Rapid Communication

Immobilization of silver nanoparticles on electropolymerized polydopamine films for metal implant applications

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ABSTRACT

Polydopamine can be formed and deposited on the surface of titanium substrates either by classical dip coating (d-pdopa) or by electropolymerization (e-pdopa), which has been recently developed. In contrast to the classical approach, dopamine electropolymerization leads to the formation of continuous, smooth films on the surface of the substrate. We further studied the applicability of the polydopamine films by examining their ability to spontaneously reduce silver ions into metal nanoparticles. A simple bacterial inhibition test was conducted with *Escherichia coli* and *Staphylococcus aureus* bacteria to examine the differences in the antibacterial efficiency of both e-pdopa and d-pdopa films coated with silver nanoparticles. The e-pdopa samples contained clearer inhibition zones than the d-pdopa samples, which is indicative of the silver nanoparticles being immobilized on e-pdopa film in a more efficient manner for the antimicrobial functionalization of metal implants.

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Bacterial infections have always been an issue for metal implants since these introduce a foreign material inside the human body. There is always a risk for an implant to result in bacterial colonization and infection, which would therefore make such an implant detrimental to the health of the patient [1]. Bacterial colonization on local tissues is a serious problem, and therefore, the surface modification of metal implants has been at the forefront of medical research [2]. Several coating techniques have been developed for metal implants, such as in Ti alloys, Chromium–Cobalt and Magnesium, to reduce the risk of bacterial infection and biofilm formation. For example, polymer coatings, surface charge modifications, and antibacterial agents have been applied to implants to reduce these risks [3].

Silver nanoparticles (AgNPs) have been proven in several studies to have intrinsic properties that inhibit bacterial growth and proliferation,

but the exact mechanism of how Ag nanoparticles kill bacteria is still up for debate [4]. Several mechanisms have been proposed for AgNPs to disrupt bacterial growth and to exhibit bactericidal effects. These include: protein inactivation, DNA association, and cell invasion [5]. Although smaller AgNPs are generally accepted to exhibit a stronger antimicrobial activity than larger ones, the agglomeration of the AgNPs also plays a significant role and could result in a quick loss of the antibacterial activity [6,7]. Many researchers have proposed that the aggregation state of the immobilized nanoparticles is highly dependent on the surface roughness of the substrates [8], which means that the same AgNPs might display different anti-bacterial efficiencies as a result of the differences in the morphology of the substrate.

Dopamine, a mussel-inspired biomolecule, can self-polymerize into polydopamine (pdopa) in an alkaline pH environment and in the presence of oxygen, which offers a simple method to coat various organic and inorganic substrates [9–11]. Another interesting feature of the surface modification with polydopamine is that these have unique reductive properties [12]. Polydopamine can be used as a reducing agent to reduce metal ions into nanoparticles without the need for any toxic components [13,14]. Despite the many advantages of surface modification by the self-polymerization of dopamine, there are also several disadvantages of using this technique, one of which is the limitation of materials that can be used, since the process needs to be undertaken in an alkaline environment, so alkaline-sensitive materials cannot be utilized. Polymerization can also be a time consuming process since

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the deposition rate of dopamine molecules is quite slow. In addition, it is very likely for the polydopamine particles to be aggregated on the substrate under alkaline condition [15,16]. To address these issues, electropolymerization is conducted in order to obtain a precise, thin, uniform surface roughness on the surface of any substrate, even those with a complex architecture [15,17,18].

In this article, we compare two techniques and evaluate their products in terms of the surface roughness and thickness, in situ Ag nanoparticle formation, and antibacterial properties. We hypothesize that the silver nanoparticles that are deposited on the electropolymerized dopamine film will exhibit a more uniform morphology and better antibacterial properties. The proposed method can therefore be easily applied on different metallic substrates and could be further developed for metal implant applications.

Multiple scanning cyclic voltammetry was used for electrochemical polymerization and film deposition. Fig. 1 shows the typical CV of dopamine polymerized at a concentration of 1 mg/mL Tris buffer saline solution (TBS, pH 7.4) on a Ti template at a scan rate of 100 mV/s. After each cycle, the area under the curve in the voltammogram decreases as a result of the deposition of polydopamine onto the working electrode. After 15 cycles, the amount of polymer that had been deposited led to an almost complete insulation of the working electrode [19].

The polydopamine coat that formed on the surface of the Ti alloy was confirmed by performing a comparison of the attenuated total reflection-Fourier transform infrared spectra (ATR-FTIR) of the pure Ti alloy and Ti alloy coated with polydopamine over 20 cycles (see Fig. 2a). Before surface modification with polydopamine, only a few bands could be seen in the spectrum. However, after the surface had been covered with polydopamine, several new absorbance signals appeared. A broad absorbance at around 3450 can be ascribed to the O–H stretching vibrations of polydopamine, and peaks at 1610 cm^{-1} and 1292 cm^{-1} correspond to the stretching vibration of the C $=$ C and the phenolic C$-$OH stretching vibration of polydopamine, respectively [20]. These ATR-FTIR results confirm the successful electropolymerization of polydopamine onto the surfaces of the Ti alloy. The changes in the surface topography of the Ti substrates after electropolymerization and dip coating were studied via AFM. The 3-D images clearly show that the dip-coated alloy has a surface morphology that is significantly rougher with a broader thickness distribution. As previously mentioned, the self-polymerization of dopamine under an alkaline condition could result in the formation of numerous unavoidable nanoparticulate aggregates of pdopa on the surface of materials, which causes a significant increase in the surface roughness [21]. In contrast, the e-pdopa samples have a much smoother surface with a much narrower, monodispersed thickness distribution. These results strongly suggest that the pdopa formed via electropolymerization leads to a
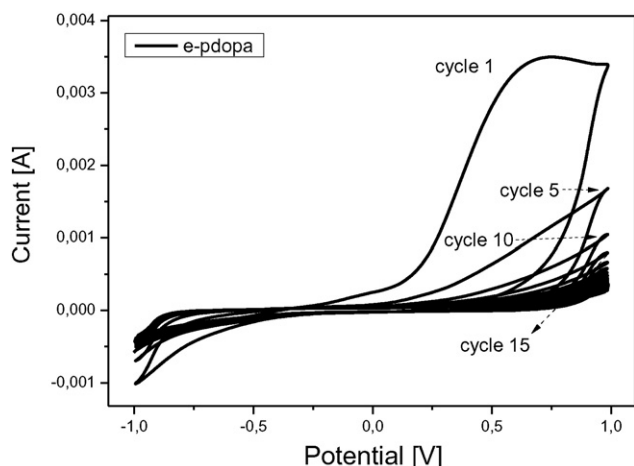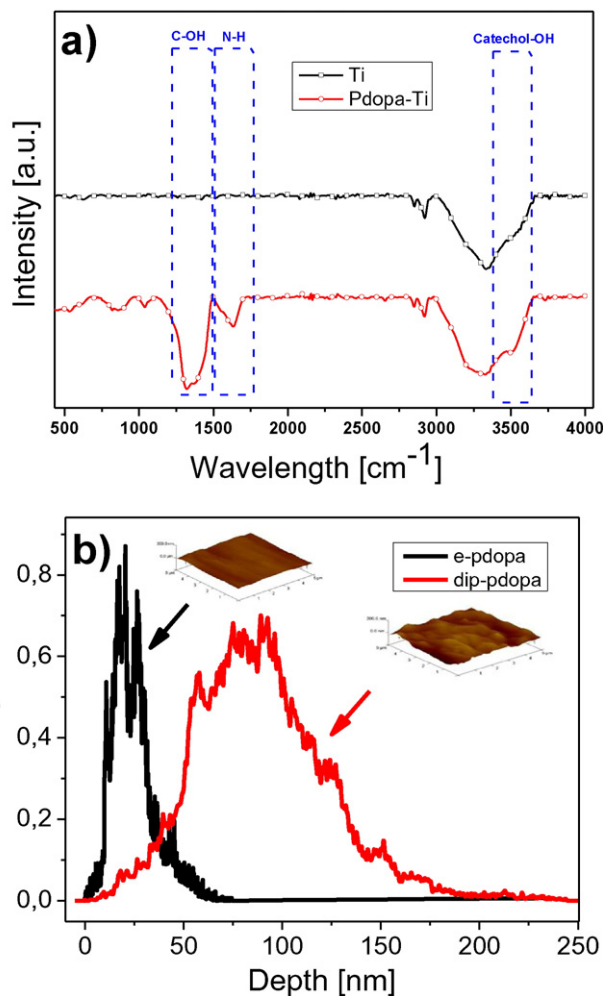

Fig. 2. (a) ATR-FTIR spectrum of Ti alloy and polydopamine deposited on Ti alloy after 20 cycles. (b) AFM results of polydopamine film produced through 20 cycles of electropolymerization and 24 h of dip coating.

highly homogeneous surface deposition, particularly when compared to that obtained through the dip coating method.

The surface morphology of the dip-coated and electropolymerized pdopa coatings on the Ti alloy substrates were further observed using FESEM. As seen in Fig. 3 (a–b), the results of the coating process through the two methods are highly different from each other. The d-pdopa-Ti alloy exhibits pdopa particles that are agglomerated, which is a consequent reaction of using an alkaline environment and of the uncontrollable self-crosslinking that occurs when using this method. These unavoidable particles not only give rise to a significant increase in the surface roughness, but also, as shown in Fig. 3c, can cause nonuniform dispersion and agglomeration of silver nanoparticles after reduction by the catechol groups. In contrast, the electropolymerized pdopa-Ti alloys exhibited an overall coating that was relatively smoother, indicating that the polymerization of the dopamine had been highly regulated by the electrochemical processes. This kind of surface morphology is favorable to obtain highly monodispersed AgNPs, as seen in Fig. 3d. The AgNPs showed a uniform distribution on the surface of the e-pdopa coating, and the majority of the particles were evenly spaced.

The surface chemical composition of d-pdopa-AgNPs and e-pdopa-AgNPs were characterized by XPS spectroscopy (Fig. 4a). The XPS results for both samples show the presence of Ag3d and Ag3p, indicating the successful reduction of silver metal ion to the silver nanoparticle [22]. As shown in the spectrum, two bands at ca. 368 and 374 eV were observed, which were ascribed to Ag3d5/2 and Ag3d3/2 binding energies, respectively [23]. The splitting of the 3d doublet of Ag is 6.0 eV

Fig. 1. Cyclovoltammetry measurements during the electropolymerization of polydopamine.