Karaagac *J Mater. Sci: Mater Eng.* (2024) 19:21
https://doi.org/10.1186/s40712-024-00166-8

Journal of Materials Science: Materials in Engineering

ORIGINAL PAPER

Open Access

Poly(ethylene glycol) patterned surfaces functionalized with gallic acid@Au nanoparticles: investigation of antibacterial activity for biomedical applications

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Abstract

Polymer patterns are promising for many applications due to their high stability and superior chemical and physical properties. By functionalizing various surfaces with polymer patterns, it is possible to detect and prevent many common infections. Treatment of resistant bacteria with antibiotics is limited and they can spread quickly. For this reason, it was designed a surface that can prevent contamination by functionalizing polymer patterns. In the study, a polymer pattern model obtained by combining gallic acid with gold nanoparticles (GA@AuNP) synthesized through green chemistry was designed. Polymer-patterned structures were obtained on silicon wafers using Poly(ethylene glycol) (PEG) polymer and were self-assembled with GA@AuNPs. Diagnosis and inhibition of bacterial cells in a short time were demonstrated with the prepared modified PEG polymer pattern. Surface-enhanced Raman scattering effects were used to optimize the stability of surfaces patterned with self-assembled GA@Au NPs. By modification of PEG polymer patterns, a biomarker design that can be used in many different bioapplications is proposed.

Keywords Polymer patterns, Gold nanoparticles, Biomarker, Antibacterial agent

Introduction

Polymer patterns are a special class of material surfaces obtained by combining polymer chains connected at one end to a substrate (Anisha et al. 2016). They are used in many practical applications with their bio adhesiveness and control of surface activity (Wagner et al. 2004; Alcantar et al.2000). Thus, it creates potential for many nano-biomedical applications. PEG polymer is frequently used in biomedical applications due to its high biocompatibility and non-toxicity (Morgase et al. 2018).

To form polymer patterns on silicon wafers, various physical and chemical effects are used. Negative conditions occurring at this interface directly affect the stability of the patterns. For this reason, it is important to activate the surface energy by increasing the silanol groups (Si–OH) on the Si wafer surface during the patterning process (Fig. 1). Silanol groups interact with the hydroxyl groups of PEG to form an ester bond (Si–O–C). Thus, it allows PEG to flow on the Si wafer. The vibrational absorption bands of C–O and –CH2 groups increase in the process. In contrast, the Si–OH absorption band decreases. This shows that Si wafer silanol groups react with PEG and are consumed simultaneously. The reaction continues until full saturation occurs (Alcantar et al. 2000; Jal et al. 2004; Leopold et al. 2013). A hydration layer is formed on the PEG surface by hydrogen bonds that create a steric barrier. This eliminates oxidation of hydrogen bonds in the presence of oxidants

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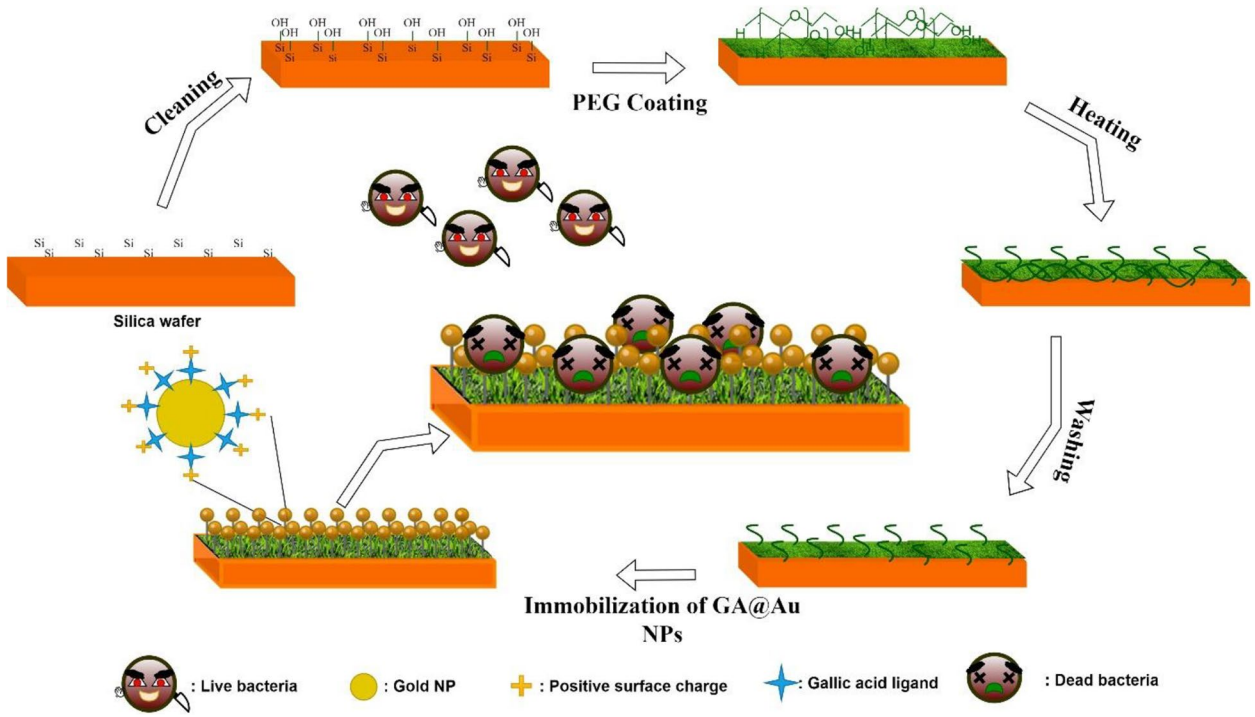

Fig. 1 Design of PEG polymer-patterned surfaces functionalized with GA@Au NPs and use of as a biomarker

and transition metals in the environment (Fundeanu et al. 2008). Additionally, PEG polymer is promising for many different applications thanks to its neutral surface (Krishnamoorthy et al.2014; Seo et al. 2017). There are antibacterial polymer-patterned structures for similar purposes in the literature. In a study, the antibacterial effect of polymer patterns obtained with Poly(2-alkyl-2-oxal) polymer was investigated. This surface that prevents bacterial adhesion was obtained (Schlenoff et al. 2014). In a study, polydimethylsiloxane (PDMS) was immobilized on the surface with PEG and tannic acid-reduced Au NPs (Au@TA NPs). It prevented bacteria from accumulating on the surface with near-infrared (NIR) irradiation (He et al. 2021). There are many studies using the superior properties of PEG and metallic NPs as antibacterial activity agents. Diagnosis and treatment of gram-negative and gram-positive bacteria are recommended by coating zinc oxide and iron oxide NPs with PEG (Keihan et al.2017; Jose et al.2018). In another study, Poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC), a zwitterionic polymer, was reported to inhibit infections on coated surfaces (Turkcan et al. 2018). Considering all these studies, it seems that it is based on the principle of removing bacteria from the surface. This did not address the increased risk of infection around the surface. When the studies are evaluated, it is not possible to kill or completely neutralize bacteria by contacting them with the surface. This will not prevent bacterial growth in the environment and will cause surfaces exposed to more bacteria to lose their activity after a while. In order to prevent this situation, a new nanomaterial design is important. Gallic acid stood out as a good candidate for this new design.

Gallic acid is a polyphenolic compound obtained from the hydrolysis of natural plant polyphenols and can be used as a reductant in the synthesis of nanomaterials (Huang et al. 2024). Gallic acid is a good biological agent due to its high catalytic activity, biocompatibility and positive surface charge (Wang et al. 2024). By combining gold and gallic acid, it is possible to achieve maximum efficiency in antibacterial activity and catalytic activity (Saeedeh et al. 2023; Liang et al. 2021). Similarly, there are many different approaches in literature to create an antibacterial effect (Jegatheeswaran et al. 2015; Habiba et al. 2015). Unlike classical antibacterial surfaces, polymer patterns create an active surface and show a three-dimensional formation due to the conformational growth of individual polymer chains (Walsh et al. 2021). Thus, it can protect its antibacterial properties more effectively by completely covering the surface.

The antibacterial activity of surfaces obtained by gold immobilization on polymer patterns can be tested using various bacteria. *Methicillin-resistant Staphylococcus Aureus* (MRSA) is a strain of *Staphylococcus Aureus* bacteria that is resistant to methicillin and other beta-lactam

antibiotics (Jinjie et al. 2023; Ganesan et al. 2023). MRSA infections can cause a variety of clinical manifestations, ranging from localized infections of the skin and soft tissues to more serious systemic infections. Especially in in vivo implant applications, infections caused by *Staphylococcus aureus* are frequently encountered on implant surfaces. In recent years, nanotechnology-based approaches have begun to be used in the treatment of (MRSA) infections (Fatima et al. 2021; Orozco et al. Karaagac et al. 2023; 2024; Zahrani et al. 2021). Studies show that targeted NPs can be effective against MRSA, overcome biological films, and exhibit anti-infective activity (Zhou et al. 2024; Iheme et al. 2024). Existing obstacles can be overcome by exploiting the antimicrobial activities of plasmonic bacteria such as gold and silver, as well as zinc and copper NPs. A previous study proved that silver NPs could be effective on MRSA. However, since Ag NPs already have high antibacterial activity, it could not explain why the process was specific to MRSA (Zdyrko et al. 2008). There is also a risk that NPs may have antimicrobial activity by being affected by environmental conditions. Therefore, it is not possible except in stable environments. Likewise, zinc oxide NPs, which have high antibacterial activity, are not suitable for use due to the same obstacles. However, as a result of these studies, it is possible to conclude that MRSA bacteria respond to nanotechnological approaches. Au NPs are promising for many bioapplications as they are biocompatible, non-toxic, and have high stability (Karaagac et al. 2020).

The study suggests that the immobilization of GA@AuNPs onto the PEG polymer pattern will be an effective biomarker. PEG containing a single hydroxyl was used to prevent adsorption resistance that may occur due to hydrogen bonds on the PEG surface. Thus, a more stable, practical, and applicable design was obtained by combining the advantages of both polymer patterns and GA@Au NPs. To systematically study the antibacterial properties of the developed PEG polymer patterns, the direct interaction of MRSA with GA@Au NP was investigated. In order to obtain a better and more detailed image, the inhibition process of MRSA was explained using STEM analysis. In addition, each step was characterized in detail to fully understand the biomarker property of the modified PEG polymer pattern.

Experimental section

Chemicals and instrumentation

N,N, dimethylformamide (DMF) (−99.5% purity), toluene (99.0% purity), chloroform (−99.0% purity), and chlorobenzene (−99.0% purity) were obtained from Merck KGaA, Germany). Si wafers (single surface polished) were purchased from Wafer World Inc. Rhodamine 6G was obtained from Sigma–Aldrich. P2VP-OH (20.0 kg/mol, polydispersity index = 1.04) and PEG (15.0 kg/mol) Polymer Source Inc. was purchased from the company. Scanning Transmission Electron Microscope (STEM), Goniometer, Raman spectroscopy, and Zeta potential instrumentations were employed to characterize the grafted polymer patterns. Distilled water was used in all experiments.

Grafting of PEG polymer patterns

Silicon wafers were cleaned in a UV-ozone device (Bioforce, procleaner) for 20 min to remove contamination. The cleaned surfaces were coated with a single hydroxyl-terminated PEG polymer (chlorobenzene (CB)-2% PEG solution) with a molecular weight of 15 kg/mol. Heating was applied to the Si substrates to make the hydroxyl ends react with the silanol groups. The substrates were heated at 180 °C for 5 min in an inert gas environment and allowed to cool after the process. It was washed with chloroform 3 times to remove excess polymers that did not react during the heating process. It was then dried with nitrogen gas and made ready for applications (Kim et al. 2003).

Synthesis of GA@Au NPs

GA@Au NPs were synthesized following a known protocol. At room temperature, with vigorous stirring and protected from light, 16.66 mM HAuCl4·3H2O solution was added to 10 mM GA solution under room conditions to obtain a mixture with a total reaction volume of 10 mL. GA@Au NPs were obtained by mixing at high speed for 30 min. Then, the final solution was centrifuged for 10 min at 5000 rpm to precipitate the NPs. The supernatant separated from the NPs was discarded, and the NPs were resuspended in distilled water. The centrifugation process was repeated 3 times to completely remove unreacted GA from the structure. GA@Au NP solution was obtained with a wine-red color as shown in Fig. 2b. It was last characterized by UV visible, STEM, DLS, and Zeta potential. (Fathy et al. 2023).

Immobilization of GA@Au NPs

Au nanoparticles were dropped onto the grafted polymer pattern surfaces and incubated for 1 h at room conditions. In order to create a humidity balance in the environment, the immobilization medium was moistened in accordance with the pattern surface area. In the application, distilled water was dropped into the petri dish containing the pattern, and the moisture value was preserved with parafilm. Polymer pattern surfaces functionalized after Au immobilization, characterized by ellipsometer, SEM, and Raman instruments.

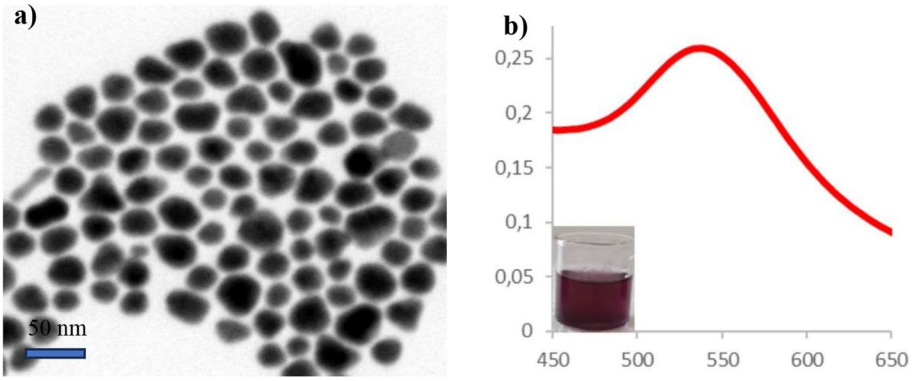

Fig. 2 Characterization of GA@Au NPs: **a** STEM analysis and **b** UV spectrum analysis

Antibacterial activity testing

To investigate antibacterial activity, MRSA (10^6 CFU) were incubated with GA@Au NP-modified polymer patterns for 5–20 min in room conditions. At the end of the incubation period, the pattern structures were characterized by STEM.

Results and discussions

Preparation of PEG polymer patterns

The design of PEG polymer patterns and the immobilization of GA@AUNPs are shown in Fig. 1. First of all, surface cleaning is required to increase silanol groups on silica surfaces. In order to increase surface activity, UV-ozone cleaning was applied to the Si surface for 20 min before the polymer chain grafting process. After that, 2%—15,000 g/mol PEG-Chlorobenzene solution was prepared and coated on the silica wafer surface by spin coating method at 3000 rpm. The surfaces prepared by the coating process were heated at 180 °C for 5 min under the inert gas atmosphere (argon). The heating process ensures the reaction of the hydroxyl groups and silanol groups on the surface. Hydroxyl and silanol groups are paired to form pattern structures. To remove the unreacted PEG polymer from the surface, washing with chloroform and ultrasonic cleaner was performed. At each stage, the pattern formed on the surface was characterized with an ellipsometer to determine its thickness. After the final washing process, the ellipsometry thickness of the surface was 9.8 nm ± 0.2 nm.

Synthesis and characterization of GA@AuNPs

In the synthesis of GA@Au NPs, it was synthesized by mixing $HAuCl_4$ $3H_2O$ and GA solutions with a magnetic stirrer at room temperature. GA@Au NPs synthesized with the green synthesis approach in a short time were analyzed by UV–visible spectrometer (Fig. 2b). A single and narrow peak was obtained at 525 nm, proving that Au was present in the structure and that the structure was spherical. Thus, information was obtained that GA formed an NP with Au. In the analysis performed with scanning electron microscopy (STEM), it was clearly seen that the NP structures were in a homogeneous size range and had uniform surface properties, confirming the UV–visible spectrometer analysis.

Zeta potential analysis was performed to determine the surface charge of GA@Au NPs. As seen in Table 1, the NP surface charge was determined to be positive. Thus, it was predicted that the MRSA membrane could be rapidly labeled with the effect of steric forces. In addition, dynamic light scattering (DLS) analysis was performed to determine the hydrodynamic diameter of NPs. As stated in Table 1, the diameter was determined to be 50 nm.

Functional pattern characterization

GA@Au NPs were directly dropped onto the prepared PEG pattern surface at the room conditions. NPs solution was incubated for 1 h. At the end of the period, silica wafers were sonicated and washed with distilled water. Polymer pattern surfaces functionalized with gold NP. It was characterized by ellipsometer, goniometer, SEM, and Raman spectrometer.

In the analysis made with an ellipsometer, it was seen that the thickness, which was around 9.8 nm ± 0.2 nm, increased to 11.5 nm ± 0.2 nm after the immobilization of GA@AuNPs. The increase in surface thickness proves that GA@AU NPs are immobilized on the polymer

Table 1 DLS and Zeta potential of GA@Au NPs

Analysis type	GA@Au NP
DLS (nm)	50 nm
ζ (mV)	10,3 mV

pattern. The surfaces were characterized with a goniometer to measure the contact angle of the surfaces. Thus, the hydrophobic or hydrophilic state of the surface was evaluated for biomedical applications. It was concluded that GA@Au NPs synthesized in aqueous solution environment would be better distributed on the surface thanks to the hydrophilic surface. On the other hand, increasing hydrophilicity will reduce protein adsorption and cell adhesion rate (Harrison et al. 2016). Thus, the surface will be protected against non-specific absorptions. Considering all these, high hydrophilic rate and stability are very important for medical sensor applications and implant technologies (Contreras et al. 2019). It is thought that these properties that PEG already has will be more stable and efficient at the nanoscale. In the goniometer analysis, it was determined as 37.6° ± 0.4° (Table 2). Thus, it was concluded that the surface was hydrophilic and could be suitable for bioapplications. In the analyses performed with STEM, it was observed that the NPs were distributed homogeneously on the surface and no agglomeration occurred (Fig. 2a). In analyses performed with Raman spectroscopy, molecules registered in the system can be detected depending on the development of signals around NP structures. The SERS activity of GA@Au NP-functionalized polymer models was measured using the reporter molecule 6G with 532 nm laser excitation. The SERS spectrum of rhodamine 6G is shown in Fig. 3b. Luminescence-induced noise signal data was obtained by taking the arithmetic average of the spectra obtained from all measured areas, and the spectrum peaks were arranged after the measurement. As a result of the characterization of PEG patterns with SERS, a specific peak is expected at 840 cm^{-1}, 1100, and 1150 cm^{-1} levels. In the analysis, shifts occurred (as expected) due to the effect of OH groups of Au NP. New peaks were detected at 790 cm^{-1}, 1100, and 1110 cm^{-1} levels. Likewise, shifts were observed in the CH2 wagging bands in

Table 2 Surface characterization of PEG pattern, ellipsometer, and goniometer analysis

PEG pattern analysis	Goniometer	Ellipsometer
After the washing	37.6° ± 0.4°	9.8 nm ± 0.2 nm
After the immobilization of GA@Au NP	52.6° ± 0.2°	11.5 nm ± 0.2 nm

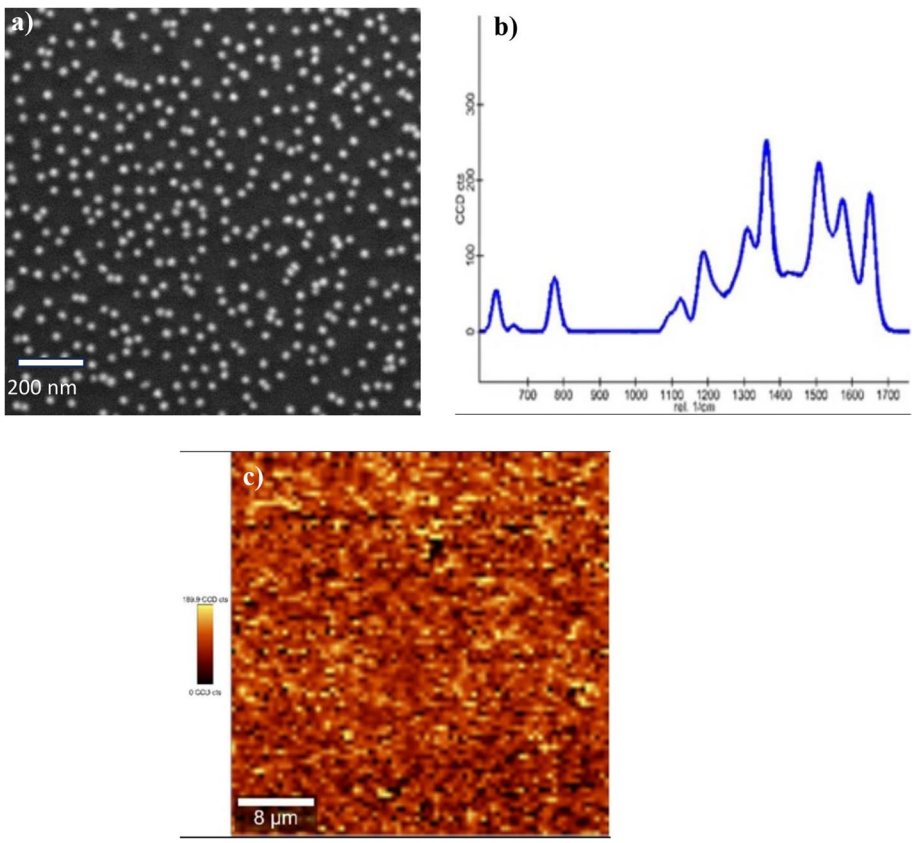

Fig. 3 Characterization of PEG pattern with GA@Au NPs. **a** SEM analysis. **b** SERS spectra of rhodamine 6G reporter molecule. **c** Raman mappings (based on SERS intensity at 1361 $cm.^{-1}$)

the range of 1280–1380 cm^{-1} and in the bending bands in the range of 1350–1450 cm^{-1} (San Juan et al. 2022). In addition, the distinct peaks in the peaks indicate that the wool Au NP was successfully immobilized to the surface. A very intense radiation was detected in proportion to the Raman intensity map SEM analysis (Fig. 3c).

Antibacterial activity of GA@AuNP

Designed a study to test the biomarker property of GA@Au NPs synthesized through biocompatible and green synthesis. GA is a phenolic compound and is known to have a wide-spectrum antibacterial activity. Antibacterial activity has been proven in a study with zinc oxide NP (Lee et al. 2017). In another study, antibacterial agent design was made using Silver (Silisyum, and Zinc Oxide, but as a result of long and complex experimental study, an effective result could not be provided. Low doses have been proposed for MRSA to be released, although the nano size is increased toxicity (Wu et al. 2020). GA@Au NP is synthesized in the distribution and narrow size range. In this study, the interaction of MRSA with GA@Au NP was systematically evaluated. Naked MRSA and GA@Au NP-interacting MRSA bacteria were compared by STEM analysis. It is given in Fig. 4a. GA@Au NP was incubated with MRSA cell solution for 5 min. Teichoic acid and lipoteichoic acids bound in the peptidoglycan layer on the MRSA surface contribute to the negative charge of the surface (Brown et al. 2013). Thanks to the electrostatic interaction between positively charged GA@AuNPs and the MRSA membrane, the NPs were quickly assembled on the membrane of MRSA cells (Fig. 4a). In addition, when the incubation time increased, GA@AuNPs completely inhibited MRSA by disrupting the cell membrane, as seen in the STEM images (Fig. 4b).

The adhesion and inhibition process of GA@Au NP on the MRSA surface was examined at minute intervals. The inhibition process of MRSA was systematically discussed with the experimental study. In addition, the disadvantages (toxicity, infection, stability problems, etc.) that occur in studies using silver, zinc, and iron NPs, which are frequently encountered in the in vivo environment, were not encountered (Nanda et al. 2009; Kadiyala et al. 2018; Yeo et al. 2022).

Conclusion

In summary, an active, effective, and biocompatible surface was presented with the PEG polymer pattern and plasmonic GA@AU NP. Proper fabrication of PEG polymer models and self-assembled immobilization of plasmonic NPs onto surfaces are demonstrated. A spectacular array was obtained by immobilizing GA@Au NPs onto the surface of the PEG polymer model via self-assembly. Although no agent was used to fix the nanoparticles on the polymer surface, long-lasting effective immobilization was achieved. This is proven by Raman spectroscopy signals and Sem images. With this study, the immobilization of a ligand other than citrate to the PEG pattern surface was successfully achieved. Additionally,

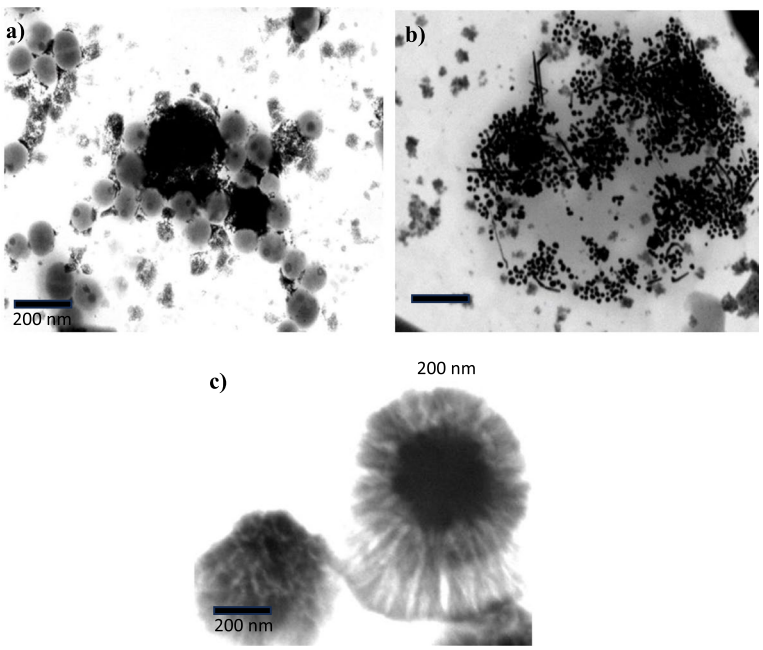

Fig. 4 GA@AuNP interaction with MRSA bacteria, **a** 5 min, **b** 10 min, **c** 20 min incubation

the developed surfaces were tested on resistant bacteria. With the STEM images taken, it was demonstrated by a time-dependent systematic experiment that GA@Au NPs rapidly inhibit MRSA bacteria. The fact that it inhibits bacteria in as little as 5 min reveals how effective it is. In conclusion, the study demonstrated the use of nanoparticles as a polymer-patterned biomarker. In the study, the way to apply active surfaces quickly and systematically under room conditions was clearly revealed. With the presented strategy, it can be expected that new patterns can be developed using different polymers. It was predicted that active surfaces can be produced by developing new nanomaterials with different ligands.

Acknowledgements
The author would like to thank Ocsoylab and its team for providing laboratory facilities to carry out the studies.

Author's contributions
Zehra Karaagac: conceptualization, data curation, ınvestigation, methodology, writing — original draft, writing — review and editing, formal analysis, funding acquisition, ınvestigation, project administration, resources, software, supervision, validation, visualization, writing — original draft.

Funding
This research received no external funding.

Availability of data and materials
All data gathered or analyzed during this study experiments is included in this article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The author declares that she has no competing interests.

Received: 25 April 2024 Accepted: 17 August 2024
Published online: 26 August 2024

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