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Anticoagulation and endothelial cell behaviors of heparin-loaded graphene oxide coating on titanium surface

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ABSTRACT

Owing to its unique physical and chemical properties, graphene oxide (GO) has attracted tremendous interest in many fields including biomaterials and biomedicine. The purpose of the present study is to investigate the endothelial cell behaviors and anticoagulation of heparin-loaded GO coating on the titanium surface. To this end, the titanium surface was firstly covered by the polydopamine coating followed by the deposition of the GO coating. Heparin was finally loaded on the GO coating to improve the blood compatibility. The results of attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), Raman spectroscopy and X-ray photoelectron spectroscopy (XPS) indicated that the heparin-loaded GO coating was successfully created on the titanium surface. The scanning electron microscopy (SEM) images indicated that a relative uniform GO coating consisting of multilayer GO sheets was formed on the substrate. The hydrophilicity of the titanium surface was enhanced after the deposition of GO and further improved significantly by the loading heparin. The GO coating can enhance the endothelial cell adhesion and proliferation as compared with polydopamine coating and the blank titanium. Loading heparin on the GO coating can significantly reduce the platelet adhesion and prolong the activated partial thromboplastin time (APTT) while not influence the endothelial cell adhesion and proliferation. Therefore, the heparin-loaded GO coating can simultaneously enhance the cytocompatibility to endothelial cells and blood compatibility of biomaterials. Because the polydopamine coating can be easily prepared on most of biomaterials including polymer, ceramics and metal, thus the approach of the present study may open up a new window of promising an effective and efficient way to promote endothelialization and improve the blood compatibility of blood-contact biomedical devices such as intravascular stents.

1. Introduction

In the past several decades, the discovery of novel materials-based therapies and medical devices for disease treatment and diagnosis significantly improves the human health. Due to their excellent mechanical properties and corrosion resistance in the physiological environment, titanium and its alloys are widely used for the cardiovascular implants such as artificial heart valve, coronary stent, blood pump and left ventricular assist device [1–4]. However, the limited bio- and blood compatibility must be first resolved prior to the safe and effective use for implants. For example, the metal leaching could result in the lack of cell adhesion, proliferation, and thrombosis when they come into contact with human tissues and/or blood [5–7]. Meanwhile, the interactions of proteins or living cells with the implant surface can arouse cascade of biochemical reactions which can adversely affect the implant performances [8]. Therefore, it is necessary to control the interfacial behaviors between the implant and its surrounding biological environment to improve the performances in vitro and in vivo. Surface modification can effectively change the physical and chemical properties of biomaterials surface to improve the properties and functionalities of the original materials, and thus reduce the adverse interfacial interactions between implant and host. Up to date, numerous surface modification methods have been developed to ultimately increase the biocompatibility and hemocompatibility of titanium based alloys, including the deposition of inorganic films (titanium oxide, titanium nitride, diamond-like carbon, etc) or polymer coatings, the immobilization of the biomolecules by wet chemical method and the fabrication of multifunctional layer by layer-by-layer (LBL) technique [9–12]. While substantial progress has been achieved in the biocompatibility and blood compatibility, it is still a standard clinic practice to mandatorily administrate anticoagulation drugs after the installation of medical devices such as stent in a blood stream. It is therefore necessary to develop new material coatings or coating systems so that anticoagulation drugs and their associated side-effects can be avoided or reduced.

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Graphene oxide (GO), which is single-layered carbon atoms packed into a two-dimensional (2D) honeycomb lattice with some active chemical groups such as carboxyl and hydroxyl on its surface or edge, has been extensively explored for the applications in a large variety of fields including biosensor, drug delivery system, gene transfection, nanocomposite materials and nanoelectronic devices [13–15]. Its ultrahigh surface area provides an excellent platform for cargo loading and attachment of various biomolecules, which reveals remarkable performances in drug delivery, cellular imaging, bone tissue, stem cell differentiation, and biosensors [16–20]. Although graphene appears to be toxic as discovered by some reports [17,18], especially when high-dose graphene is applied [19], its derivatives with well-controlled biocompatible surface show no significant side effects in vitro to cells and in vivo to animals in the tested dose ranges [20,21]. Another work reported by Liao. et al shows that the hemolysis and cytotoxicity of GO is related with the extent of exfoliation, particle size, particulate state, oxygen content and surface charges. The toxicity of GO also depends on the exposure environment and mode of interaction with cells [22]. Although GO exhibits cytotoxicity in some cases, the chemical groups can be further bound with the bioactive molecules to improve the biocompatibility. The GO or functionalized GO can be mixed with other materials to fabricate a platform suitable for cell attachment and growth [23,24]. On the other side, the negative charged property of GO make it easily adsorb on the positive surface, which provides a possible approach to fabricate multilayer nanocomposites on the substrates via LBL technique for biosensor, biomaterials or biomedical devices [25–27]. Recently, some reports suggested that graphene oxide was suitable for growth of neuronal cells and human osteoblasts [28,29]. However, up to now, barely any reports have focused on using GO to construct a bio-interface for blood contacting materials. The effects of graphene oxide coating on endothelial cell behaviors were barely investigated.

Heparin is one of glycosaminoglycans that has been proved to have excellent anticoagulation, preventing the formation of clot and extension of existing clots within the blood [30]. It has been widely applied to improve the blood compatibility of biomaterials. Due to the ultrahigh negative charges and the existing carboxyl groups, electrostatic adsorption on the positive charged surface and partial esterification of heparin with the substrates having hydroxyl are the two common ways to immobilize heparin on the biomaterials. However, it is not possible to immobilize heparin on the GO surface via electrostatic adsorption due to the negative charged property of GO. The esterification between GO and heparin need to optimize the reaction conditions to maintain activity of pentasaccharide sequence within the heparin chains. In this report, in order to improve the bio- and hemo-compatibility of the titanium surface, the polydopamine coating was firstly prepared on the titanium surface and then the GO coating was fabricated, heparin was finally loaded onto the GO coating. The results of ATR-FTIR, Raman spectroscopy, XPS, water contact angle and scanning electron microscopy (SEM) demonstrated that the heparin-loaded GO coating was fabricated on the titanium surface successfully. The cell behaviors and platelet adhesion experiments indicated that as compared to the blank titanium surface the GO coating can enhance the endothelial cell adhesion and proliferation and effectively inhibit platelet attachment. Loading heparin on the GO coating can significantly enhance the blood compatibility while not influence the cell adhesion and proliferation obviously.

2. Materials and methods

2.1. Preparation of polydopamine coating

The pure titanium (TA2) rod with a diameter of 10 mm was cut into the titanium plates. The plate thickness was about 1 mm. The plates were polished and then cleaned ultrasonically by acetone, ethanol and distilled water in sequence. After dried by the compressed air flow, the plates were immersed into 2 g/L dopamine solution (10 mM Tris buffer, pH 8.5) for 12 h. The deposition process was carried out three times to obtain polydopamine (DOPA) coating on the surface.

2.2. Fabrication of heparin-loaded GO coating

The GO sheets, supplied by Nanjing XFNANO Materials Tech Co.,Ltd, were dispersed into ethanol to obtain the 0.5 mg/ml GO solution. The purity of GO is >99% and the GO thickness is about 0.8–1.2 nm. The titanium plate with polydopamine coating was immersed into the GO solution for 4 h to adsorb GO. After rinsed with ethanol, the sample was dried by the blowing nitrogen gas. Another 1 mL GO solution was then dropped on the substrate to get the sufficient thickness. The GO coating was finally formed through the evaporation of the solvent at room temperature, and then baked at 120 °C under nitrogen stream to make it stable. The GO-coated titanium plate was finally immersed into 1 mg/mL heparin solution for 4 h. The sample was dried by nitrogen gas. The adsorption process was performed three times to obtain the heparin-loaded GO coating.

2.3. Characterization of the modified titanium surface

Attenuated total reflectance Fourier transformation infrared spectra (ATR-FTIR, TENSOR 27, Bruker, Germany) was used to examine the chemical changes of the titanium surface. The measurements were carried out at room temperature and the scanning range was 650 cm^{-1} to 4000 cm^{-1}.

Raman analysis of the GO and heparin-loaded GO coatings was conducted by Renishaw Invia Plus laser Raman spectrometer (Renishaw, UK, RM 2000) at room temperature.

The XPS measurements were carried out by a Quantum 2000 XPS Apparatus (PHI Co., Chanhassen, MN) with a focused monochromatic Al Kα X-ray source (1486.6 eV) for excitation. The power of the X-ray source was kept at 25.7 W. The surface element concentration was obtained from the XPS survey spectra. Multipak software was provided by the manufacture for data analysis.

The surface hydrophilicity was characterized by water contact angel measurement (DSA25, Krüss GmbH, Germany). The test was done by the sessile drop method at room temperature using the distilled water. Five parallel samples were measured and the values were averaged.

The surface morphologies of GO and heparin-loaded GO coatings were observed by scanning electron microscopy (SEM, FEI Quanta 250) after spray-coated gold.

2.4. Anticoagulation

2.4.1. Platelet adhesion

The fresh whole blood collected from a healthy volunteer was centrifuged at 1500 rpm for 15 min to obtain the platelet-rich plasma (PRP). 200 μL PRP was dropped on each sample surface to cover the whole surface. After incubated 2 h at 37 °C, the samples were rinsed with 0.9 wt% NaCl solution. The adhered platelets were fixed by 2.5% glutaraldehyde (*v*/*v*, in PBS) for 24 h, rinsed again with 0.9 wt% NaCl solution and then dehydrated by 50%, 50%, 75%, 90%, 100% ethanol solutions for 10 min each in sequence. The samples were coated gold in vacuum and then observed by a scanning electron microscopy (SEM, FEI Quanta 250).

The relative number of the adhered platelets on different samples was measured by LDH (lactate dehydrogenase) assay. Briefly, the samples were firstly immersed into PBS solution for equilibrating 12 h. After drying, 100 μL PRP was covered onto each sample and incubated for 1 h at 37 °C. The sample was rinsed by 0.9 wt% NaCl solution followed by adding 40 μL of Triton-X-100 (diluted to 1%) on the surface. Taking 25 μL of the lysates to mix with a substrate solution of 200 μL NADH and sodium pyruvate in a 96-well plate, the absorbency at