The effect of reduction of graphene oxide on the formation of hydroxyapatite and tricalcium phosphate

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ABSTRACT

This study reports on biomimetic mineralization route employing reduced graphene oxide (RGO) at different rates as a template material for biomineralization of biphasic hydroxyapatite/tricalcium phosphate (HAP/TCP). RGO-HAP/TCP hybride composites were synthesized via biomineralization in Simulated Body Fluid (SBF). GO was prepared from pristine graphite by Hummers and Offeman method. GO nanoparticles were reduced with sodium borohydride ($NaBH_4$) before soaking in 5xSBF for 3 weeks at 37 °C and 7.4 pH to performing biomineralization. Surface area and average size distribution of fabricated GO were found as 813.2 m^2 g^{-1} and 1.2968 μm by using BET and DLS technique, respectively. All surfaces are overall negatively charged and zeta potentials of GO, R_1GO, R_2GO, R_3GO were found to be −35.05, −30.01, 29.5, −29.01 and −28.9, respectively in the neutral pH. The contact angles of GO, R_1GO, R_2GO and R_3GO are 33.5°, 55.3°, 57.4° and 61.1°, respectively. The ID/IG values determined by Raman are 1.07, 1.04, 1.02 and 1.01 for GO, R_1GO, R_2GO and R_3GO, respectively. After the reduction, its maximum peak at 236 was clearly shifted to 260, 264 and 267 nm for R_1GO, R_2GO and R_3GO. The precipitation of biphasic HAP/TCP on R_1GO and R_2GO was confirmed by XRD, FTIR and TGA analysis.

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1. Introduction

Biphasic HAP/TCP consisting of HAP (Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$) and TCP (Tricalcium phosphate, $Ca_3(PO_4)_2$) with more effective bone regeneration ability, controllable degradation rate, perfect bioactivity, biocompatibility, osteoconduction and osteoinduction ability is widely used in medicine as a bone graft substitute and which makes it a perfect implant biomaterial that promotes repair bone defects and binds to the bone tissue [1–9]. Nevertheless, the mechanical properties of ceramic phases such as HAP/TCP are inadequate for large bone defects [10], major long-term and load-bearing implant applications [11]. Weak wear resistance of HAP/TCP is another disadvantage for its implementation in orthopedic implants [12].

Graphene could be an ideal and promising nanoscale reinforcement material for biomimetic mineralization due to chemical and physical properties (high surface area (2620 m^2 g^{-1}), low mass, high electrical and thermal conductivity), high mechanical strength, and proven great biocompatibility [13–20]. Graphene oxide (GO) which can be considered as graphene sheets decorated with oxygen containing hydrophilic functional groups (epoxide, hydroxyl and carboxyl group), supported proliferation and adhesion of osteoblasts [21]. In this respect, GO has recently gained much attention.

Combining GO and HAP/TCP is an attractive option for tissue engineering as well as represents another option as to form materials that can mimic natural bone tissues. It is difficult to make a completely homogeneous composition of the HAP/TCP-GO composites by using conventional mixing technology. By contrast, biomimetic biomineralization having resemblance to materials of biological origin via synthesis of inorganic-organic hybrid materials has attracted enormous attention. These bioinspired biomimetic method, which can induce the formation of bone like apatite, has promoted a great innovation on the design of developed functional materials for human wellbeing [22–26].

It was shown in the previous studies about GO [27] that oxygen containing hydrophilic functional groups can act as influential

nucleation sites for precipitating Ca^{2+} ions and thus, it effects the subsequent both crystallization and growth process of HAP/TCP [28]. On the other hand, it was also shown that HAP/TCP particles could nucleate on RGO surface, which should lack oxygen containing hydrophilic functional groups [29]. This is an attractive situation, reveals the need for clarifying the influence of type and density of oxygen containing hydrophilic functional groups of GO on the nucleation of HAP/TCP.

In this study, the effect of hydrophilic functional groups containing oxygen in GO on the biomineralization process has been investigated. For this purpose, we fabricated and reduced GO at different ratios and biomineralization was conducted on as-synthesized samples by biomimetic method.

2. Experimental section

2.1. Materials

Sodium chloride (NaCl, Merck, CAS: 7647-14-5), sodium hydrogen carbonate (NaHCO$_3$, Sigma Aldrich, CAS: 144-55-8), potassium chloride (KCl, Sigma Aldrich, 7447-40-7), potassium hydrogen phosphate trihydrate (K$_2$HPO$_4 \cdot$3H$_2$O, Merck, CAS: 16788-57-1), magnesium chloride hexahydrate (MgCl$_2 \cdot$6H$_2$O, Merck, CAS: 7791-18-6), calcium chloride (CaCl$_2$, Merck, CAS: 10043-52-4), sodium sulfate (Na$_2$SO$_4$, Merck, CAS: 7757-82-6), trishydroxymethyl aminomethane [TRIS, (CH$_2$OH)$_3$CNH$_2$, Sigma Aldrich, CAS: 77-86-1], hydrochloric acid (HCl, %37, Sigma Aldrich), natural graphite flakes (carbon content: 99.9%, 325 mesh, Sigma Aldrich, CAS: 7782-42-5), potassium persulfate (K$_2$S$_2$O$_8$, Sigma Aldrich, CAS: 7727-21-1), phosphorus pentoxide (P$_4$O$_{10}$, Sigma Aldrich, CAS: 1314-56-3), potassium permanganate (KMnO$_4$, Sigma Aldrich, CAS: 7722-64-7), hydrogen peroxide (H$_2$O$_2$, 30%, Sigma Aldrich) and sodium borohydride (NaBH$_4$, Sigma Aldrich, CAS: 16940-66-2) were used for preparation of SBF and, synthesis and reduction of GO. All chemicals were analytical grade and were used without further purification.

2.2. Synthesis and reduction of GO

GO was synthesized from natural graphite flakes by the method of Hummers and Offeman [30]. The graphite powder was put into an 80 °C solution of concentrated H$_2$SO$_4$, K$_2$S$_2$O$_8$, and P$_2$O$_5$ to obtain pre-oxidized graphite. After cooling filtering and drying processing, GO was obtained by the oxidation of pre-oxidized graphite with of H$_2$SO$_4$ and the gradual addition of 18 g of KMnO$_4$. During oxidation, the color of the mixture changed from dark purplish-green to dark brown. The suspension was cooled and diluted with 350 mL of ice. Then, H$_2$O$_2$ (30%) was added until the gas evolution ceased to ensure that residual permanganate was reduced to soluble manganese ions. The GO was repeatedly washed with dilute 1 M HCl and deionized (DI) water until a pH of 4–5 was achieved in order to remove metal ions. The product was separated from the mixture by centrifugation at 10,000 rpm. Prepared GO samples were diluted in DI water to form a suspension of 0.5 mg/mL. These suspensions were ultrasonically dispersed for 1 h. In order to obtain R$_1$GO, R$_2$GO and R$_3$GO (reduced samples at different rates), in turn 1.89 g, 5.68 g and 9.46 g of NaBH$_4$ was added to a 500 mL dispersed GO suspensions. The mixtures were kept stirring at room temperature for 12 h to get RGOs (R$_1$GO, R$_2$GO and R$_3$GO). The RGOs were filtered and washed by DI water until neutral pH, before drying at 60 °C for 24 h.

2.3. Biomineralization

In order to achieve a rapid biomineralization rate a concentrated

SBF solution (5xSBF) was prepared by adding certain amounts of concentrated solutions of NaCl, NaHCO$_3$, KCl, K$_2$HPO$_4 \cdot$3H$_2$O, MgCl$_2 \cdot$6H$_2$O, CaCl$_2$ and Na$_2$SO$_4$ into DI water [31]. The solution was buffered at pH of 7.2 at 37 °C using TRIS and HCl. RGOs at different rates and reference sample (GO) were immersed in 5xSBF for 3 weeks at 37 °C in a water bath. Fifty milliliter of 5xSBF were used for the same amount of each GO and RGOs which is 50 mg. After immersion in 5xSBF the samples were gently rinsed with DI-H$_2$O and dried at 60 °C for 24 h.

2.4. Characterization methods

The morphology and microstructure of as-synthesized products were characterized by scanning electron microscopy (SEM, JEOL, JMS 6060) and elements in the synthesized materials determined by energy-dispersive X-ray spectroscopy (EDS) analysis. All the signals were corrected for atomic number, absorption and fluorescence (ZAF correction routine). For the phase characterization of the synthesized GO-HAP hybrid composites, X-ray diffractometer (XRD, D/Max 2200 LV) were used with Cu Kα radiation ($\lambda = 0.15406$ nm) operating at 40 kV, 30 mA and step size was 0.04°. Thermogravimetric analysis (TGA, Netzsch) was performed in an alumina crucible under a nitrogen atmosphere with heating rate of 10 °C/min. Wettability and hydrophilicity of the samples was characterized by measuring the contact angle using a video based contact angle device (Attention optical tensiometer). Raman spectra were recorded using Kaiser Raman Rxn. Fourier transform infrared (FTIR) analysis was performed on Perkin Elmer-(Spectrum-Two) in the wave number range of 400–4000 cm^{-1}, with a resolution of 4 cm^{-1}. UV–Visible spectra were measured on a Shimadzu-2600 UV–Vis spectrophotometer. Dynamic light scattering (DLS) and zeta potential measurements of fabricated GO were performed with Nano Plus instrument at natural pH after re-dispersion in DI water. Surface area measurements of synthesized GO were carried out by physical adsorption of N$_2$ at 77 K (Micromeritics ASAP 2010) and obtained by the Brunauer-Emmett-Teller (BET) method.

3. Results and discussion

Fabricated GO is promising nanoscale reinforcement material for biomimetic mineralization due to obtained large specific surface area (813.2 m^2 g^{-1}) determined using BET method by measuring the amount of nitrogen physisorbed to GO surface. This surface area below the theoretical value of GO surface area (2620 m^2 g^{-1}) [32]. This could be due to incomplete exfoliation during sonication of the GO. The particle size distribution of aqueous dispersion of GO was determined by dynamic light scattering (DLS) analysis at the scattering angle $\theta = 90°$. Five minutes of ultrasonication with a micro tip was carried out to disperse the GO before subjecting to size distribution by DLS. DLS theory is a well-established technique for measuring round-particle size over a size range from a few nanometers to a few microns. Although DLS is more suitable for spherical particles rather than for planar sheets like GO, it serves to indicate whether uniformly sized dispersion of GO was produced. As shown in Fig. 1, the mean size of fabricated GO was 1.2968 μm and successfully obtained with scattered in a narrow range GO.

Fig. 2 shows SEM images of the morphology GO, R$_1$GO, R$_2$GO and R$_3$GO before (Fig. 2a) and after biomineralization (Fig. 2b). Before immersion in 5xSBF, Both GO and RGOs consist of randomly aggregated thin crumpled sheets, corrugations and scrolling are observed because the 2D membrane structure becomes thermodynamically stable via blending [33]. The GO sheet showed a relative wrinkled sheet-like structure and reduced lateral size compared with the RGO sheets shown in Fig. 2a. The reduction of