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Bioactive hydroxyapatite/graphene composite coating and its corrosion stability in simulated body fluid

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ABSTRACT

The hydroxyapatite/graphene (HAP/Gr) composite was electrodeposited on Ti using the electrophoretic deposition process to obtain uniform bioactive coating with improved mechanical strength and favorable corrosion stability in simulated body fluid (SBF). Incorporation of Gr was verified by Raman spectroscopy, X-ray diffraction, Fourier transform infrared spectroscopy, thermogravimetric analysis, and X-ray photoelectron analysis. The HAP/Gr composite coating exhibited reduced surface cracks, nearly double the hardness, and elastic modulus increased by almost 50% compared to pure HAP coating, as estimated by a nanoindentation test. The bioactive HAP/Gr composite coating provided a newly formed apatite layer in SBF with enhanced corrosion stability, as evidenced by electrochemical impedance spectroscopy. The thermal stability of the HAP/Gr coating was improved in comparison to the pure HAP coating, and the Ca/P ratio was closer to the stoichiometric value. No antibacterial activity against *Staphylococcus aureus* or *Escherichia coli* could be verified. The HAP/Gr composite coating was classified as non-cytotoxic when tested against healthy peripheral blood mononuclear cells (PBMC).

1. Introduction

Carbon nanomaterials with two-dimensional (2D) morphologies as a single layer of sp^2-hybridized carbon atoms packed in a honeycomb form, known as graphene (Gr), have been reported recently. The extraordinary electrical, thermal, and mechanical properties (e.g., tensile strength 130 GPa and Young's modulus 0.5–1 TPa) and very high specific surface area (up to 2630 $m^2\,g^{-1}$) have drawn great attention as a reinforcement in the composite field of material science [1–3]. Graphene materials possess physical properties identical to those of carbon nanotubes (CNTs) but have a larger surface area. It has been reported that inclusion of Gr into polymer or ceramic matrices leads to remarkable improvements in the properties of the host materials [1]. Furthermore, graphene nanosheets (GNSs), formed by several layers of Gr with a thickness of up to 100 nm [4], are much easier to produce than other graphene materials and successfully use as nanofillers for polymers [5], metals [6], and ceramics [3,7] to produce composites with exceptional mechanical properties.

Biomaterials used in orthopedic surgery usually encounter complex service environments and therefore require versatile performances from the materials [8]. As a major player in orthopedic surgery, synthetic hydroxyapatite ($Ca_{10}(PO_4)_2(OH)_2$, HAP), chemically similar to bone mineral, has been developed in various forms and shapes. Metallic implants, such as Ti and its alloys, have insufficient biocompatibility and lack bioactivity, which means they usually cease to function over the long term because of wear, disease, or injury [8,9] or release metallic ions with a high potential to corrode in their biological environments [9]. HAP provides bioactivity, biocompatibility and an ability to initiate osteogenesis, but on the other side it lacks good mechanical properties. Because of its poor mechanical properties, such as an intrinsic brittleness, low fracture toughness (0.8–1.2 MPa), low flexural strength (<140 MPa), and wear resistance [10], the main focus of HAP research has been to improve its mechanical performance by combining it with various reinforcements.

The focus of the latest published research has been the fabrication of Gr or its derivatives to create reinforced HAP biocomposites

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because of the exciting findings regarding the biological performance of Gr [8]. Nonetheless, the mechanical properties of hydroxyapatite limit its use in the regeneration of various parts of the bone systems, especially those under significant mechanical tension. The incorporation of Gr or its derivatives as reinforcing materials in HAP composites has been studied and reported using *in situ* synthesis [11,12], spark plasma sintering (SPS) [13], biomimetic mineralization [14,15], chemical vapor deposition [16], and electrospinning [17]. The general idea of using Gr as nanofiller is to minimize the brittleness of HAP and gain an improved composite. Any reinforcement material for HAP should not only significantly improve the mechanical properties, but also retain HAP's original biocompatibility. Latest published reports on graphene materials aimed to demonstrate that crack deflection is more effective for sheet-like reinforcement than for tubular-like reinforcement, suggesting that Gr exhibits a more pronounced toughening effect on brittle materials than do carbon nanotubes (CNTs) [18]. Also, reports on CNTs cytotoxicity in organic environments are disconcerting [19]. Unlike CNTs, Gr is synthesized in relatively pure ways and is therefore expected to show little cytotoxicity, since few metallic catalyst particles are associated with its production [20]. Also, recent reports have discussed the qualities of Gr and Gr-based composites, including low toxicity toward human osteoblasts [21], excellent antibacterial properties [22], and its potential to initialize apatite mineralization [23]. Therefore, our aim was to explore the potential of implementing Grs as HAP reinforcement for load-bearing orthopedic applications.

Electrophoretic deposition (EPD) is a special colloidal processing technique widely used to apply bioactive ceramic or composite coatings on various metal surfaces, such as Ti and its alloys, stainless steel, and Co—Cr alloys, for orthopedic applications with improved osteoconductivity, bioactivity, biocompatibility, and corrosion resistance. EPD emerged as the method of choice due to its many advantages (e.g., high deposition rate) and good control of deposition parameters that affect coating thickness, crystallinity, and desirable uniformity even on substrates of complex shape [24–30]. Briefly, factors influencing the EPD process are electrical conditions (voltage and time) and parameters related to suspension (particle charging, solid loading, dispersants, suspension viscosity, particle size distribution). According to the proposed mechanism [31], the deposition process occurs in several steps. Charged particles attract oppositely charged ions (counterions) around the particles. In the case of cataphoretic deposition, positively charged particles migrate toward the cathode. The rate of migration that the particles can achieve depends on the applied electric field, suspension viscosity, particle radius and particle charge. Because the particles are close enough to the cathode, attractive forces dominate, and coagulation/deposition occurs. The primary process is OH^- ion generation and hydrogen evolution on the cathode by H_2O discharge, followed by electrocoagulation of the ceramic particles at the cathode surface by neutralization of positively charged groups with electrochemically generated OH^- ions. Evolved hydrogen on the cathode goes out through the coating, leaving vacancies inside the deposited film and causing its porous structure.

In this study, the novel hydroxyapatite/graphene (HAP/Gr) composite was electrodeposited on Ti using the EPD process to obtain uniform bioactive coating with improved mechanical strength and favorable corrosion stability in simulated body fluid (SBF).

2. Experimental

2.1. Materials

For synthesis of nanosized HAP powder, we used a modified chemical precipitation method that required the reaction of calcium oxide (obtained by aerobic calcination of $CaCO_3$ for 5 h at 1000 °C) and phosphoric acid, according to our previously reported protocol [32,33]. The final suspension was spray-dried at 120 ± 5 °C into granulated powder. Ti from Aldrich (foil, thickness 0.25 mm, 99.7% trace metals basis) was used as a substrate for electrophoretic deposition of HAP/Gr coatings. Ti samples were cut in various sizes depending on the intended type of analysis (25 mm × 10 mm for surface analysis, 40 mm × 20 mm for impedance measurements, and 10 mm × 5 mm for cell based assays). Mechanical pretreatment of Ti plates was done according to a previously established protocol [32,34]. Grit emery paper was used for initial polishing and finalized with 0.3 μm wet alumina. Subsequently, plates were degreased in acetone and ethanol for 15 min each in an ultrasonic bath.

2.2. Electrophoretic deposition of HAP/Gr composites

The Gr nanopowder (AO-3), purity 99.2%, was purchased from Graphene Supermarket, USA. The average diameter of the Gr nanoflakes was 12 nm. Approximately 30–50 layers of Gr monolayers were overlapped. Electrophoretic deposition was done from 100 mL of absolute ethanol suspension containing 1 wt.% of nanosized HAP and 0.01 wt.% of Gr at pH = 2.00. The prepared HAP/Gr suspension was ultrasonicated for 30 min to maximize homogeneity. During deposition, the suspension was stirred constantly.

A three-electrode cell arrangement was used for cathodic electrodeposition. A pretreated Ti plate served as a working electrode and a substrate for HAP/Gr composites deposition. The counter electrodes were two pure platinum panels, placed parallel to the Ti 1.5 cm away, thus assuring uniform coating on both sides of the foil. The HAP/Gr composites were deposited using the constant voltage method at 60 V and a deposition time of 2 min at room temperature. Electrodeposited HAP/Gr coatings were dried at room temperature.

2.3. Characterization

The surface morphology of HAP/Gr composite coating, before and after immersion in SBF, was analyzed by field-emission scanning electron microscopy (FE-SEM) using a LEO SUPRA 55, Carl Zeiss, Germany, operated at an acceleration voltage of 200 kV and a TESCAN MIRA 3 XMU. Fourier transform infrared (FT-IR) spectroscopy was carried out using KBr pellets in a Perkin Elmer (spectrum one system) spectrophotometer. The scan was carried out in the range of 450–4000 cm^{-1} with a spectral resolution of 0.5 cm^{-1}. X-ray photoelectron spectroscopy (XPS) measurements were carried out using a K-Alpha System (Thermo electron) equipped with Al Kα X-ray radiation (1486.6 eV) and a micro-focused monochromator. The elemental profile was carried out using Ar-ion sputtering. HR-Raman analysis was carried out using a Renishaw inVia Raman spectrophotometer equipped with a 514 nm argon laser, operating at 10% of total power (50 mW). Thermogravimetric and differential thermal (TGA/DTA) analysis was conducted using a TA instrument (TGA Q5000 IR/SDT Q600) from 30 °C to 1000 °C under N_2 (50 mL/min) at a heating rate of 20 °C/min. A Philips PW 1051 Powder Diffractometer with Ni-filtered Cu Kα radiation ($\lambda = 1.5418$ Å) was used to assess the phase composition of the HAP/Gr composite coatings before and after immersion in SBF. X-ray diffraction intensity was measured using the scan-step technique ($2\theta = 8$–$80°$). The scanning step width was 0.05° with an exposure time of 50 s per step. The phase analysis was done with the commercially available computer program EVA V.9.0 implementing the PDF-2 database.

2.4. Nanoindentation test

All deposited HAP/Gr composite and pure HAP coatings on Ti plates were analyzed to measure the hardness and Young's modulus of the material. A nanoindentation test was conducted on a Hysitron TriboScope nanomechanical system with an *in situ* imaging mode equipped with a Berkovich indenter at an applied force of 10,000 μN. Loading and unloading rates were kept at 2000 μN/s without holding time. Eight to twelve indentations at different spots of the coating were made for each sample.

2.5. Biomimetic mineralization

The bioactivity of the HAP/Gr composite coatings was assessed by immersion in SBF solution, which was refreshed every 24 h. The composition of the SBF solution was similar to human blood plasma. Reagent-grade salts were dissolved in deionized water up to Na^+ 142.0 mM, K^+ 5.0 mM, Mg^{2+} 1.5 mM, Ca^{2+} 2.5 mM, HCO_3^- 4.2 mM, Cl^- 147.8 mM, HPO_4^{2-} 1.0 mM, and SO_4^{2-} 0.5 mM. The final solution was buffered in Tris and pH adjusted to 7.40 with 1 M hydrochloric acid. Samples were kept in a thermostatic bath at 37 °C. After 7 days, plates were taken out, rinsed in deionized water, and dried at room temperature for further analysis.

2.6. Electrochemical impedance spectroscopy (EIS)

Corrosion stability of the HAP/Gr composite coatings was analyzed based on EIS measurements during 15 days exposure in SBF at 37 °C. A standard three-electrode setup arrangement was used for the EIS experiments. The working electrode, the coated Ti plate, had a testing surface area of 1 cm^2. The counter electrode was a platinum mesh, and the reference electrode was a saturated calomel electrode