticles on the antibacterial activity of PMMA specimens reinforced with SiO_2 and ASS against Streptococcus mutans (S. mutans) and Staphylococcus aureus (S. aureus) bacteria were assessed by observing the change in the inhibition zone surrounding each of the test samples in Figs. 8a, and b, respectively. The results show that no inhibition zone was observed around pure PMMA. Including nanoparticles within the PMMA matrix led to the formation of an inhibition zone around each test sample towards S. mutans and S. aureus compared to pure PMMA. The greater inhibition zone appeared to indicate the existence of antimicrobial activities. The antibacterial activity increased as the concentration was increased, which means the highest antibacterial activity of samples obtained from (PMMA-1.5% SiO_2), (PMMA-7.5% ASS), and (PMMA-1.5% SiO_2-7.5%

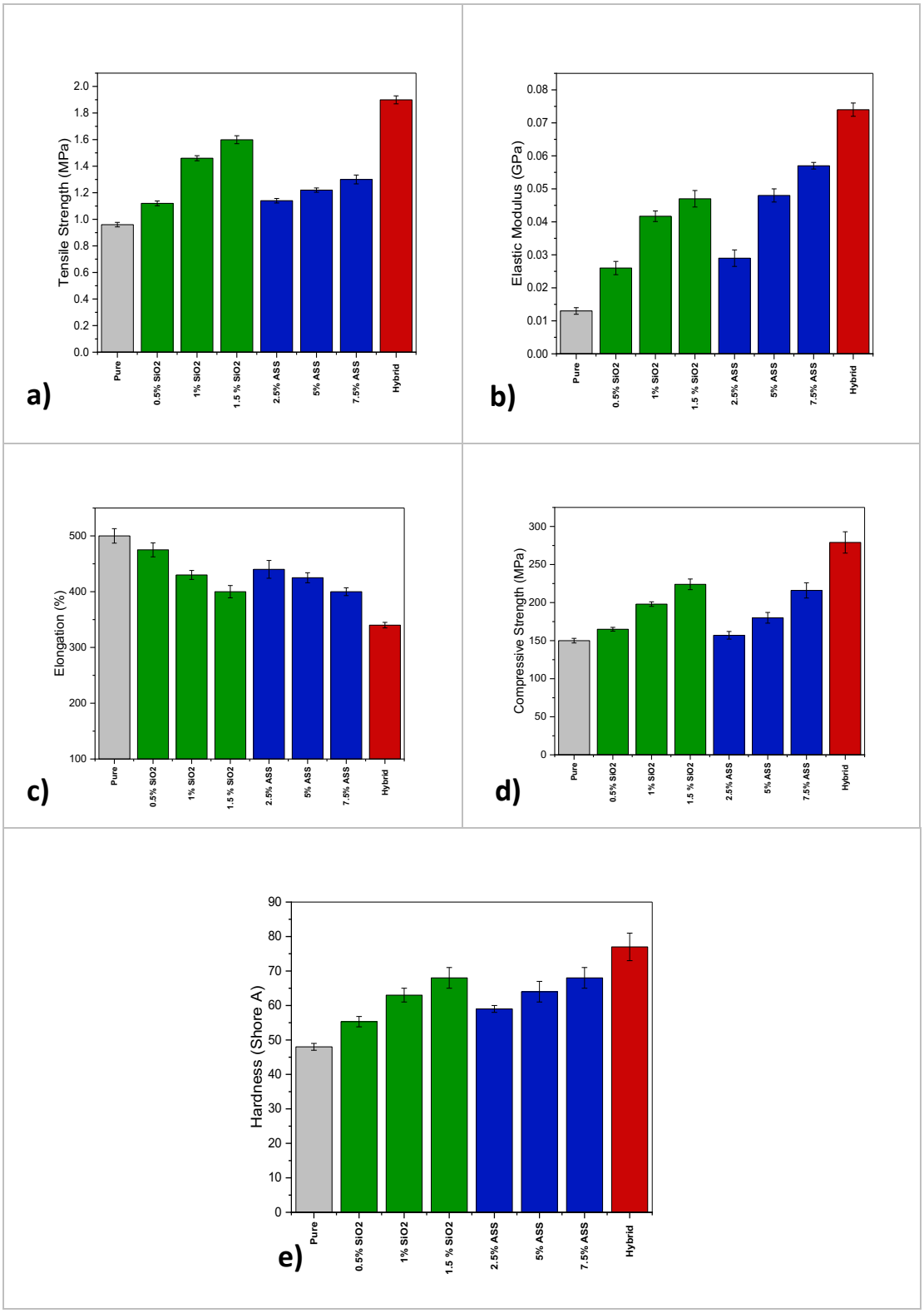

Fig. 7. Mechanical Tests Results a-Tensile Strength, b-Elastic Modulus, c-Elongation, d-Compressive Strength and e-Hardness.

ASS) samples. PMMA composite samples with 1.5% SiO_2 exhibited better antibacterial properties than)PMMA- 7.5%ASS(and)PMMA-1.5% SiO_2-7.5% ASS(hybrid nanocomposite samples. The presence of metal oxide elements, which affect the cellular structure of bacteria, is thought to have an inhibitory influence on bacterial growth. Additionally, the reduced size of SiO_2 particles makes effective penetration into bacterial cell membranes and nuclei possible. When SiO_2 is reduced to nanoscale dimensions, it demonstrates prominent antimicrobial surface properties that allow for interactions with bacterial surfaces and subsequent intracellular penetration, resulting in distinctive bactericidal mechanisms. Silicon ions are released and interact with negatively charged components of bacterial cell membranes in a humid environment, such as an agar plate, which obstructs respiratory processes and causes bacterial cell demise [30]. The variation in effectiveness of prepared samples against bacteria is determined by ingredient nature, and the specific kind of bacteria utilized in this test [31].

To evaluate the potential cytotoxic activity of the pure PMMA, PMMA-1.5% SiO_2, PMMA-7.5% ASS, and PMMA-1.5% SiO_2-7.5% ASS samples against chondrocyte cells, an MTT assay was conducted on the 3rd day of culture at 37°C. The MTT assay was used to measure the cytotoxicity of these samples and compare them against control cells that were initially seeded without any sample treatment. The percentage viability of the cells was calculated after noting the optical density (OD) values of the control during the culture period. Fig. 9a shows the results of optical density, and Fig. 9b shows the results of cell viability. Fig. 9b plots a graph by taking the cell count of the control group as 100%. The results indicated that the chondrocyte cell line was significantly attached to the samples, mimicking the extracellular matrix (ECM) structure. These figures reveal that the inclusion of SiO_2NP, ASSNP, or SiO_2NP and ASSNP together shows non-cytotoxic effects against chondrocyte cell lines due to an increase in the optical density of cells that leads to increased cell viability, where the cells can seed on the surface of all the samples.

The ability of the reinforced PMMA samples to support cell adhesion was examined by SEM analysis. Fig. 10 shows the SEM images of the chondrocyte cells seeded on the pure PMMA, PMMA-1.5% SiO_2, PMMA-7.5% ASS, and PMMA-1.5% SiO_2-7.5% ASS samples. The appearance of these cells in PMMA-1.5% SiO_2 NP(and)PMMA-7.5%ASS NP(nanocomposite samples in a denser ECM (extracellular matrix) substrate Additionally, in the)PMMA-1.5% SiO_2NP-7.5% ASSNP(hybrid nanocomposite sample, the cells formed the ECM structure. It is clear from these figures that PMMA samples reinforced with SiO_2 and ASS nanoparticles

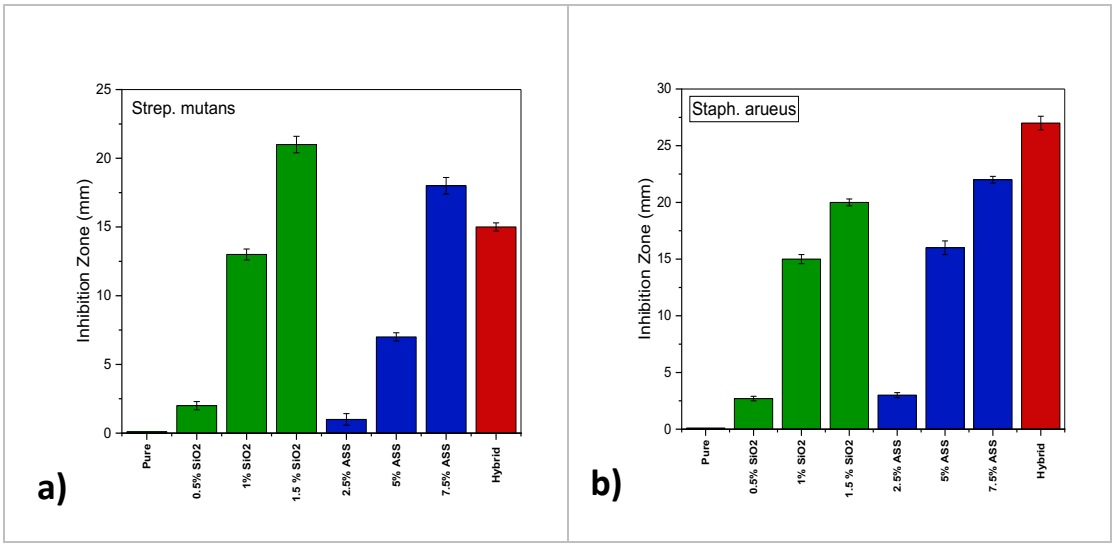

Fig. 8. Inhibition Zone of the Nano-composite and Hybrid PMMA Polymeric Specimens against: a) Streptococcus mutans (S. mutans) and b) Staphylococcus aureus (S. aureus) bacteria.

increased cell proliferation when compared to unreinforced PMMA samples, demonstrating that they have great biological compatibility and are acceptable materials for implant manufacturing. It also demonstrated no negative effects on human cells, allowing researchers to investigate the favorable reactions of prepared samples. Furthermore, neither unreinforced nor reinforced

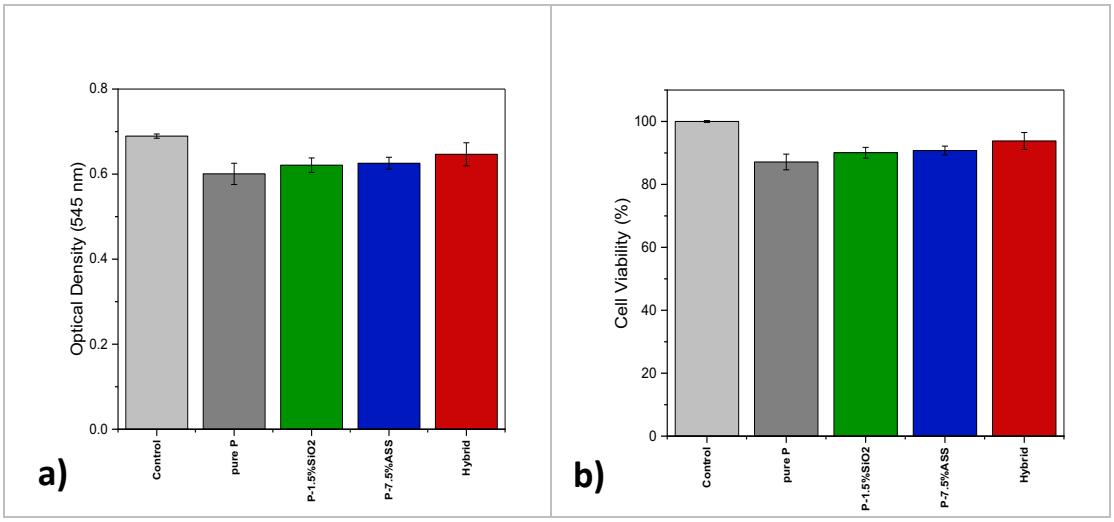

Fig. 9. a) Optical Density and b) Cell Viability of Chondrocyte Cells on PMMA the Nano-composite and Hybrid samples measured using MTT assays in 3 Day of Cell Culture.

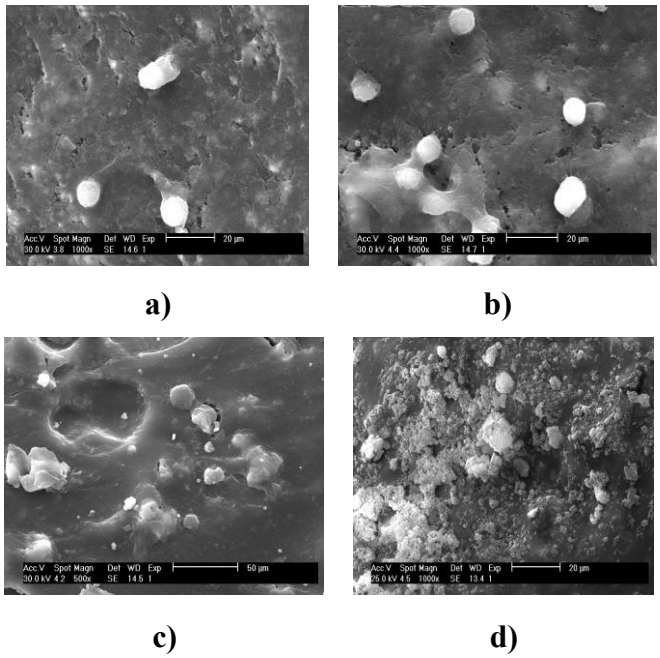

Fig. 10. SEM Images of Chondrocytes Cells Cultured on: a) Pure PMMA, b-PMMA-1.5%SiO_2, c) PMMA-7.5%ASS and d) PMMA-1.5%SiO_2-7.5%ASS Samples for 3 days.

PMMA samples display any signs of symptoms [32]. Further confirmation that more cells spread and adhere to)PMMA-1.5%SiO_2-7.5% ASS(than pure PMMA is provided by the DAPI nuclei staining of chondrocytes cells in Fig. 11. The dissolution of nano-additive, which stimulates cellular activity, is related to this.

The histological test was used to estimate the effect of unreinforced and reinforced PMMA samples by implantation in a rabbit model for 45 days. During the experiment, all animals remained in good health and achieved uneventful healing. No prominent signs of inflammation, allergic reactions, or other complications around the surgical site were observed postoperatively in all groups throughout the experimental periods. Figs. 12a, and b indicate the images of histological analysis for unreinforced PMMA sample at two magnifications (40 X) and (400X), respectively. Fig. 12a revealed a very thickened articular surface plate (Red arrow), narrows joint cavity (Asterisk) & normal epiphyseal ends (black arrows). Fig. 12b showed thickening of the articular plate associated with figures of parallel columns of migrated newly formed chondrocytes (Black arrows), narrows joint cavity (Asterisk) & normal cartilage plate of opposite ends (Red arrows).

The histological images of the PMMA sample reinforced with hybrid nanoparticles (SiO_2, ASS) at magnification 40X showed normal articular surface (Red arrows), normal joint cavity (Asterisk), normal epiphyseal ends (Black arrow) in Fig. 13a. Fig. 13b at magnification 400x revealed that normal fibrous cytoarchitecture of the synovial membrane (Red arrow) and a normal joint cavity (Asterisk) & fibrous joint capsule (Black arrow).

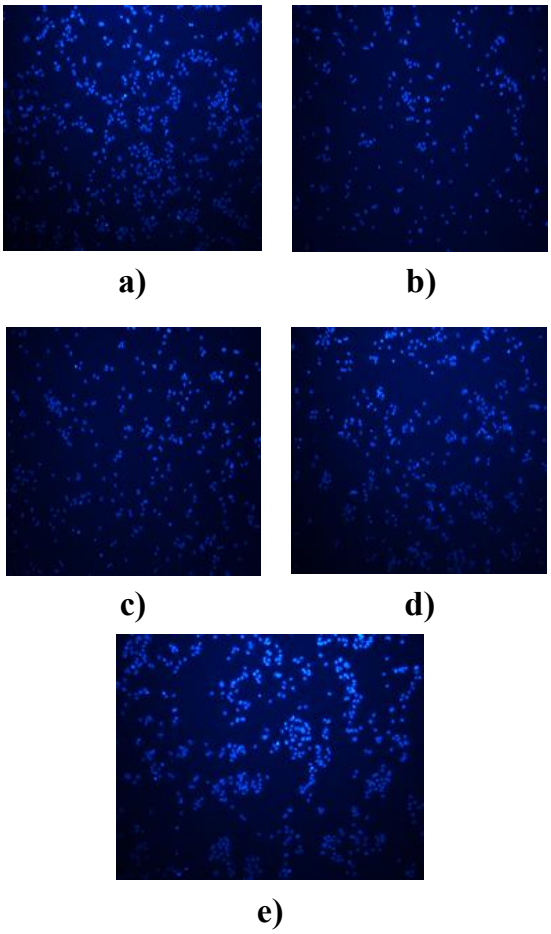

Fig. 11. DAPI Nuclei Staining of Chondrocytes Sells on a) Control, b) Pure PMMA, c) PMMA-1.5%SiO_2, d) PMMA-7.5%ASS and e) PMMA-1.5%SiO_2-7.5%ASS Samples.

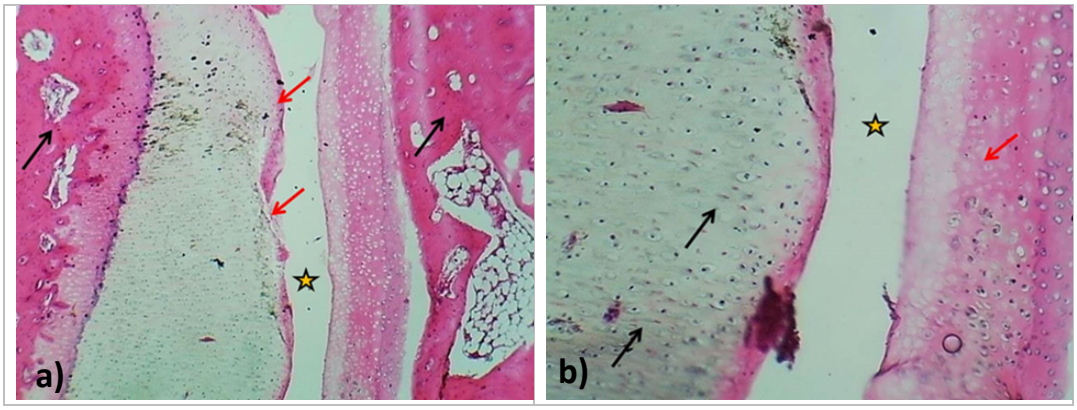

Fig. 12. Section of Joints for Unreinforced PMMA at: a) (40X) and b) (400X) Magnification

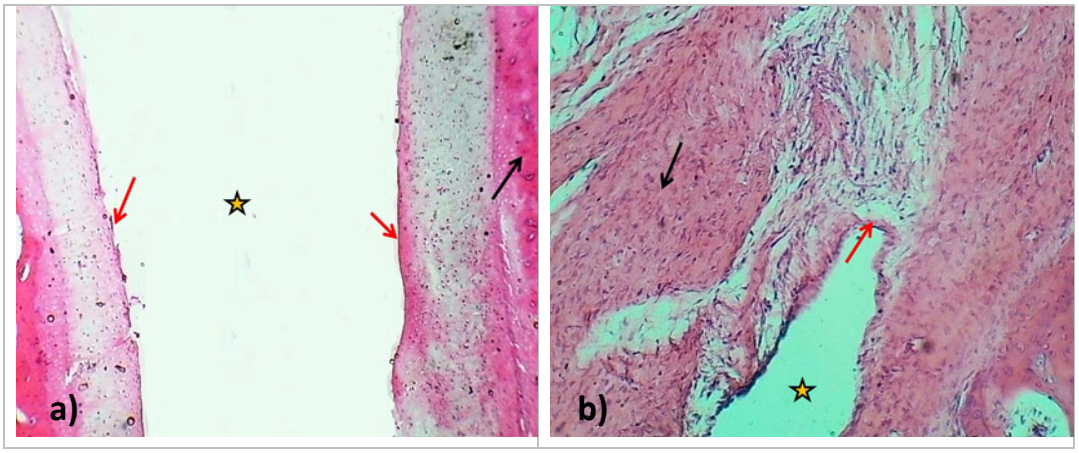

Fig. 13. Section of Joint for Reinforced PMMA with 1.5% SiO_2 and 7.5% ASS Nanoparticles PMMA at: a) (40X) and b) (400X) Magnification.

CONCLUSION

From the experimental results of the manufacture of polymer composite and hybrid samples with the addition of SiO_2NP and ASSNP, it was concluded that:

1) Specimens made of pure polymethyl have lower mechanical properties and antibacterial activity than specimens reinforced with SiO_2 or ASS nanoparticles. So, the properties of the polymer specimens improved with the increasing inclusion of nano-powder into the polymer matrix PMMA.

2) The optimum value of mechanical properties of composite samples found for PMMA-1.5% SiO_2NP improved at a rate of 66.52% tensile strength, 261.5% elastic modulus, 49.5% compressive strength, and 41.66% hardness compared with the pure sample. In contrast, the PMMA composite samples reinforced with 7.5% ASSNP boosted at a rate of 62.5% tensile strength, 356% elastic modulus, 36.02% compressive strength, and 41.66% hardness compared with the pure sample.

3) When comparing the results of composite samples, it showed that the samples reinforced with SiO_2NP had better results than the samples reinforced with ASSNP.

4) The mechanical properties of PMMA hybrid samples reinforced with SiO_2NP+ASSNP were boosted at 97.82% tensile strength, 469.23% for elastic modulus, 86% for composite strength, and 60.42% for hardness.

5) When compared between composite and

hybrid samples, results show that hybrid samples have better results than composite samples.

6) The antibacterial results revealed an increased inhibition zone of PMMA samples, including SiO_2 and ASS nanoparticles against S. mutans and S. aureus bacteria.

7) MTT results indicate that reinforced PMMA nanocomposite and hybrid specimens did not show cytotoxic effects on the chondrocyte cells examined. As a consequence, (PMMA-SiO_2-ASS) demonstrates unique in vivo and in vitro response compared with pure PMMA sample.

8) This study focuses on the requirement for novel, inexpensive, and environmentally acceptable materials for medicine and the creation of materials utilized for cartilaginous joints.

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CONFLICT OF INTEREST

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

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