Applied Materials Today 4 (2016) 31–39

Contents lists available at ScienceDirect

Applied Materials Today

journal homepage: www.elsevier.com/locate/apmt

Biomimetic polymer-based Ag nanocomposites as a antimicrobial platform

Mei-Lang Kung[a], Pei-Ying Lin[a], Shi-Wei Peng[a], Deng-Chyang Wu[b,c,d,e], Wen-Jeng Wu[d,f,g,h,i], Bi-Wen Yeh[d,g,h], Ming-Hong Tai[d,j,k,l], Huey-Shan Hung[m,n], Shuchen Hsieh[a,d,o,*]

[a] Department of Chemistry, National Sun Yat-sen University, 70 Lien-hai Rd., Kaohsiung, Taiwan
[b] Division of Gastroenterology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[c] Department of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[d] Center for Stem Cell Research, Kaohsiung Medical University, Kaohsiung, Taiwan
[e] Department of Internal Medicine, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[f] Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[g] Department of Urology, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[h] Department of Urology, School of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[i] Department of Urology, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung Medical University, Kaohsiung, 80708, Taiwan
[j] Institute of Biomedical Sciences, National Sun Yat-sen University, Kaohsiung, Taiwan
[k] Department of Biological Sciences, National Sun Yat-sen University, Kaohsiung, Taiwan
[l] Center for Neuroscience, National Sun Yat-Sen University, Kaohsiung, 804, Taiwan
[m] Center for Neuropsychiatry, China Medical University Hospital, Taichung, Taiwan
[n] Graduate Institute of Basic Medical Science, China Medical University, Taichung, Taiwan
[o] School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan

ARTICLE INFO

Article history:
Received 8 March 2016
Received in revised form 6 May 2016
Accepted 28 May 2016

Keywords:
Dopamine
AFM
Silver nanoparticles
Antibacterial efficiency
Biocompatibility

ABSTRACT

Dopamine, a component of marine mussel adhesive proteins, plays an important role in various fields such as biosensors, bioelectronics, as well as tissue and pharmaceutical engineering. In this study, we describe a simple approach to prepare a dopamine-functionalized Ag nanocomposite. Using AFM, XPS, and SEM analysis, we demonstrated that dopamine is effectively coated onto a glass substrate and that silver (Ag) nanoparticles are successfully attached and well dispersed on the dopamine-covered glass. Furthermore, absorbance analysis, adhesion assay, and Kirby-Bauer disc diffusion tests are show that the dopamine/Ag nanocomposite exhibits good antibacterial efficiency and inhibits bacterial cell adhesion. The biocompatibility of the nanocomposites towards endothelial cells was also assessed using the MTT assay. The present data show that dopamine/Ag nanocomposites are selective compatible with endothelial cells and toxic for bacterial cells. The findings of the present study can thus support a safe and efficient strategy to develop medical devices and intra-devices or intra-catheters for the prevention of nosocomial and catheter-mediated blood-stream infections and/or endothelial injury complications.

1. Introduction

Bacterial biofilms are the surface-associated communities of undesirable attached microorganisms encased in a hydrated extracellular polymeric substances (EPS) matrix [1,2]. They represent a widespread and expensive problem, because such accumulation of bacteria could occur everywhere in water-based equips in contact with different materials, and also because once the biofilm state is established, it is almost impossible to eliminate with an inherently external resistance. The formation of bacterial biofilms on indwelling medical devices is of particular concern, as these bacteria hold a significantly increased (10–1000 fold) resistance to antibiotics, [3,4] which makes them a major cause of morbidity and mortality in nosocomial infections (NI) worldwide [5,6]. In order to reduce the growing incidence of NI cases and associated hospitalization costs, there is an immediate need for the development of antimicrobial surfaces able to resist microorganism attachment and prevent bacterial infection.

A variety of antimicrobial surfaces have been generated through impregnation of materials with active biocides or adsorption of antimicrobial agents onto substrate surfaces, allowing the slow release of the agents into the near surroundings or their direct targeting to the adhered cells [7,8]. However, the destabiliz-

* Corresponding author at: Department of Chemistry and Center for Nanoscience and Nanotechnology, National Sun Yat-Sen University, Kaohsiung 80424, Taiwan.
E-mail address: shsieh@faculty.nsysu.edu.tw (S. Hsieh).

http://dx.doi.org/10.1016/j.apmt.2016.05.003
2352-9407/

ing effect, potential toxicity, and increased antibiotic resistance of antimicrobial agents limit their biomedical applications [6,9]. Recently, several studies have explored a mussel-inspired method involving polydopamine (PDA) as a biomimetic surface modifier on various organic and inorganic substrates, including metals, semiconductors, polymers, and ceramics. Owing to the multiple functional groups present in the PDA thin film formed on the substrate surface, the PDA-functionalized surfaces could be easily conjugated with organic components, promoting electroless metal depositions and nanomaterials coating [10–14]. In addition, PDA-functionalized hybrid metal nanoparticles (NPs) such as Ag NPs, and have shown antibacterial potency. Sureshkumar et al. have reported that dopamine coating anchoring silver (Ag) NPs onto magnetic nanofibers grants antibacterial activity to the magnetic Ag nanocomposite [15]. Xu et al. fabricated a microfiltration membrane deposited with dopamine and then functionalized with Ag and Au nanoparticles, and demonstrated its antibacterial efficacy against gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* [16]. Therefore, alternative antimicrobial strategies based on nanoparticles, typically Ag, have the potential to overcome the limitations of antimicrobial agents, including antibiotic resistance [1,17,18]. However, such synthetic processes often need harsh conditions and multi-step synthesis procedures, and may be harmful for cell survival [19,20]. Thus, facile and simple methods for preparing biocompatible and antimicrobial nanomaterials are being pursued.

In this study, we introduce an approach to create an antibacterial surface by simple immersion and coating of a biomimetic molecular dopamine onto glass substrates, a process which enhances the adsorption of Ag NPs. We evaluate the dopamine/Ag nanocomposite exhibits antimicrobial characteristics against model microbes *E. coli* and also possess anti-biofilm formation potential. Moreover, we demonstrate that the selective biocompatibility of the prepared dopamine/Ag nanocomposite towards endothelial cells. The present results offer a potential strategy for preventing the formation of bacterial biofilms on indwelling medical devices and for decreasing the incidence of nosocomial infections, exploiting the remarkable adhesion properties of dopamine for anchoring Ag on a variety of substrates.

2. Materials and methods

2.1. Sample preparation

Before the experiments, organic contaminants (such as dust particles, grease or silica gel) were washed away from the micro cover glass (12 mm, *Matsunami*) substrate surface by using an Extran neutral cleaning agent (Merck). Next, we removed any oxide layer that may have built up on the substrate surface using ethanol (ACS HPLC grade). Finally, the substrates were rinsed rigorously with Milli-Q water (18.2 MΩ cm at 25 °C) and dried under a stream of nitrogen gas to eliminate any ionic or heavy metal contaminants. A stock Tris solution (1 M) was prepared by dissolving Tris (3.6342 g) (Sigma-Aldrich) in deionized (DI) water and adjusting the pH value to 8.5 using 6 M HCl. The dopamine solution (10 mM) was obtained by dissolving 2 mg dopamine hydrochloride (Sigma-Aldrich) in 1 ml Tris buffer (10 mM, pH 8.5). In order to prepare dopamine-coated (labeled just "Dopamine" hereafter) or dopamine/Ag-coated ("Dopamine/Ag") glass substrates, the glasses were directly immersed in freshly dopamine solution for 6 h, rinsed with Milli-Q water, and dried under a stream of nitrogen gas. The glass-coated dopamine was further deposited with 0.5 M $AgNO_3$ for different time intervals (3, 6, 16, or 24 h) at room temperature. The fabricated Dopamine/Ag substrates were finally rinsed with Milli-Q water and dried in a nitrogen gas stream, and subsequently subjected to characterization.

2.2. Characterization

Each sample (Dopamine and Dopamine/Ag (3 h) nanocomposites) was measured by AFM (Asylum Research, MFP-3D^{TM}). All AFM topography images were acquired in AC mode under ambient condition and were used a silicon cantilever (Olympus AC240TS) with a normal spring constant of 2.0 N/m. The image resolution is 512 × 512 pixels. X-ray photoelectron spectroscopy (XPS) investigated the elemental composition of dopamine or dopamine-deposited silver (Dopamine/Ag) onto cover glass using a JEOL JPS 9010 MX equipped with a monochromatic Mg Kα X-ray radiation source. We used Fityk (0.9.8), an open-source spectra modelling program, for peak fitting. The intensity and position of the peaks corresponding to the Ag, C—O, C—N, C=O, C—OH, C—C, C—H and R—NH_2 bonds in these substrate were quantified by fitting Gaussian functions to features in the pair distribution function within Fityk.

2.3. Bacterial growth activity

To determine the effects of the Dopamine/Ag nanocomposite in bacterial growth, different nanocomposites were prepared by depositing $AgNO_3$ on the dopamine-coated glass for various time intervals (3, 6, 16, and 24 h). Bacterial *E. coli* cells (1×10^8 cells/ml) were co-incubated with cover glasses (Control), Dopamine nanocomposite and/or Dopamine/Ag nanocomposite at 37 °C for 3 h with shaking. The bacterial growth activity was further determined by measuring the supernatant *E. coli* turbidity using absorbance detection at 570 nm.

2.4. Biofilm formation

Bacterial biofilms were prepared on flat-bottomed 24-well plates (Nunc), as described below [21]. Briefly, an *E. coli* suspension was prepared in Luria-Bertani (LB) broth from 16 h shaking cultures. The suspensions were diluted in a ratio of 1:20 with normal LB broth. Samples (1×10^8 cells/ml) were introduced into the wells and incubated at 37 °C for 3 h without shaking cultures. Each well was washed twice with double-distilled water to remove non-adherent bacteria. The *E. coli* biofilms were analyzed quantitatively using crystal violet assay. Crystal violet solution 0.2% crystal violet (Sigma-Aldrich) and 4% paraformaldehyde in phosphate-buffered saline (PBS) was added (300 μl/well) and incubated at room temperature for 10 min, followed by removal of supernatant fluid, rinsing twice with double-distilled water, and drying in air. The bacterial residue, stained with crystal violet, was re-dissolved with a 300 μl crystal violet elution buffer (ethanol absolute 50% and acetic acid 0.1% in double-distilled water) and shaken for 15 min. A 100 μl aliquot from each well was transferred onto a micro-titer plate, and the spectrometric absorbance at 595 nm was measured using a micro-plate reader (Anthos 2010).

2.5. Microscopy and imaging

To validate the efficacy of the nanocomposites for bacterial adhesion, the *E. coli* suspension (1×10^8 cells/ml) was introduced into 24 wells containing either Dopamine or Dopamine/Ag nanocomposite and incubated at 37 °C for 3 h without shaking. The cultured plate was then washed twice with PBS to remove non-adherent bacteria, and adhered bacterial cells were further incubated with 200 μl DiI working dye (Invitrogen, Cat. No. D3899) at 37 °C for 30 min. After twin washes with double-distilled water, images were acquired by a Leica DM 6000B inverted fluorescence

microscope equipped with an OLMPLUS digital CCD camera. The red fluorophores images were monitored using R-DIL channels, and the original magnification was 400×. The DiI dye was dissolved in dimethyl sulfoxide (DMSO) (stock solution: 20 mg/ml), and the working solution (2 μg/ml) was prepared by diluted DiI stock solutions using pre-warmed PBS.

2.6. Scanning electron microscope (SEM)

E. coli was cultured on glass surfaces coated with dopamine or dopamine-Ag at 37 °C for 3 h. The bacterial cells were washed with PBS and fixed in 10% glutaraldehyde solution (Sigma, 25%) for 30 min. The cells were then prepared with platinum coating using platinum-coated conductive substrates (JEOL, JFC-1600). The scanning electron microscopy images were obtained with a JEOL SEM 7000F microscope at an accelerating voltage of 5 kV.

2.7. Disc diffusion test

E. coli suspensions were prepared in LB broth from 16 h shaking cultures. The suspensions were diluted with LB broth, and 100 μl (10^8 cfu/ml) were swabbed onto microfiber filter paper discs (6 mm) impregnated with dopamine solution (10 mM) or dopamine (2 mg/ml) deposited with $AgNO_3$ (0.5 M). The discs were placed on top of an agar plate. The plates were then incubated at 37 °C for 16 h, and the zone of inhibition of bacterial growth was used as a measure of susceptibility.

2.8. Cytotoxicity assay

EA926 endothelial cells, a human endothelial cell (HEC) variant from American Type Culture Collection (ATCC), were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco), 2 mM L-glutamine (Hyclone), 100 mg/ml streptomycin (Hyclone), and 100 U/ml penicillin (Hyclone). Cells were maintained in a humidified air incubator (5% CO_2, 37 °C) [22]. For the biocompatibility assay, cells were maintained in 3-cm petri dishes (1×10^5 cells/dish, 1 ml) containing the dopamine-coated or dopamine/Ag-coated substrate and incubated for 24 h; the cell viability, either upon or beyond these substrates, were then recorded and measured using optical microscope and MTT assay, respectively.

2.9. Statistical analysis

All results are expressed as the mean ± standard error (SE) of at least three independent experiments. $P < 0.05$ was considered significant.

3. Results and discussion

3.1. Surface characterization of Dopamine/Ag nanocomposites

Dopamine, whose chemical structure mimics adhesive proteins in mussels, can be deposited through self-polymerization to form thin, surface-adherent polydopamine (PDA) films onto either inorganic or organic materials surfaces [11,14,23,24]. The characterization of these materials surfaces has highlighted their potential application in various fields, including biosensors [7,25] and tissue engineering [26–28]. Recently, PDA-functionalized hybrid nanomaterials have been developed, which showed great potential in catalysis [29], medical metallic implants [30], and anti-fouling applications [16]. The present study introduces a simple method for preparing dopamine-functionalized hybrid silver nanoparticle substrates, and examines their efficacy towards microorganism inhibition, their anti-biofouling properties, and their biocompatibility. First, we characterize the morphology of the Dopamine/Ag substrate, using atom force microscopy (AFM). Fig. 1 shows AFM topographical images of various modifications of cover glass. Fig. 1a shows that the bare cover glass exhibits a flat surface. Following coating with dopamine, which self-polymerizes and form a thin film on the glass support surface (Fig. 1b), the surface morphology was obviously altered and the corresponding root-mean-square roughness (RMS, 0.81 nm) increased in comparison with Fig. 1a (RMS = 0.20 nm). The larger size of the particles in Fig. 1b may be due to polydopamine particles adsorbed onto the substrate. Furthermore, Fig. 1c shows the morphology of the Dopamine/Ag substrate obtained after depositing with $AgNO_3$ for 3 h on the polydopamine thin film. The bright spots in the image indicate that Ag NPs were successfully deposited and well-dispersed on the PDA film, and the corresponding RMS roughness values were dramatically increased (12.35 nm) over those of the control groups (Fig. 1a and b). The approximate size of the Ag NPs can be identified in about 37 nm by the $1 \times 1\ \mu m^2$ image line scan shown in the lower panel of Fig. 1c. Fig. 1d shows the size distribution of Ag NPs, determined from the AFM topographical images in an area containing 1516 nanoparticles. The width of the size distribution of Ag nanoparticles deposited on the dopamine-coated substrate is 28.8 ± 7.2 nm.

Fig. 2 presents the XPS spectra of the Dopamine and Dopamine/Ag substrates. The survey spectrum shows the presence of oxygen, nitrogen, silver, and carbon in the substrate surface (Fig. 2a). More information about the chemical composition of the two substrates is given by the high-resolution spectra. As shown in Fig. 2b, the doublet peaks at 378.2 and 372.2 eV match the reported data for Ag $3d_{3/2}$ and Ag $3d_{5/2}$ of elemental silver, thus confirming the successful reduction of Ag nanoparticles on the dopamine layer [31]. This conclusion agrees well with that based on the AFM topography images. The binding energies at 531.6 eV in Fig. 2c corresponds to the O 1s peak, indicating that catechol was oxidized into quinone groups (C═O), whereas the strong oxygen O 1s peak at 535 eV represents the contribution from hydroxyl groups (C—OH) of the catechol. The shift of the O 1s of C—OH to higher energy (536.2 eV) can be attributed to the catechol or quinone groups in dopamine chelating Ag^+ ions and further inducing the reduction of Ag^+ ions to Ag nanoparticles [30]. As illustrated in Fig. 2d, the peaks located at 285.2 and 287.2–288.6 eV can be attributed to C—C/C—H and C═O groups, respectively [32]. While the peak at 291.6 eV is due to π–π transitions in the aromatic ring. [30], Fig. 2e shows the N 1s spectrum, with the binding energy at 402.2 attributed to the R-NH_2 groups on the dopamine layer. The N 1s binding energy shift to 403.6 eV can be attributed to the spontaneous reduction and chelation of Ag and Ag nanoparticles on the surface [16]. Dopamine is a major component of marine mussels; it is a catecholamine that also plays an important role in medical and physiological processes. Several functional groups such as amino, imino, hydroxyl, and catechol, as well as π–π interactions, [11,33] confer to PDA good adhesiveness for many solid materials surfaces in hydrated environments. Altogether, our results demonstrate that a PDA layer can be formed on the glass substrate surface, and that Ag NPs can be successfully functionalized on the PDA layer. These findings can attract considerable interest in the potential applications of dopamine/silver nanocomposites.

3.2. Dopamine/Ag nanocomposite exhibit antimicrobial activity and anti-biofilm formation

Next, we detect the effects of the Dopamine/Ag nanocomposite for bacterial growth activity. As shown in Fig. 3a, our data reveal that Dopamine/Ag nanocomposites (3, 6, 16, and 24 h of Ag NPs deposition) possessed an excellent antimicrobial activity (81.4 ± 1.9%, 64 ± 3.4%, 36 ± 5.8%, and 28.4 ± 3.2%, respec-

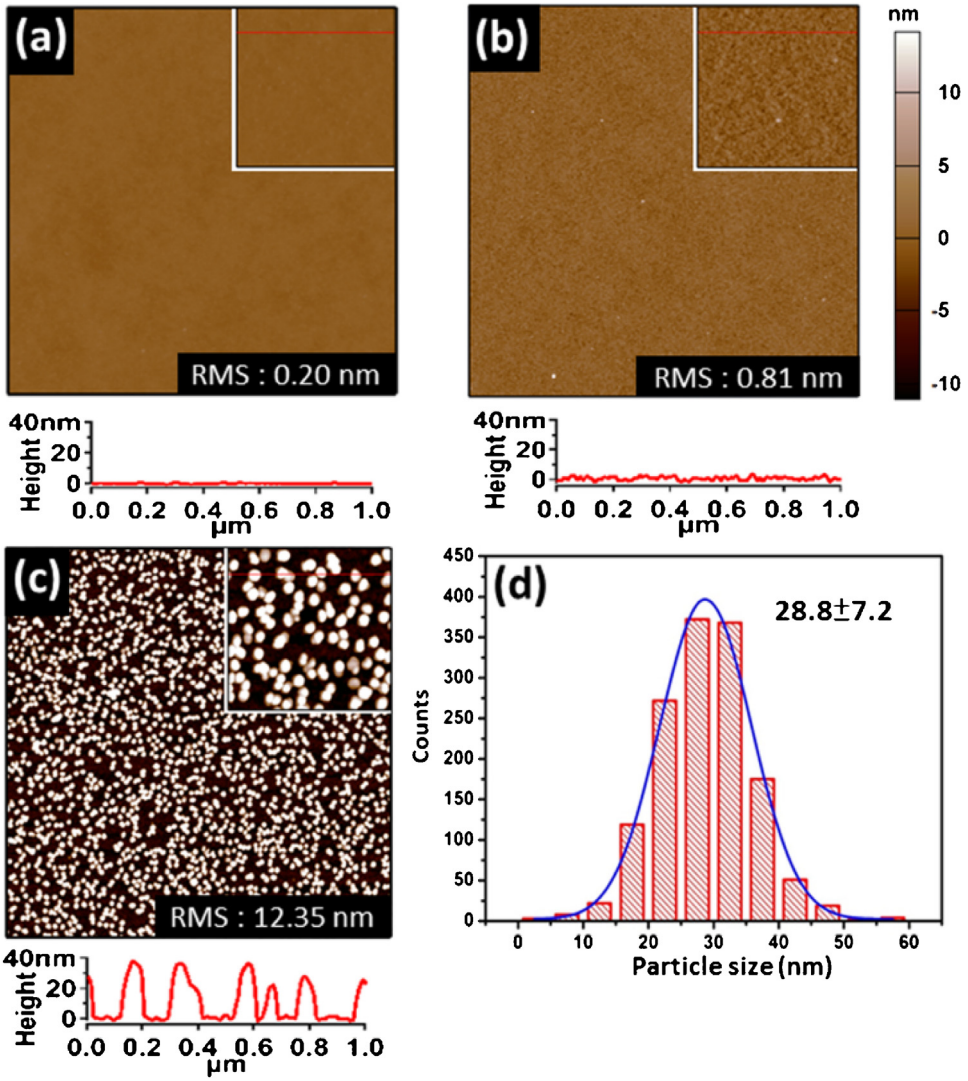

Fig. 1. AFM topography image ($5 \times 5\ \mu m^2$) of (a) bare cover glass surface, (b) polydopamine film on cover glass, (c) Ag nanoparticles (NPs) deposited on polydopamine film. The inset image ($1 \times 1\ \mu m^2$) in each figure is a magnification of the $5 \times 5\ \mu m^2$ image. Line scans below each image show substrate profiles from the $1 \times 1\ \mu m^2$ image. (d) Distribution of Ag NPs deposited on polydopamine film.

tively), as compared with control and dopamine groups ($100 \pm 7.9\%$ and $101.5 \pm 1.5\%$, respectively). We further examined the effect of Dopamine/Ag nanocomposites on *E. coli* biofilm formation using crystal violet assay. Fig. 3b shows that the Dopamine/Ag nanocomposites exhibit an excellent inhibition of bacterial adhesion ($65.7 \pm 1.1\%$, $48 \pm 2.3\%$, $41.5 \pm 2.2\%$, and $38.8 \pm 0.7\%$ for 3, 6, 16, and 24 h of Ag NPs deposition, respectively), as compared with control and dopamine groups ($100 \pm 4\%$ and $162.6 \pm 1.4\%$, respectively). Furthermore, the fluorescence image obtained using DiI dye staining also highlights a significant inhibition of bacterial adhesion by Dopamine/Ag nanocomposites (Fig. 3c). The bacterial adhesion is further quantified in Fig. 3d. Dopamine/Ag groups show attachment inhibition of 29.8 ± 4.7, 24.2 ± 6.1, 8.6 ± 1.1, and 2.3 ± 1.6 ($\times 10^4$ cells/cm^2) for 3, 6, 16, and 24 h of Ag NPs deposition, respectively, as compared with 40.1 ± 7 and 94.5 ± 13.4 ($\times 10^4$ cells/cm^2) of control and dopamine groups, respectively. A similarly results for antimicrobial against E. coli adhesion also has demonstrated with dopamine/Ag-coated onto culture plate (Fig. S1). These results indicate that the Dopamine/Ag nanocomposite suppresses not only bacterial adhesion and further inhibits the biofilm onset, but also disturbs bacterial growth activity. Moreover, the antimicrobial efficiency and inhibition of biofilm formation appear to time-dependent of Ag NPs deposition. Previously studies have demonstrated that antibacterial mechanism of silver is may owing to ionic silver (Ag^+) releases from Ag NPs and interacts with thiol groups (present in cysteine and other compounds) and formation of S-Ag or disulfide bonds, therefore destroy bacterial proteins, interrupt the electron transport chain, and DNA lost its replication ability. In addition, the cell death of Ag NPs exposed bacteria may be caused by cell membrane osmotic collapse and the release of intracellular material [34–36]. Indeed, our data also support that ionic silver (Ag^+) can be released into aqueous solutions with an Ag-deposited time dependent manner (354.6 ± 0.7, 607.4 ± 2.1, 1850.5 ± 5.0 and $2755.5 \pm 3.8\ \mu g\ l^{-1}$ of Ag^+ ion releasing form 3 h, 6 h, 16 h and 24 h of Ag deposited-times of Ag/dopamine nanocomposites, respectively.) (Table S1), and believe that the released ionic silver is toxicity to bacterial cells during 3 h incubation. We therefore propose the ionic silver (Ag^+) releases from Dopamine/Ag nanocomposite can interfere the bacterial growth and inhibit bacterial adhesion.

Next, we investigated the antimicrobial activity of the Dopamine/Ag nanocomposite using SEM. Fig. 4 shows that bacterial cells reveal entirety morphology and exhibit a good adhesive ability to the Dopamine nanocomposite (Fig. 4a). Conversely, bacte-

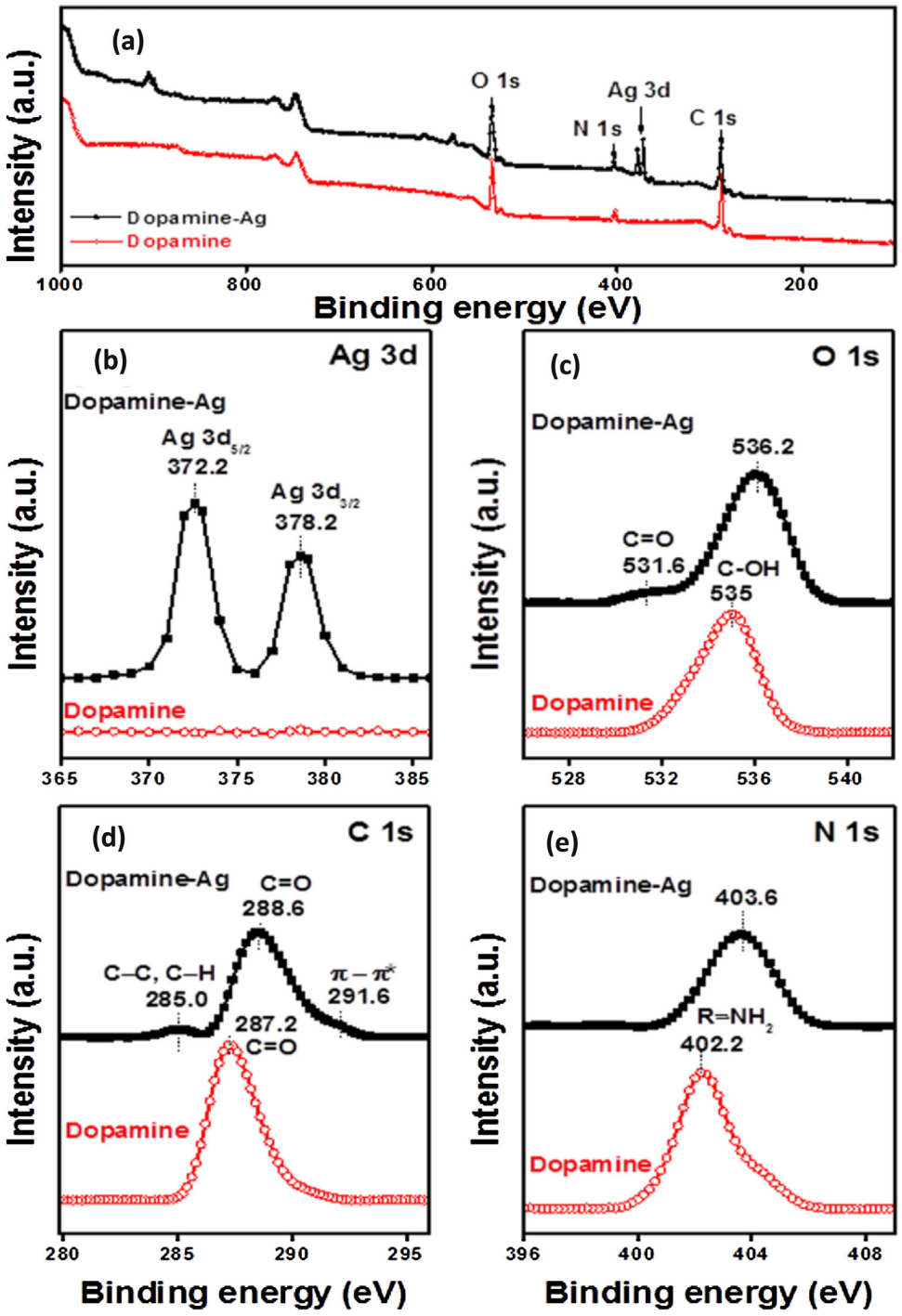

Fig. 2. Near-surface XPS elemental analysis of dopamine and Ag-deposited dopamine substrates. (a) XPS survey scans; each peak is associated to the corresponding element. (b–e) High-resolution spectra of Ag 3d, O 1s, C 1s, and N 1s.

rial cells were almost absent on the Dopamine/Ag nanocomposite, which shows well dispersed Ag NPs on the dopamine-deposited glass substrate (Fig. 4b). In addition, the antimicrobial efficacy of Dopamine/Ag was further examined using the Kirby-Bauer disc diffusion assay. Bacterial cells were swabbed onto LB agar plates and allowed to grow for 16 h. Primary screening revealed that Dopamine/Ag nanocomposites exhibit a superior antimicrobial activity (Fig. 5). In addition, the significant antimicrobial activity of Dopamine/Ag were further demonstrated and recorded (13.9 ± 0.4, 14.2 ± 0.3, 14.7 ± 0.6, and 15.4 ± 0.58 mm for 3, 6, 16, and 24 h of Ag NPs deposition, respectively) (Table 1). These results show that the

Table 1
Zone inhibition measurements using the Kirby-Bauer disc diffusion test to assess the antibacterial activity of dopamine-deposited with $AgNO_3$.

	Control	Dopamine				
		$AgNO_3$				
		0 h	3 h	6 h	16 h	24 h
Inhibitory activity (mm)[a]	–	–	13.9 ± 0.4	14.2 ± 0.3	14.7 ± 0.6	15.4 ± 0.58

[a] Each value is the mean of three replicates of the diameter (mm) of the inhibition zone in the bacterial layer. Values are mean of three independent experiments.

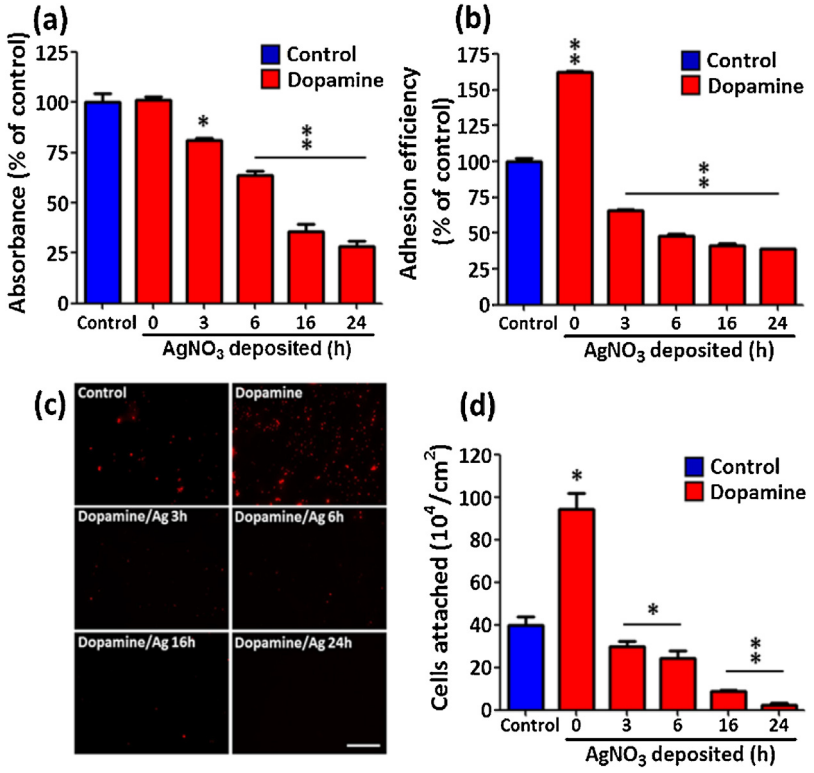

Fig. 3. Antimicrobial performance of dopamine/Ag coated glass surfaces against *E. coli* biofilm formation. The *E. coli* cultures (1×10^8 cells/ml) were seeded on the glass surface for 3 h at 37 °C. *E. coli* (a) turbidity and (b) biofilms were determined quantitatively at an optical density of 570 nm. (c) Image of *E. coli* adhesion on glass surfaces as examined by inverted fluorescence microscope. (d) Amount of attached cells on each substrate. ('*' denotes a significant difference from the control group, $^*P < 0.05$; $^{**}P < 0.001$). Scale bar = 50 μm.

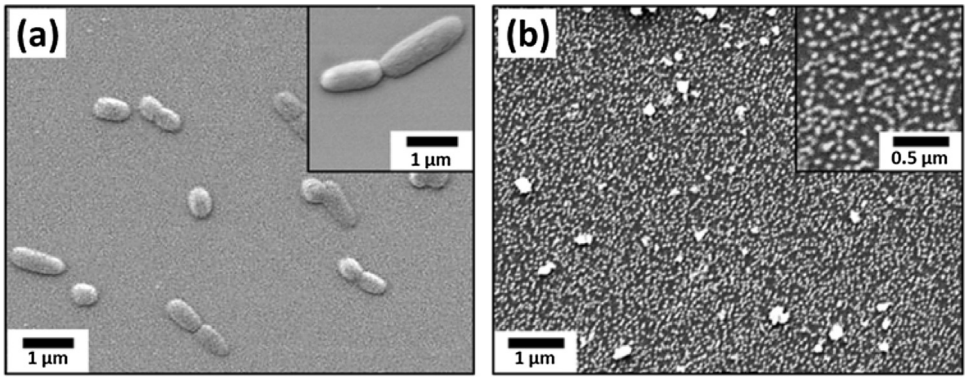

Fig. 4. SEM images of cells grown onto glass surfaces coated with dopamine or dopamine deposited with $AgNO_3$. (a) Adherent *E. coli* cells on the dopamine-coated glass recorded after 3 h of incubation; the inset panel shows the cell morphology. (b) *E. coli* cells were almost absent on the dopamine/Ag coated glass. The inset panel shows well-dispersed Ag NPs on the dopamine-coated glass.

Dopamine/Ag nanocomposite has good potential in applications aimed at biofouling prevention.

Microfouling, the undesirable attachment and subsequent growth of microorganisms on submerged surfaces, is a ubiquitous problem in many areas including medicine and health care [37,38]. The inhibition of microorganism-mediated biofilm formation in medical catheters or implanted devices would decrease nosocomial infection and complications. In the present study, we developed a dopamine-coated substrate deposited with Ag NPs, and demonstrated its antimicrobial efficiency through the inhibition of bacterial growth and attachment. We found that the bioinspired dopamine surfaces can assist the deposition of Ag NPs onto the glass substrate in a short-term deposition reaction (3 h), which can lead to excellent antimicrobial efficacy.

3.3. Dopamine-based Ag nanocomposite selective retains the biocompatibility for human endothelial cells

Silver nanoparticles have shown cytotoxicity both in microorganisms [39] or in mammalian cells [40], but different biological influences are also observed during cells exposed to adequate concentrations [41,42]. To investigate the influence of the Dopamine/Ag nanocomposites for cell survival, the viability of EA926 endothelial cells were detected. EA926 cells were co-incubated with the Dopamine/Ag nanocomposites for 24 h and the cytotoxicity was recorded and evaluated using microscopy and MTT assay. We examine the cell viability on both the surface and the surrounding of Dopamine/Ag nanocomposites. Fig. 6a shows that cells were plated on the different dopamine nanocomposites,

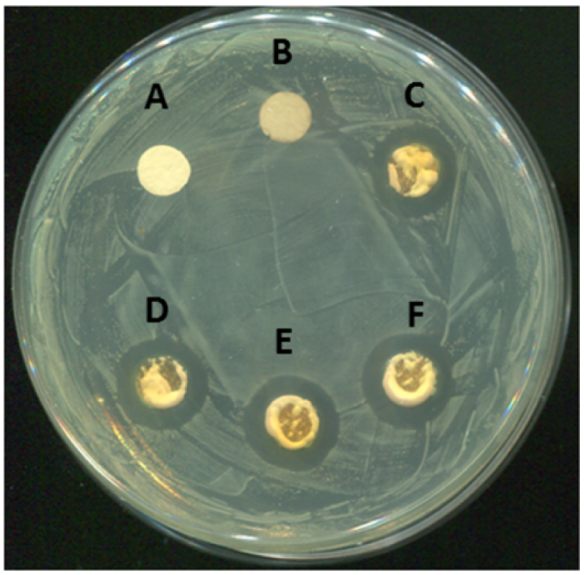

Fig. 5. Antimicrobial efficacy of Ag-deposited dopamine measured using the Kirby-Bauer disc diffusion test. (A) Control; (B) Dopamine; (C) Dopamine/Ag 3 h; (D) Dopamine/Ag 6 h; (E) Dopamine/Ag 16 h; (F) Dopamine/Ag 24 h.

and a well biocompatibility was observed on the control group and the dopamine group. However, we also observe a severe cytotoxicity on the surface of Dopamine/Ag nanocomposites (either short (3 h) or long time (24 h) deposition of Ag NPs). Owing to our data has demonstrated that the *E. coli* turbidity could be inhibited during bacterial cells co-incubated with Dopamine/Ag nanocomposites (Fig. 3a), we further detect the cell viability beyond the Dopamine/Ag nanocomposites. Our result shows that the Ag NPs on the Dopamine/Ag nanocomposite does not obviously affect the systematic cell viability and cell morphology (Fig. 6b). The cell viability was quantitated using MTT assay, and suggests that Dopamine/Ag nanocomposite does not induce a culture systemic cell death (92.2 ± 5.5, 92.2 ± 5.5, 90.0 ± 6.2, 89.7 ± 4.5, and 88.6 ± 6.9 for 0, 3, 6, 16, and 24 h of Ag NPs deposition, Fig. 6c). These results suggest that the Dopamine/Ag nanocomposite is a selective biocompatible and harmless for endothelial cells. Ag NPs are toxic for many types of cells, including endothelial cells in aqueous systems. [40,43,44] Here, we showed that the Dopamine/Ag nanocomposite prepared by depositing Ag NPs on a glass substrate using a mussel-inspired method is selective harmless for endothelial cells and toxic for bacterial cells (Fig. 7). Previously documents have demonstrated that catheter-related bloodstream infection (CRBSI) is the most common cause of nosocomial bacteraemia and can poten-

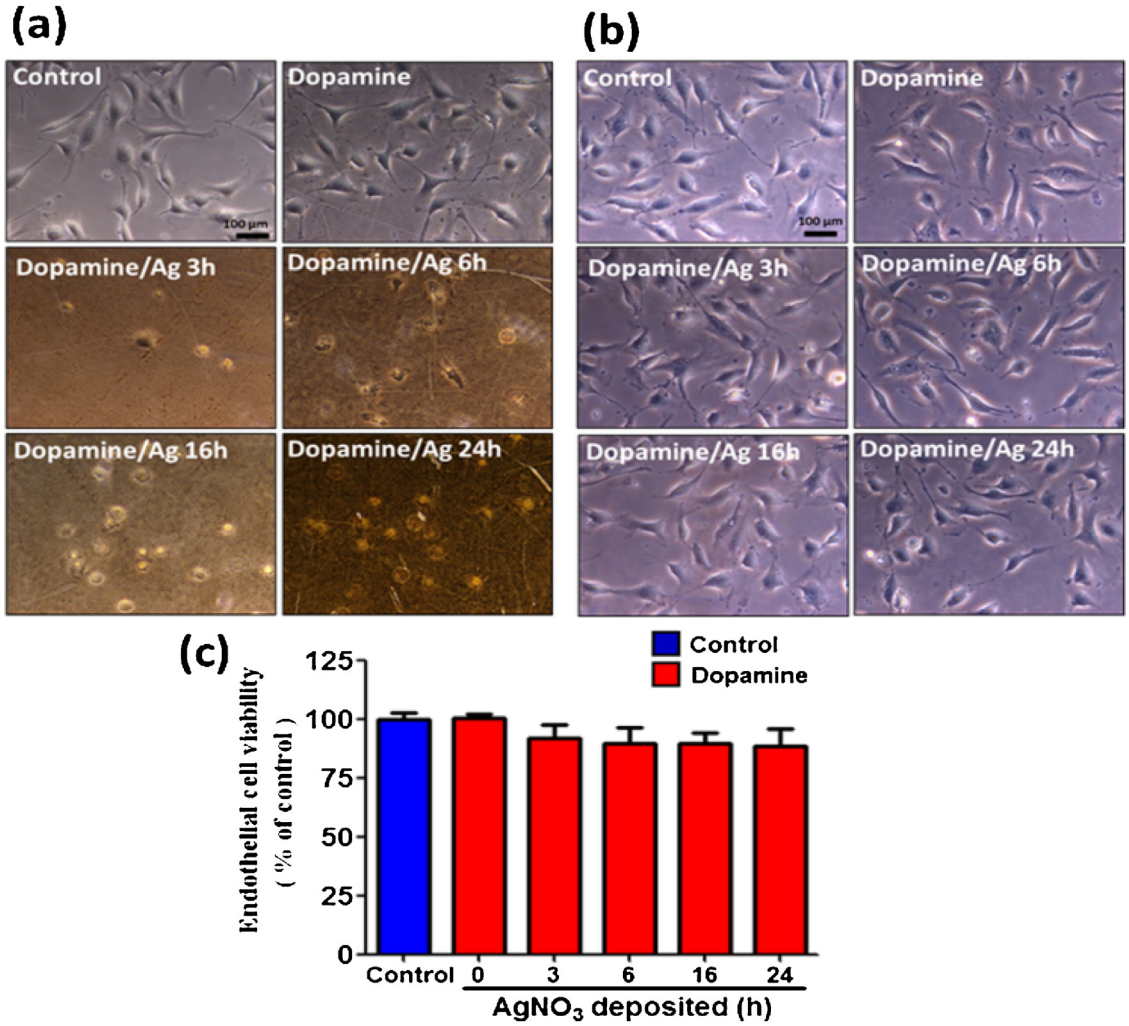

Fig. 6. *in vitro* cytotoxic effects of Dopamine/Ag nanocomposite on the endothelial cell line EA926. Cells were grown on 3 cm petri dishes and co-cultured with dopamine/Ag nanocomposite for 24 h. The cell viability on the dopamine/Ag nanocomposite surface (a) and/or on the culture dish (b) was imaged using microscopy. These results show that cells would not survive at the dopamine/Ag nanocomposite surface, while they can maintain their good morphology and viability around the dopamine/Ag nanocomposite. (c) The cell viability of (b) was further determined quantitatively using MTT assay. Control group is indicates that cells co-culture with cover slide substrate.

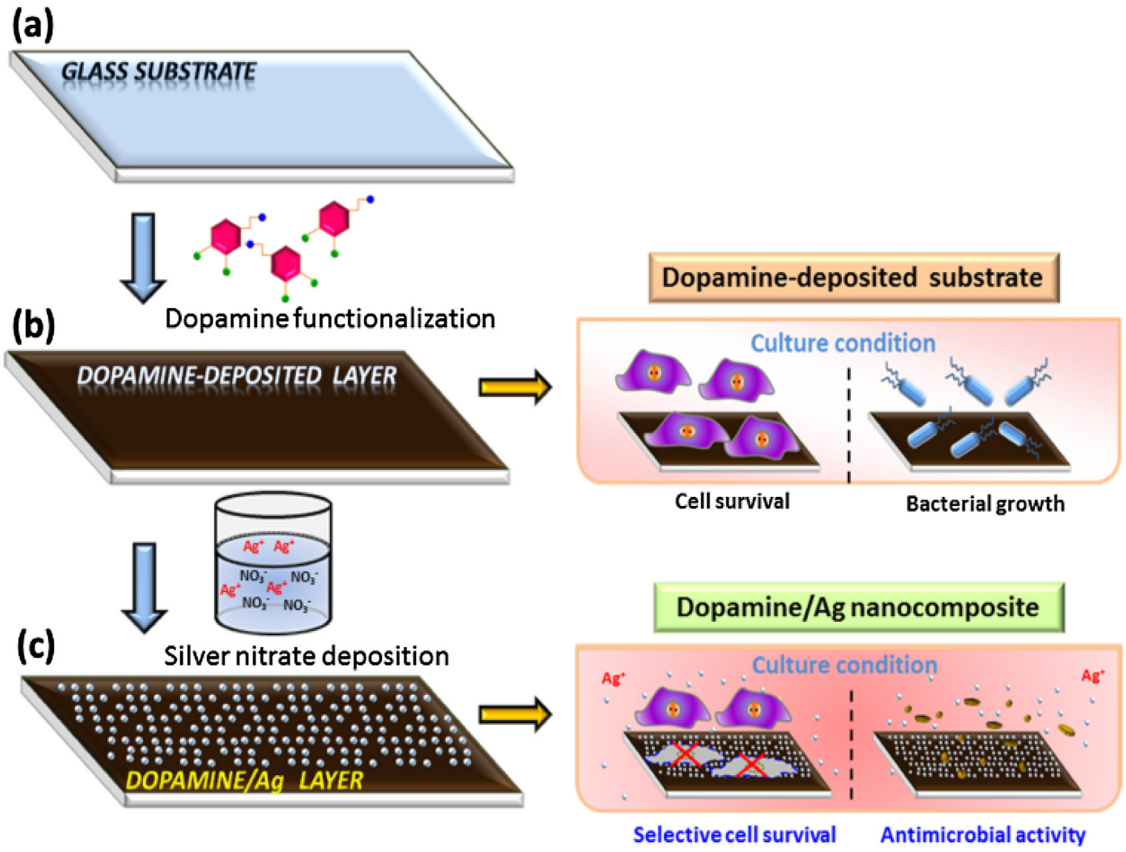

Fig. 7. The characteristics of antibacterial and biocompatible for Dopamine/Ag nanocomposites could be a potential substrate for medical devices. (a) The glass substrate as a control. (b) Dopamine is deposited onto the glass substrate, which reveals a well bacterial growth and adhesion as well as biocompatibility. (c) Ag NPs was modified onto the dopamine-deposited substrate (Dopamine/Ag nanocomposite), which shows the excellent toxicity for bacterial cells and selective retains the biocompatible and harmless for endothelial cells.

tially lead to increased mortality and costs. Therefore, once patients were suffered from CRBSI, it represents that patients have a serious threat from sepsis. Currently, the main treatment strategies for catheter-related sepsis involve removing the infected catheter from the patient and/or administering an antibiotic therapy [38,45]. Thereby, our dopamine-deposited Ag NPs nanocomposites is selective harmless for endothelial cell and can provide a novel route for reducing the incidence of catheterization-mediated microorganism infections and catheter-related endothelial injury complications.

4. Conclusions

The analysis of dopamine-deposited Ag nanocomposites revealed a small size (28.8 ± 7.2 nm) and good dispersion of silver nanoparticles on the substrate surface. The Dopamine/Ag substrate shows excellent antimicrobial activity and reduced biofilm formation, which suggests good antifouling potency and microorganism inhibition properties for these nanocomposites. According to previous studies [38,45], catheter-mediated bloodstream infections play an important role in nosocomial bacteraemia and increased complications. Here, we demonstrate that the Dopamine/Ag nanocomposite is selective harmless for endothelial cells and toxic for bacterial cells, and can therefore underpin a safe and efficient strategy towards medical devices and intra-devices or intra-catheters for the prevention of nosocomial and catheter-mediated blood-stream infections or endothelial injury complications.

Acknowledgements

The authors would like to thank the Ministry of Science and Technology of Taiwan (NSC 104-2628-M-110-001-MY3), and Kaohsiung Medical University "Aim for the Top Universities Grant, KMU-TP104G00, KMU-TP104G01, KMU-TP104G03 and KMU-TP104G04, as well as the National Sun Yat-sen University Biochip Research Group for financial support of this work. Prof. Hsieh also thanks Dr. David Beck for helpful discussions.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apmt.2016.05.003.

References

[1] T.S. Sileika, H.D. Kim, P. Maniak, P.B. Messersmith, Antibacterial performance of polydopamine-modified polymer surfaces containing passive and active components, ACS Appl. Mater. Interfaces 3 (2011) 4602–4610.
[2] H.-C. Flemming, J. Wingender, The biofilm matrix, Nat. Rev. Microbiol. 8 (2010) 623–633.
[3] R.C. Evans, C.J. Holmes, Effect of vancomycin hydrochloride on *Staphylococcus epidermidis* biofilm associated with silicone elastomer, Antimicrob. Agents Chemother. 31 (1987) 889–894.
[4] T.F. Mah, G.A. O'Toole, Mechanisms of biofilm resistance to antimicrobial agents, Trends Microbiol. 9 (2001) 34–39.
[5] J.F. Acar, Consequences of bacterial resistance to antibiotics in medical practice, Clin. Infect. Dis. 24 (Suppl. (1)) (1997) S17–S18.
[6] T. Shalev, A. Gopin, M. Bauer, R.W. Stark, S. Rahimipour, Non-leaching antimicrobial surfaces through polydopamine bio-inspired coating of

quaternary ammonium salts or an ultrashort antimicrobial lipopeptide, J. Mater. Chem. 22 (2012) 2026–2032.
[7] S. Krishnan, C.J. Weinman, C.K. Ober, Advances in polymers for anti-biofouling surfaces, J. Mater. Chem. 18 (2008) 3405–3413.
[8] J.C. Tiller, C.-J. Liao, K. Lewis, A.M. Klibanov, Designing surfaces that kill bacteria on contact, Proc. Natl. Acad. Sci. 98 (2001) 5981–5985.
[9] H. Murata, R.R. Koepsel, K. Matyjaszewski, A.J. Russell, Permanent, non-leaching antibacterial surfaces—2: how high density cationic surfaces kill bacterial cells, Biomaterials 28 (2007) 4870–4879.
[10] K.J. Jeong, L. Wang, C.F. Stefanescu, M.W. Lawlor, J. Polat, C.H. Dohlman, R.S. Langer, D.S. Kohane, Polydopamine coatings enhance biointegration of a model polymeric implant, Soft Matter 7 (2011) 8305–8312.
[11] H. Lee, S.M. Dellatore, W.M. Miller, P.B. Messersmith, Mussel-inspired surface chemistry for multifunctional coatings, Science 318 (2007) 426–430.
[12] F. Yu, S. Chen, Y. Chen, H. Li, L. Yang, Y. Chen, Y. Yin, Experimental and theoretical analysis of polymerization reaction process on the polydopamine membranes and its corrosion protection properties for 304 stainless steel, J. Mol. Struct. 982 (2010) 152–161.
[13] H. Lee, N.F. Scherer, P.B. Messersmith, Single-molecule mechanics of mussel adhesion, Proc. Natl. Acad. Sci. 103 (2006) 12999–13003.
[14] F.K. Yang, B. Zhao, Adhesion properties of self-polymerized dopamine thin film, Open Surf. Sci. J. 3 (2011) 115–122.
[15] M. Sureshkumar, D.Y. Siswanto, C.-K. Lee, Magnetic antimicrobial nanocomposite based on bacterial cellulose and silver nanoparticles, J. Mater. Chem. 20 (2010) 6948–6955.
[16] L.Q. Xu, J.C. Chen, R. Wang, K.-G. Neoh, E.-T. Kang, G.D. Fu, A poly(vinylidene fluoride)-graft-poly(dopamine acrylamide) copolymer for surface functionalizable membranes, RSC Adv. 3 (2013) 25204–25214.
[17] A. Kumar, P.K. Vemula, P.M. Ajayan, G. John, Silver-nanoparticle-embedded antimicrobial paints based on vegetable oil, Nat. Mater. 7 (2008) 236–241.
[18] R. Gottesman, S. Shukla, N. Perkas, L.A. Solovyov, Y. Nitzan, A. Gedanken, Sonochemical coating of paper by microbiocidal silver nanoparticles, Langmuir 27 (2011) 720–726.
[19] P.W. Li, T.H. Kuo, J.H. Chang, J.M. Yeh, W.H. Chan, Induction of cytotoxicity and apoptosis in mouse blastocysts by silver nanoparticles, Toxicol. Lett. 197 (2010) 82–87.
[20] E. Oliveira, H.M. Santos, J. Garcia-Pardo, M. Diniz, J. Lorenzo, B. Rodriguez-Gonzalez, J.L. Capelo, C. Lodeiro, Synthesis of functionalized fluorescent silver nanoparticles and their toxicological effect in aquatic environments (Goldfish) and HEPG2 cells, Front. Chem. 1 (2013) 29.
[21] R.R. Kulesus, K. Diaz-Perez, E.S. Slechta, D.S. Eto, M.A. Mulvey, Impact of the RNA chaperone Hfq on the fitness and virulence potential of uropathogenic *Escherichia coli*, Infect. Immun. 76 (2008) 3019–3026.
[22] P. Vandrangi, S.C. Gott, R. Kozaka, V.G. Rodgers, M.P. Rao, Comparative endothelial cell response on topographically patterned titanium and silicon substrates with micrometer to sub-micrometer feature sizes, PLoS One 9 (2014) e111465.
[23] H. Lee, Y. Lee, A.R. Statz, J. Rho, T.G. Park, P.B. Messersmith, Substrate-independent layer-by-layer assembly by using mussel-adhesive-inspired polymers, Adv. Mater. 20 (2008) 1619–1623.
[24] H. Lee, J. Rho, P.B. Messersmith, Facile conjugation of biomolecules onto surfaces via mussel adhesive protein inspired coatings, Adv. Mater. 21 (2009) 431–434.
[25] S. Kang, M. Elimelech, Bioinspired single bacterial cell force spectroscopy, Langmuir 25 (2009) 9656–9659.
[26] J. Kang, S. Tada, T. Kitajima, T.I. Son, T. Aigaki, Y. Ito, Immobilization of bone morphogenetic protein on DOPA- or dopamine-treated titanium surfaces to enhance osseointegration, Biomed. Res. Int. 2013 (2013) 265980.
[27] S.H. Ku, J.S. Lee, C.B. Park, Spatial control of cell adhesion and patterning through mussel-inspired surface modification by polydopamine, Langmuir 26 (2010) 15104–15108.
[28] S.H. Ku, J. Ryu, S.K. Hong, H. Lee, C.B. Park, General functionalization route for cell adhesion on non-wetting surfaces, Biomaterials 31 (2010) 2535–2541.
[29] Y. Xie, B. Yan, H. Xu, J. Chen, Q. Liu, Y. Deng, H. Zeng, Highly regenerable mussel-inspired Fe3O4@polydopamine-Ag core–shell microspheres as catalyst and adsorbent for methylene blue removal, ACS Appl. Mater. Interfaces 6 (2014) 8845–8852.
[30] S. Saidin, P. Chevallier, M.R. Abdul Kadir, H. Hermawan, D. Mantovani, Polydopamine as an intermediate layer for silver and hydroxyapatite immobilisation on metallic biomaterials surface, Mater. Sci. Eng. C 33 (2013) 4715–4724.
[31] Y.W. Lu, X.W. Du, J. Sun, X. Han, S.A. Kulinich, Influence of surface SiAg bonds on photoluminescence of porous silicon, J. Appl. Phys. 100 (2006) 063512.
[32] R.A. Zangmeister, T.A. Morris, M.J. Tarlov, Characterization of polydopamine thin films deposited at short times by autoxidation of dopamine, Langmuir 29 (2013) 8619–8628.
[33] H. Lee, N.F. Scherer, P.B. Messersmith, Single-molecule mechanics of mussel adhesion, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 12999–13003.
[34] R.L. Davies, S.F. Etris, The development and functions of silver in water purification and disease control, Catal. Today 36 (1997) 107–114.
[35] Q.L. Feng, J. Wu, G.Q. Chen, F.Z. Cui, T.N. Kim, J.O. Kim, A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*, J. Biomed. Mater. Res. 52 (2000) 662–668.
[36] A. Panacek, L. Kvitek, R. Prucek, M. Kolar, R. Vecerova, N. Pizurova, V.K. Sharma, T. Nevecna, R. Zboril, Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity, J. Phys. Chem. B 110 (2006) 16248–16253.
[37] L. Hall-Stoodley, J.W. Costerton, P. Stoodley, Bacterial biofilms: from the natural environment to infectious diseases, Nat. Rev. Microbiol. 2 (2004) 95–108.
[38] S.I. Blot, P. Depuydt, L. Annemans, D. Benoit, E. Hoste, J.J. De Waele, J. Decruyenaere, D. Vogelaers, F. Colardyn, K.H. Vandewoude, Clinical and economic outcomes in critically ill patients with nosocomial catheter-related bloodstream infections, Clin. Infect. Dis. 41 (2005) 1591–1598.
[39] Q. Li, S. Mahendra, D.Y. Lyon, L. Brunet, M.V. Liga, D. Li, P.J.J. Alvarez, Antimicrobial nanomaterials for water disinfection and microbial control: potential applications and implications, Water Res. 42 (2008) 4591–4602.
[40] M. Ahamed, M.S. AlSalhi, M.K.J. Siddiqui, Silver nanoparticle applications and human health, Clin. Chim. Acta 411 (2010) 1841–1848.
[41] C. Greulich, J. Diendorf, J. Geßmann, T. Simon, T. Habijan, G. Eggeler, T.A. Schildhauer, M. Epple, M. Köller, Cell type-specific responses of peripheral blood mononuclear cells to silver nanoparticles, Acta Biomater. 7 (2011) 3505–3514.
[42] C. You, C. Han, X. Wang, Y. Zheng, Q. Li, X. Hu, H. Sun, The progress of silver nanoparticles in the antibacterial mechanism, clinical application and cytotoxicity, Mol. Biol. Rep. 39 (2012) 9193–9201.
[43] H. Rosas-Hernández, S. Jiménez-Badillo, P.P. Martínez-Cuevas, E. Gracia-Espino, H. Terrones, M. Terrones, S.M. Hussain, S.F. Ali, C. González, Effects of 45-nm silver nanoparticles on coronary endothelial cells and isolated rat aortic rings, Toxicol. Lett. 191 (2009) 305–313.
[44] K. Kalishwaralal, E. Banumathi, S.R.K. Pandian, V. Deepak, J. Muniyandi, S.H. Eom, S. Gurunathan, Silver nanoparticles inhibit VEGF induced cell proliferation and migration in bovine retinal endothelial cells, Colloids Surf. B 73 (2009) 51–57.
[45] S. Fletcher, Catheter-related bloodstream infection, continuing education in anaesthesia, Crit. Care Pain 5 (2005) 49–51.