Assembly of Gentamicin and Zn2+ Loaded Coatings on TiO2 Nanotubes to Synergistically Improve the Blood Compatibility, Endothelial Cell Growth and Antibacterial Activities

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Abstract

Titanium and its alloys are widely used in blood contacting implantable and interventional medical devices; however, their biocompatibility is still facing great challenges. In this study, with the aim of improving the biocompatibility and antibacterial activities of titanium, TiO$_2$ nanotubes with a diameter of about 30 nm were firstly prepared on the titanium surface by anodization, followed by the introduction of polyacrylic acid (PAA) and gentamicin (GS) on the nanotube surface by layer-by-layer method, and finally zinc ions were loaded into the surface to improve the bioactivities. The nanotubes have excellent hydrophilic properties and special nanotube-like structure, which can selectively promote the albumin adsorption and enhance the blood compatibility and promote the growth and functional expression of endothelial cells to a certain extent. After the introduction of PAA and GS, although the superhydrophilicity cannot be achieved, the results of platelet adhesion, cGMP activity, hemolysis rate and partial thromboplastin time (APTT) showed that the blood compatibility was improved, and the blood compatibility was further enhanced after zinc ions loading on the surface. On the other hand, the surface modified materials showed good cytocompatibility to endothelial cells. The introduction of PAA and zinc ions not only promoted the adhesion and proliferation of endothelial cells, but also up-regulated expression of vascular endothelial growth factor (VEGF) and nitric oxide (NO). The slow and continuous release of GS and Zn$^{2+}$ for more than 14 days, which can significantly improve the antibacterial properties of the materials. Therefore, the present study provides an effective method for the surface modification of titanium-based blood-contacting materials to simultaneously endow with good blood compatibility, endothelial growth behaviors and antibacterial properties.

1 Introduction

Blood-contacting implantable and interventional medical devices, such as artificial heart valves, thrombus filters, vascular stents, etc., have saved thousands of lives [1, 2]. Titanium and its alloys are widely used in blood-contacting medical devices, but they still face great challenges in clinical applications, such as inflammation, thrombosis and infection. Generally speaking, ideal medical implants should have the abilities to integrate and communicate with surrounding tissues or cells, trigger specific cell responses and maintain the function of tissues and organs, and prevent infections caused by microorganisms after the implantation [3, 4]. In this regard, surface functionalization represents one of the straightforward and effective methods to endow biomaterials with excellent properties and functions [5, 6]. According to the mechanism of the interaction between the implant and the surrounding physiological environment, the introduction of bioactive factors on the surface by physical or chemical conjugation can endow the inert biomaterial with good biological activities, so as to regulate the cell-material interaction behaviors, induce specific cell responses, and prevent the infection caused by implantation and related biological effects [7–10].

Although great progress has been made in the surface functionalization of titanium-based blood-contacting materials, there are still issues to be solved, including the delayed surface endothelialization, thrombosis and infection caused by implantation [11, 12]. Studies have shown that the surface properties
of implantable and interventional medical devices are related not only to the surface bioactivities, but also to the surface topographies. In recent years, the application of nanomaterials with special tubular structure in the blood-contacting biomaterials has attracted great attention [13]. Anodization is a surface modification technology that can in-situ prepare nanotubes on the titanium surface [14]. The nanotubes prepared by anodization not only do not change the mechanical properties of bulk materials, but also provide an excellent platform for loading bioactive molecules for surface functionalization to enhance the surface bioactivities [15–17]. Our previous results showed that the anodized TiO$_2$ nanotubes with different dimensions have different effects on the behaviors of human blood and endothelial cells, demonstrating that the interfacial biological behaviors between implanted materials and tissues can be regulated by regulating the surface morphologies [18]. Therefore, loading the bioactive factors into the nanotubes can further regulate the biocompatibility from two aspects of surface bioactivities and surface morphologies.

Zinc is the second most abundant trace element in the human body, which participates in a large number of physiological reactions and is an important substance involved in cell growth behavior and cell function expression [19]. Zinc ion also plays an important role in the cardiovascular system, it can prevent local vascular ischemia and vascular infarction [20]. Zinc deficiency is closely related to atherosclerosis, and zinc can protect the integrity of vascular endothelium by preventing nuclear factor apoptosis and inflammation-related genes [21]. Moreover, zinc ions can induce bacterial apoptosis by changing the charge balance of bacteria [22]. Therefore, the loading of zinc ions into the anodized TiO$_2$ nanotubes can improve the anticoagulant and promote the growth of endothelial cells.

In addition, intravascular devices should not only have good blood compatibility and the ability to promote endothelial growth, but also have good antibacterial properties because the implant-centered infection is often one of the important reasons of the implantation failure [23]. Loading or immobilization of antibacterial substances on the implant surface is an important approach to endow devices with antibacterial properties [24]. Polyacrylic acid (PAA) is a cheap and environmentally friendly water-soluble organic polymer. The carboxylic acid groups of PAA can absorb a large number of metal ions, drugs and other positively charged substances, so it is widely used in the field of biomaterials [25]. Gentamicin (GS) is a kind of aminoglycoside, which is widely used in antibacterial therapy in clinic. It has broad spectrum of antimicrobial activity and especially has excellent antibacterial activity for Gram-negative bacteria [26]. Therefore, in the present study, we first prepared TiO$_2$ nanotubes on the titanium surface by anodization, and then PAA was further introduced on the nanotubes surface followed by loading GS by layer-by-layer (LBL) and zinc ions with the help of carboxylic acid groups of PAA. The results indicated that PAA and the continuous released zinc ions can significantly improve the anticoagulant and promote endothelial cell growth, and the excellent and long-lasting antibacterial properties can be achieved through the release of GS and zinc ions.

2 Materials And Methods

2.1 Preparation of TiO$_2$ nanotubes on titanium surface
Titanium plates (TA2) with a diameter of 15 mm and a thickness of 2 mm were successively polished with sandpapers of 400#, 800#, 1200#, 1500# and 2000#, respectively, and then polished to the mirror with a polishing machine. After ultrasonically cleaning for 10 min with acetone, ethanol and deionized water, the titanium plates were immersed in 50 ml electrolyte (ethylene glycol solution containing 0.25%wt NH₄F and 6 ml deionized water) to anodize 3 hours at 30V using the plate as the anode. The plates were cleaned ultrasonically for 30 min in ethylene glycol solution and for 5 min in ethanol. The titanium oxide nanotube array was dried and heat-treated at 500°C in air for 3 hours, and it was named TNT.

2.2 Loading GS and Zn²⁺ on TiO₂ nanotubes

The TNT samples were firstly immersed in 2 mg/ml dopamine solution (pH8.0) for 12 hours and then the samples were washed by the deionized water. The process was repeated for three times, and the as-prepared samples were named as TNT-Dopa. The TNT-Dopa samples were further immersed in polyethyleneimine (PEI) solution (5mg/ml, pH10.0) for 30 minutes. After cleaning, the sample was immersed in 1mg/ml PAA solution (pH7.4) for 10 minutes, and the obtained sample was labelled as TNT-PAA. For loading gentamicin (GS), the TNT-PAA sample was alternately immersed in GS (1mg/ml) and PAA solution (1mg/ml), and the process was repeated 10 times, the obtained sample was named as TNT-PAA/GS and the outermost layer was PAA. Finally, the sample was immersed in 1M ZnSO₄ for 2 hours to load Zn²⁺, and the sample was labelled as TNT-PAA/GS-Zn.

2.3 Sample characterization

The surface morphologies of the samples before and after modification were observed by scanning electron microscopy (SEM, FEI Quanta 250). The changes of chemical groups on the titanium surface were examined by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR, TENSOR 27, Bruker, Germany), the measurements were carried out at room temperature and the scanning range was from 650 cm⁻¹ to 4000 cm⁻¹. The surface atomic concentrations of the different samples were measured by X-ray photoelectron spectroscopy (XPS, VG Science, East Grinstead, UK). Water contact angle measurement (DSA25, Krüss GmbH, Germany) was used to characterize the surface hydrophilicity, and five parallel samples were measured and averaged.

2.4 Protein adsorption

The adsorption behaviors of fibrinogen (FIB) and bovine serum albumin (BSA) were measured by BCA method. The samples were firstly immersed in ethanol for 30 minutes, and then in phosphate buffer (PBS) for 10 hours. After that, the samples were placed into 1mg/ml BSA solution and 1mg/ml FIB solution for 2 h at 37 °C, respectively. After washing twice by PBS, the sample was put into 2 ml SDS (sodium dodecyl sulfate, 1%wt) solution for ultrasonically desorbing 30 min. Taking 150 μl eluent and 150 μl BCA working solution (reagent A: reagent B: reagent C=25: 24: 1) to react 1 hour at 60 °C, and then 200 μl mixing solution was transferred into a 96-well plate to measure the absorbance at 562nm by the
micro-plate reader (Bio-Tek, Eons), and the adsorption amount of protein was calculated according to the standard curve.

2.5 Release of GS and Zn2+

The TNT-PAA/GS-Zn sample was immersed in 5 ml 37°C PBS solution for 1 h, 3 h, 5 h, 7 h, 1 d, 3 d, 7 d and 14 d, and then 200 μl solution was transferred into 96-well plate. The absorbance at 562nm was measured by a micro-plate reader (Bio-Tek, Eons), and the release amount of GS was calculated according to the standard curve. The concentration of zinc ions was measured by inductively coupled plasma emission spectrometer (Optima 7000 DV), and release concentration was calculated according to the standard curve. Three parallel samples were measured and averaged, and the release profiles were further plotted.

2.6 Blood compatibility

2.6.1 Hemolysis assay

The hemolysis rate was measured according to ISO10993-4 standard. Fresh human blood from a healthy volunteer was centrifuged at 1500 r/min for 10 min to obtain the red blood cells. The red blood cells were prepared into 2% suspension with physiological saline. The samples were immersed in the suspension solution of red blood cells for incubating 1 hour at 37 °C. The solution was then centrifuged at 3000 rpm for 5 min. Taking 100 μl the supernatant into a 96-well plate, and the absorbance (A) was measured at 450 nm by the micro-plate reader. Under the same conditions, the absorbance value (B) of the mixed solution of 2% red blood cells and 98% normal saline was measured as negative control, and the absorbance value (C) of the solution of 2% red blood cells and 98% deionized water was recorded as the positive controls. Three parallel samples were measured and the values were averaged. The hemolysis rate was calculated according to the following formula.

Hemolysis rate (%) = (A -B)/(C-B) × 100%.

2.6.2 Platelet adhesion and activation

Fresh human whole blood of a healthy volunteer was centrifuged at 1500 rpm for 15 min to obtain platelet-rich plasma (PRP). 200 μl PRP was fully covered on each sample surface to incubate 2 h at 37 °C, and then the samples were rinsed with PBS for three times. The adherent platelets were fixed with 2.5% glutaraldehyde (in PBS buffer) for 24 hours, and then rinsed with PBS solution. The samples were successively dehydrated with 30%, 50%, 75%, 90%, 100% ethanol solutions for 10 min each, and the samples were dried in the air. After spraying gold on the surface, the morphologies of the platelets were observed by SEM (FEI Quanta 250). Five SEM images with small magnification were randomly selected for calculating the number of platelets adhered to the surface, and the values were averaged and expressed as platelets per mm².
For platelet activation assay, enzyme linked immunosorbent assay (ELISA) method was used to measure the activity of cGMP (cyclic guanosine monophosphate) secreted by platelets. In brief, 200 μl PRP was dropped on each sample surface to cover the entire surface. After incubating at 37 °C for 2 hours, the plasma on the surface was diluted 5 times, subsequently, the plasma was transferred to the enzyme plate for culturing 30 min at 37 °C. 50 μl of enzyme-labeled reagent was added to each well, cultured at 37 °C for 30 minutes. Then 50 μl chromogenic agent A and 50 μl chromogenic agent B were added to each well and kept away from light for 10 minutes at 37 °C. Finally, the terminating solution was added to stop the reaction. The absorbance at 450 nm was measured and the concentration of cGMP was calculated according to the standard curve.

### 2.6.3 APTT

Fresh human anticoagulant whole blood was centrifuged for 15 min at 3000 rpm to obtain platelet-poor plasma (PPP). 100 μl PPP was covered onto the sample surface and cultured at 37 °C for 15 min. Subsequently, 50 μl PPP and 50 μl APTT reagent (Sysmex, Japan) were added into the test tube and cultured at 37 °C for 3 min. 50 μl 0.025 M CaCl$_2$ solution was finally added. The clotting time was measured by automatic coagulation meter (CA-1500, Sysmex, Japan), and the average values of three parallel samples were measured.

### 2.7 Endothelial cell growth behaviors

#### 2.7.1 Cell adhesion and proliferation

The samples were placed in a 24-well culture plate and sterilized overnight with ultraviolet on the super-clean table, and then 0.5 ml endothelial cell suspension (5×10$^4$ cells/ml) and 1.5 ml cell culture medium were added to each sample surface. After incubating at 37 °C and 5% CO$_2$ for 1 and 3 days, respectively, the samples were washed with PBS for 3 times. Each sample surface was stained with 200 μl rhodamine (in PBS, 1: 400) for 20 minutes, and then washed with PBS for 3 times. Finally, 200 μl of DAPI (PBS, 1: 400) was added to the surface for 3 min. After washed 3 times with PBS, the cells were observed by fluorescence microscopy (Zeiss, inverted A2).

For cell proliferation, endothelial cells were cultured in the same way as mentioned above. After 1 and 3 days, the samples were washed with PBS for 3 times. 0.5 ml CCK-8 solution (10% in cell culture medium) was added and incubated in 37 °C incubator for 3.5 hours. After that, 200 μl medium was transferred into a 96-well plate, and the absorbance at 450nm was measured by a micro-plate reader (Bio-Tek,Eons) to determine the proliferation activity of endothelial cells.

#### 2.7.2 NO and VEGF expression

The NO release from endothelial cells was measured by Griess method. The endothelial cells were cultured on the sample surface for 1 day and 3 days, respectively, and the supernatant was added to the 96-well plate, and then Griess Reagent I and II were added successively. The absorbance at 540 nm was
determined by a microplate reader, and the NO concentration was calculated according to the standard curve.

For VEGF assay, according to the instructions of the enzyme-linked immunosorbent assay kit, endothelial cells were cultured on the sample surface for 1 day and 3 days, respectively, and then the supernatant was absorbed to dilute 5 times and added to the enzyme plate. After incubated at 37 °C for 30 min, 50 μl of enzyme labeled reagent was added and incubated at 37 °C for another 30 min, and then 50 μl chromogenic agent A and 50 μl chromogenic agent B were added to each well to react 10 min at 37 °C in the dark. Finally, the terminating solution was added to stop the reaction, and the OD value at 450nm was determined by a micro-plate reader. Three parallel samples were measured and averaged. The VEGF concentration was determined according to the standard curve.

2.8 Antibacterial activities

2.8.1 Bacterial adhesion

Escherichia coli was cultured in liquid medium for 20 hours, 10ml solution was centrifuged at 2000 rpm for 3 minutes, and then dispersed evenly. The bacterial solution was diluted 10 times and 50 μl bacterial suspension was dropped on the sample surface to culture 2 hours. The sample was then washed 3 times with PBS and fixed for 2.5 hours with 2.5% glutaraldehyde solution, followed by washing the sample with PBS, and finally dehydrated with 50%, 70%, 90%, 100% ethanol solutions for 10 minutes each time. After spraying gold on the sample surface, the adhered bacteria were observed by SEM (FEI Quanta 250).

2.8.2 Antibacterial activities

Escherichia coli was cultured overnight in liquid medium, 10 ml bacteria solution was taken and centrifuged at 1300 rpm for 5 minutes to determine the survival and number of bacteria. The sample was placed into a 12-well plate and then 500 µl bacterial solution was added. After cultured at 37 °C for 30 minutes, 1500 ml sterilized deionized water was added and continued to culture at 37 °C for 24 hours. Taking 50 µl bacterial liquid was evenly covered on the surface of the solid medium. After being cultured at 37 °C overnight, the bacterial was observed by taking pictures using Huawei Mobile (Nova 6).

3 Results And Discussion

3.1 Surface characterization

Figure 1 shows the representative SEM images of the titanium dioxide nanotubes modified by the different bioactive factors. It is obvious that after different surface modification processes, the surface nanotube structure remains intact. Compared with TNT-Dopa, the surface immobilization of PAA and the loading of GS and Zn^{2+} gradually reduced the diameter of the nanotube and increased the thickness of the tube wall. Furthermore, the chemical group changes on the surface were examined by ATR-FTIR. It can be seen from Fig. 2a that there was almost no infrared absorption on the unmodified titanium
surface. Our previous work showed that the anodized TiO2 nanotubes have a small amount of hydroxyl on the surface [18], but it cannot be directly used to immobilize the bioactive molecules. To solve this problem, a polydopamine coating was prepared on the surface of TiO2 nanotubes, followed by the grafting of polyethyleneimine (PEI) to create a positively charged surface, PAA with negative charges was then immobilized on the surface by electrostatic interaction to construct a negatively charged surface. Finally, in order to improve the biocompatibility and antibacterial properties, gentamicin and zinc ions were introduced on the PAA-modified surface by layer-by-layer technique and ion chelation, respectively. The results of Fig. 2a show that the stretching vibration and in-plane bending vibration of -NH bond and -OH bond appeared on TNT-Dopa surface at 1590 cm$^{-1}$ and 3300 cm$^{-1}$, and the stretching vibration of -OH bond occurred around 3700 cm$^{-1}$, indicating that the polydopamine coating had been successfully prepared on the surface. After grafting of PEI, the in-plane bending vibration and stretching vibration of -CH$_2$ bond can be observed at 1462 cm$^{-1}$ and 2832 cm$^{-1}$, and the bending vibration of -NH bond of primary amine and secondary amine and the stretching vibration of C-N bond can be detected at 1200 cm$^{-1}$ and 1656 cm$^{-1}$, indicating that PEI had successfully covalently linked with polydopamine coating. For TNT-PAA/GS, the stretching vibrations of C = O bond and -COOH bond appeared at 1519 cm$^{-1}$ and 1694 cm$^{-1}$, respectively, suggesting that PAA and GS were successfully self-assembled onto the PEI-modified surface. In order to further clarify the surface element composition, the surface element composition of the modified sample was further analyzed by XPS. Fig. 2b is the XPS diagram of the different samples, and the element compositions are shown in Table 1, it can be seen that the characteristic peaks of C$_{1s}$ (285.2eV) and N$_{1s}$ (400.3eV) appeared on the TNT-Dopa. After the immobilization of PAA, the characteristic peak of O1s (531.8eV) increased obviously, concurrently the characteristic peak of C$_{1s}$ (285.2eV) and carbon content on the surface was reduced, indicating that PAA was successfully grafted onto the surface. For the TNT-PAA/GS, the increased nitrogen content indicated that GS was successfully loaded on the surface. The occurrence of Zn$_{2p}$ peak (1020.9eV) on TNT-PAA/GS-Zn proved that the Zn ions were successfully chelated to the surface.

### Table 1 The surface element concentration of the different samples measured by XPS.

| Sample         | Atomic concentration at.% |
|----------------|---------------------------|
|                | Ti  | O  | C  | N  | Zn |
| Ti             | 61.12 | 29.86 | 9.02 | 0  | 0  |
| TNT-Dopa       | 9.28 | 14.30 | 71.83 | 4.59 | 0  |
| TNT-PAA        | 9.97 | 30.42 | 56.41 | 3.20 | 0  |
| TNT-PAA/GS     | 3.27 | 31.77 | 56.20 | 8.76 | 0  |
| TNT-PAA/GS-Zn  | 1.86 | 28.78 | 59.73 | 6.26 | 3.37 |
3.2 GS and Zn$^{2+}$ release profile, surface hydrophilicity and protein adsorption

In order to characterize the release profile of gentamicin and Zn$^{2+}$, TNT-PAA/GS-Zn was immersed in PBS solution for different times, and gentamicin and Zn ions were collected to measure the release kinetics curves. As can be seen from the release curve of Fig. 3a, both gentamicin and zinc ions released for more than 14 days. After immersed in PBS solution for 4 hours, the release concentration of gentamicin reached 3.9 μg/ml, and the total release concentration was 13.54 μg/ml at 14th days. Previous studies have shown that the working concentration of gentamicin is 4-20 μg/ml [27], therefore the continuous antibacterial activities can last at least 14 days. At the same time, it can also be seen from Fig. 3a that there was an obvious burst release period of one day for gentamicin and Zn$^{2+}$. The release rate was relatively large before 1 day, and gentamicin reached 5.5 μg/ml, more than 40% of the total release in 14 days. After one day, the release rates of gentamicin and zinc ions became stable. After 14 days, the total concentration of zinc ion was 0.63 mg/l. It was reported that zinc ion concentration of 0.49-5.2mg/l can promote cell viability, proliferation, adhesion and migration, therefore the released Zn$^{2+}$ content was within the range of cell physiological concentration.

Biomaterials should have good surface properties to avoid adverse host reactions after contact with organisms [28], in which wettability is an important factor affecting interface biological reactions [29]. The surface hydrophilicity/hydrophobicity is closely related to the protein adsorption and biocompatibility. Generally speaking, the good wettability is helpful to prevent the non-specific protein adhesion and promote the cell adhesion and proliferation [30]. As can be seen from Fig. 3b, the water contact angle of the blank titanium decreased obviously after anodizing, it was considered that the introduction of a large amount of oxygen elements and the formation of the special nano-porous structure can contribute to the excellent hydrophilicity. Due to the introduction of hydrophilic amine groups after the immobilization of dopamine, TNT-Dopa still had excellent hydrophilicity. However, after further the immobilization of PAA, the water contact angle increased significantly, this was mainly because the porous structure on the surface of the material was filled to some extent after the fixation of PEI and PAA (as shown in Fig. 1), which partially changed the surface morphology and made it difficult for water molecules to enter the interior of the nanotubes, so the contact angle increased. After further GS loading, the porous structure of the surface was further filled, and thus the water contact angle also increased although the hydrophilic carboxyl groups were introduced. Finally, because the positively charged zinc ions can chelate with the hydrophilic -COOH groups on the surface, combining with the further porous filling, the water contact angle increased further.

It is well known that protein adsorption is the first event when biomaterials contact blood, and it plays a decisive role in the blood compatibility [31]. Albumin and fibrinogen are the two main proteins in the blood. In general, albumin adsorption can reduce platelet adhesion. On the contrary, the adsorption of fibrinogen could increase the platelet adhesion and activation. Fig. 3c and d shows the adsorption concentrations of bovine serum albumin (BSA) and fibrinogen (Fib) on the different surfaces. As compared to the pristine titanium, the anodized titanium surface can enhance albumin adsorption, while
the fibrinogen adsorption decreased to a certain extent, indicating that the anodized TiO$_2$ nanotube array can selectively adsorb albumin. It was considered that the behaviors of protein adsorption were related to the surface wettability, surface morphologies and surface charges. The study showed that when the water contact angle is less than 110 °, fibrinogen would preferentially be adsorbed on the hydrophobic surface [32]. The increase of surface hydrophilicity after anodizing and a small amount of negatively charged hydroxyl groups on the surface were beneficial to the adsorption of positively charged albumin on the surface, but not conducive to the adsorption of negatively charged fibrinogen. The polydopamine have very strong stickiness to lysine-rich proteins [33], so the contents of BSA and fibrinogen adsorbed on TNT-Dopa surface increased significantly. It has been shown that the immobilization of polyacrylic acid on the surface can repel the non-specific protein adsorption because of the hydration layer formed by PAA and the negatively charged character of PAA [34]. Therefore, after the immobilization of PAA on TNT-Dopa, both BSA and fibrinogen adsorption decreased significantly. However, it was worth noting that BSA adsorption returned to the level of titanium oxide nanotubes, while fibrinogen adsorption was slightly higher than that of TNT. Furthermore, after loading GS by layer-by-layer technique, due to the further increase of hydrophobicity, the adsorption content of BSA protein did not change significantly, but the adsorption amount of fibrinogen decreased, indicating that with the increase of self-assembly layers, the introduction of a large number of PAA increased the content of negative charges on the surface, so that the negatively charged fibrinogen was not easily adsorbed on the PAA surface, thus reducing the fibrinogen adsorption. The zinc ions loaded on the surface can chelate with the carboxyl group of PAA to reduce the surface hydrophilicity, at the same time, zinc ion loading reduced the content of negative charges on the surface, so BSA adsorption increased slightly, while the adsorption capacity of fibrinogen further decreased.

3.5 Blood compatibility

Blood compatibility refers to the required response of blood to exogenous substances or materials, which generally refers to the compatibility between materials and various components of blood [35]. Generally speaking, blood compatibility includes three aspects: the interaction between materials and plasma proteins (such as albumin and fibrinogen), the interaction between materials and blood cells (red blood cells, white blood cells, platelets, etc.), and the interaction between materials and coagulation factors.

Platelets are one of the main components of human blood. Its main functions are clotting and hemostasis as well as repairing damaged blood vessels. The platelet adhesion to the biomaterials surface is a key event of coagulation. Platelet adhesion, aggregation and activation will promote blood coagulation. Therefore, the biomaterials with good blood compatibility should have the role of maintaining normal platelet physiological function, and can effectively prevent platelet adhesion, aggregation and activation [36]. At the same time, the increase of cGMP released from platelets can inhibit platelet activation [37]. In this paper, the adhesion and aggregation of platelets were observed by SEM, and the platelet activation was evaluated by measuring the cGMP. The results are shown in Fig. 4a, b and c. There were a large number of platelets adhered to the blank titanium surface, and the adhered platelets displayed spread state and extended pseudopodia, indicating that the platelets on the titanium
surface may have been activated, the results of cGMP further proved this point. After anodization, the number of platelets adhered to the surface was significantly reduced. On the one hand, the anodized TiO$_2$ nanotube arrays had excellent hydrophilic properties, which can reduce platelet adhesion and aggregation; on the other hand, the selective adsorption of albumin by TiO$_2$ nanotube not only contributed to reduce platelet adhesion and aggregation, but also promote the expression of cGMP (Fig. 4c), leading to the improved blood compatibility. For TNT-Dopa, although its hydrophilicity was still excellent, compared with TNT, the number of platelets adhered to the surface was still significantly increased, while the expression of cGMP was also decreased, which could be due to the fact that the polydopamine coating had the ability of non-specific protein adsorption which can significantly increase the fibrinogen adhesion to the surface so as to enhance platelet adhesion and activation. Compared with the blank titanium and TNT, when the composite film of PAA and GS was prepared on the surface, the number of platelets on the surface decreased sharply, and the expression of cGMP also increased, indicating that the anticoagulation was enhanced. It was considered that PAA itself is a substance with good blood compatibility and can effectively reduce fibrinogen adsorption [38]. When zinc ions were loaded on the surface, the adsorption amount of fibrinogen further decreased, while the albumin adsorption increased, which not only reduced the adhesion and aggregation of platelets, but also increased the cGMP release and inhibited platelet activation. On the other hand, zinc ions can make platelets produce more NO signals which can inhibit platelet adhesion and aggregation by inhibiting thromboxane A$_2$ (TXA-2) receptor [39]. Therefore, the loading of zinc ions on the surface further improved the blood compatibility.

The effects of the different materials on red blood cells were further studied. Hemolysis rate is one of the important methods to characterize the interaction between materials and red blood cells. According to the international ISO10993-4 standard, the hemolysis rate (HR) below 5% means that the material meets the requirements, on the contrary, the material with HR more than 5% is not suitable to be used as a blood contacting material. Fig. 4d shows the hemolysis rates of the different samples. The hemolysis rates of all samples were less than 5%, indicating that none of them could cause severe hemolysis. The hemolysis rate of TiO$_2$ nanotubes was lower than that of pure titanium, but the hemolysis rate increased slightly after the preparation of polydopamine coating. When the composite film of PAA and GS was prepared and the zinc ion was loaded, the hemolysis rate further decreased significantly, indicating that PAA and zinc ions can improve the blood compatibility.

Generally speaking, when the biomaterial interacts with human blood, blood coagulation may happen. Blood coagulation is a complex chain process involving a series of stimulus responses in conjunction with coagulation factors and enzymes, whose intent is to stop blood fluxes when a vascular tissue injury occurs [40]. According to the difference of the initial pathway and participating factors, blood coagulation can be divided into two pathways: endogenous coagulation and exogenous coagulation. Among them, the endogenous coagulation pathway is initiated by the activation of factor XII [41], and the activated partial thromboplastin time (APTT) is an important method reflecting the endogenous coagulation pathway, especially the activity of coagulation factor XII [42]. Figure 4e shows the APTTs of the different
samples. It can be seen that the clotting time of blank titanium was shorter than that of normal plasma, indicating that it may promote blood coagulation to a certain extent. However, the clotting time of the anodized titanium was longer than that of normal plasma, suggesting that the surface of TiO$_2$ nanotube had good anticoagulation performance. Although the clotting time decreased after dopamine surface modification, the clotting time was significantly prolonged after the immobilization of PAA and the loading of GS and Zn$^{2+}$, indicating that the subsequent surface modification improved the anticoagulant properties of the materials.

### 3.6 Endothelial cell growth behaviors

Fig. 5a and b shows the fluorescent images and the CCK-8 values of endothelial cells adhered to the different surfaces. It can be clearly seen that the number of cells adhered to the surface of the blank titanium was less than that of other modified samples. After anodizing, the number of adherent cells on the surface increased, and the morphologies of adherent cells displayed spread state, its proliferation was also improved (Fig. 5b). It can be concluded that the surface of pure titanium became more hydrophilic after anodizing, which can improve cell adhesion and proliferation on the surface through the exchange and adsorption of extracellular matrix proteins [43], moreover, the special nanostructure also contributed to cell adhesion and spreading. Dopamine is a chemical substance produced by the central nervous system of the human body. The polydopamine coating on the surface can promote the adhesion and proliferation of endothelial cells [44]. Therefore, the number of endothelial cells adhered to TNT-Dopa increased significantly, and the proliferation performance was further improved. After the preparation of the first PAA layer on PEI-modified TNT surface, due to the cytotoxicity of PEI and the hydrophobicity of PAA, the adhesion of cells to the material surface was reduced, and the morphologies of cells did not spread well, so the proliferation of endothelial cells decreased slightly. When PAA and GS were deposited alternately for 10 times, the thickness of the coating increased, which eliminated the effect of PEI on cells. Therefore, the number of endothelial cells attached on the surface increased again, and the spreading and proliferation of endothelial cells were better than TNT-Dopa. Zinc is an essential micronutrient for human health, and Zn$^{2+}$ homeostasis in cells is essential for cell function and survival [45]. Zn$^{2+}$ acts as the first or second messenger and is the signal pathway that triggers physiological functions. In our previous work, zinc ions were doped into TiO$_2$ nanotubes by hydrothermal method. The results showed that the release of zinc ions not only increased the anticoagulant properties of the materials, but also promoted the adhesion and proliferation of endothelial cells [46]. In this study, the zinc ions were loaded on the TNT surface by chelation with PAA. The results of Fig. 5 showed that the release of zinc ions from the surface can significantly promote the adhesion and proliferation of the endothelial cells.

Furthermore, the function expression of endothelial cells was studied. Figure 5c shows the results of VEGF secretion of endothelial cells on the different samples. It can be seen that, compared with the blank titanium, anodization can promote the expression of VEGF in endothelial cells. When the polydopamine coating was prepared on the surface, due to the enhancement of the coating on cell adhesion and
proliferation, endothelial cells could express more VEGF. For TNT-PAA and TNT-PAA/GS, the amount of VEGF decreased slightly as compared to TNT-Dopa, but it was not significant. After zinc ion loading, the content of VEGF increased significantly, indicating that the release of zinc ions can promote the up-regulated expression of VEGF in endothelial cells, which was conducive to maintaining the growth of endothelial cells and promoting the surface endothelialization of the implanted materials. Fig. 5d shows the NO secretion of endothelial cells grown on different samples. The NO content released by endothelial cells on the surface increased after anodizing. The NO content for TNT-Dopa was larger because the polydopamine coating can promote the growth of endothelial cells. After further preparation of PAA film and alternating deposition of PAA and GS, the growth state of cells was slightly worse (Fig. 5a). At the same time, the content of VEGF release could also indirectly affect the production of eNOS in endothelial cells, thus affecting the release of NO [47], therefore the NO content decreased slightly. However, it was worth noting that the loading zinc ions can significantly promote the activity of intracellular NOs enzyme and promote the release of NO, and thus enhance the NO expression of endothelial cells.

3.7 Antibacterial activities

Bacterial adhesion is widespread in nature, and there are usually two strategies of antibacterial strategies: killing bacteria and reducing bacterial adhesion [48]. In this study, gentamicin and zinc ions were loaded into titanium oxide nanotubes on the titanium surface to prevent infection. Gram-negative bacteria—Escherichia coli was used as a test strain to evaluate the antibacterial properties of the modified material surface. Fig. 6a shows the SEM images of bacterial adhesion on the different sample surfaces. It can be seen that although titanium had good biocompatibility to endothelial cells, its anti-bacterial adhesion property was poor, and there were a large number of Escherichia coli bacteria adhering to the surface. In contrast, the property of non-specific protein adsorption caused by the excellent hydrophilicity of the nanotubes and a small amount of negative charges on the surface could contributed to inhibit the adhesion of negatively charged Escherichia coli. As the same with cell results, polydopamine coating could make Escherichia coli easier to adhere to the surface, therefore, compared with TNT sample, the adsorption of bacteria increased on TNT-Dopa. When PAA was prepared on the surface, the amount of negative charges increased, which reduced the adhesion of Escherichia coli to the surface. Moreover, after the multilayer film of PAA and GS was prepared, the adsorption of bacteria increased on TNT-Dopa. When PAA was prepared on the surface, the amount of negative charges increased, which reduced the adhesion of Escherichia coli to the surface. Moreover, after the multilayer film of PAA and GS was prepared, the increased negative charges on the surface and the continuous GS release could further prevent the negatively charged Escherichia coli to stay on the surface. Due to the antibacterial and bactericidal activity of releasing zinc ions [49], there was almost no bacterial adhesion on TNT-PAA/GS-Zn. Fig. 6b shows the antibacterial properties of the different samples, from which it can be seen that pure titanium, TiO2 nanotubes and dopamine coatings had poor bactericidal properties. After further immobilization of PAA, the number of Escherichia coli colonies decreased significantly, indicating that the PAA coating had certain antibacterial properties. When the PAA and GS composite coating was prepared, the release of GS could effectively kill bacteria, so the number of observed colonies decreased significantly. Finally, zinc ion can also inactivate the protein needed by bacteria and cause the condensation of DNA [50], so the antibacterial activity was further improved after Zn$^{2+}$ loading.
4 Conclusion

In this paper, PAA/GS-Zn multi-functional bioactive coatings were successfully prepared on the titanium surface with nanostructure. The specific nanotube structure and the following functionalization significantly influenced the surface hydrophilicity, protein adsorption, blood compatibility and endothelial cell growth behaviors of the materials. TiO$_2$ nanotubes had excellent hydrophilicity, but after the immobilization of PAA and loading of GS and zinc ions, the hydrophilicity became worse due to the decrease of the diameter of TiO$_2$ nanotubes. The super-hydrophilic TiO$_2$ can selectively promote albumin adsorption, while the immobilization of PAA and the loading of GS and zinc ions can prevent the non-specific protein adsorption. At the same time, combining the good blood compatibility and negative charged characteristics of PAA with the physiological activity of zinc ions, PAA/GS-Zn coating can not only significantly prevent platelet adhesion, aggregation and activation, but also reduce hemolysis rate and increase partial thromboplastin time, thus significantly improve the blood compatibility. In addition, the anodized nanotube array can promote endothelial cell adhesion, proliferation and up-regulated expression of VEGF and NO. Although PAA/GS coating can promote cell adhesion and proliferation and up-regulate NO expression, it cannot significantly promote VEGF expression. Loading Zn$^{2+}$ can not only significantly promote endothelial cell adhesion and proliferation, but also up-regulate NO and VEGF expression. Finally, due to the continuous and slow release of GS and zinc ions, the surface modified materials showed good antibacterial and germicidal efficacy to Escherichia coli.

Declarations

Ethics approval and consent to participate

Not Applicable.

Consent for publication

All authors agreed to submit this manuscript for publication.

Availability of data and material

All data generated or analyzed during this study are included in this article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Changjiang Pan and Ya Yang conceived and designed the experiments, and wrote the manuscript; Youdong Hu and Li Quan analyzed the data; Yanchun Wei and Sen Liu contributed reagents/materials/analysis tools, Zhongmei Yang and Yuebin Lin performed the experiments.

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References

1. Lavery KS, Rhodes C, McGraw A, Eppihimer MJ. Antithrombotic technologies for medical devices. Adv Drug Delivery Rev. 2017;112:2–11.

2. Gbyli R, Mercaldi A, Sundaram H, Amoako KA. Achieving totally local anticoagulation on blood contacting devices. Adv Mater Interfaces. 2018;5:1700954.

3. Nguyen PQ, Courchesne NMD, Duraj-Thatte A, Praveschotinunt P, Joshi NS. Engineered living materials: prospects and challenges for using biological systems to direct the assembly of smart materials. Adv Mater. 2018;30:1704847.

4. Bacakova L, Filova E, Parizek M, Ruml T, Svorcik V. Modulation of cell adhesion, proliferation and differentiation on materials designed for body implants. Biotechnol Adv. 2011;29:739–67.

5. Maitz MF, Martins MCL, Grabow N, Matschegewski C, Huang N, Chaikof EL, Barbosa MA, Werner C, Sperling C. The blood compatibility challenge. Part 4: Surface modification for hemocompatible materials: passive and active approaches to guide blood-material interactions. Acta Biomater. 2019;94:33–43.

6. Xiao Y, Wang W, Tian X, Tan X, Yang T, Gao P, Xiong K, Tu Q, Wang M, Maitz MF, Huang N, Pan G, Yang Z, A versatile surface bioengineering strategy based on mussel-inspired and bioclickable
peptide mimic, Research, 2020 (2020) 7236946.

7. Rodda AE, Meagher L, Nisbet DR, Forsythe JS. Specific control of cell–material interactions: targeting cell receptors using ligand-functionalized polymer substrates. Prog Polym Sci. 2014;39:1312–47.

8. Pan G, Guo B, Ma Y, Cui W, He F, Li B, Yang H, Shea KJ. Dynamic introduction of cell adhesive factor via reversible multicovalent phenylboronic acid/cis-diol polymeric complexes. J Am Chem Soc. 2014;136:6203–6.

9. Cao L, Qu Y, Hu C, Wei T, Zhan W, Yu Q, Chen H. A universal and versatile approach for surface biofunctionalization: layer-by-layer assembly meets host–guest chemistry. Adv Mater Interfaces. 2016;3:1600600.

10. Li X, Gao P, Tan J, Xiong K, Maitz MF, Pan C, Wu H, Chen Y, Yang Z, Huang N. Assembly of metal-phenolic/catecholamine networks for synergistically anti-inflammatory, antimicrobial, and anticoagulation coatings. ACS Appl Mater Interfaces. 2018;10:40844–53.

11. Wu XD, Liu CJ, Chen HP, Zhang YF, Li L, Tang N. Layer-by-Layer deposition of hyaluronan and quercetin-loaded chitosan nanoparticles onto titanium for improving blood compatibility. Coatings. 2020;10:256.

12. Kushwaha M, Anderson JM, Bosworth CA, Andukuri A, Minor WP, Lancaster JR, Anderson PG, Brott BC. Jun H.W. A nitric oxide releasing, self assembled peptide amphiphile matrix that mimics native endothelium for coating implantable cardiovascular devices. Biomaterials. 2010;31:1502–8.

13. Junkar I, Kulkarni M, Benčina M, Kovač J, Mrak-Poljšak K, Lakota K, Sodin-Šemrl S, Mozetič M. A. Iglič,Titanium dioxide nanotube arrays for cardiovascular stent applications. ACS Omega. 2020;5:7280–9.

14. Minagar S, Berndt CC, Wang J, Ivanova E, Wen C. A review of the application of anodization for the fabrication of nanotubes on metal implant surfaces. Acta Biomater. 2012;8:2875–88.

15. Khudhair D, Bhatti A, Li Y, Hamedani HA, Garmestani H, Hodgson P, Nahavandi S. Anodization parameters influencing the morphology and electrical properties of TiO2 nanotubes for living cell interfacing and investigations. Mater Sci Eng C. 2016;59:1125–42.

16. Zhong S, Luo RF, Wang X, Tang LL, Wu J, Wang J, Huang RB, Sun H, Huang N. Effects of polydopamine functionalized titanium dioxide nanotubes on endothelial cell and smooth muscle cell. Colloids Surf B Biointerfaces. 2014;116:553–60.

17. Roguska A, Pisarek M, Belcarz A, Marcon L, Holdynski M, Andrzejczuk M, Janik-Czachor M. Improvement of the bio-functional properties of TiO2 nanotubes. Appl Surf Sci. 2016;388:775–85.

18. Gong Z, Hu Y, Gao F, Quan L, Liu T, Gong T, Pan C. Effects of diameters and crystals of titanium dioxide nanotube arrays on blood compatibility and endothelial cell behaviors. Colloids Surf B Biointerfaces. 2019;184:110521.

19. Krężel A, Maret W. The biological inorganic chemistry of zinc ions. Arch Biochem Biophys. 2016;611:3–19.
20. Zalewski PD, Beltrame JF, Wawer AA, Abdo Al. C. Murgia, Roles for endothelial zinc homeostasis in vascular physiology and coronary artery disease. Crit Rev Food Sci. 2019;59:3511–25.

21. Choi S, Liu X, Pan Z. Zinc deficiency and cellular oxidative stress: prognostic implications in cardiovascular diseases. Acta Pharmacol Sin. 2018;39:1120–32.

22. Cai X, Dai GJ, Tan SZ, Ouyang Y, Ouyang YS, Shi QS. Synergistic antibacterial zinc ions and cerium ions loaded a-zirconium phosphate. Mater Lett. 2012;67:199–201.

23. Alzahrani T, Liappis AP, Baddour LM, Karasik PE. Statin use and the risk of cardiovascular implantable electronic device infection: a cohort study in a veteran population. Pacing Clin Electrophysiol. 2018;41:284–9.

24. Khatoon Z, McTiernan CD, Suuronen EJ, Mah TF, Aralcon EI. Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon. 2018;4:e01067.

25. Yan R, He W, Zhai T, Ma H. Anticorrosion organic–inorganic hybrid films constructed on iron substrates using self-assembled polyacrylic acid as a functional bottom layer. Electrochim Acta. 2019;295:942–55.

26. Chen C, Chen Y, Wu P, Chen B. Update on new medicinal applications of gentamicin: Evidence-based review. J Formos Med Assoc. 2014;113:72–82.

27. Ballarre J, Aydemir T, Liverani L, Roether JA, Goldmann WH, Boccaccini AR. Versatile bioactive and antibacterial coating system based on silica, gentamicin, and chitosan: Improving early stage performance of titanium implants. Surf Coat Technol. 2020;381:125138.

28. Engberg AE, Nilsson PH, Huang S, Fromell K, Hamad OA, Mollnes TE, Rosengren-Holmberg JP, Sandholm K, Teramura Y, Nicholls IA, Nilsson B, Ekdahl KN. Prediction of inflammatory responses induced by biomaterials in contact with human blood using protein fingerprint from plasma. Biomaterials. 2015;36:55–65.

29. Martino S, Angelo FD, Armentano I, Kenny JM, Orlacchio A. Stem cell-biomaterial interactions for regenerative medicine. Biotechnol Adv. 2012;30:338–51.

30. Mumtaz F, Chen C, Zhu H, Pan C, Wang Y. Controlled protein adsorption on PMOXA/PAA based coatings by thermally induced immobilization. Appl Surf Sci. 2018;439:148–59.

31. Brash JL, Horbett TA, Latour RA, Tengvall P. The blood compatibility challenge. Part 2: Protein adsorption phenomena governing blood reactivity[J]. Acta Biomater. 2019;94:11–24.

32. Feller T, Kellermayer MSZ, Kiss B. Nano-thrombelastography of fibrin during blood plasma clotting. J Struct Biol. 2014;186:462–71.

33. Jia LL, Han FX, Wang H. Polydopamine-assisted surface modification for orthopaedic implants. J Orthop Transl. 2019;17:82–95.

34. Lv Q, Cao C, Zhu H. Blood compatibility of polyurethane immobilized with acrylic acid and plasma grafting sulfonic acid. J Mater Sci Mater Med. 2004;15:607–11.

35. Ratner BD. Blood compatibility-a perspective. J Biomat Sci Poly E. 2000;11:1107–19.
36. Gorbet M, Sperling C, Maitz MF, Siedlecki CA, Werner C, Sefton MV. The blood compatibility challenge. Part 3: Material associated activation of blood cascades and cells. Acta Biomater. 2019;94:25–32.

37. Danielewski O, Schultess J, Smolenski A. The NO/cGMP pathway inhibits Rap 1 activation in human platelets via cGMP-dependent protein kinase I, Thromb. Haemostasis. 2005;93:319–25.

38. Ghavamzadeh R, Haddadi-Asl V. H. Mirzadeh, Bioadhesion and biocompatibility evaluations of gelatin and polyacrylic acid as a crosslinked hydrogel in vitro. J Biomat Sci Poly E. 2012;15:1019–31.

39. Cortese MM, Suschek CV, Wetzel W, Kröncke KD, Kolb-Bachofen V. Zinc protects endothelial cells from hydrogen peroxide via Nrf2-dependent stimulation of glutathione biosynthesis. Free Radical Bio Med. 2008;22:2002–12.

40. Frishman WH, Burns B, Atac B, Alturk N, Altajar B, Lerrick K. Novel antiplatelet therapies for treatment of patients with ischaemic heart disease: inhibitors of the platelet glycoprotein IIb/IIa integrin receptor, Am. Heart J. 1995;130:877–92.

41. Woodruff RS, Xu Y, Layzer J, Wu W, Ogletree ML, Sullenger BA. Inhibiting the intrinsic pathway of coagulation with a factor XII–targeting RNA aptamer. J Thromb Haemost. 2013;11:1364–73.

42. Nie S, Qin H, Li L, Zhang C, Yan W, Liu Y, Luo J, Chen P. Influence of brush length of PVP chains immobilized on silicon wafers on their blood compatibility. Poly Advan Technol. 2018;29:835–42.

43. Lai Y, Pan F, Xu C, Fuchs H, Chi L. In situ surface-modification-induced superhydrophobic patterns with reversible wettability and adhesion[J]. Adv Mater. 2013;25:1682–6.

44. Yang Z, Tu Q, Zhu Y, Luo R, Li X, Xie Y, Maitz MF, Wang J, Huang N. Mussel-inspired coating of polydopamine directs endothelial and smooth muscle cell fate for re-endothelialization of vascular devices. Adv Healthc Mater. 2012;1:548–59.

45. Kambe T, Tsuji T, Hashimoto A, Itsumura N. The physiological, biochemical, and molecular roles of zinc transporters in zinc homeostasis and metabolism. Physiol Rev. 2015;95:749–84.

46. Pan C, Hu Y, Gong Z, Yang Y, Liu S, Quan L, Yang Z, Wei Y, Ye W. Improved blood compatibility and endothelialization of titanium oxide nanotube arrays on titanium surface by zinc doping. ACS Biomater Sci Eng. 2020;6:2072–83.

47. Kroll J, Waltenberger J, Induces VEGF-A. Expression of eNOS and iNOS in Endothelial Cells via VEGF Receptor-2 (KDR), Biochem. Bioph Res Co. 1998;252:743–6.

48. Yu Q, Wu Z, Chen H. Dual-function antibacterial surfaces for biomedical applications. Acta Biomater. 2015;16:1–13.

49. Fang J, Zhao J, Sun Y, Ma H, Yu X, Ma Y, Ni Y, Zheng L, Zhou Y. Biocompatibility and antibacterial properties of zinc-ion implantation on titanium. J Hard Tissue Biol. 2014;23:35–44.

50. Jin G, Cao H, Qiao Y, Meng F, Zhu H, Liu X. Osteogenic activity and antibacterial effect of zinc ion implanted titanium, Colloids Surf. B Biointerfaces. 2014;117:158–65.
Figure 1

The representative SEM images of surface modified titanium oxide nanotubes on the titanium surfaces.
Figure 2

The ATR-FTIR spectra (a) and XPS spectra (b) of the different samples.
Figure 3

(a) GS and Zn2+ release profiles of TNT-PAA/GS-Zn; (b) The water contact angles of the different samples. (c) and (d) shows BSA and fibrinogen adsorption of the different samples, respectively.
Figure 4

SEM images (a) and the number (b) of the platelets adhered on the different samples; (c), (d) and (e) show the cGMP concentration of the attached platelets, hemolysis rate and APTT of the different samples, respectively.
Figure 5

The fluorescent pictures of endothelial cells adhered to the surfaces of different samples. CCK-8 values (a), VEGF (b) and NO (c) activities of endothelial cells grown on the different sample surfaces for 1 and 3 days, respectively.
Figure 6

The bacterial adhesion (a) and antibacterial properties (b) of the different samples.