Research Article

Antibacterial and cytotoxicity enhancement of 316L stainless steel alloy via epsilon-polylysine and graphene oxide/silver composite coating

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Abstract: Background: The rising demand for dental and biomedical equipment with effective antibacterial qualities requires the development of highly functional coatings. Aim: This study investigated the effectiveness of coating 316L stainless steel (SS) alloy with ε-polylysine (ε- PLL) and graphene oxide (GO) /silver (Ag) nanoparticle (NP) composites to enhance antibacterial and cytotoxic properties. Materials and methods: Coatings were deposited on 316L SS substrates via 10 min of electrophoretic deposition at 10 V. Three coating formulations (0.5% ε-PLL, 0.5% ε-PLL / 0.02% GO composite and 0.5% ε-PLL / 0.02% GO / 0.001% Ag NP composite) were prepared. The coated samples were characterized by X-ray diffraction, Fourier transform infrared spectroscopy, field- emission scanning electron microscope, and energy dispersive x -ray spectroscopy. The antibacterial efficacy against *Streptococcus mutans and Aggregatibacter actinomycetem comitans*, as well as cytotoxicity were evaluated. Results: Characterisation confirmed the successful deposition of all coating formulations. The ε-PLL - GO/Ag NP composite coatings demonstrated the most potent antibacterial effects, with inhibition zones of 27 and 24 mm, respectively, followed by the ε-PLL–GO composite (25 and 22 mm) and ε-PLL (22 and 20 mm) against *S. mutans* and *A. actinomycetem comitans* respectively. All the coated samples exhibited a survival rate over 90% after 72 h with no cytotoxic effects observed. Conclusion: All the coated samples, especially the ε-PLL-GO /Ag NP composite, demonstrated promising biomedical characteristics, indicating potential for use in coatings for medical and dental equipment.

Keywords: epsilon polylysine, graphene oxide, electrophoretic deposition, biomedical devices, antibacterial properties.

Introduction

Bacterial infections and associated biofilm production pose a significant threat to human health. For decades, antibiotics have served as the primary therapeutic approach for managing infections. However, the emergence of multidrug resistance has considerably exacerbated the current situation, representing a complicated global issue that transcends national boundaries [(1)].

Biofilms, composed of microorganisms embedded in extracellular matrices and affixed to solid surfaces, are crucial in infections [2]. Extracellular matrices produced by bacteria provide protection against host immune responses and antimicrobial agents [3]. Thus, methods that efficiently inhibit biofilm formation and bacterial adhesion are urgently required. Biomaterials are designed to interact with biological systems, serving a crucial role in various medical applications [4]. The selection of appropriate metallic materials for dental and biomedical applications is determined by several factors, such as biocompatibility, corrosion behavior, mechanical properties, manufacturability and cost. Recent advancements in surface engineering have provided numerous novel opportunities for the development of functional biomaterials. Surface coating and multifunctional surface topography have emerged as innovative techniques to reduce biofilms formation. Current techniques facilitate the fabrication of nanoscale structured biomaterial surfaces, thereby unlocking innovative prospects for biomedical applications [5]. In recent years, various materials such as polymers [6] hydrogels [7], antimicrobial peptides [8],inorganic nanomaterials have been extensively investigated as coating materials. Electrophoretic deposition (EPD) is a rapid, simple, and versatile coating method for producing homogeneous coatings on complex geometries and coatings with high microstructural uniformity, homogeneity, and controlled thickness [9]. The brief processing duration, and minimal equipment requirements facilitate the modification of deposition time.

ε-polylysine (ε-PLL) is a natural polymer produced by microorganisms. It possesses unique properties such as broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria and fungi [10]. Its minimum inhibitory concentration (MIC) for bacterial growth is below 8-32 μg/mL [11]. ε-PLL has attracted considerable interest because of its biodegradability, biocompatibility, water solubility and stability under high temperature, acid, and alkaline conditions. It has been extensively used in the advancement of biomaterials for medical applications and the production of various ε-PLL-based structures [12,13] .The US Food and Drug Administration has recognized ε-PLL as a safe antimicrobial agent. It has been used in food industry as a preservative in many countries, such as the United States, China and Japan, because of its potent antibacterial and antifungal effects [14].

Nanotechnology and nanoscience have experienced rapid advancement in recent decades. Graphene has emerged as a highly promising material in the field of biomaterial research since its initial isolation in 2004 [15]. The antibacterial properties of graphene oxide were initially documented in 2010, garnering substantial interest in medical research. Graphene and its derivatives have been investigated for various applications including the coating of medical devices, surface modifications, cosmetics, catalysts, textiles and electronics, theragnostic advanced dressings, and sensors [16]. Furthermore, they show potential as drug delivery systems, antimicrobial agents, and in cancer treatment. In the study of novel antimicrobial strategies, Ag nanoparticles (NPs) have been extensively investigated in the field of antimicrobial surface development. Their antimicrobial properties have long been acknowledged, and the emergence of nanotechnology has facilitated the utilization of Ag NPs as an innovative approach to address pathogenic microorganisms and reduce antibiotic resistance [17,18].

The surface modification of 316L stainless steel alloys (SS) have been extensively studied. However, the majority of the existing literature primarily addresses mechanical properties and biocompatibility, with limited research dedicated to antibacterial modifications [19]. 316 L SS has attracted significant attention for its extensive application in prosthetic knee and hip joints, orthopedics' devices, orthodontics appliances, and heart valve parts because of its availability, low-cost, and simple manufacturing process [20].

ε-PLL a natural polymer renowned for its superior antibacterial and biocompatibility features has been utilized in several dental and biomedical applications [21–23]; however, its use as a coating material on 316L SS remains inadequately investigated. Moreover, research on the combined effect of ε-PLL-GO/Ag composites on the biocompatibility and antibacterial performances is limited. To the best of our knowledge, this work is the first application of EPD to coat the surface of 316L SS with ε- PLL- GO/Ag composites. The coatings were characterized, and their cytotoxic effects were assessed. The antibacterial and anti-adhesion effects of coatings against *S. mutans* (Gram positive) and *A. actinomycetem comitans* (Gram negative) bacteria were compared. This study aimed to provide a novel approach for improving the clinical performance of coated materials and enhancing their use in dental biomedical fields applications.

Materials and Methods

316L SS alloys sheets used for substrate preparation were purchased from Wuxi Haoguang Special Steel Co., Ltd. (China). ε-PLL (molecular weight:3800-4200 Da) was procured from Med Chem Express (USA). GO (purity: >98%; thickness:< 5nm) was purchased from Platonic Nanotech Private Limited (India). Ag NPs (purity: 99.99%; particle size:2-20 nm) were obtained from Hongwu International Group Ltd. (China).

316L SS alloys were sectioned into 10 mm x 2 mm samples for antibacterial tests(N=104) and 6mmx2mm for cytotoxicity tests (N=120,10 per each test) using cutting machine, followed by mechanical polishing (400, 600, and 1200 grits) with diamond paste. Prior to each experiment, the samples underwent ultrasonic cleaning in a series of deionized water - anhydrous ethanol - acetone - deionized water for 20 minutes at each step, followed by drying with N_2 stream [23], The total sample (N=224) was divided into four groups: three groups were coated with ε-PLL(N=56), ε-PLL- Go composite(N = 56), and ε-PLL- Go/Ag composite(N=56) respectively, whereas one group (N=56) was used as the control.

The optimization parameters for ε-PLL coating on the substrate surface using ε-PLL 0.5% solution were as follows: voltage of 10 V; time interval of 10 min, and current, 80 –140 mA was set for EPD at room temperature. The samples were affixed to the cathode (working electrode) and SS electrode sample (anode), positioned 10mm apart. Both electrodes were immersed parallel to each other in the suspension inside a glass beaker to optimize deposition settings. After the coating process, the coated samples were carefully cleaned with deionized water and dried under N_2 flow. The surface of the modified samples was marked as ε-PLL. The same parameters and processes were used in the preparation of ε-PLL- GO composite coating; 0.02% of GO was added to the 0.5% ε-PLL solution. The produced samples were marked as ε-PLL- GO. The third group (ε-PLL- GO /Ag composite) was prepared by adding 0.001% of Ag NP into the prepared ε-PLL- GO composite solution under the same EPD parameters. The coated samples were marked as ε-PLL- Go/Ag. All the coated samples were air-dried at room temperature for 24h and then thoroughly characterized. Detailed analysis of the structural, chemical, and physical properties of the materials, were conducted by (XRD; Lab X, XRD- 6000, Shimadzu; Kyoto, Japan) and (FTIR; ABB/SPECTROLAB MB300/UK), (FESEM; Inspect TM F50, FEI company, Netherlands) and (EDS; Thermo Scientific Company, Axia Chemi SEM, Netherlands) to investigate the surface morphology and elemental composition of the coatings.

The antibacterial properties and cytotoxicity of the coating materials were assessed via anti-bacterial and biological tests, respectively.

Antibacterial Experiments

The influence of the elution of coated materials on the antibacterial efficacy was assessed using the inhibition zone method. Mueller-Hinton agar plates were uniformly inoculated with 1.5×10^8 *S. mutans* and *A. actinomycetem comitans* bacterial solutions. The bacterial strains were isolated from plaque samples obtained from the oral cavity of patients and examined under a light microscope for morphological characteristics. Bacterial identification was subsequently confirmed by Gram staining and verified by VITEK 2 (BioMérieux, France). The coated samples were gently positioned and arranged on the agar surface using sterile tweezers, and four different samples were allocated to each plate. The disks were positioned at a sufficient distance from one another and the edge of the plate. The plates were inverted and incubated for 24 hours at 37°C in a 5% cardon dioxide incubator, and the inhibition zone of bacterial growth was measured in millimetres around each sample using a digital vernier caliper [24].

Anti-adhesion assay:

The bacterial cell pellets were activated with 2 mL of nutrient broth and incubated for 2 h at 37°C in a sterile tube under aerobic conditions. The bacterial suspension (100 μL) was inoculated into the nutrient broth (10 mL) and incubated for 24. About 1 mL of bacterial suspension was added to 9 mL of nutrient broth containing the sample and incubated for an additional 24 h. Bacterial turbidity was adjusted to 0.5 McFarland (1.5×10^8 CFU/mL) by using a spectrophotometer at 600 nm. Each sterilized sample was rinsed twice with PBS to remove non-adherent cells, then immersed in normal saline, and subjected to ultrasonication for 10 min to detach adherent bacteria. Serial dilution (to 10^{-8}) was conducted, and CFUs were calculated as follows:

$$\textbf{CFUs = colony number in the plate} \times \textbf{dilution factor.} \quad (1)$$

Cytotoxicity Experiments.

L929 fibroblasts (ATCC CCL-1, NCTC clone 929) were cultured in Modified Eagle's Medium (DMEM) supplemented with 10% FBS, 100 IU/mL penicillin and 0.1 μg/mL fungizone at 37 °C in 5% CO_2. The cells were cultured in sterile flasks, and the medium was replaced every 3 days until 80–90% confluence was achieved [25] Cells were detached using 0.125% trypsin and 0.025% EDTA and monitored with an inverted microscope. The L929 cells were inoculated at a density of 3×10^4 cells/well in a 96-well plate, sealed with sterile parafilm. and incubated for 48 h at 37°C in 5% CO_2. The cells were then treated with 100 μL of sample extracts for 24, 48, and 72h. For extract preparation, the coated samples were immersed in 300 mL DMEM and incubated at 37°C in 5% CO_2. An untreated culture medium served as the negative control. After treatment, the extracts were replaced with 50 μL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reagent (5 mg/mL) and 50 μL of fresh medium. The Plates were wrapped in foil and incubated for 4 h at 37°C. The medium was discarded, the cells were rinsed with PBS, and formazan crystals were dissolved in 150 μL MTT solvent or dimethyl sulfoxide (DMSO). Optical absorbance at 550 nm was measured using a microplate reader, and cell viability was calculated as follows:

$$\textbf{Cell viabilities (\%)} = \textbf{(A/B)} \times \textbf{100} \quad (2)$$

where A and B represent the optical densities of the tested samples and the negative control, respectively.

Statistical analysis

Sample size calculation was performed using the G*Power software. An F-test/ANOVA model was selected for four independent groups. Expected means and standard deviations obtained from a pilot study were used to estimate the effect size with an α-error probability of 0.05 and a statistical power of 0.95, the minimum required sample size was calculated to be 10 specimens per group per test.

IBM SPSS 28.0 software was used for statistical analysis. Quantitative data were presented as mean values ± standard deviation, and normality was assessed using the Shapiro–Wilk test. Then, differences between the study groups were analysed by one-way ANOVA if normally distributed, or Kruskal–Wallis if not. Homogeneity of variance was tested using Levene's test. Post hoc comparisons were performed using Tukey's HSD for equal variances or Games–Howell for unequal variances, and $p < 0.05$ was considered significant.

Results

characterization of the coated samples

The presence of distinct FTIR peaks associated with characteristic vibrational modes indicates the successful uniform deposition of coating materials onto the substrates, offering insights into the chemical composition of the coated layers. Distinct peaks related to functional groups of ε-PLL were observed at 1690–1653 cm^{-1}, which corresponding to C=O stretching vibrations.

Characteristic peaks associated with CH2 stretching were detected at 2908 cm^{-1} and 2971 cm^{-1} in substrates coated with ε-PLL. Samples coated with ε-PLL- GO composite exhibited characteristic peaks corresponding to C-O stretching vibrations at 1140 cm^{-1}, C=O stretching vibrations at 1690 cm^{-1}, CH2 stretching vibrations at 2900 cm^{-1} and 2969 cm^{-1} and O-H stretching vibrations at 3480 cm^{-1} confirming the incorporation of GO in the samples. Samples coated with ε-PLL- GO/ Ag composite exhibited peaks corresponding to (O-H) stretching vibration appear at 3432 cm^{-1}, and asymmetric stretching vibration in NH_2 groups was detected at 3080 cm^{-1}. The bands at 2913 and 2841 cm^{-1} were attributed to (C-H) stretching vibration bond at CH_2.The stretching vibration bands for the amid I group (C=O), amide II group (N-H) and Amid III group (C-N) were observed at 1653 cm^{-1}, 1561 cm^{-1} and 1361 cm^{-1}, respectively. Moreover C-H stretching vibration bands appeared at 1224 and 649 cm^{-1} and stretching vibration bands of C-O bond were noted at 1081 cm^{-1}.

These diagnostic peaks confirmed the successful deposition of the composite coating layer of ε-PLL -Go/Ag composite. Given that Ag NPs cannot be directly measured by FTIR, EDXS analysis was performed. The results indicated the successful incorporation of Ag NPs into the composite layer.

XRD analysis revealed SS peeks only at 2θ = 43.58,50.79 and 74.69 degree which corresponded to JCPDS standard card no. 00-033-0397 for SS for all groups, with a plateau morphology of ε-PLL. Two additional peeks of GO were identified at 2θ = 12.8 and 44.04 degree in the samples coated with ε-PLL- GO, whereas Ag NPs exhibited peeks at 2θ= 38.2, 44.4, 64.6 and 77.59 degree corresponding to JCPDS standard card no. 01-087-0718 for Ag in samples coated with ε- PLL -Go/Ag NPs composite.

Changes in the composition of SS substrate after coating were investigated by EDS analysis. The concentration of Cr, Fe and other SS alloy in the groups decreased. The C content increased by 53.5%, 65.1% and 66.8% in samples coated with ε-PLL, ε- PLL- GO and ε- PLL- GO /Ag composites respectively. By contrast, the O content increased by 6.2%,10.3% and 10.4% in the coated samples. Moreover, the presence of 3.2% Ag NPs in samples coated with Ag NPs demonstrated the successful coating of all three coating. FESEM images showed the homogenous and uniform deposition of the coatings on the substrates.

Inhibition zone test

The inhibitory efficacy of the coated materials on the growth of both *S. mutans* and *A. actinomycetemcomitans* was investigated using the inhibition zone test (Figure1). Bacteriostatic rings were observed around all the experimental coated samples, indicating considerable difference from the control group. None of the agar samples from the control group for either bacterial group exhibited a bacteriostatic ring. The mean diameters of bacteriostatic ring in *S. mutans* agar samples for ε-PLL, ε-PLL- GO, ε-PLL-GO/Ag were 32 ± 1.155, 35 ± 1.247, 37 ± 0.816 mm respectively; in *A. actinomycetemcomitans* agar samples, the diameters were 30 ± 0.667, 32 ± 1.333, 34 ± 0.943 mm, respectively, as illustrated in Figure 2. Significance testing conducted by multiple comparison (post-hoc: Tukey's HSD for *S. mutans*, and Kruskal-Wallis for *A. actinomycetemcomitans*) demonstrated significant pairwise differences ($p < 0.01$) among the four sample groups in each bacterial category.

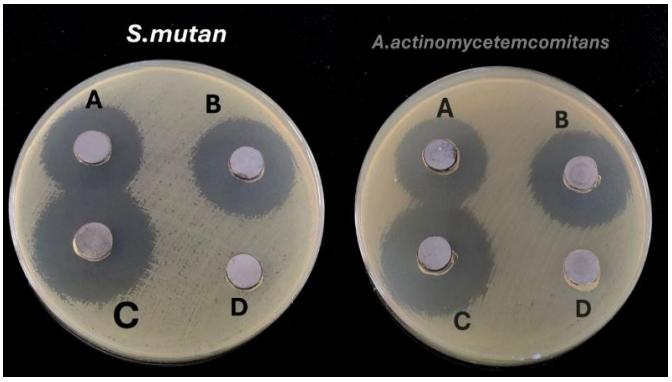


Figure 1: Inhibition zone test (A: ε-PLL, B: ε-PLL&GO, C: ε-PLL&GO/Ag, D: Control)

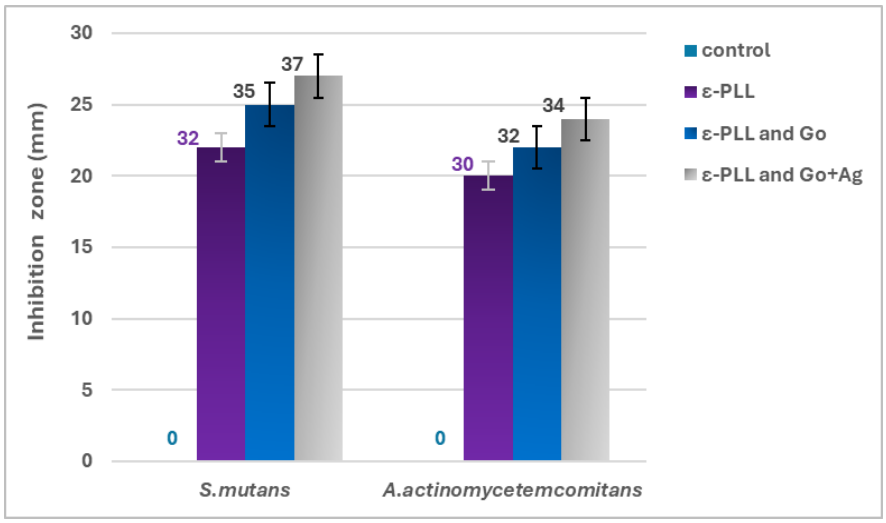


Figure 2: Multiple comparison of inhibition zones around the experimental samples between *S. mutans* and A. *actinomycetecomitans* bacteria

Anti-adhesion assay

The anti-adhesion effect of the coated samples was evaluated using the plate counting method. The mean colony counts for *S. mutans* agar samples in the control, ε-PLL, ε-PLL- GO, and ε-PLLGO/Ag groups were 59 ± 3.5, 20 ± 2.5, 10 ± 2.1, 4 ± 1.5 colonies, respectively. The mean colony counts for *A. actinomycetemcomitans* agar samples in the control, ε-PLL, ε-PLL- GO, and ε-PLLGO/Ag groups were 123 ± 2.6, 25 ± 0.4, 11 ± 0.2, 6 ±0.) colonies, respectively (Figures 3 and 4). Significance testing through multiple comparisons (post hoc: Tukey HSD) showed that pairwise differences ($p<0.01$) among the four study groups per each bacterial group were significant, with the exception of the mean colony counts of ε-PLL-GO and ε-PLL-Go/Ag samples which were insignificant ($p = 0.17$, $p = 0.07$) for *S. mutans* and *A. actinomycetemcomitans* samples, respectively.

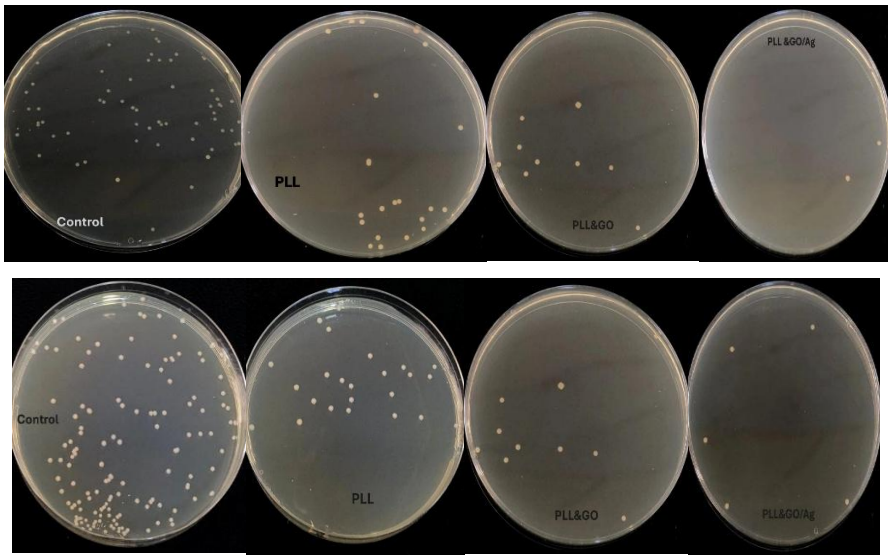


Figure 3: Anti-adherent test (upper series: *S. mutans*, lower series: *A. actinomycetemcomitans*)

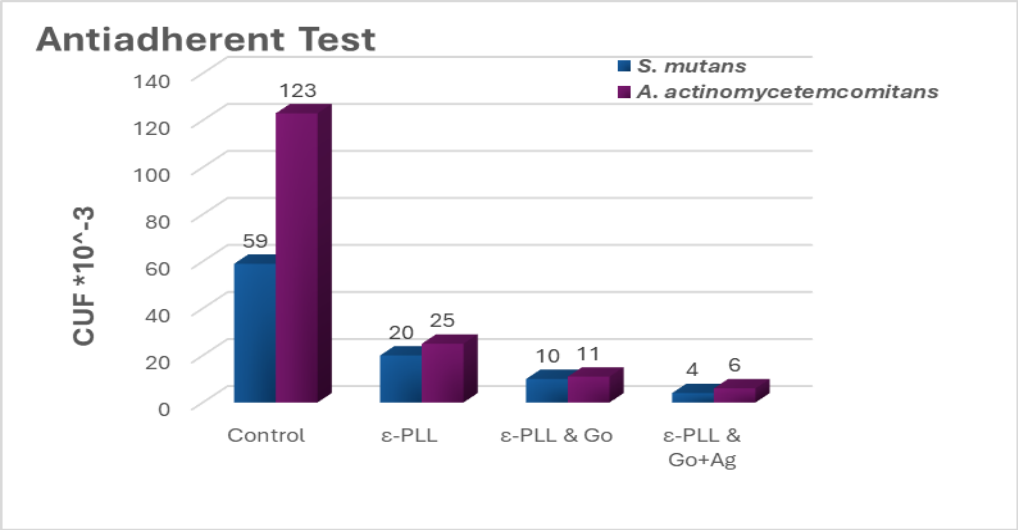
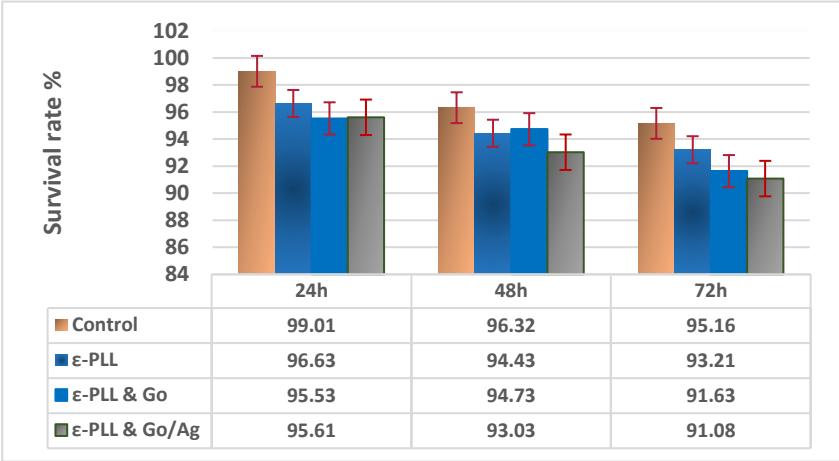


Figure 4: Measurement of CFUs to evaluate the antibacterial properties of the experimental groups.

Cytotoxicity assay

Figure 5 illustrates that all the coated samples showed a survival rate over 90%. The ε-PLL group showed a higher cell survival rate compared with the other groups, but the cell viability of the coated samples decreased at 48h and 72h. Significant differences (Tukey's HSD) were revealed between control and ε-PLL-GO/Ag groups after 48h (p =0.01), and between the control and both ε-PLL-GO, ε-PLL- GO/Ag groups after 72h (p=0.005, p=0.001) .The mean survival rates of all study samples at 24, 48, 72h are depicted in Figure 5.



	24h	48h	72h
Control	99.01	96.32	95.16
ε-PLL	96.63	94.43	93.21
ε-PLL & Go	95.53	94.73	91.63
ε-PLL & Go/Ag	95.61	93.03	91.08

Figure 5: Cell viability of the experimental groups 24 hours,48 hours and 72 hours immersion times

Discussion

Metallic materials composed of precious alloys are widely utilized in orthopaedic surgery, research on the circulatory system, and dentistry. 316L SS alloys are considered promising materials to produce cost-effective devices with superior biomedical compatibility because of their diverse physiochemical properties [26]. Several mechanisms facilitate bacterial adhesion, colonization, and formation of biofilm on biomaterials surfaces such as hydrophobic glycoprotein-mediated forces, electrostatic interactions, and van der Waals [27]. A growing number of research has adopted various strategies to coat dental and medical devices with biomaterials that demonstrate enhanced bacterial resistance and hypersensitivity, inhibit the adhesion of pathogenic bacteria and biofilm formation, and kill bacteria in the early stages of development, utilizing safe and natural substances [23,28,29].

ε-PLL, GO and Ag NPs are materials that exhibit broad-spectrum antibacterial characteristics and are extensively used as coatings for various alloys such as NiTi [29] and CrCoMo alloys [23] which are used in biomedical applications. This study involved the deposition of several combinations of these coatings on the 316L SS alloy, commonly utilized in the production of various dental and biomedical appliances. GO exhibits a negative charge [30], whereas ε-PLL displays a positive charge, resulting in the formation of highly stable solutions, as confirmed by zeta potential test: 60.52mVfor ε-PLL solution, 85.47mV for ε-PLL-CO composite, and 54.74mV for the ε-PLL- GO /Ag composite .all three were deemed highly stable solution [31] for successful deposition on the 316L SS via EPD .After successful coating, the antibacterial activity of the coated materials was evaluated using disk diffusion assay and colony counting methods to determine the antibacterial efficacy of each group against *S. mutans* and *A. actinomycetemcomitans* bacteria by monitoring their inhibitory effects.

ε- PLL showed significant inhibitory effects on *S. mutans* and *A. actinomycetemcomitans* producing a clear ring with inhibition zone diameters 22, 20 mm respectively. These findings were consistent with another study, which reported an inhibition zone diameter of 10 ± 0.5 mm for *E. coli* (Gram negative) and 12± 0.1mm for *Staphylococcus aureus* (Gram positive) under 200 μg/ml ε-PLL, the MIC for both types of bacteria was 12.5 μg/ml, and ε-PLL was observed to have damaged their cell morphology [32]. This finding may be attributed to ε- PLL, an antimicrobial natural polymer that combats various bacteria responsible for numerous oral and body infections. Our findings provide further evidence that ε- PLL serves not only as a natural food preservative but also an efficient coating layer on 316 L SS which is utilized in production of various dental and biomedical devices. In the ε-PLL-GO group, antibacterial efficacy improved to 25 and 22mm respectively, ε-PLL mostly eradicated the bacteria through electrostatic interaction and disruption permeability of cell membrane, leading to cell disintegration. Simultaneously, GO diminished bacterial activity via physical disruption; the incorporation of GO increased the antibacterial efficacy compared with samples coated with ε-PLL alone. This result was aligned with previous studies indicating that GO is an efficient antibacterial agent for pharmaceutical, biomedical, and dental applications; thus, the combination of ε-PLL and GO significantly enhanced antibacterial efficacy particularly against gram positive bacteria compared with gram negative bacteria [27,33]. However, these results were inconsistent with the findings reported by Guo *et al* [23] who reported the absences of inhibition zones surrounding samples coated with ε-PLL and ε-PLL-GO, despite a significant reduction in adhesion rate. They asserted that the coated samples killed or repelled bacteria because of the intrinsic properties of the coatings; they found that the surfaces demonstrated anti-adhesive and contact-killing or inhibitory properties. By contrast, the present study attributed the bactericidal action to the release ε-PLL, as evidenced by HPLC analysis, which will be detailed in a future publication. Orthodontic adhesive containing 3 wt.% of PLL-carbon dots exhibits significant antibacterial efficacy against *S. mutans*, and the antibacterial ring gradually increased in concentration -dependent manner, without altering the physicochemical [34]. The inhibition zones increased to 27 and 24 mm in the samples coated with ε-PLL-GO/Ag composite. This effect may be attributed to Ag NPs, which exhibit significant antimicrobial activity and low cytotoxicity, even at low concentrations, for improving the quality of dental appliance [35].

By evaluating the number of individual bacterial colonies in each group (Figure 4) The results demonstrated a significant reduction in number of colonies among the experimental groups with the control group, with the ε-PLL-GO/Ag composite group exhibiting the least colonies. A prior study [21] reported the efficacy of ε-PLL in inhibiting bacterial adhesion to *S. mutans*, thereby reducing the risk of pathogen colonization, which was aligned with our findings. In addition to *S. mutans*, this study showed that ε-PLL exhibited a satisfactory antibacterial effect against *A. actinomycetemcomitans*. Numerous studies have reported the inhibitory effect of ε- PLL on Gram negative bacteria, with similar results noted against *S. mutans and P. gingivalis* [28]. Our findings not only confirm previous studies but also demonstrated that ε-PLL-GO/Ag composite could serve as an effective antibacterial coating. Our results revealed that the antibacterial efficacy of ε- PLL against *S. mutans* was higher than that against *A. actinomycetemcomitans*. This result was consistent with previous studies [36,37] demonstrating a similar effect on Gram positive and Gram-negative bacteria. A clinical trial indicated that a low concentration mouthwash comprising combination of ε-PLL, FP, and Domiphen effectively inhibits the growth of *S. mutans, P. gingivalis, A. actinomycetemcomitans, and F. nucleatum*, exhibiting a higher MIC against *S.mutan* compared with *A. actinomycetemcomitans* [38]. Another clinical study reported that toothpaste containing ε-PLL and FP exhibits anti-halitosis

and anti-plaque properties due to the synergistic interaction between ε-PLL and FP in enhancing antibacterial efficacy, thereby supporting the antibacterial effect of ε- PLL [39].

However, some contradicting investigations indicated that ε-PLL is more effective against Gram negative than Gram positive bacteria at the same concentrations when the surface charge of the cell membrane surface is considered [40]. A considerable reduction in bacterial colonies was observed in the samples coated with ε-PLL-Go composite, consistent with previous studies [23] that demonstrated a noticeable decrease in bacterial activity in the ε- PLL- GO coated samples compared with those coated with ε-PLL. The current research showed that the incorporating of Ag NPs into the ε- PLL- Go composite enhanced the antibacterial activity, considerably decreasing bacterial colonies compared with the other coatings which may be attributed to the synergistic effect of these antibacterial components. This composite coating was used as a coating material for the first time. The clinical significance of this coating is related to its role in enhancing the functional performance of metallic medical implants and orthodontic appliances by improving antibacterial efficacy, thereby reducing biofilm accumulation known to cause peri-implant infections, white spot lesions around orthodontic brackets, and gingival inflammation.

Biocompatibility is a fundamental requirement in the fabrication of materials designed for direct contact with living tissues [41]. Thus, the cytotoxicity verification of coated materials requires verification; the interaction of these materials with L929 fibroblasts was investigated using the MTT assay, a widely employed approach for evaluating biocompatibility.

ε-PLL is considered safe and can degrade into amino acids essential for the body. Therefore, the cytotoxicity of the coated samples is correlated with the incorporation of GO and Ag NPs; numerous studies suggested that cytotoxicity associated with GO is concentration dependent [42]. Our work builds upon this discovery by using a minimum concentration of GO for the coatings, and all the samples coated with GO exhibited a survival rate exceeding 90% even after 72 h. These rates were considerably higher than the 70% cutoff established by ISO standard standards requirement for optimal cellular viability. The samples coated with ε-PLL- GO /Ag composite met the ISO requirement. Similar results were reported by other studies [22, 29] that used combinations of ε-PLL with GO or ε-PLL and Ag NPs. This agreement may be attributed to similar experimental settings by coating titanium implants in contrast to the present study focused on coating 316L SS to enhance the antibacterial effects.

The study limitations are**:** Limited bacterial spectrum (only 2 strains), single cell line for cytotoxicity, lack of long-term stability and release studies and no in vivo validation.

Conclusion

This study successfully prepared and optimized a coating of ε-PLL, GO and Ag NPs on the surface of 316L SS by EPD. Results demonstrated the excellent antibacterial performance of ε-PLL coatings, and the addition of Go/Ag NPs enhanced their antibacterial effectiveness. The coated materials exhibited safe biological properties without causing cytotoxicity. Considering the trade-off between antibacterial efficacy and biological toxicity, we propose the coating composite of ε-PLL-GO/ Ag NPs as promising new strategy for the coating of 316L stainless steel -based orthodontic, dental, and medical equipment. Further research should extend these findings to other bacterial strains and cell lines to obtain an accurate evaluation of the

antibacterial efficacy of these coated materials. Other composites should also be investigated for their antibacterial effects.

Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

R.A.R. contributed to the conception and design of the work and was responsible for the acquisition of data. R.A.R. and A.M.A. contributed to the interpretation of results. A.M.H. and K.V.K. edited and drafted the work. All authors approved the final version of the manuscript and are responsible for all aspects of the work.

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تعزيز الفعالية المضادّة للبكتيريا وخفض السُمّية الخلوية لسبيكة الفولاذ غير القابل للصدأ 316L بواسطة طلاء مركّب من الإبسيلون-بوليلايسين وأكسيد الغرافين/الفضة

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المستخلص

الخلفية: تزداد الحاجة إلى طلاءات حيوية ذات فعالية عالية مضادة للبكتيريا للاستخدام في الأجهزة الطبية والسنية. الهدف: تهدف هذه الدراسة إلى تقييم تأثير طلاء سبيكة الفولاذ غير القابل للصدأ 316L بمركبات الإبسيلون-بوليلايسين وأكسيد الغرافين/الجسيمات النانوية الفضية في تحسين الفعالية المضادة للبكتيريا والتوافق الخلوي. المواد وطرائق العمل: حُضّرت ثلاث صيغ من الطلاءات ورُسّبت على سطح السبيكة بتقنية الترسيب الكهربائي الجسيمي عند 10 فولت لمدة 10 دقائق، وهي: 0.5% إبسيلون-بوليلايسين، و0.5% إبسيلون-بوليلايسين مع 0.02% أكسيد الغرافين، و0.5% إبسيلون-بوليلايسين مع 0.02% أكسيد الغرافين و0.001% جسيمات نانوية فضية. وتم توصيف العينات باستخدام XRD وFTIR وFESEM وEDS. كما جرى تقييم الفعالية المضادة للبكتيريا ضد *Streptococcus mutans* و*Aggregatibacter actinomycetemcomitans*، إضافة إلى اختبار السمية الخلوية. النتائج: أظهرت النتائج نجاح ترسيب الطلاءات على سطح السبيكة. وسجّل الطلاء المركب من الإبسيلون-بوليلايسين وأكسيد الغرافين/الجسيمات النانوية الفضية أعلى فعالية مضادة للبكتيريا، بأقطار تثبيط بلغت 27 مم و24 مم، يليه طلاء الإبسيلون-بوليلايسين وأكسيد الغرافين بقيم 25 مم و22 مم، ثم طلاء الإبسيلون-بوليلايسين بقيم 22 مم و20 مم ضد الجرثومتين على التوالي. كما أظهرت جميع العينات المطلية معدل بقاء خلوي بلغ 90% فأكثر بعد 72 ساعة من دون تأثير سمي خلوي ملحوظ. الاستنتاج: أظهرت الطلاءات المحضرة، ولا سيما الطلاء المركب من الإبسيلون-بوليلايسين وأكسيد الغرافين/الجسيمات النانوية الفضية، خصائص مضادة للبكتيريا وتوافقًا خلويًا واعدين، مما يدعم إمكانية استخدامها في طلاء الأجهزة الطبية والسنية.