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Nucleation and characterization of hydroxyapatite on thioglycolic acid-capped reduced graphene oxide/silver nanoparticles in simplified simulated body fluid

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ABSTRACT

Herein hydroxyapatite (HA) has been synthesized by the nucleation on the surfaces of reduced graphene oxide/silver nanoparticles (rGO/AgNPs) chemisorbed with thioglycolic acid (TGA). The self-assembled monolayer of TGA formed on rGO/AgNPs was immersed in simplified simulated body fluid under gentle growth conditions, forming rGO/AgNPs/TGA/HA biocomposite. The phase structures and functional groups of biocomposite were analyzed by X-ray diffraction spectroscopy, Fourier transform infrared spectroscopy and Raman spectroscopy. Enhanced Raman spectrum of TGA on prepared rGO/AgNPs was obtained with excitation at 633 nm, showing that TGA was chemisorbed on AgNPs through S atom and TGA molecular plane exhibited a tilted orientation with respect to AgNPs. The morphologies of biocomposite were investigated by means of atomic force microscope and transmission electron microscope coupled with energy dispersive spectrum. Analysis shows that the AgNPs uniformly distributed on the rGO nanosheets with the size of about 15–20 nm and HA formation initiated through Ca^{2+}-adsorption upon complexation with $—COO^-$ groups of TGA on AgNPs. The results obtained indicated that the rGO/AgNPs/TGA/HA biocomposite may have immense potential application in bone tissue engineering fields for its outstanding and stable activities.

1. Introduction

Synthetic hydroxyapatite (HA: $Ca_{10}(PO_4)_6(OH)_2$) is attracting much attention for its good biology compatibility and firm bond with the bone, making it one of the most bioactive and biocompatible materials in using of artificial bones [1], scaffolds for tissue engineering [2], and chromatographic packing for proteins [3]. Disadvantage of HA is their poor mechanical properties such as brittleness and low fatigue resistance, hence, the use of HA is limited in the clinic applications. Recently, researchers are focusing on creating HA composites with metal implants and various methods have been developed: sol–gel coating [4], cathodic deposition [5], electrodeposition [6,7], pulse laser deposition [8], plasma spraying [9], biomimetic precipitation [10]. Among them biomimetic method seems to be the most hopeful since it mimics mineralization process of bone which is based on the use of simulated body fluid (SBF) with semblable ionic content like human body fluid. Using the biomimetic method provides formation of HA with structural and morphological natures very similar to the natural bone.

Traditional biomedical metallic implants are titanium and its alloys for their favorable corrosion resistance as well as their low toxicity, biocompatibility, and good mechanical properties such as high strength, durability, wear resistance [11,12]. Compared with titanium, graphene is an ideal two-dimensional monolayer sheet of hexagonal carbon atoms, which has received great attention because of its unique structure and exceptional physiochemical properties, including doubled external surface area, easy modification, planar support for biomaterials, chemical inertness, biocompatibility, high mechanical strength and thermal conductivity [13–16]. Moreover, graphene could also act as a promising substrate for providing remarkably high specific surface area to accommodate highly concentrated metal nanoparticles [17], in particular silver nanoparticles (AgNPs), which has been attracted increasing interests in inhibitory and bactericidal field for its stability, durability, low toxic, biocompatibility without genotoxicity or cytotoxicity and broad-spectrum antibacterial activity [18–20]. Recent studies have shown that AgNPs/graphene sheets hybrids not only demonstrate excellent surface-enhanced Raman scattering (SERS) activity but also exhibit good antimicrobial properties at low concentrations [21–23]. Therefore, incorporating AgNPs seems the best strategy for inhibiting initial bacterial attachment onto biomaterials to avoiding possible infections during surgical operation or after implantation.

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Several studies have pointed out the important role played by the carboxylic terminal group for the nucleation of HA [24–26]. In this study, we have developed a facile method based on biomimetic techniques to nucleate HA choosing the self-assembled monolayer (SAM) of thioglycolic acid (TGA). TGA is an interesting compound from a biological point of view given that it has been used to synthesize some antiviral drugs [27] and also it is a functional molecule whose $-COO^-$ groups strongly induced HA formation in SBF by electrostatic adsorption of Ca^{2+} and subsequently adsorption negatively charged PO_4^{3-}. In addition, the $-SH$ group of TGA is easily cleavaged to form Ag—S bond when adsorbed on AgNPs.

The aim of this research is to synthesize HA biocomposite with favorable antibacterial activity, corrosion stability, biocompatibility and high mechanical strength. Here we report a facile method to prepare graphene oxide (GO) nanosheets using modified Hummers method and ultrasonication. The reduced graphene oxide/AgNPs (rGO/AgNPs) hybrid was synthesized by reduction of silver nitrate ($AgNO_3$) with excessive hydrazine hydrate ($N_2H_4{\cdot}H_2O$) as a reducing agent on GO nanosheets at normal atmospheric condition and rGO/AgNPs/TGA/HA biocomposite were fabricated in simplified SBF with assistance of TGA and rGO/AgNPs. The morphology and compositions of as-prepared biocomposite were analyzed by a series of characterizations, indicating that these biomimetic HA coatings on the surfaces of rGO/AgNPs acted as a bonding layer to bone and therefore, HA layers were expected to improve fixation of uncemented prosthesis through osseointegration and to promote higher healing rates for their potential antibacterial activity, high biocompatibility, enhanced corrosion resistance and high mechanical strength.

2. Experimental

2.1. Materials and reagents

Graphite powder, potassium permanganate ($KMnO_4$), sodium nitrate ($NaNO_3$), concentrated sulfuric acid (H_2SO_4), hydrogen peroxide (H_2O_2, 30%), $AgNO_3$, $N_2H_4{\cdot}H_2O$ (50%), hydrochloric acid (HCl, 5%), barium chloride ($BaCl_2$), calcium chloride ($CaCl_2$), sodium chloride (NaCl), dipotassium phosphate (K_2HPO_4), trisaminomethane (Tris) were all purchased from Sinopharm Chemical Reagent Co., Ltd. (China). TGA was purchased from Sigma–Aldrich (Germany). Deionized (DI) water was obtained from Millipore water purification system.

2.2. Preparation of GO nanosheets

Graphite oxide was prepared from powder graphite adopting modified Hummer method [28]. Typically, graphite was added to a flask with 50 ml of 98% H_2SO_4 in an ice bath, followed by slow addition of $KMnO_4$ and $NaNO_3$ under stirring and cooling to keep the temperature of the reaction mixture below 5 °C for 2 h. The temperature was then raised to 35 °C with a water bath, and the mixture was stirred for 30 min. After that, excess DI water was added to the above mixture with the temperature increasing to 98 °C and then the mixture was stirred for 30 min. Subsequently, 30% H_2O_2 was added to the deep brown mixture until the color of the mixture changed to bright brown-yellow. The resultant suspension was intensively washed, first with a diluted solution of HCl (5%) and then washed with DI water repeatedly by centrifugation until it became free of sulfate anion which could not be detected with $BaCl_2$. The resulting solid was dried in a vacuum oven at 60 °C overnight. Finally, the graphite oxide was suspended in DI water. The stable GO nanosheets aqueous suspension was obtained with the aid of ultrasonication for 3 h.

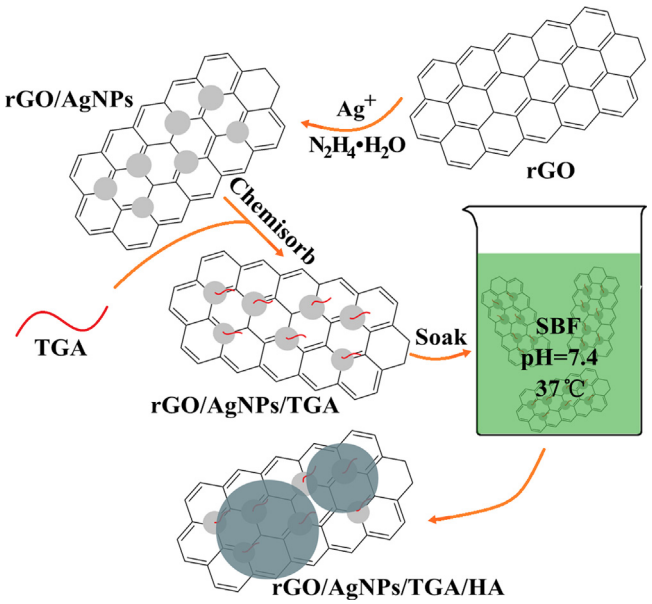

Fig. 1. Schematic illustration of the formation of HA in SBF solution on the surfaces of rGO/AgNPs hybrids chemisorbed with TGA.

2.3. Fabrication of rGO/AgNPs and rGO/AgNPs/TGA hybrids

The hybrids were synthesized based on a wet chemical method. 6.5 mg/ml of GO suspension was dispersed in DI water (100 ml), and a pre-calculated amount of $AgNO_3$ (300 mg) was added to the reaction vessel under vigorous stirring for 4 h. Then 10 ml of dilute $N_2H_4{\cdot}H_2O$ (2%) was conducted dropwise into GO/$AgNO_3$ mixture in ambient conditions, during which the color of the solution changed from bright brown-yellow to nigger-brown. The resulting homogeneous dispersion was under gentle stirring for 6 h for the maximum reduction of $AgNO_3$ and GO to obtain rGO/AgNPs. Finally, the dispersion was centrifuged and washed with DI water three times to remove the remaining reagents.

The rGO/AgNPs/TGA hybrid was simply prepared by injecting 0.2 ml of TGA into the above-mentioned rGO/AgNPs solution and the mixture was stirred with a mechanical stir bar for 2 h. During this time the TGA was chemisorbed onto the surfaces of the AgNPs, forming a rGO/AgNPs/TGA hybrid.

2.4. Synthesis of rGO/AgNPs/TGA/HA biocomposite

The synthesis of HA/rGO/AgNPs/TGA biocomposite was based on a biomimetic method in simplified SBF. Typical simplified SBF in this study contained 140 mM NaCl, 2.5 mM $CaCl_2$ and 1 mM K_2HPO_4 and the pH of the solutions was adjusted to a specific value at 7.4 by the addition of Tris and HCl. The concentration of each reagent and the pH were the same as that of human body fluid. For the nucleation of HA, the as-prepared rGO/AgNPs/TGA hybrids were immersed into SBF solution contained in a plastic beaker, which was kept at 37 °C in a shaking water bath for 120 h. Then the biocomposite were taken out of the solutions after immersion, rinsed with DI water several times, followed by drying in an oven at 37 °C. The preparation process of rGO/AgNPs/TGA/HA biocomposite was interpreted in Fig. 1.

2.5. Characterization

Morphological characterizations of rGO/AgNPs and rGO/AgNPs/TGA/HA were performed by transmission electron microscopy (TEM, JEM-2010, JEOL, Japan), high-resolution