Brazilian Journal of Microbiology (2021) 52:619–625
https://doi.org/10.1007/s42770-021-00455-w

CLINICAL MICROBIOLOGY - RESEARCH PAPER

Antimicrobial effect of gold nanoparticles in the formation of the *Staphylococcus aureus* biofilm on a polyethylene surface

Lorena Dafnee Villa-García[1] · Raúl Márquez-Preciado[1] · Marine Ortiz-Magdaleno[2] · Olga Araceli Patrón-Soberano[3] · Marco Antonio Álvarez-Pérez[4] · Amaury Pozos-Guillén[2] · Luis Octavio Sánchez-Vargas[5]

Received: 21 September 2020 / Accepted: 6 February 2021 / Published online: 22 February 2021

Abstract

The main of this study was to evaluate the inhibitory effect on the in vitro formation of the *Staphylococcus aureus* biofilm formed on a polyethylene (PE) surface with a nanostructured Gold (Au) coating for medical devices. An experimental in vitro study was carried out using PE discs with an Au nanoparticle coating (AuNPs) on one side (experimental group) and without coating on the other (control group); the discs were mounted in the CDC biofilm reactor adding broth of yeast-dextrose-peptone (YPD) sterile culture inoculated with *S. aureus* in a cell suspension (5×10^8 cells/ml). The specimens were evaluated at different times (6, 12, 24, 48, 72 h) and stained with the Live/Dead Bacterial Viability Kit (Invitrogen) for observation, analysis, and quantification with confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). The results showed that as evaluation time passed an increasing of *S. aureus* biofilm formation was observed in the control group, in the experimental group, a statistically significant biofilm inhibition was observed with respect to the AuNPs uncoated specimens ($p \leq 0.05$) and showed a ratio of almost 4:1 viable/nonviable in the biofilm of the uncoated surfaces, with a difference > 5 Log_{10} in the CFU counts. The PE with AuNP coating showed an inhibitory effect on the biofilm formation of *S. aureus*.

Keywords Polyethylene, · Biofilm, · *Staphylococcus aureus*, · Gold nanoparticles, · Biofilm reactor

Introduction

Infections associated with biomaterials used in the human body have a low incidence; however, when they happen, this represents a serious complication with high mortality rates. Among the materials implanted daily with biomedical purposes in the medical and dental area, we find various biocompatible materials that can have a natural or synthetic origin, and the most used are polymers, which have multiple uses in these areas and are essential for these materials that they do not allow colonization of pathogenic microorganisms and cause an infection in the patient [1].

A highly used polymeric material is high-density polyethylene (PE) due to its dimensional stability against high sterilization temperatures. Its applications range from surgical instruments to temporary and permanent biomedical devices, such as catheters, sutures, and implants for facial and cranial reconstructions [2, 3]. The coating and characterization of biomedical materials surface with nanoparticles makes it possible to inhibit the growth of bacterial biofilm and has been continuously investigated. Gold (Au) is a noble metal used in

Responsible Editor: Fernando R. Pavan.

✉ Luis Octavio Sánchez-Vargas
octavio.sanchez@uaslp.mx

[1] Paediatric Dentistry Postgraduate Program, Faculty of Stomatology, Universidad Autónoma de San Luis Potosí, San Luis Potosí, SLP, Mexico

[2] Basic Science Laboratory, Faculty of Stomatology, Universidad Autónoma de San Luis Potosí, San Luis Potosí, SLP, Mexico

[3] Molecular Biology Division, Instituto Potosino de Investigación Científica y Tecnológica, San Luis Potosí, SLP, México

[4] Tissue Bioengineering Laboratory, Faculty of Stomatology. Postgraduate and Research Division, Universidad Nacional Autónoma de México, Mexico City, Mexico

[5] Biochemical and Microbiology Laboratory, Faculty of Stomatology, Universidad Autónoma de San Luis Potosí, Av. Dr. Manuel Nava #2, Zona Universitaria, 78290, San Luis Potosí, SLP, Mexico

the form of nanoparticles to coat surfaces of biomedical materials; these nanostructured coatings of Au have low toxicity and a great bioaffinity, have photothermal properties, and influence the immune system, in addition to being excellent antimicrobials, inhibiting the formation and development of bacterial biofilms [4–7].

A biofilm is an assemblage of surface-associated microbial cells that is enclosed in an extracellular polymeric substance matrix [8]. Such structures can be replicated in vitro employing different biofilm formation models, which can be aerobic, anaerobic, dynamic, or static and are used to evaluate the efficacy of antimicrobials agents; these models can be monocultures under static or dynamic growth conditions [9]. In vitro models are routinely applied to examine the adhesion of specific bacterial species to any biomaterial surface; study the nature and pattern of biofilm formation on a particular substrate, as well as the interaction between different biofilm bacteria; and evaluate the efficacy of antimicrobial agents or antimicrobial treatment strategies [10, 11].

There is little reported evidence of the methods that can be used to decrease and inhibit the development of biofilm that forms on the surface of biopolymeric materials used in the biomedical and dental area; that is why it is important to investigate if the gold nanoparticles (AuNPs) added to the surface of these materials prevent the growth of microorganisms with high pathogenic potential such as *S. aureus* that is a facultative gram-positive anaerobic bacterium that has a high degree of pathogenicity and is responsible for a wide range of diseases and is the main cause of nosocomial infections; its biofilm can be established on living and inert surfaces [12, 13].

Surfaces are a key factor in Biology and Medicine. In particular, surfaces are modified with nano-/microstructuring in order to improve biomedical devices. Nanoparticles (NPs) possess completely different mechanisms of microbicidal action from those of traditional antibiotics, thus providing a new alternative to increasing resistance. The objective of this study was to evaluate the inhibitory effect of the in vitro formation of *S. aureus* biofilm on a PE surface with an AuNP coating at different bacterial growth times using the CDC biofilm reactor.

Materials and methods

The specimens (polyethylene CDC; BioSurface Technologies) had a diameter of 12.7 ± 0.50 mm and a thickness of 3.8 ± 0.15 mm and were divided into two groups, a group with coating (n = 50) and one without the coating (n = 50) of AuNPs as the control group; each group was evaluated in 5 time periods 6, 12, 24, 48, and 72 h at 36 °C.

AuNP coating on PE

To cover the specimens, these were cleaned with 100% methanol for 40 min on ultrasound and dried in a vacuum chamber. The deposition of the AuNP coating was done by sputtering (Balzers SCD050) with the use of a gold target with a purity of 99.99% (JEOL Dantum LTD) and the deposition conditions were standardized to control the thickness of the deposited film by 10 min of deposit times, with argon plasma (99.995% of purity) followed by a discharge power of 7.5 W with a flow of approximately 0.3 L/s with a pressure of 5 Pa and with an electrode distance of 50 mm and an electrode area of 48 cm^2 with a 1000-cm^3 volume reaction chamber.

Formation of *S. aureus* biofilm

Staphylococcus aureus biofilm adhesion was evaluated in a dynamic model using a CDC bioreactor (model CBR 90–1, BioSurface Technologies Corp.). For the preparation of the inoculum, the yeast for bacteria *S. aureus* ATCC 25923 in sheep blood agar was incubate at 37 °C for 24–48 h. After that, a fresh colony was propagated in yeast extract, peptone, and dextrose (YPD) broth (1% yeast extract, 2% peptone, 2% dextrose) and incubated for 48 h at 37 °C; the cells were washed twice with sterile phosphate-buffered saline solution (PBS) (pH 7.2) by agitation and centrifugation at 3500 RPM for 5 min. For the CDC bioreactor system, a *S. aureus* suspension was standardized to a final concentration of 0.5 a 2×10^3 cells/ml in YPD culture broth. After a primary incubation period of 16 h at orbital agitation of 80 RPM, this was followed by an additional 24-h period of a continuous flow rate of 1.8 ml/min with YPD broth, regulated by a peristaltic pump (AUTO SCIENCE ATP-3200 model) and 60 RPM constant agitation. The specimens were aseptically removed at the different times evaluated (6, 12, 24, 48, 72 h) and washed with PBS to remove nonadhered cells.

The viability of the *S. aureus* biofilm cells adhering to PE surfaces was evaluated with Live/Dead fluorescent stain (Molecular Probes, Live/Dead Bacterial Viability Kit) incubated for 30 min in the dark at room temperature. The stained biofilm specimens were examined using an argon ion laser with 480-nm excitation wavelength and 520- and 650-nm emission wavelength with a CLSM (DMI 4000B, Leica). The quantification of colony-forming unit (CFU) assay was carried out with the biofilm recovered from the surfaces of PE specimens, using a sterilized cell scraper and rubbing the surface of each specimens, immediately after the scraper with the biofilm recovered was transferred to a tube containing 5 ml of broth YPD culture broth and incubated at 37 °C for 1 h. After the incubation period, 1 ml of each sample was serially diluted (10^{-1} to 10^{-7}) and of each dilution, 100 μl were inoculated onto trypticase soy agar plates and incubated at 37 °C for 24 h. The number of CFUs was determined, and the logarithm of CFUs per milliliter (log CFU/ml) was calculated by the same examiner.

Biofilm morphology using SEM

To identify the morphology of the *S. aureus* biofilm on the PE specimens, the surfaces were observed by SEM (Jeol, JSM-7401F) at ×800 and ×1000 magnifications and images were obtained in 50- to 100-μm scale, with voltage operation of 15 kV.

Statistical analysis

The mean and standard deviations of the UAF (arbitrary fluorescence units) of the 2 study groups were calculated at 6, 12, 24, 48, and 72 h. The *S. aureus* biofilm formation of the specimens coated or uncoated within each formation period (6 vs 12 vs 24 vs 48 vs 72) was analyzed by the nonparametric Kruskal–Wallis test, and a significance level of $p \leq 0.05$ was used.

Results

AuNP coating effect on the biofilm formation of *S. aureus* with CLSM

At 6-h intervals in the control group, a scarce biofilm was formed on the surface and was distributed sparingly with defined coconut forms and a thin uniform base layer. As the bacteria that were observed in isolation moved away (Fig. 1a) in the experimental group with the surfaces covered with AuNP, it was observed that there was less biofilm formation (Fig. 1b).

At 12 h of incubation in the control group, the biofilm increased; a uniform base layer of medium thickness was observed. As the bacteria moved away from the base, they were still isolated and separated, corresponding to a bacterial settlement phase-in process (Fig. 1c). On the other hand, in the experimental group, there was still no development of the biofilm; some isolated coccoid forms were observed (Fig. 2d).

At 24 h of the evaluation period, the base layer was uniform and with bacterial clusters; the thickness of the biofilm was greater. Outside of the base, medium-density conglomerates were distributed and nonviable cells were observed on the surface of the PE discs with conglomerates of developing bacteria (Fig. 1e). In the case of the experimental group specimens, the distributed biofilm was increased on the surface of the coated PE, observing some nonviable cells on the surface (Fig. 1f).

At 48 h and 72 h in the control group, biofilm formation continued to increase, with a higher fluorescent green intensity, and no viable cells were observed. At the 48-h period, a uniform base layer was observed; along with the extension of this, conglomerates in the form of dense granules were observed toward the surface. The latter were distributed in the medium-density cluster and, in cluster structures with intertwined nonviable bacteria, an increase was notably observed (Fig. 1g). In contrast, for the 48-h time period, a decrease in viable biofilm cells was observed in the experimental group, with the number of nonviable cells with red fluorescence considerably increasing, referring to a damaged bacterial membrane (Fig. 1h).

The last period was evaluated at 72 h in the experimental group. The mass of the biofilm was observed as uniform in the majority of the surface of the PE specimens, leaving only some cracks without their being invaded as yet. Along with

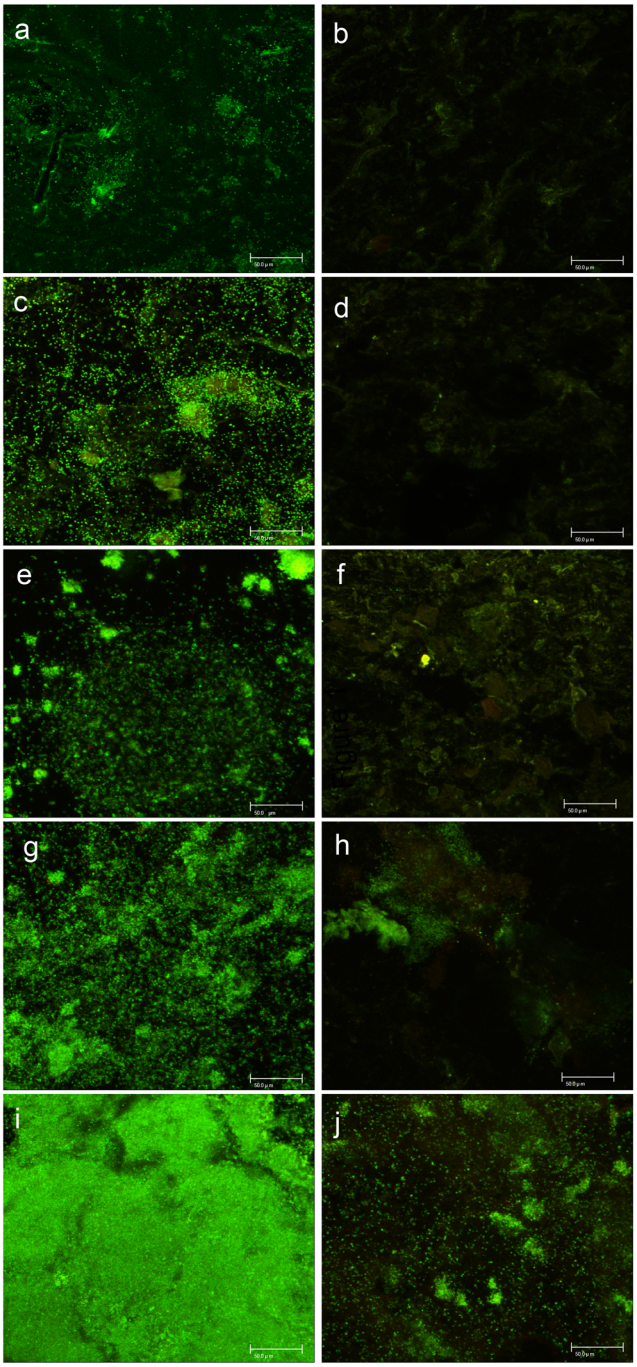

Fig. 1 Images of the formation and growth of *S. aureus* biofilm on the uncoated PE surfaces with CLSM at 6 h (**a**), 12 h (**c**), 24 h (**e**), 48 h (**g**), and 72 h (**i**) and on the coated surface with AuNPs at 6 h (**b**), 12 h (**d**), 24 h (**f**), 48 h (**h**), and 72 h (**j**)

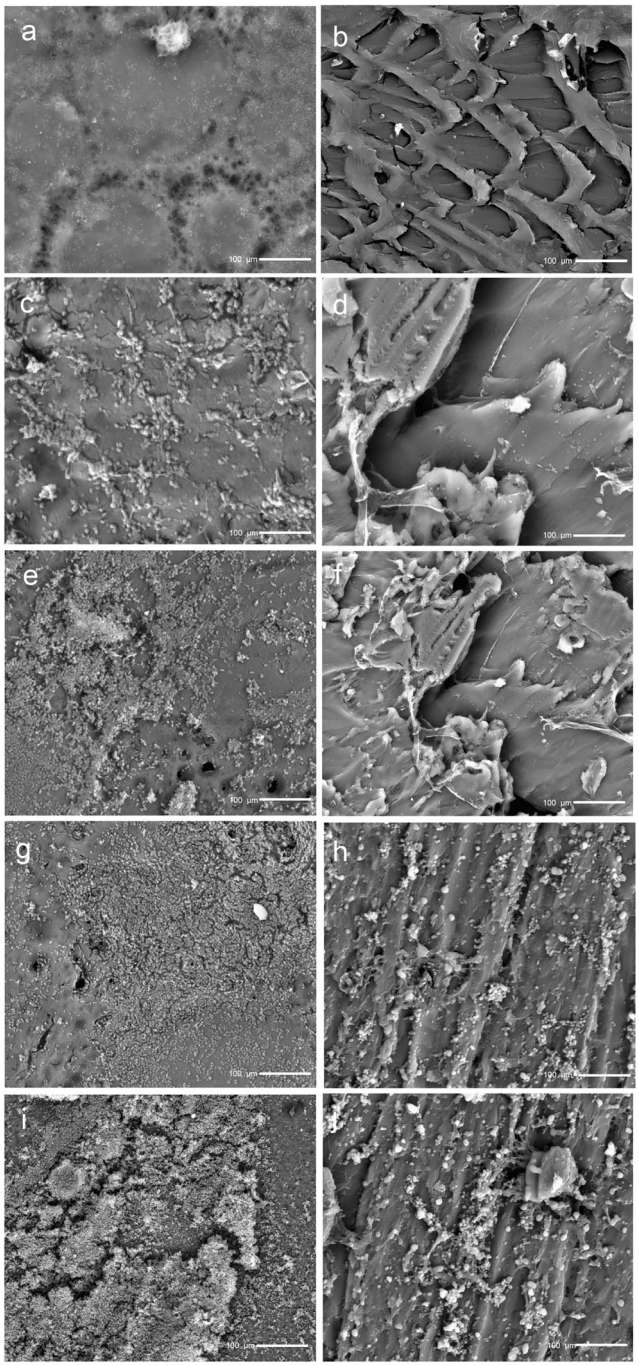

Fig. 2 Images obtained with SEM of the morphology of *S. aureus* biofilm on the uncoated PE surfaces at 6 h (**a**), 12 h (**c**), 24 h (**e**), 48 h (**g**), and 72 h (**i**) and on the coated surface with AuNPs at 6 h (**b**), 12 h (**d**), 24 h (**f**), 48 h (**h**), and 72 h (**j**)

the extension of this, conglomerates were observed in the form of dense granules, with greater extension, but spaced further from each other (Fig. 1i). In the case of the experimental group, at 72 h, the biofilm of *S. aureus* decreased, as well as the number of nonviable cells; there were a few isolated conglomerates (Fig. 1j).

AuNP coating effect on the morphology of the biofilm of *S. aureus* with SEM

At 6 h in the control group, the uniform base layer was observed, which corresponds to the primary phases of settlement of the biofilm (Fig. 2a). In contrast, in the experimental group of specimens with AuNP, no primary settlements were observed, the base layer or conglomerates were unable to be seen, and the bacteria were very scarce and were distributed in isolation or in pairs (Fig. 2b).

At 12 h of the incubation time, localized clusters of low growth were observed in clusters or groups (Fig. 2c) and, in the experimental group, the formation of a dense base layer was observed (Fig. 2d). At 24 h, higher conglomerates of developing bacteria were observed (Fig. 2e), while in the experimental group, the beginning of an isolated and localized primary settlement phase was observed (Fig. 2f).

At 48 h, a large number of bacteria were observed that completely covered the surface in the control group (Fig. 2g), while in the experimental group, in the images obtained with SEM, numerous clustered bacteria were observed. These were deposited in areas of greater porosity or where, presumably, the Au layer was not uniform or did not coat the surface (Fig. 2h).

In the last period of evaluation, at 72 h in the control group, there was biofilm growth on the total surface of the PE specimens (Fig. 2i), while in the experimental group, it was observed that greatest growth occurred in the retentive areas of the surface of the PE specimens (Fig. 2j).

The dynamics of biofilm formation of *S. aureus* on uncoated PE surfaces showed an increasing trend in the quantification of viable cells from 6 h of biofilm formation, with sustained and constant growth until 72 h (Table 1). Moreover, the values obtained show that the biofilm onto the experimental surface group coated with AuNPs had a smaller biofilm growth, although it showed a lower increase in the evaluation times compared to the surfaces of the specimens uncoated with AuNPs (Fig. 3).

AuNP coating effect on the viability of *S. aureus*

The analysis of the cell viability of the biofilm by UAF showed a ratio of almost 4:1 viable/nonviable in the biofilm of the uncoated surfaces, while in the biofilm of the surfaces coated with AuNPs, the average viable/nonviable ratio is of 1:1, which indicates that cells that developed in the biofilm lost viability affected by the AuNPs (Table 1). The CFU count of the cells recovered from the biofilm at 72 h was 10.941759 Log_{10} for the control group compared to the surfaces of the specimens uncoated with AuNPs with 5.562292 Log_{10}.

Table 1 Description of the evolution of the values of AFU and mean differences between the study groups at 6, 12, 24, 48, and 72 h and their respective *p* values

Times (h)	Media values of AFU	Fluorescence green viable cells	
		Difference (Exp-Ctrl)	*p**
6	23.8/10.8 SD 13.7/5.1	13.98	< 0.05
12	22.8/11.5 SD 5.9/2.5	14.63	< 0.05
24	19.0/10.2 SD 5.2/1.8	8.01	< 0.05
48	37.2/11.7 SD 16.2/3.2	25.98	< 0.04
72	59.8/18.2 SD 49.0/11.8	34.32	< 0.03
Times (h)	Media values of AFU	Fluorescence red viable cells	
		Difference (Exp-Ctrl)	*p**
6	9.9/7.0 SD 2.5/1.3	3.83	> 0.05
12	8.2/6.7 SD 3.9/2.7	4.81	< 0.05
24	11.0/8.5 SD 6.1/4.6	1.69	> 0.05
48	11.3/9.0 SD 3.4/3.8	2.74	> 0.05
72	25.5/17.6 SD 10.7/5.0	0.58	> 0.05

*Student's T for independent samples

*Indicates significant differences between both groups ($p \leq 0.05$) (SD standard deviation)

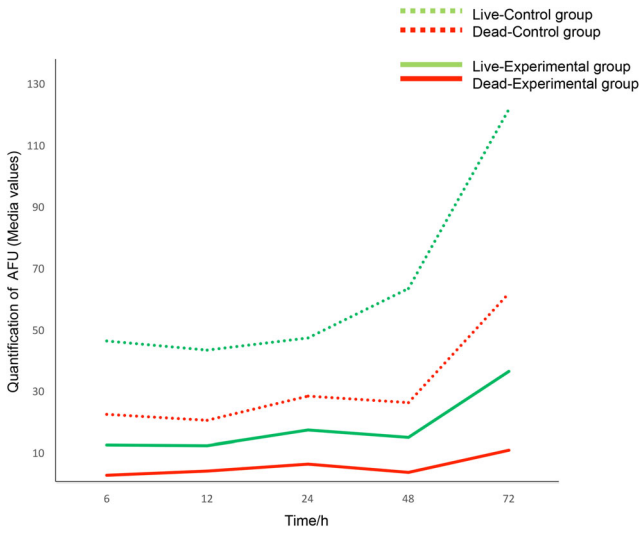

Fig. 3 Biofilm growth curve of *S. aureus* on the uncoated (control group) and coated (experimental group) PE surfaces, media UAF values corresponding to viable and dead cells

Discussion

In this study, a nanostructured coating with AuNPs to a PE surface was evaluated, resulting in an effective antimicrobial alternative that inhibits the formation of a biofilm of *S. aureus* under controlled dynamic conditions; less settlement of *S. aureus* during biofilm formation on a surface of PE with an AuNP coating was shown, observing a decrease in the bacterial viability compared to an uncoated surface nanostructured with AuNPs.

AuNPs have advantages over other types of nanoparticles; previous studies have shown high biocompatibility with low cytotoxicity [14], additionally, it has been shown that AuNPs can be conjugated with cefaclor a second-generation antibiotic, what it provides a potent antimicrobial activity on *S. aureus* and *E. coli*, proposing its use in hospital surfaces [15]. The Au and silver nanoparticles (AuAgNPs) are another successful conjugation since they act as antibacterial/antibiofilm activities [16]. Another feature previously reported is its use in the nanocomposites functionalization with Au-chitosan and the small molecule 2-mercapto-1-methylimidazole, with a high bactericidal effect and low toxicity, was demonstrated by proposing its use in medical devices [17]. According to the previous works and ours results, the AuNPs are an alternative to coat the biomedical PE devices surfaces, thus avoiding bacterial settlements and the formation of biofilm of nosocomial bacteria of such as *S. aureus* that directly affect the integral health of patients, it has even been reported that it can reduce the biofilm of *S. aureus* and *Pseudomonas aeruginosa* with high concentrations of gold and iron-oxide nanoparticles [18].

Our study showed that AuNPs behaved like an agent with antimicrobial activity of *S. aureus* biofilm; our results are confirmed by other articles that prove AuNPs may serve as potential therapeutic agents against the biofilm-forming bacterial pathogens since the essentially inert and nontoxic nature of Au makes it an attractive material as an antimicrobial agent [6].

Our results are in agreement with the previous study; Yu et al. [19] who reported the strong inhibitory effect of AuNPs on pathogenic biofilm formation and invasion to host cells conclude that this effect does not result from growth inhibition but is mediated by the strong electrostatic interaction between AuNPs and pathogenic cells. Boda et al. [20] demonstrate potential therapeutic activity of ultrasmall AuNPs with core diameters of 0.8 and 1.4 nm as an effective treatment option against staphylococcal infections. Bing et al. [20] reported that the AuNPs exhibited striking antibacterial properties against both gram-positive and gram-negative bacteria and excellent ability to disperse bacterial biofilms.

The PE is a high-weight polymer. molecular, thermoplastic, with a variable crystalline structure, with high-performance applications in medical devices. In this study, a

coating of AuNPs demonstrated that have inhibiting the growth of *S. aureus* biofilm on a PE surface, which is a high-weight polymer. molecular, thermoplastic, with a variable crystalline structure, with high-performance applications in medical devices.

Staphylococcus aureus is one of the most frequent causes of nosocomial and medical device-related biofilm infections. The development of biofilm deposited by *S. aureus* is characterized by a complex architecture [8]; in our results, the growth of *S. aureus* biofilm on the uncoated PE disks of AuNPs was increased at 48- and 72-h biofilm biomass and was observed throughout the surface, while in the experimental group with AuNP coating, the formation and growth of the biofilm were considerably lower, in addition to a greater amount of nonviable bacteria compared to the control group, coinciding with other authors in that AuNPs as an effective treatment option against staphylococcal infections [7, 20].

An important variable of this work was the biofilm formation model; the use of a model for in vitro tests of bacterial biofilm growth depends on the control of several factors; the importance of considering differences between experimental conditions in different model systems has been demonstrated [21]. In this study, we use the CDC biofilm reactor that offers a standardized system that controls and establishes the flow and temperature parameters for bacterial biofilm growth in conditions similar to clinical environments.

Additionally, the technique to cover the surface of the PE with AuNPs was an important variable; for this, the sputtering technique was used, which has been used successfully to deposit thin films of material on to a surface, which allowed to uniformly coat the PE specimens. Besides, under these conditions, the AuNPs incorporated to the surface maintain their antimicrobial properties, for example, previous work demonstrated a photothermal ablation in the biofilm of *S. aureus* of high virulence and resistant to methicillin under infrared light irradiation [7], or it has even been shown that gold nanoclusters have shown excellent therapeutic effect [22].

The lack of reports on the effect of the AuNPs incorporated in PE surfaces to inhibit the formation of the biofilm of *S. aureus* allows establishing with the results of this study guide for the development of new dental and biomedical biomaterials with antibiofilm effect. The effect on the formation dynamics of bacterial biofilm in vitro or in vivo, on other polymers commonly used in medicine and dentistry on various devices, is a broad field of future research; since, as demonstrated in this study, polymers added with Au nanostructured coatings can prevent the colonization of microorganisms, it will also be necessary to study whether this effect is maintained in the long term.

Among some strategies used for the elimination of bacterial biofilms are the modulation of biofilm signaling and the dissolution of DNA and cationic materials that dissolve the biofilm. Currently, the design of smart surfaces to eliminate the biofilm formation on biomaterial surfaces is under continuous investigation. It has been described that AuNPs are excellent carriers of drugs and by themselves have an antibacterial effect by nanothermolysis, as well as a possible effect on intracellular enzyme systems [23].

In addition to the antimicrobial characteristics, the AuNPs represent a novel nanomaterial used among other things, for the diagnosis and treatment of infections and diseases such as cancer, as well as drug transport systems with biocompatible characteristics, and can be functionalized with antibodies, carbohydrates, and pharmacological agents that allow greater selectivity on bacteria or malignant cells. The production cost of gold (Au) NPs is compared to NPs of other metals and materials; recent investigations have been focused on the utilizations of metal NPs including Au and copper as antibacterial agents for bacterial infections. AuNPs have exhibited unique optical and structural properties for the biomedical applications and devices [19, 24]. The AuNPs have been conjugated with various surface ligands for antimicrobial agents, due to their high biocompatibility, easy modification, and photothermal stability [25, 26]. In addition, recently, low cost and high biocompatibility biological gold nanoparticles with superior antimicrobial properties have been obtained [27]. That is why when studying nanoparticles and their different applications, new fields of research in nanomedicine are opened that will allow new and effective ways of treating diseases in the near future in order to improve the quality of life of patients.

Conclusions

The PE surface with a nanostructured coating of AuNPs showed an inhibitory effect on the formation, maturation, and viability of the *S. aureus* biofilm. It was shown that the formation of the biofilm of *S. aureus* on a PE surface without AuNP coating in a dynamic training model has an increasing trend from 6 h of training, with its greatest development at 72 h, while when the surface of the PE was coated with AuNPs, the development was limited and after 48 h, it began to increase, so it follows that the inhibitory effect of the coating is more effective in the early stages of settlement delaying the development of the *S. aureus* biofilm.

Authors' contributions Contributions to the manuscript were as follows: Lorena Dafnee Villa-García: conceptualization, methodology, data curation. Raúl Márquez-Preciado, Olga Araceli Patrón-Soberano, and Amaury Pozos-Guillén: supervision, conceptualization, data curation. Marco Antonio Álvarez-Pérez, Marine Ortiz-Magdaleno, and Luis Octavio Sánchez-Vargas: writing (original) and final draft preparation, approved the version to be published.

Funding Marco Antonio Álvarez-Pérez want to thanks to the financial support by the Dirección General de Personal Academico-Universidad Nacional Autonoma de Mexico: PAPIIT IT203618 project.

Declarations

Conflicts of interest/competing interests All the authors declare that they have no conflict of interest.

References

1. Houacine C, Yousaf SS, Khan I, Khurana RK, Singh KK (2018) Potential of natural biomaterials in nano-scale drug delivery. Curr Pharm Des 24(43):5188–5206. https://doi.org/10.2174/1381612825666190118153057
2. Jarząb N, Walczak M, Smoliński D, Sionkowska A (2019) The impact of medicinal brines on microbial biofilm formation on inhalation equipment surfaces. Biofouling 34(9):963–975. https://doi.org/10.1080/08927014.2018.1515353
3. Hempel JM (2015) Single-stage ear reconstruction and hearing restoration using polyethylene implant and implantable hearing devices. Facial Plast Surg 31(6):604–610. https://doi.org/10.1055/s-0035-1567887
4. Capek I (2017) Polymer decorated gold nanoparticles in nanomedicine conjugates. Adv Colloid Interf Sci 249:386–399. https://doi.org/10.1016/j.cis.2017.01.007
5. Hamouda IM (2012) Current perspectives of nanoparticles in medical and dental biomaterials. J Biomed Res 26(3):143–151. https://doi.org/10.7555/JBR.26.20120027
6. Ahiwale SS, Bankar AV, Tagunde SBP, Kapadnis (2017) A bacteriophage mediated gold nanoparticles synthesis and their antibiofilm activity. Indian J Microbiol 57(2):188–194. https://doi.org/10.1007/s12088-017-0640-x
7. Hu D, Li H, Wang B, Ye Z, Lei W, Jia F, Jin Q, Ren KF, Ji J (2017) Surface-adaptive gold nanoparticles with effective adherence and enhanced photothermal ablation of methicillin-resistant staphylococcus aureus biofilm. ACS Nano 11(9):9330–9339. https://doi.org/10.1021/acsnano.7b04731
8. Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8(9):881–890. https://doi.org/10.3201/eid0809.020063
9. Azeredo J, Azevedo NF, Briandet R, Cerca N, Coenye T, Costa AR, Desvaux M, di Bonaventura G, Hébraud M, Jaglic Z, Kačániová M, Knøchel S, Lourenço A, Mergulhão F, Meyer RL, Nychas G, Simões M, Tresse O, Sternberg C (2017) Critical review on biofilm methods. Crit Rev Microbiol 43(3):313–351. https://doi.org/10.1080/1040841X.2016.1208146
10. Goeres DM, Loetterle LR, Hamilton MA, Murga R, Kirby DW, Donlan RM (2015) Statistical assessment of a laboratory method for growing biofilms. Microbiology 151(Pt 3):757–762. https://doi.org/10.1099/mic.0.27709-0
11. Percival SL, Suleman L, Vuotto C, Donelli G (2015) Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. J Med Microbiol 64(Pt 4):323–334. https://doi.org/10.1099/jmm.0.000032
12. Moormeier DE, Bayles KW (2017) Staphylococcus aureus biofilm: a complex developmental organism. Mol Microbiol 104(3):365–376. https://doi.org/10.1111/mmi.13634
13. Kennedy CA, O'Gara JP (2004) Contribution of culture media and chemical properties of polystyrene tissue culture plates to biofilm development by Staphylococcus aureus. J Med Microbiol 53(Pt 11):1171–1173. https://doi.org/10.1099/jmm.0.45764-0
14. Priya MRK, Lyer PR (2015) Anticancer studies of the synthesized gold nanoparticles against MCF 7 breast cancer cell lines. Appl Nanosci 5:443–448. https://doi.org/10.1007/s13204-014-0336-z
15. Rai A, Prabhune A, Perry AA (2010) Antibiotic mediated synthesis of gold nanoparticles with potent antimicrobial activity and their application in antimicrobial coatings. J Mater Chem 20:6789–6798. https://doi.org/10.1039/C0JM00817F
16. Prabhawathiac V, Malliappan PS, Boobalana T, Manohara CM, Doblea M (2019) Design of antimicrobial polycaprolactam nanocomposite by immobilizing T subtilisin conjugated Au/Ag core-shell nanoparticles for biomedical applications. Mater Sci Eng C Mater Biol Appl 94:656–665. https://doi.org/10.1016/j.msec.2018.10.020
17. Lu B, Lu F, Ran L, Yu K, Xiao Y, Li Z, Dai F, Wu D, Lan G (2018) Imidazole-molecule-capped chitosan-gold nanocomposites with enhanced antimicrobial activity for treating biofilm-related infections. J Colloid Interface Sci 531:269–281. https://doi.org/10.1016/j.jcis.2018.07.058
18. Sathyanarayanan MB, Balachandranath R, Genji Srinivasulu Y, Kannaiyan SK, Subbiahdoss G (2013) The effect of gold and iron-oxide nanoparticles on biofilm-forming pathogens. SRN Microbiol 25:272086. https://doi.org/10.1155/2013/272086
19. Yu Q, Li J, Zhang Y, Wang Y, Liu L, Li M (2016) Inhibition of gold nanoparticles (AuNPs) on pathogenic biofilm formation and invasion to host cells. Sci Rep 6:26667. https://doi.org/10.1038/srep26667
20. Boda SK, Broda J, Schiefer F, Weber-Heynemann J, Hoss M, Simon U, Basu B, Jahnen-Dechent W (2015) Cytotoxicity of ultrasmall gold nanoparticles on planktonic and biofilm encapsulated gram-positive Staphylococci. Small 11(26):3183–3193. https://doi.org/10.1002/smll.201403014
21. Roy R, Tiwari M, Donelli G, Tiwari V (2018) Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. Virulence 9(1):522–554. https://doi.org/10.1080/21505594.2017.1313372
22. Xie Y, Liu Y, Yang J (2018) Gold nanoclusters for targeting MRSA in vivo. Angew Chem Int Ed Eng 57(15):3958–3962. https://doi.org/10.1002/anie.201712878
23. Jain PK, Huang X, El-Sayed IH, El-Sayed MA (2008) Noble metals on the nanoscale: optical and photothermal properties and some applications in imaging, sensing, biology and medicine. Acc Chem Res 41(12):1578–1586. https://doi.org/10.1021/ar7002804
24. Yougbare S, Chang TK, Tan SH, Kuo JC, Hsu PH, Su CY et al (2019) Antimicrobial gold nanoclusters: recent developments and future perspectives. Int J Mol Sci 20(12):2924. https://doi.org/10.3390/ijms20122924
25. Lomelí-Marroquín D, Medina Cruz D, Nieto-Argüello A, Vernet Crua A, Chen J, Torres-Castro A et al (2019) Starch-mediated synthesis of mono- and bimetallic silver/gold nanoparticles as antimicrobial and anticancer agents. Int J Nanomedicine 14:2171–2190. https://doi.org/10.2147/IJN.S192757
26. Peña-González CE, Pedziwiatr-Werbicka E, Martín-Pérez T, Szewczyk EM, Copa-Patiño JL et al (2017) Antibacterial and antifungal properties of dendronized silver and gold nanoparticles with cationic carbosilane dendrons. Int J Pharm 528(1-2):55–61. https://doi.org/10.1016/j.ijpharm.2017.05.067
27. Bing W, Sun H, Wang F, Song Y, Ren J (2018) Hydrogen-producing hyperthermophilic bacteria synthesized size-controllable fine gold nanoparticles with excellence for eradicating biofilm and antibacterial applications. J Mater Chem B 6(28):4602–4609. https://doi.org/10.1039/c8tb00549d