



Mechanical and antibacterial investigations of TiO₂ and MgO nanoparticles for medical application

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The research examines the effect of reinforcing silicone rubber (SR) with TiO₂ nanoparticles (TiO₂ NPs) and MgO nanoparticles (MgO NPs) at a selected ratio 2wt.%, 3wt.% and 4wt.%. silicone rubber is prepared as matrix and reinforced by these two types of NPs separately. The evaluation on the effect of some physical, mechanical and biological property of the prepared bio-composite are performed. Including hardness, wettability, and bacterial colony formation (CFU) testing, along with field emission scanning electron microscopy (FESEM) examination to monitor the distribution of nanoparticles on the surface of the samples. wettability test results showed a gradual increase in contact angle values with increasing NPs concentration. silicone rubber sample SR/MgO-4 recorded the highest value at 101.6°, while the control sample recorded 94.91°. This indicates an increased hydrophobicity of the rubber surface after the addition of nanoparticles. A gradual increase in hardness values is observed with ratio portion for both NPs used. Bacteriological testing also revealed a significant decrease in the number of bacterial colonies growing on the enriched samples compared to the control sample, indicating that the nanoparticles used possess antibacterial activity. Finally, FESEM images further supported these findings by showing the NPs distribution and the variation in the nature of the agglomerations depending on effect of NPs proportion.

Key words: TiO₂ NPs; MgO NPs; Hardness; Contact angle; Antibacterial efficacy.

1. INTRODUCTION

Silicone rubber is a significant synthetic polymer in biomedical applications, owing to its distinctive physical and chemical properties. Its characteristics depend on polymer chain length and the degree of

cross-linking, while the high strength of the Si–O bond imparts thermal stability and chemical resistance, making it suitable for medical applications [1]. As a result of these outstanding properties, silicone rubber has been widely used as a preferred material in the maxillofacial prostheses, finger joints, soft tissue replacements, as well as catheters, heart valves, cannulas and tracheal stents, manufacture of orthopedic prostheses [2]. In recent years, there has been a growing global need to develop advanced materials based on silicone rubber elastomers with enhanced performance, by reinforcing them with nanoparticles that possess antimicrobial, antibacterial, and antifungal properties [3]. The properties of polymers are significantly improved when reinforced with nanoparticles, and the type of filler and the filling technique greatly influence these improvements [4-66]. NPs are of great scientific importance, as they form an effective link between the substrate and atomic or molecular structures [7,8]. According to a prior study, adding TiO₂ and SiO₂ NPs to silicone rubber improved hydrophobicity and raised the contact angle, which decreased the adhesion of microbes and contaminants to the surface [9]. Previous work has shown that blending other types of silicone rubber and PMMA with nanopowders of pomegranate peel, date pits, and TiO₂ is suitable for improving certain physical and biological properties [10]. According to another study, adding TiO₂ and ZnO NPs to reinforce silicone rubber provided it extremely potent antibacterial action against a variety of bacterial. Additionally, the results demonstrated that the addition of the nanoparticles did not significantly alter the material's wettability or surface contact angle [11]. A previous study showed that adding MgO NPs to silicone rubber improved antibacterial activity against *S. aureus* and *E. coli* bacteria, and also reduced the contact angle and improved wettability compared to unreinforced samples [12]. Finally, in a previous study of authors', silicone rubber is reinforced with TiO₂ and MgO NPs at selected concentrations, examined various mechanical properties, the results showed that the 2% concentration achieved the best improvement in mechanical properties compared to the higher concentrations [13]. Although numerous studies have used TiO₂ or MgO separately to reinforce silicone rubber, a comprehensive comparison of the effects of these two types of nanoparticles on physical, mechanical, and surface properties, as well as antibacterial activity, remains limited. Therefore, this study focuses on a comparative evaluation to fill the gap and evaluate the effect of using NPs that could advance the performance of silicone rubber for medical industries applications. This research aims to develop silicone rubber nanocomposites reinforced with TiO₂ and MgO NPs in specific weight ratios. In order to evaluate the nanocomposite's surface behavior and its affinity or repulsion to water, wetting ability is examined by measuring the contact angle. In order to assess how the inclusion of NPs affected the composite's surface toughness, hardness is also assessed. The antibacterial activity is examined to confirm that they could stop the growth of bacterial colony formation using CFU testing. Along with field emission scanning electron microscopy (FESEM) examination.

2. MATERIALS AND METHOD

The foundation material for this investigation is room-temperature vulcanized silicone rubber (RTV). A rubber component and a hardener are combined in a 100:2 ratio to form this two-component system. MgO and TiO₂ of NPs are added to the material's structure to improve its characteristics. At the first, reference samples without additives are made using the conventional rubber-to-hardener ratio. 2.94 g of hardener and 143.94 g of silicone rubber are combined, and the liquid is stirred until there are few trapped air bubbles. The weight percentages of the nanoparticles (2, 3, and 4 wt%) are calculated relative to the mass of the silicone rubber base. And to ensure full cross-linking of the material, the liquid is then put into a glass mold and left to solidify at room temperature for 48 hours. After the curing period is over, the samples are taken out of their molds and kept in a lab setting until the required tests could be carried out. Three weight percentages of TiO₂ and MgO NPs 2wt.%, 3wt.%, and 4wt.%, are used to prepare the nanocomposite samples. TiO₂ and MgO NPs weighed 2.94 g, 4.48 g, and 5.88 g, respectively, for each of these percentages. The average particle size measured to be 106.1 nm for MgO and 82.2 nm for TiO₂.

Then, in accordance with the necessary ratio, these amounts are progressively added to the rubber component at weights of 141 g, 139.5 g, and 138.18 g, respectively.

To guaranty uniform dispersion of the NPs throughout the polymer matrix and reduce agglomeration, mixing is done by hand using a glass rod within a glass beaker for ten minutes of constant stirring. After verifying the homogeneity of the mixture, the hardener is added according to the established ratio (100:2) as shown in Table 1, with weights of 2.94 g, 2.9 g, and 2.82 g for the three ratios respectively. Manual stirring continued until a homogeneous mixture is obtained. In the final step, the finished mixtures are poured into standard glass molds of appropriate dimensions for each test and left to solidify at room temperature for 48 hours to ensure complete structural crosslinking.

Table 1 Composition of silicone rubber and different reinforced NPs.

Sample Code	SR proportion% (w/w)	SR weight (g)	NPs proportion% (w/w)	NPs Weight (g)
control	100%	146.88	-	-
SR/TiO ₂ -2	98%	141	2 %	2.94
SR/TiO ₂ -3	96%	139.5	3%	4.48
SR/TiO ₂ -4	94%	138.18	4%	5.88
SR/MgO-2	98%	141	2%	2.94
SR/MgO-3	96%	139.5	3%	4.48
SR/MgO-4	94%	138.18	4%	5.88

3. EXPERIMENTAL

4.1 Wettability test

The angle of contact is the angle formed between the surface of a material and a liquid droplet at their point of contact, and is used to assess the surface's wettability and its hydrophilic or hydrophobic nature. Static contact angles are measured using an optical contact angle meter and a surface tension meter (Creating Nanotechnology). The sample is placed on the instrument's sample holder. A 2.5 mL drop of distilled water is then placed on the composite material's surface. The contact angle measurement is recorded immediately after immersion, and the droplet shape is recorded using a digital camera. This is done in accordance with the principles of ASTM D5946-04, which is based on measurements of water contact angles with polymeric materials.

4.2 Hardness test

Hardness measures material resistance to a small rigid object forced over the material surface. Shore A hardness test according to ASTM D2240 for measuring rubber hardness. A Shore A hardness tester is used [14]. This type of hardness tester is typically used for soft vulcanized rubber and thermoplastic elastomers. The samples are 3 mm thick. The hardness of two samples is measured at each reinforcement ratio, and measurements are taken at three locations: the sample face, the sample base, and a hard horizontal surface to ensure accuracy.

4.3 Antibacterial Activity Evaluation

After adjusting the bacterial suspension using the McFarland scale, it is diluted to a concentration of 1×10^9 colony-forming units (CFU)/ml. Then, 1 ml of the 1×10^9 diluted solution is mixed with each of the following substances individually: control, SR/ TiO₂ at 2%, SR / TiO₂ at 4%, SR / Mg at 2%, and SR / Mg at 4%. The samples are then incubated at 37°C for 24 hours. After the incubation period, the incubated material with the bacterial suspension is poured into a Petri dish, and the nutrient medium is added once it had reached approximately room temperature. The mixture is then thoroughly stirred and allowed to solidify before being placed in the incubator. Finally, the colony-forming units (CFU) are calculated using ImageJ software. And as follows in the equation below to extract the reduction:

$$\text{Reduction (\%)} = \frac{\text{CFU Control} - \text{CFU Sample}}{\text{CFU Control}} \times 100 \quad (1)$$

where CFU control is number of colony-forming units recovered from pure silicone rubber (the control sample) and CFU sample is number of colony-forming units recovered from nanocomposite silicone rubber samples (containing TiO₂ or MgO).

4.4 Morphological analysis

SR/TiO₂ and SR/MgO composite samples are prepared for SEM investigation, using gold palladium powder, micrographs of the selected areas are obtained using a Leo Evo 40 SEM 7 device with a capacity of 20 kV.

4. RESULTS AND DISCUSSION

4.1 Wettability test results

A crucial metric for assessing the wettability and surface characteristics of silicone rubber and samples supplemented with nanoparticles is contact angle. The contact angle as shown in Figure 2 of the control sample is 94.91°, while all of the reinforced samples had higher values, suggesting increased hydrophobicity when the nanoparticles are added [15]. The reinforced samples had contact angle values between 95.394° and 101.6°. For TiO₂ reinforced samples, the contact angle increased from 95.394° at a 2% concentration to 99.2° at 3%, and further to 100.1° at 4%. This demonstrates an increase in the contact angle corresponding to the rise in TiO₂ concentration from 2% to 4%. Regarding MgO reinforced samples, the contact angle is 99.877° at a 2% concentration, rising to 100.671° at 3%, and reaching a maximum of 101.6° at 4%. This indicates a continuous increase in the contact angle with rising MgO concentration, with the highest value among all samples recorded at the 4% concentration. In all three concentrations, the MgO reinforced samples showed greater contact angle values than the TiO₂ reinforced samples when comparing the two types of nanoparticles; the SR/MgO-4 sample recorded the maximum contact angle of 101.6° [16]. Overall, the findings demonstrated that the addition of both TiO₂ and MgO increased the contact angle; the degree of this increase varied depending on the kind and concentration of particles. This shift is explained by the impact of rising nanoparticle concentration on surface roughness, which is connected to rising contact angles [17]. Furthermore, according to earlier data, the average particle size is 106.1 nm for MgO and 82.2 nm for TiO₂ [13].

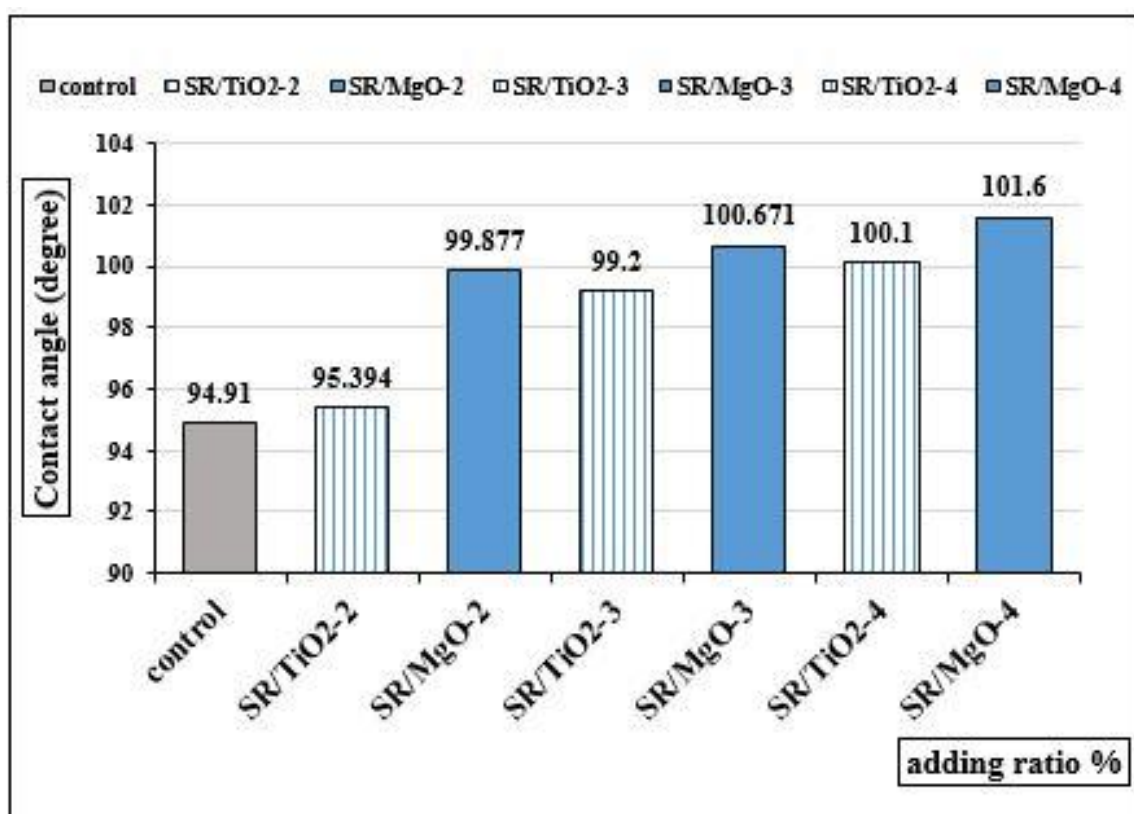


Figure 1 Effect of adding TiO₂ and MgO nanoparticles on the contact angle of silicone rubber.

4.2 Hardness test results

As shown in Figure 2, the Shore A hardness test results for silicone rubber reinforced with TiO₂ and MgO NPs at varying loading levels revealed that the control sample exhibited the highest hardness value (65.2), whereas all reinforced samples showed lower hardness values than the control. For the TiO₂ reinforced samples, the hardness value is 47.15 at a 2% loading level, rising to 53.25 at 3% and reaching 56.7 at 4%. It is observed that the addition of TiO₂ initially caused a decrease in hardness compared to the control sample, followed by an increase in hardness as the loading level increased. Regarding the

MgO reinforced samples, the hardness value is 45.7 at a 2% loading level, rising to 58 at 3% and reaching a peak of 60.2 at 4%. Thus, the MgO samples also demonstrated an initial drop in hardness relative to the control, followed by an increase in hardness as the nanoparticle content increased. In general, hardness ratings dropped when nanoparticles are added. This could be explained by the particles' integration into the silicone matrix, which can slow down the polymerization rate of the material and prevent polymer chain cross-linking [18]. This is consistent with research [19], which shows that although nanoparticles' small size makes it easier for them to enter between polymer molecules, they can occasionally experience agglomeration. Hardness values consequently rose when more nanoparticles are added. This is explained by the fact that increasing the number of nanoparticles further limits the mobility of the chains in the composite material, increasing the measured surface hardness; the load is transferred from the silicone rubber matrix to the nanoparticles, improving the material's resistance to deformation. This result is consistent with earlier studies showing that adding nanoparticles can reduce the mobility of polymer chains and make composite materials harder [20,21].

When comparing the two types of nanoparticles, at concentrations of 3% and 4%, MgO based composites had greater hardness values than TiO₂ based ones, whereas the TiO₂ sample at 2% concentration had a higher value than the comparable MgO sample. Among the reinforced samples, the SR/MgO-4 sample had the highest hardness (60.2), but it is still less than the reference sample's hardness (65.2).

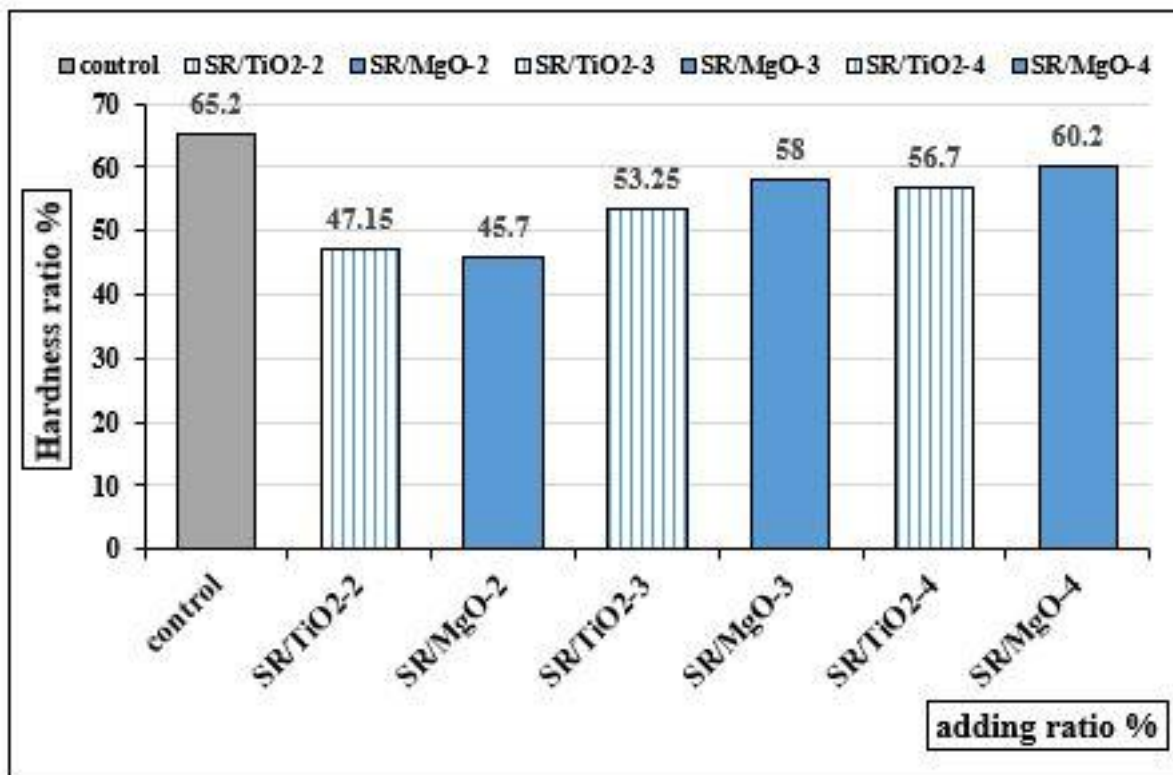


Figure 2 Effect of adding TiO₂ and MgO nanoparticles on the hardness ratio of silicone rubber.

5.3 Antibacterial activity evaluation results

The antimicrobial activity of silicone rubber composites containing TiO₂ and MgO NPs is evaluated against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* after 24 h of incubation at 37 °C. Bacterial viability is determined by counting colony-forming units (CFU) using ImageJ, as shown in Figure 3 and Table 2. The results showed a reduction in viable *E. coli* cells of 73.39% and 50.14% for SR/TiO₂-2 and SR/TiO₂-4, respectively, while SR/MgO-2 and SR/MgO-4 showed reductions of 53.64% and 29.55%, respectively. For *S. aureus*, the reductions are 15.26% and 43.93% for SR/TiO₂-2 and SR/TiO₂-4, and 16.20% and 49.84% for SR/MgO-2 and SR/MgO-4, respectively. The raw CFU results are presented in the corresponding figures and tables. The SR/TiO₂ samples showed a more pronounced antibacterial effect against *E. coli* than *S. aureus*. This difference may be related to the structural differences between Gram-negative and Gram-positive bacterial cell walls, particularly the presence of a lipopolysaccharide layer in *E. coli*, whereas Gram-positive bacteria lack this layer. Hydrophobic interactions between bacterial cells and polymer surfaces also play an important role in microbial adhesion [22]. The incorporation of TiO₂ and MgO NPs resulted in different antibacterial responses compared with the control. According to the results obtained, the antibacterial activity can be associated with changes in the physicochemical properties of the composites and the interaction of the nanoparticles with the silicone rubber matrix [23][24]. The antibacterial activity of TiO₂ may also be related to its photocatalytic effect under UVA and visible light. This effect is associated with the generation of reactive oxygen species (ROS), which contributes to bacterial inactivation [25]. Overall, the tested SR/TiO₂ and SR/MgO composites exhibited antibacterial activity against both *E. coli* and *S. aureus*. The highest reduction is observed for SR/TiO₂-2 against *E. coli* (73.39%), while SR/MgO-4 showed the highest reduction against *S. aureus* (49.84%).

Table 2 Antibacterial activity of silicone rubber nanocomposites against *E. coli* and *S. aureus*.

Sample code	<i>E. coli</i> CFU	<i>E. coli</i> Reduction(%)	<i>S.aureus</i> CFU	<i>S.aureus</i> Reduction (%)
Control	714×10^{12}	-	321×10^9	-
SR/TiO ₂ -2	190×10^{12}	73.39%	272×10^9	15.26%
SR/TiO ₂ -4	356×10^{12}	50.14%	180×10^9	43.93%
SR/MgO-2	331×10^{12}	53.64%	269×10^9	16.20%
SR/MgO-4	503×10^{12}	29.55%	161×10^9	49.84%

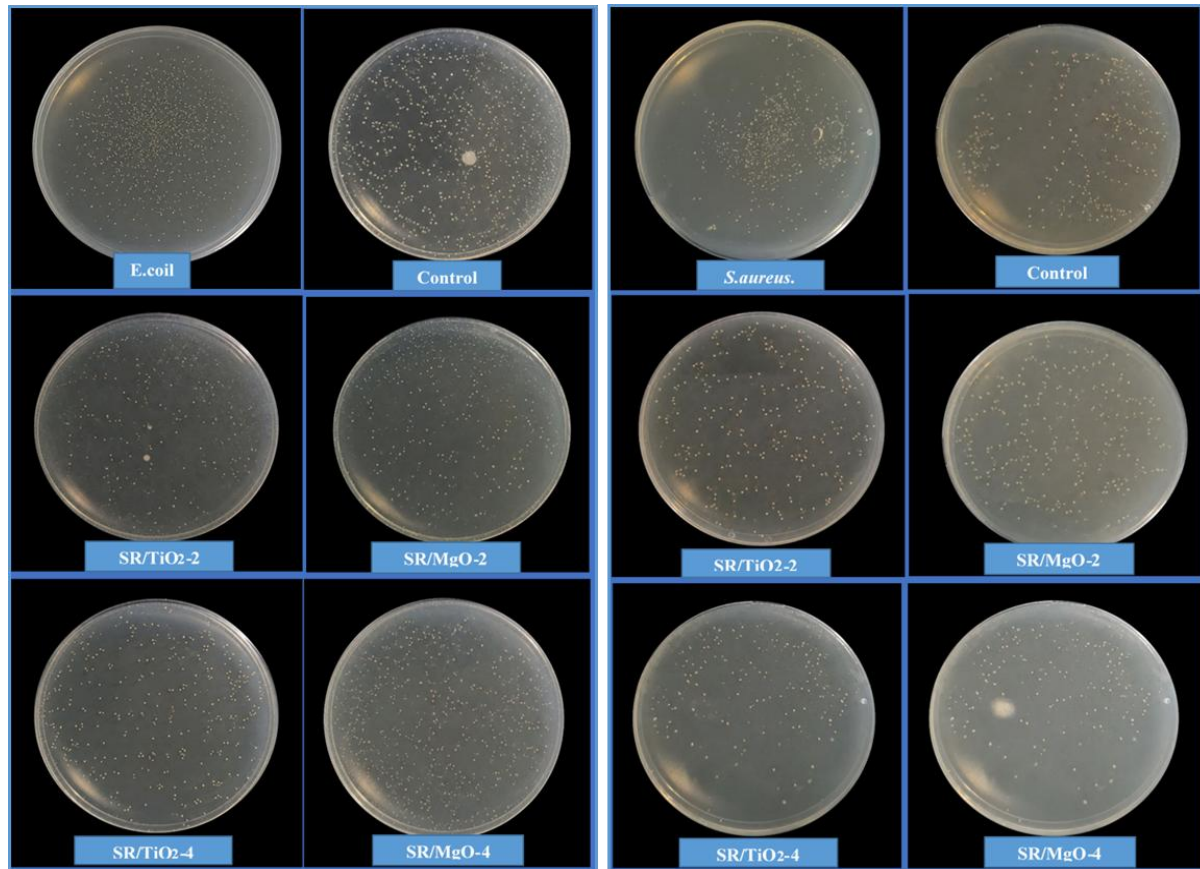


Figure 3 Colony formation of (a) *E. coli* and (b) *S. aureus* on silicone rubber samples after incubation.

5.4 Morphological analysis results

FESEM is utilized to explore the morphological features of silicone rubber nanocomposites. As in Figure 4 (a,b,c,d) which represents SR/TiO₂-2, SR/MgO-2 and SR/TiO₂-4, SR/MgO-4, respectively. A clear distribution of fillers in silicone rubber composites without any agglomeration and observation of much smoother surface plane, as in Figure 4 a and b for 2wt.% ratio. On the other hand, Figure 4 (c,d) presented the use of 4wt.% of TiO₂ and MgO in silicone rubber nanocomposites. A clear observation that both nanoparticles type dispersed well in the matrix at lower ratio compared to Figure 4 (a,b). Increased agglomerations are very obvious, also the aggregation of nanoparticles. That in turn increase stress concentrations transfer between the matrix and fillers and then increasing the surface hardness as tested before to some extent. qualitative homogeneity is poor during increasing nanoparticles.

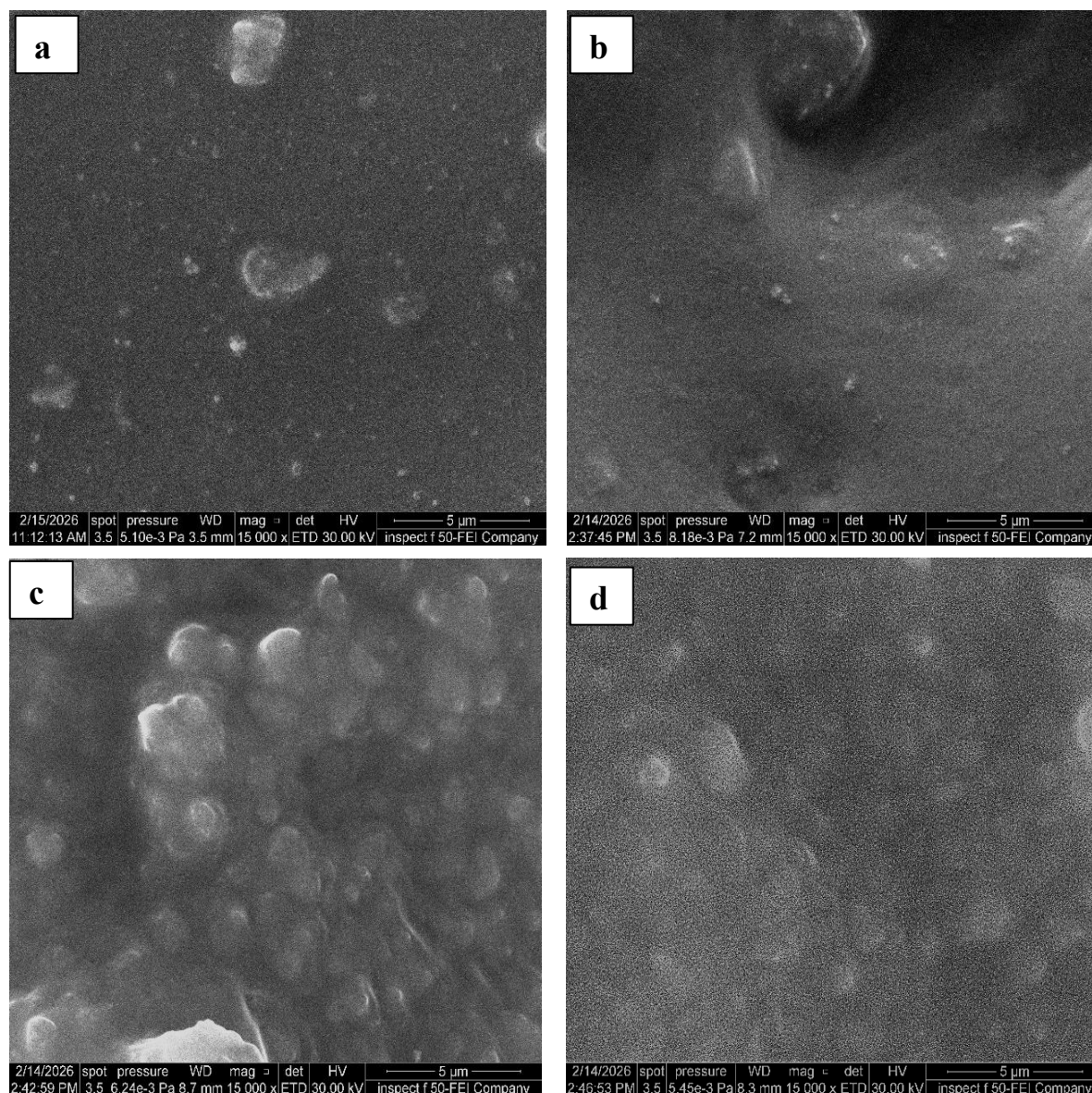


Figure 4 FESEM micrograph of SR/nanocomposite at (5 μm X): a) (SR/TiO₂ 2wt.%), b) (SR/MgO 2wt.%), c) (SR/TiO₂ 4wt.%), d) (SR/MgO 4wt.%).

5. CONCLUSIONS

1. The addition of TiO₂ and MgO nanoparticles resulted in an increase in the contact angle compared to the reference sample (SR), which measured 94.91°. Values for MgO-loaded samples were higher than those for TiO₂-loaded samples at corresponding concentrations; the SR/MgO-4 sample exhibited the highest contact angle at 101.6°, indicating enhanced hydrophobicity upon the addition of nanoparticles.
2. The reference sample recorded the highest hardness at 65.2 Shore A. Following an initial drop at the 2% concentration, hardness increased with higher nanoparticle concentrations; the SR/MgO-4 sample recorded the highest hardness among the reinforced samples at 60.2 Shore A, though this remained lower than that of the reference sample.

3. The variations in antibacterial activity show that bacterial species and nanoparticle composition both influenced the antimicrobial response. Even at the lower tested dose, TiO₂ shown strong action against *E. coli*, which may be related to its capacity to produce reactive oxygen species and cause oxidative damage to bacterial cells. On the other hand, at the greater concentration, MgO demonstrated more effective antibacterial action against *S. aureus*, possibly as a result of the combined effects of membrane disruption, reactive oxygen species production, and the alkaline environment associated with MgO.

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