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Antibacterial Activity of Hydrophobic TaN-Ag Nanocomposite Thin Film

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ABSTRACT

This study investigates the antibacterial activity of TaN-Ag nanocomposite thin film as well as hydrophobic properties. TaN-Ag nanocomposite thin film was deposited on cleaned 316 stainless steel, which is suggested for surgical tools. The samples were synthesized using DC co-sputtering technique. After deposition, the heat treatment was done at 350 °C at different times. The crystalline structure, topography, and morphology of the thin films were characterized by X-ray diffraction, atomic force microscopy, and scanning electron microscopy, respectively. Also, self-cleaning characteristics and hydrophilic properties were studied using contact angle tests. After four months, antibacterial test was performed using E.coli bacteria. The number of colonies was decreased up to 50%, after 6 hours without using UV irradiation during the incubating time. The results showed that the average size of nanoparticles was less than 50 nm and the self-cleaning properties of the TaN-Ag nanocomposite thin films were improved by surface roughness; so, the bacterial adhesion was reduced.

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1. INTRODUCTION

Surgical site infections associated with a surgical instrument remain a serious and common complication in surgeries. Therefore, today, the incidence of infections causes requires greater attention to the use of instrumental devices with antibacterial coating. Hence, using a suitable antibacterial coating on surgical instrument can solve the problem of infection, which accrues after operations [1-4]. Antibacterial coating should have some properties such as good mechanical properties (wear-resistance, oxidation-resistance), antiadhesion, self-cleaning, and biocompatibility. Current strategies to inhibit the adhesion of bacteria onto devices inside other properties mentioned above involve the use of proper coating; for example, TiN and ZrN coatings exhibit high antibacterial performance with respect to the oral micro flora and streptococcus [3]. In addition, Ta alloys are known for their excellent biocompatibility that makes an excellent protective coating in biomedical applications [3,5]. Researchers have reported that deposition of TaN thin film on Ti substrate is too hydrophobic [4]. The first step for infection is bacterial adhesion; a hydrophobic surface avoids bacterial adhesion and biofilm formation [1]. Therefore, using a coating with suitable hydrophobic property is useful. Moreover, J. H. Hsieh et al. [6] demonstrated that TaN thin films deposited using

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DC co-sputtering technique had great mechanical properties including wear resistance, oxidation resistance, and high hot hardness. Surface modification of Ta with the coating or addition of antibacterial metals nanoparticles or nanoalloys to reduce the number of bacteria and adhesives is an efficient way to increase the benefit of clinical process. Ag and Cu are known as efficient antibacterial agents because of their specific antimicrobial and non-toxic activities [6]. It is noticeable that as a doping element, Ag can increase the antibacterial activities without light irradiation. [6]. M. Yoshinari et al. [7] reported that Ag nanoparticles avoided synthesis of bacterial DNA and had greater antibacterial activities against bacteria; therefore, Ag nanoparticles are good candidates. In addition, using high concentrations of Ag nanoparticle could be harmful because of its poisonous property [8]. To characterize the antibacterial layer, controlling the variation of some properties such as hydrophobic, roughness, and surface energy has received focus. According to reports, the roughness of TaN-Cu layer depends on the emergence of nanoparticles on the surface [9]. According to [2], surface energy reduction results in bacterial adhesion reduction. Another effective parameter in antibacterial activity of TaN-Ag layer is the average size of nanoparticles. Reduction in average size of nanoparticles can increase antibacterial effects of the TaN-Ag nanocomposite thin film [3]. In additional to the above, Riekkinen et al. [10] investigated the effect of TaN_x on electrical and optical properties of annealed TaN_x/Ag and TaN_x films to find more complete information about the useful layers. In general, TaN-Ag thin film has enough useful properties to apply it to the antibacterial thin film. It is noticeable that the performance of systems such as antibacterial, photocatalyst or self-cleaning structures is related to synthesis method. Chemical methods like ultrasound irradiation can be one of the best methods for fabrication of new photocatalyst systems [11]. For example, Cu(I) is considered for photocatalyst nanocomposite [12] due to the collecting agent and efficient catalyst in organic photoreaction. Recent reports have shown that the mechanical properties of Ag-doped TaN films could be enhanced after thermal annealing. This is due to the formation of nano-sized Ag particles in the nitride matrix and their diffusion toward the surface by increasing the annealing temperature [3].

This study synthesizes and modifies TaN-Ag nanocomposite thin film. Therefore, useful properties of TaN-Ag coatings make it a good candidate as an antibacterial nanocomposite thin film for coating on surgical instrument. The aim of this study is the modification of anti-adhesion and self-cleaning properties by producing a TaN-Ag nanocomposite thin film with high hydrophobic property. In our study, we tried to save the hydrophobic property of a surface by modifying the surface parameters which is not interesting enough in other papers.

2. MATERIALS AND METHODS

TaN-Ag nanocomposite thin films were deposited on pre-cleaned 316 stainless steel substrates with 1×1 $(cm)^2$ using the reactive DC co-sputtering technique. The deposition chamber was evacuated to a base pressure of about 2.4×10^{-4} torr. A schematic and geometrical view of the sputtering system can be seen in Figure 1. To synthesize TaN-Ag nanocomposite thin film, Ag wire (12 cm length and 1 mm diameter) was placed on cylindrical Ta target (20 cm length and 3.1 cm diameter). The Ta-Ag target was sputtered using 150 W power at a pressure of 2.2×10^{-2} torr in mixed Ar-N_2 (50%-50%) discharge gas to diffuse nanoparticles on the surface. The deposited samples were annealed at 350 °C for 3, 5, and 7 minutes by electrical oven (A.R.K. Co) with a ramping rate of 35 °C/min.

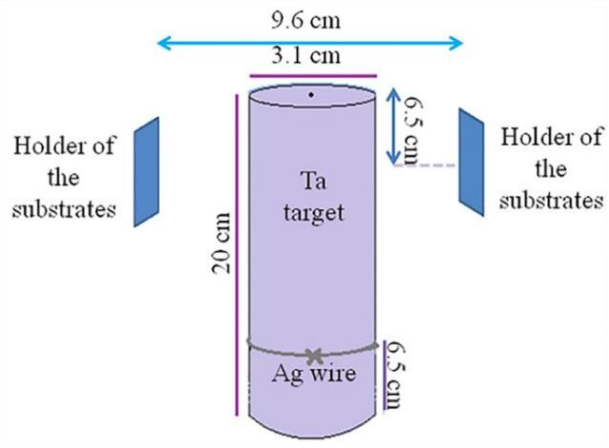


Figure 1. The schematic setup for deposition of TaN-Ag nanocomposite thin film

Electron Dispersive X-ray (EDX), (VEGAII TESCAN) was used to measure the percentage of materials involved in as-deposited thin films. To determine the crystalline structure of TaN-Ag thin film, deposited samples were tested by X-Ray Diffraction (XRD), (CLRPD 3000, made in Italy) system before and after the annealing process. Topography and surface properties of the thin films were investigated by Atomic Force Microscopy (AFM). The AFM images of the samples were analyzed using image analyzer 2.1 (IP). To investigate the morphology of layers, Scanning Electron Microscopy (SEM) (VEGAII TESCAN) was used. After heat treatment, the samples were kept on no-sterile plates at room temperature for four months. The antibacterial activity of thin films against Escherichia coli (E.coli, ATCC25922) bacteria was evaluated by the Colony Forming Account (CFU) method. The test was performed with 6.9×10^8 CFU for 6 h at 7.5 pH and 37 °C. It is noticeable that all of our

antibacterial tests were done without UV irradiation, which can improve the ability of our coating in the surgical instrument. In order to determine the hydrophobic character of the prepared films, Contact Angle (CA) technique was used. The water contact angle measurements were performed in atmospheric air at room temperature after two weeks of the annealing process using the commercial contact angle meter (Data physics OCA 15 plus) with ± 1° accuracy. A water droplet was injected on several spots of the surface using a 2 ml micro-injection.

3. RESULTS AND DISCUSSION

The crystalline structure of the deposited thin films was studied by XRD (Figure 2(b)). After heat treatment, the peaks attributed to TaN with a hexagonal structure appeared at 34.54°. As reported by others, Hexagonal Crystalline Structure is formed in a partial flow of N_2 (N_2 /{Ar + N_2}), equal to 10% [10]. In addition, XRD peaks belong to the diffraction line of the TaN phase (referring to standard card No. #71-0265). Based on our XRD result, no peaks belonging to Ag were observed in the X-Ray Diffraction due to its low concentration, but EDX (Figure 2(a) and 2(c)) analysis confirmed the presence of Ag nanoparticles in the matrix of TaN layers and antibacterial test implied the presence of these nanoparticles on the TaN surface. As expected, in the sputtering process, when the partial pressure of the reactive gases increased, the concentration of Ta particles vapor decreased. Of note, in all the films produced with reactive gases, the partial pressure lower than 3 mTorr, N/Ta ratio exceeds Ar/Ta ratio. For these films, the difference between N/Ta and Ar/Ta ratios is small, but as will be seen in the structural and properties analysis, they exhibit nitride like behaviors that lead to TaN formation.

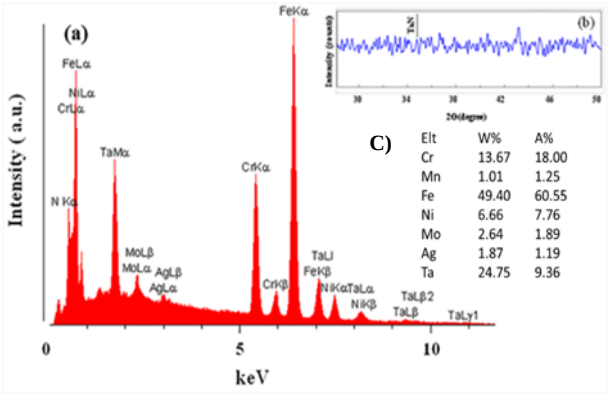
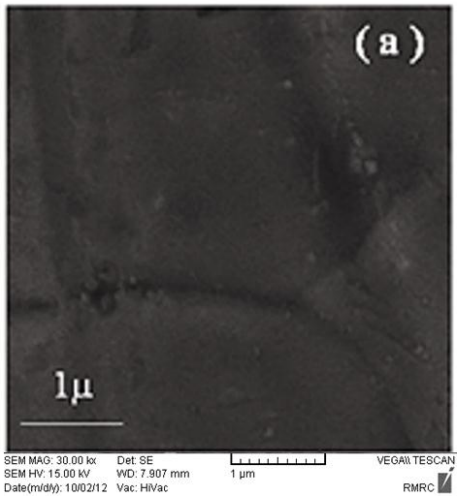
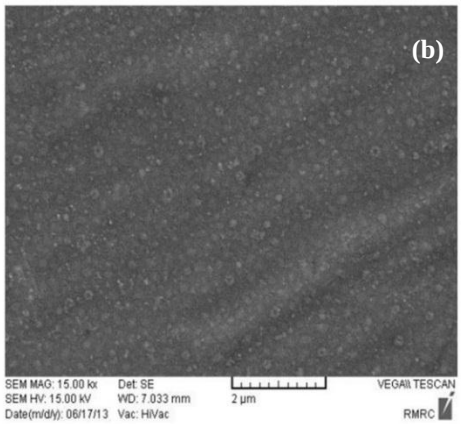


Figure 2. (a) Diagram of EDX, (b) XRD pattern, and C) Atomic percent of elemental analysis of as-deposited TaN-Ag thin film on 316 stainless steel substrate

Figure 3 shows the SEM image of TaN-Ag nanocomposite films after and before the annealing process at 350 °C for 5 min. According to Figure 3(a), for as deposited film, no feature with nano size was observed on the surface. However, during heat treatment, nanoparticles homogeneously became visible on the surface of the film. Due to the lower mobility of Ag nanoparticles, at low temperatures, the sputtered Ag atoms were aggregated in TaN and Ag clusters formed. Therefore, upon increasing the annealing temperature to 350 °C, Ag clusters in TaN received enough energy to diffuse toward the surface and formed particles. Based on our results, with increase in temperature, the higher number of metal (Ag) atoms could diffuse out on the surface due to the evidence of its greater tendency to lose electrons and higher catalytic and bioactivity, as reported elsewhere [13]. According to [14], Ag nanoparticles tend to form in rod shape. However, in Figure 3(b) and 3(c) (with different scale bar), Ag nano particles have fine and round shapes.





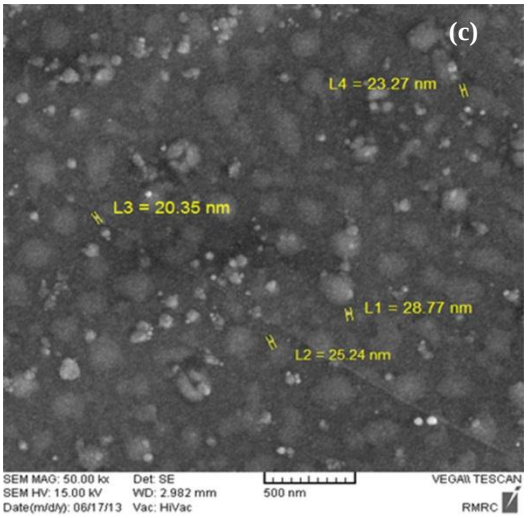


Figure 3. The yielded SEM images of TaN-Ag layer: (a) before annealing process; (b) and (c) surface of films annealed at 350 °C for 5 min

To determine the average size of the Ag particles and their distribution, AFM technique was utilized. Figure 4 shows the surface roughness and average size of nanoparticles after heat treatment at 350 °C at different annealing times (3, 5, and 7 min). According to Figure 4, the average size of the nanoparticles is about 50 nm. It is noticeable that the average grain size of nanoparticles obtained in our work is smaller than that in other similar studies [6]. This is because of the application of Cylindrical DC co-sputtering systems. According to Fig. 1, the substrates were placed on the upper section compared to the Ag target; thus, the nanoparticles with a larger size could not reach the substrates because of their heavy weights. Hence, the average size was reduced. Obviously, in Figure 4, with a rise in the annealing time, the average size of nanoparticles increased. The same result was reported by others [3,6]. In addition, as shown in Figure 4, the average roughness as a function of annealing time increased with a rise in the annealing time.

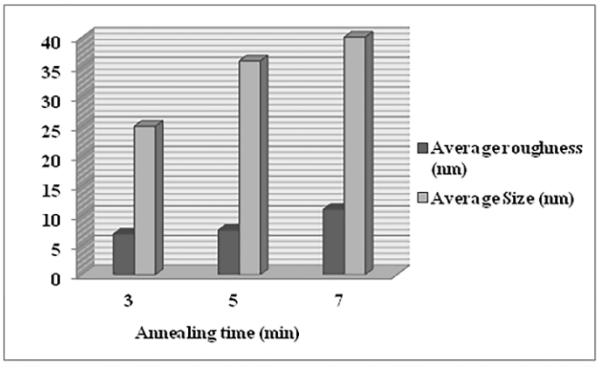


Figure 4. Average size of Ag nanoparticles and average roughness of TaN-Ag layers at 350 ºC was measured by AFM results

Figure 5 shows the 3D AFM images before and after heat treatment (annealing time of 3 min). As can be seen in the figures, by performing heat treatment and diffusing the particles on the surface of the thin layer, the concentration of particles increased and increased the surface roughness, which confirmed the SEM images.

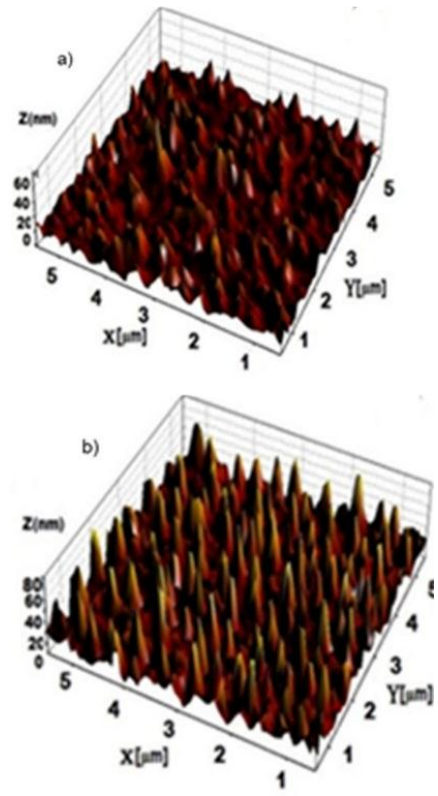


Figure 5. 3D AFM images of TaN-Ag layers: (a) before and (b) after annealing at 350 °C (3 min)

After surface characterization, we have studied our films by measuring surface contact angle, which has an important role of antibacterial activity. Heng-Li Huang et al. [4] observed that the emergence of Ag nanoparticles decreased the contact angle of the TaN layer. However, in our samples, after the emergence of Ag nanoparticles, the contact angle increased. Roughness could explain this contrast. Table 1 shows the contact angle measurements of TaN-Ag thin films.

TABLE 1. Contact angle, roughness factor, (CA/r) ratio, and antibacterial activities of the samples annealed at 350 ºC with different annealing times and Ag concentrations

Annealing time (min)	**7**	**5**	**3**
(Ag/TaN) percent ratio	7%	8%	8.5%
Antibacterial activity (CFU)	52%	46%	39%
Contact angle	95.6°±3	92°±3	93°±3
Roughness factor (r)	1.04	1.03	1.04
CA/r	92.8°	88.5°	89.423°

Results showed that the presence of Ag nanoparticlesdid not reduce the CA; this is due to increasing surface roughness by adding Ag particles on the surface film. We found that the contact of a droplet with thin film surface was attenuated upon increasing the surface roughness which resulted in a greater contact angle. It is similar with butterfly wing effects. The butterfly wings decorated as bumps, which made them super hydrophobic surface [15]. Because of the lower adhesion of water and air than water and solids, roughness of surfaces can reduce the adhesive force on water droplets and liquid-to-solid contact area.

Figure 6. shows that the contact of water droplets on TaN-Ag thin films was annealed at 350 °C with different annealing times (without irradiation UV). Thus, based on our investigation, the negative effect of Ag nanoparticles on hydrophobic property of thin film was reduced by increasing surface roughness. Four months after the annealing process, antibacterial tests were performed for investigation of antibacterial longevity. To determine the effects of annealing time and Ag concentration on antibacterial activity, the layer with a higher concentration ratio of Ag/TaN was annealed at a lower time frame (3 min) and the layer with lower Ag/TaN ratio was annealed at a longer time frame (7 min). The results are summarized in Table 1. Antibacterial efficiency of the TaN-Ag nanocomposite against E.coli is shown in Table 1 for different Ag concentrations and annealing times. According to Table 1, thin films annealed at 350 °C for 7 min had better antibacterial behavior than either TaN-Ag nanocomposite. One of the reasons for this behavior is that by increasing the annealing time, Ag concentration on ion surface films increased. In other words, Ag nanoparticles need time to cross the inner matrix of TaN. After increasing annealing time, more Ag nanoparticles can approach the surface. Therefore, the sample with lower Ag/TaN percentage ratio and longer annealing time has the best antibacterial efficiency. In addition, a rough surface generally has a larger surface area, which increases the possibility of bacterial contact and thereby, promotes colonization by bacteria as well as spherical Ag nanoparticles. Therefore, TaN-Ag annealed at 350 °C for 7 min has greater surface roughness and antibacterial activity, as reported by others [16]. Wang et al. [17] demonstrated that a smooth Ti surface exhibited a low S.aureus bacterial adherence, which resulted in a low probability of infection. On the other hand, in this case, less adherence of bacteria to the surface occurs. Therefore, the most significant short-term antibacterial effect can be observed on the surface with 95° CA. The samples were annealed again at the same time and temperature, i.e., 5 min and 350 °C.

Figure 7 exhibits the results of antibacterial test with respect to (Ag/TaN) percentage ratios of 1, 3, 6, and 24 h. As is implied, with increase in Ag concentration, antibacterial activities increased. The result was emphasized by other studies [1,3,4,6].

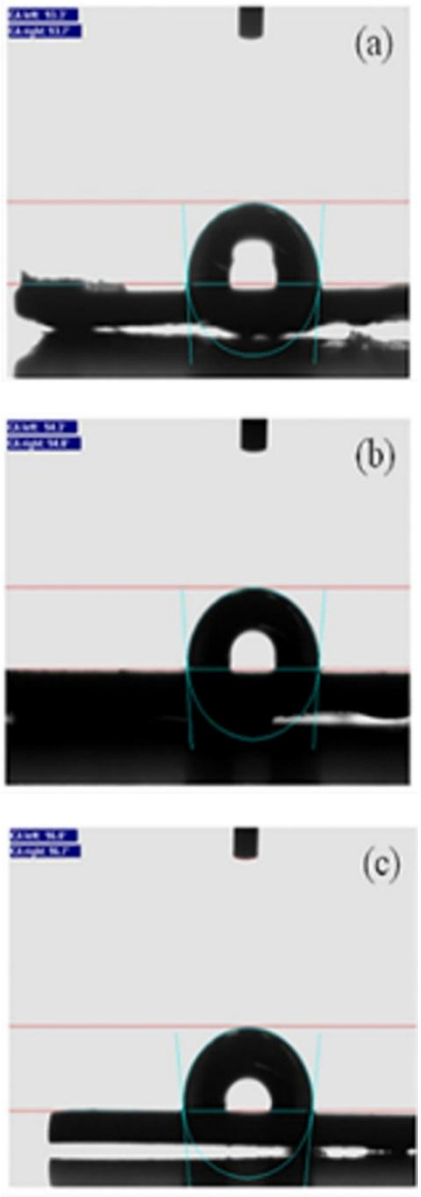


Figure 6. Contact angle images of TaN-Ag layers, which were annealed at (a) 3 min (8.5% Ag), (b) 5 min (8% Ag), and (c) 7 min (8.5% Ag).

Generally, the concentration of nanoparticles, annealing time, contact time with bacteria, and contact area of the antibacterial layer with bacteria play important roles in attaining good antibacterial activities.

For antibacterial mechanism, there are many reports in the literature showing that the electrostatic attraction between negatively charged bacterial cells and positively charged nanoparticles is crucial to the activity of nanoparticles as bactericidal materials [18]. Recently,

Amro et al. [19] demonstrated that metal depletion might cause the formation of irregular shaped pits in the outer membrane and changed membrane permeability, as caused by progressive release of Lipopolyscaceharide (LPS) molecules and membrane proteins. We may speculate that a similar mechanism causes the degradation of the membrane structure of E.coli bacteria during treatment with silver nanoparticles. In addition, it is believed that DNA loses its replication ability and cellular proteins inactivate Ag^+ treatment [20]. In addition, it was also shown that Ag^+ would bind to the functional groups of proteins, causing protein denaturation [21]. It is noticeable that the mechanism of antibacterial properties of Ag nanocomposite is still not fully understood [22].

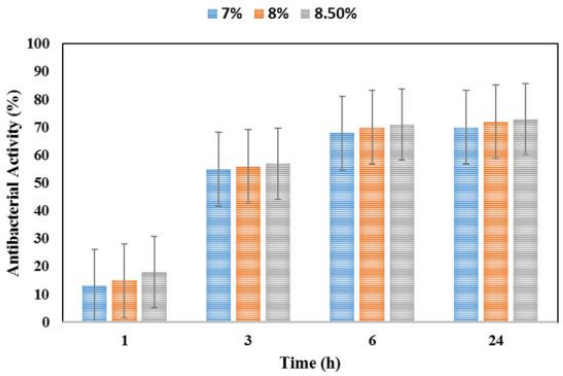


Figure 7. Variation of antibacterial activities by incubating time respect to the different Ag concentrations

4. CONCLUSION

The TaN-Ag nanocomposite thin films were deposited on 316 stainless steel substrates using reactive cylindrical DC co-sputtering system. The layers have suitable advantages which make them viable as coating for surgical instruments. Hexagonal crystalline structure was attained for TaN layer by a 50%-50% gaseous mixture of Ar-N_2. Increasing the number of Ag nanoparticles on the surface causes an increase in its roughness. The Ag nanoparticles have fine and round shapes. The modification of the contact angle occurs due to increased roughness. On the other hand, increasing the contact angle decreases bacterial adhesion. Antibacterial activity efficiency depends on both Ag concentration and annealing time. Hence, the sample with lower Ag/TaN percentage ratio and the longer annealing time presents a better antibacterial activity. At the same annealing time for all three samples, the best antibacterial activity belonged to the layer with the highest Ag concentration. With a rise in annealing time at a constant temperature, the average size of nanoparticles increased.

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