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Graphene oxide/hydroxyapatite composite coatings fabricated by electrochemical deposition

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ABSTRACT

As novel nanomaterials, graphene and its derivations have been applied into hydroxyapatite as reinforcements for biomedical applications. However, graphene/hydroxyapatite composites serving as implant coating have rarely been studied. In this study, graphene oxide (GO)/hydroxyapatite (HA) composite coatings have been firstly fabricated by electrochemical deposition technique on titanium (Ti) substrate. Then, the microstructure, phase constituents, bonding strength and in vitro cellular responses of composite coatings were researched. Raman spectroscopy and transmission electron microscopy corroborated that graphene oxide was successfully incorporated into the composite coatings. Results revealed that addition of GO have enhanced both the crystallinity of deposited apatite particles and the bonding strength of the as-synthesized composite coatings. Moreover, in vitro cell culture assessment showed better biocompatibility of composite coatings compared with the pure HA coating and pure Ti substrate. These results suggested that GO/HA composite coatings might be a promising candidate in the field of biomaterials, especially for implant coatings.

1. Introduction

Hydroxyapatite (HA), which owns similar chemical composition to the mineral component in nature bone and teeth, has been of great interest in bone and tissue engineering [1]. HA also possesses superior osteoconductivity and bioactivity [2], which makes it a powerful candidate for the regeneration of bone tissue in the field of orthopedic surgery. However, the inherent brittleness (low tensile strength and fracture resistance) of HA restricts its practical application [3]. To overcome this drawback, second reinforcement phases were incorporated, such as TiO_2 [4], Al_2O_3 [5] and yttria stabilized ZrO_2 [6], to improve its mechanical performance.

Recently, hydroxyapatite/carbon nanomaterial composites and composite coatings have attracted much attention, for example, hydroxyapatite/carbon nanotubes (HA/CNTs) composites [7,8]. Previous research suggested that the incorporation of CNTs could enhance the mechanical properties of HA and improve the bioactivity of carbon nanotubes simultaneously [9]. Graphene, another carbon nanomaterial, has also been utilized as reinforcement filers of polymers [10], metals [11] and ceramics [12,13] to improve the tensile strength and Young's modulus of host materials, since it owns unique structural features and exceptional electronic, thermal and mechanical properties. Studies on its biomedical applications, such as biosensors [14], nanoprobe [15, 16] and drug delivery [17,18], have further denoted superior biocompatibility and biostability of graphene. Compared to CNTs, graphene and its derivatives can be produced in a relatively pure way with little cytotoxicity [19], and they also showed distinct mechanical characteristic and bioactivity in HA composite materials [20,21]. Graphene oxide is the oxygenated derivative of graphene, which contains abundant chemical functional groups on the edge (carboxyl and carbonyl groups) and planes (hydroxyl and epoxide groups) [22]. The specifically structured graphene oxide possesses desirable dispersion behavior and can be stably dispersed in aqueous media [23]. Recent investigations denoted that the incorporation of GO could significantly enhance the mechanical performance of graphene oxide based composites [24,25]. It was also reported that GO could promote the adhesion and proliferation of

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osteoblasts [26,27] and had potential antibacterial activity [28,29]. Besides, the function of inducing apatite nucleation might be another merit of GO in implant application [30]. Since GO owns these exceptional advantages, it has emerged as an attractive precursor for HA-based composite coatings of implant.

Many techniques were utilized to fabricate HA based composite coatings. The most common strategy employed was spark plasma spraying, which involved a high temperature process. However, the thermal spraying is usually associated with the dissolution of HA particles, weakened mechanical stability and accelerated degradation of the coatings in their early lifetime of implantation [31]. Additionally, because of the low thermal stability of graphene oxide, the procedure of plasma spraying might introduce much change into GO structure. Li et.al [32] suggested that GO sheets have suffered 49.5% weight loss at 600 °C, which might be caused by the pyrolysis of the labile oxygenic functional groups. In comparison, electrochemical deposition (ECD) is an economical coating technique and can be conducted at absolutely lower temperature. This method also has many other advantages such as easy deposition on complex geometries and convenient control of the coating properties [33,34].

To our knowledge, study on the electrochemical deposition of graphene oxide/hydroxyapatite (GO/HA) composite coatings is completely rare. Therefore, the present study was devoted to the synthesis of GO/HA composite coatings using electrochemical deposition. The microstructure, phase composition, adhesion strength and in vitro cellular responses of the composite coatings were investigated. It was expected that GO/HA composite coatings might be able to improve the mechanical properties, bioactivity and osseointegration of titanium implants.

2. Materials and methods

2.1. Deposition of GO/HA composite coatings

Commercially available titanium of 99.9% purity (Locke Titanium, Chengdu, China) and a dimension of 10 × 10 × 1 mm were used as substrates for electrochemical deposition (ECD). Their surfaces were polished with silicon carbide sandpapers (grit range of 400–1200), etched in 4% HF solution for 2 min, ultrasonically cleaned in acetone, ethyl alcohol and ultrapure water for 15 min, respectively, and finally dried in air. Edges of titanium substrates were rounded to avoid an edge effect during ECD.

To achieve a well-dispersed and stable suspension, ultrasonic treatment at 570 W was applied to graphene oxide (Nanjing XFNANO Materials Tech Co., Ltd., China) for 2 h in ultrapure water according to the instruction, and golden, transparent GO suspension was obtained finally.

The electrolyte used for deposition contained 0.042 M $Ca(NO_3)_2$, 0.025 M $NH_4H_2PO_4$, 0.15 M $NaNO_3$ and 6% H_2O_2 (mass fraction) [35, 36]. The electrolyte was prepared with analytical reagent grade chemicals (Kelong Chemical Reagent Corporation, Chengdu, China) and ultrapure water was used as solvent. The pH of electrolyte was adjusted to 6.0 at 25 °C. For the fabrication of GO/HA composite coatings, GO suspension was added and ultimate concentration of GO in the electrolyte was 25 μg/mL, 50 μg/mL, 100 μg/mL, 200 μg/mL, respectively.

According to the concentration of GO in electrolyte, experimental groups were referred to as 25 μg/mL GO/HA, 50 μg/mL GO/HA, 100 μg/mL GO/HA, 200 μg/mL GO/HA, respectively. For comparison, pure HA coating was designated as control.

Based on our preliminary experiments, the ECD process was carried out at 15 °C in a three-electrode cell fitted with a Ti sheet as the cathode, a graphite rod acting as the counter-electrode and a saturated calomel electrode as the reference electrode. A magnetic agitation (Electrical Appliances Co., Changzhou, China) was utilized to stir the electrolyte. The current density was kept at 6 mA/cm^2 for 30 s at the beginning of ECD, and then at 0.6 mA/cm^2 for 30 min using an electrochemical workstation.

After the coating process, pretreated specimens were rinsed in ultrapure water, soaked in 0.1 M NaOH solution at 60 °C for 48 h, dried in air at room temperature for 24 h, and finally calcined in vacuum at 450 °C (heating rate = 30 °C/min, dwell time = 2.5 h, cooling rate = 30 °C/min).

2.2. Coating characterization

Phase composition of the synthesized coatings was performed by X-ray diffraction (XRD, DX-1000, Dandong Fangyuan Instrument CO., LTD.), Fourier transform infrared spectroscopy (FTIR, MX-1E, Nicolet Co.) and X-ray photoelectron spectroscope (XPS, PHI 5600, Physical Electronics). Raman spectra (LabRAM HR, HORIBA Jobin Yvon SAS, Longjumeau, France) were recorded in the backscattering configuration using an Argon ion laser with a wavelength of 532 nm. The surface and cross-sectional morphologies of the samples were characterized by scanning electron microscope (SEM, KYKY-2800 microscope, KYKY Technology Development LTD.). Transmission electron microscopy (TEM, FEI, Hillsboro, OR, USA) and atomic force microscope (AFM, SPM-9600, Japan) were employed to analyze the particle size of GO and HA.

2.3. Bonding strength test

Bonding strength between coating and Ti substrate was measured using Universal Instron Mechanical Testing System (Instron 5569, Instron Co.) according to ASTM F1044-05 standard. The pure HA coated and GO/HA coated Ti substrates were bonded to another uncoated Ti sheet respectively with epoxy resin, and then cured at 60 °C for 2 h in an oven. Shear load parallelized to the plane of coatings was applied to each test specimen at a constant rate of 1.0 cm/min cross-head speed. The test was continued until complete separation of the coatings has been achieved. Five samples in each group were tested and the average adhesion strength was calculated as the maximum load/contact area.

2.4. In vitro cellular assessment

2.4.1. Cell culture

Osteoblast-like MG63 cells were employed to evaluate the biocompatibility of the prepared coatings. Pure Ti, pure HA coating and GO/HA composite coatings were previously sterilized in 120 °C water steam for 1 h and then placed into a 24-well plate (Corning, NY). Before cells seeding, these specimens were immersed in culture medium for 24 h.

MG63 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, HyClone) containing 10% fetal bovine serum (FBS, HyClone) and 1% penicillin/streptomycin. Cultures were maintained at 37 °C in a humidified incubator in the presence of 5% CO_2 and subcultures were performed using a 0.01% trypsin solution in phosphate buffered saline (PBS) at pH 7.4. Cells were observed daily under an inverted microscope and the culture media were changed every other day. For the in vitro assessment of coatings, cells were seeded on experiment specimens at a density of 1×10^4 cells/well.

2.4.2. Cell adhesion

The cellular attachment and spread morphology of osteoblast-like MG63 cells on pure Ti, pure HA coating and GO/HA composite coatings were evaluated by SEM. Basically, after the 24 h incubation, cells were thoroughly washed with PBS twice to remove most apoptotic or necrotic cells and fixed with 2.5% glutaraldehyde at 4 °C in the refrigerator overnight, followed by dehydration in gradient ethanol solution (30%, 50%, 75%, 80%, 90%, and 100%) series for 15 min each, and then subjected