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***In-situ* deposition of hydroxyapatite on graphene nanosheets**

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Abstract

Graphene nanosheets were effectively functionalized by *in-situ* deposition of hydroxyapatite through a facile chemical precipitation method. Prior to grafting of hydroxyapatite, chemically modified graphene nanosheets were obtained by the reduction of graphene oxide in presence of ethylenediamine. The resulting hydroxyapatite functionalized graphene nanosheets were characterized by attenuated total reflection IR spectroscopy, X-ray diffraction, field emission scanning electron microscopy, transmission electron microscopy, X-ray energy dispersive spectroscopy, Raman spectroscopy and thermogravimetric analysis. These characterization techniques revealed the successful grafting of hydroxyapatite over well exfoliated graphene nanosheets without destroying their structure.

Keywords

Graphene; Hydroxyapatite; Graphene oxide; Functionalization

1. Introduction

In recent years, carbon nanomaterials have attracted considerable attention owing to their unique properties and wide range of applications [1–6]. Among the carbon nanomaterials, graphene (GR) is a new member, in which single layer of sp^2 -hybridized carbon atoms are packed together into a honeycomb two-dimensional (2D) lattice [7]. The extraordinary electrical, thermal, and mechanical properties of GR have drawn current interest in materials science [8–10]. In comparison with carbon nanotubes (CNTs), GR not only possesses identical physical properties but also larger surface area. In addition, the production cost of GR is much lower than that of CNTs [11,12]. However, the applications of GR are limiting [13] because of its hydrophobicity and tend to form agglomerates or even re-graphitized to graphite. For which the interfacing of secondary materials with GR is an ideal option, which may not only prevent the restacking of GR sheets but also sensitively regulate the properties

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of GR based nanocomposites. It has been reported that inclusion of GR into polymer or ceramic matrices lead to remarkable improvement in the properties of host materials [14–17]. Owing to this, the GR and inorganic nanocomposites, derived from decoration of GR sheets with inorganic nanomaterials drawn special interest. Also, it is demonstrated that the innovative properties achieved from GR-inorganic nanocomposites were not found in either of their individual components [18–20]. Therefore, the combination of GR with secondary nanomaterials integrates unique characters and functions of the two components.

Hydroxyapatite (HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is a form of calcium phosphate that bears close chemical resemblance with the mineral component of bones and teeth tissues [21]. Thus, HA is one of the few materials those are classified as bioactive materials, that is, it can bind to bone tissue and promote bone repair when applied in orthopedic, dental and maxillofacial fields [22,23]. However, the poor mechanical properties of HA such as lower fracture toughness and higher elastic modulus restricted its applications [24]. This drawback can be effectively defeat by combining HA with mechanically stiffed material like GR.

The chemical reduction of graphene oxide (GO) to GR, offers a low cost method of producing and functionalizing GR on industrial scale [25–26]. The most widely used reducing agents in chemical reduction method includes toxic reagents like hydrazine, dimethylhydrazine, NaBH_4 etc., trace amount of these agents could have detrimental effect on their applications, especially in biomedical field. In consideration of this, to avoid the use of harmful reducing agents, herein, we report a simple method for reduction of GO to GR in presence of bio-compatible, ethylenediamine (EDA). The EDA reduced GR nanosheets were further utilized for *in-situ* decoration of HA. The HA functionalized GR nanosheets were characterized using attenuated total reflection infrared (ATR-IR) spectroscopy, X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), X-ray energy dispersive spectroscopy (EDS), Raman spectroscopy and thermogravimetric analysis (TGA).

2. Experimental

2.1. Materials

All the reagents were purchased from Aldrich and used without further purification unless otherwise noted. All the aqueous solutions were prepared with ultrapure water obtained from Milli-Q Plus system (Millipore).

2.2. Synthesis of GR nanosheets

Graphene oxide (GO) was prepared from graphite powder according to the Hummers and Offeman method [27]. Then GO (50 mg) was dispersed in EtOH (50 mL) by sonication for 5 mins and the dispersion was subjected to centrifugation and EtOH was removed. The obtained GO was re-dispersed in ethylenediamine (EDA) to yield the yellow-brown suspension, which was subjected to refluxing for 1 hr at 80 °C. The resulting black suspension of GR nanosheets was then centrifuged, subsequently washed with EtOH and DI water and dried under vacuum at 40 °C for 5h.

2.3. In-situ deposition of HA over GR nanosheets

The GR (40 mg) nanosheets were dispersed in 40 mL DI water by sonication for 5 mins, to this an aqueous solution of $\text{Ca}(\text{OH})_2$ (0.01 mol L^{-1}) was added and the suspension was stirred under ambient conditions for 1 h. Then pH of the suspension was adjusted to ≈ 9 by slow addition of H_3PO_4 under constant stirring. The resulting HA functionalized GR nanosheets (*f*-GR) was separated by centrifugation, washed with DI water and dried under vacuum at 40°C for 7h.

2.4. Characterization

FTIR spectra were recorded using Smiths ChemID diamond attenuated total reflection (DATR) spectrometer and TGA were performed with a Perkin Elmer Diamond TG/DTA instrument at a heating rate of $10^\circ\text{C}/\text{min}$. Powder XRD patterns were recorded on Scintag X-ray diffractometer (PAD X), equipped with Cu K α photon source (45 kV, 40 mA) at scanning rate of $3^\circ/\text{min}$. SEM measurements were carried out on a JEOL JXA-8900 microscope and TEM images were obtained with a JEOL 2010 microscope. EDS measurements were made with a FEI Tecani F 20 system attached to TEM. Raman spectra were recorded with Renishaw R-3000QE system in the backscattering configuration using an Argon ion laser with wavelength 785 nm.

3. Results and discussion

The oxygen functionalities on the surface of GO and the deoxygenation of the surface of GR, as well as the deposition of HA on GR, were confirmed by IR spectroscopy. Fig. 1 shows the ATR-IR spectra of GO, GR, *f*-GR and HA. The spectrum of GO (Fig. 1a) depicts a broad band around 3395 cm^{-1} and a intense band at 1619 cm^{-1} due to O-H stretching and aromatic C=C vibrations, respectively. Also, it displayed the bands owing to C=O (1719 cm^{-1}), carboxy C-O (1377 cm^{-1}), epoxy C-O (1161 cm^{-1}), and alkoxy C-O (1039 cm^{-1}) groups exists on the surface of GO nanosheets. The spectrum of GR (Fig. 1b) exhibited significant differences from that of GO. The characteristic absorption bands of oxygen functionalities such as O-H, C=O and C-O disappeared in Fig. 1b. In addition, a new band appeared in the spectrum of GR at 1509 cm^{-1} , which is ascribed to the skeletal vibration of GR nanosheets [28]. These conformational changes demonstrate the successful reduction of GO to GR in presence of EDA. The spectrum of *f*-GR (Fig. 1c) displayed the characteristic absorption bands of natural HA, such as the P-O bending of phosphate group was observed at 563 and 599 cm^{-1} and the vibrations of O-P-O carbonate ions of hydroxyl sites were appeared at 1021 cm^{-1} . The band, observed at 1422 cm^{-1} was attributed to vibrations of carbonate ions. The spectrum of *f*-GR is similar to that of free HA (Fig. 1d), which suggests that HA retained the essential feature of its native structure in *f*-GR.

The XRD patterns, shown in Fig. 2 were utilized to monitor the structural changes that occurred in GO, GR and *f*-GR. The pattern of GO (Fig. 2a) displayed a characteristic peak at 2θ value of 13.2° , corresponding to (0 0 1) reflection of stacked GO nanosheets [29]. After reduction of GO with EDA, two distinctive peaks at 26.4° and 44.4° appeared in the pattern of GR (Fig. 2b) corresponding to (0 0 2) and (1 0 0) reflections of GR, respectively. The basal spacing (*d*-spacing) calculated for GO and GR was found to be 6.7 and $3.37 \text{ \AA}$,

respectively. The higher value of d -spacing for GO nanosheets can be ascribed to presence of oxygen containing functional groups such as carboxyl (-COOH), hydroxyl (-OH), and epoxy groups, inserted H₂O molecules, and other structural defects [30]. The reduction in the value of d -spacing for GR demonstrate the successful conversion of GO to GR in presence of EDA by effective removal of oxygen containing functional groups situated on the surface of GO. Also, the broadening of (0 0 2) diffraction peak in Fig. 2b indicates smaller size of GR nanosheets. The principal peaks appeared at 27.5°, 33.4° and 39.9° for f -GR (Fig. 2c) were attributed to (0 0 2), (2 1 1) and (3 1 0) reflections of HA, respectively (JCPDS file No. 09-0432). The remaining peaks observed at 26.3° and 44.9° for f -GR were assigned to (0 0 2) and (1 0 0) reflections of GR. The XDR pattern of f -GR reveals that the HA deposited on GR is low crystalline in nature. The graphitization index (G) for GR and f -GR was determined according to equation: $G = (3.440 - d_{002}) / (3.440 - 3.354)$, where d_{002} is value of d -spacing [10,31]. The value of G calculated for GR and f -GR was found to be 91.8 and 69.8%, respectively, which indicates existence of highly ordered graphitic structure.

The morphology of GO and f -GR was studied by FESEM. Figs. 3a–c shows the micrographs of GO, these images shows the loosening of GO nanosheets and their porous structure due to the opening of planer carbon networks wedged at the edge surface of crystallite by oxidation and exfoliation. At higher magnification (Figs. 3a and b), it is clearly visible that GO is effectively exfoliated into thin nanosheets and their surface is wrinkled. The effective deposition and distribution of HA onto GR nanosheets in f -GR can be clearly observable in the Figs. 3d–f. In Fig. 3f, it is easily recognizable that both, the surfaces of GR nanosheets and also their inter layers are densely packed with HA, which resulted to the formation of sandwich-like layered structure between GR nanosheets and HA. The GR nanosheets exist in f -GR are curled and entangled. The successive washings of f -GR with water does not remove the HA from GR nanosheets, so the HA deposited on GR nanosheets is strongly adhered. The morphology of f -GR was further analyzed by TEM images shown in Figs. 4a–d. It can be clearly seen that the GR nanosheets are efficiently decorated by nano HA and there is no free standing HA was present in the void space, hence a strong interaction exists between HA and GR nanosheets. It can be seen that the wrinkled or crumpled silk wave-like GR nanosheets in Figs. 4a and b, which is a characteristic feature of existence of single-layer GR nanosheets. It has been reported that corrugation and scrolling are part of the intrinsic nature of GR, which result from the fact that the 2D membrane structure becomes thermodynamically stable through bending [32]. Furthermore, the energy dispersive X-ray spectroscopy (EDS) measurements (Fig. 5) confirm the elemental composition of f -GR. Fig. 5 shows the presence of C, Ca, P, and O in f -GR, which demonstrate the successful deposition of HA over GR nanosheets. The ratio of Ca to P estimated from the EDS was found to be 1.64, which is close to the stoichiometric ratio (1.67) exists in natural bone tissue [24]. The additional signals from Cu were originated by copper grid used to support the sample during TEM measurement.

The Raman spectrum of GR shown in Fig.6a displayed the Raman peaks of G-band at 1589 cm⁻¹ and the D-band at 1309 cm⁻¹, whereas, the spectrum of f -GR (Fig.6b) exhibited the G- and D-bands at 1582 and 1299 cm⁻¹, respectively. The G-band is a characteristic feature of graphitic carbon layers corresponding to tangential vibration of carbon atoms and the D-

band is a typical sign of presence of defective graphitic carbon [33]. When the spectrum of GR was compared with that of *f*-GR, it was observed that both G- and D-bands were shifted to downward. The downward shifting of bands in *f*-GR might be due to increase in number of GR nanosheets after deposition of HA. This observation is in accordance with previous results that the position of G-band in single layered GR sheets shifts to lower wavenumber after stacking into number of GR layers [34–37]. Hence, the position of G-band for GR at 1589 cm^{-1} could be attributed to existence of single layered GR nanosheets. Also, the appearance of high intense G-band in Fig. 6a indicates the high graphitization of prepared GR nanosheets. The extent of graphite mode in the carbon material can be quantified by the intensity ratio of the D- to G-bands (ID/IG). The ratio of ID/IG for GR and *f*-GR was found to be 0.79 and 1.02, respectively. It can be seen that the ratio of ID/IG increases after deposition of HA, which shows that the graphitic planes in GR were affected by deposition of HA.

The thermal property of GO, GR and *f*-GR were studied by TGA and the profiles are shown in Fig. 7. The initial weight loss of 10% below $100\text{ }^{\circ}\text{C}$ for GO (Fig. 7a) is attributed to removal of water molecules adsorbed on the surface of GO. The successive major weight loss of 88% taken place around $200\text{ }^{\circ}\text{C}$ is ascribed to decomposition of labile oxygen containing functional groups situated on the surface of GO. Between 200 and $800\text{ }^{\circ}\text{C}$, a minute weight loss was observed, which can be attributed to the removal of more stable oxygen functionalities. GR (Fig. 7b) exhibits the higher thermal stability than GO. The weight loss of 7% displayed below $100\text{ }^{\circ}\text{C}$ for GR is owing to elimination of adsorbed water. The following weight loss of 22% between 200 and $580\text{ }^{\circ}\text{C}$ illustrates the residual oxygen containing functional groups on GR. The significant weight loss of 19% occurs between 590 and $605\text{ }^{\circ}\text{C}$ could relate to decomposition of GR nanosheets. Moreover, there is no considerable weight loss occurred around $200\text{ }^{\circ}\text{C}$ for GR, which indicates the complete reduction of GO to GR in presence of EDA. While, the initial weight loss below $200\text{ }^{\circ}\text{C}$ and the successive weight reduction below $580\text{ }^{\circ}\text{C}$ for *f*-GR (Fig. 7c) were attributed to the removal of the adsorbed water and the residual oxygenate groups, respectively. The consecutive weight loss onset at $584\text{ }^{\circ}\text{C}$ corresponds to the decomposition of GR nanosheets. So, the deposition of HA over GR nanosheets provides the high thermal stability to *f*-GR. The residual weight measured for GR and *f*-GR at $800\text{ }^{\circ}\text{C}$ was found to be 49.7 and 58.8%, respectively.

From the previous studies, it is already known that GR exhibits good biocompatibility [38–41]. Lahiri et al. assessed the cytotoxicity of graphene nanoplatelets by culturing the osteoblasts in medium with different concentrations of graphene nanoplatelets and assessing the cell morphology and viability [38]. It was found that the cytotoxicity of graphene nanoplatelets to osteoblast is dependent on its concentration and is also influenced by agglomeration of particles. Kim et al. illustrated that, compared to pristine graphene oxide and graphene, the hybrid materials: graphene oxide- CaCO_3 and graphene- CaCO_3 exhibits remarkably enhanced hydroxyapatite formation when incubated in a SBF solution. Thus formed graphene oxide-hydroxyapatite and graphene-hydroxyapatite composites support high viability of osteoblast cells with elongated morphology [39]. Biris et al. demonstrated that the multicomponent nanocomposite material formed out of graphene layers, Au

nanoparticles supported on the surface of hydroxyapatite nanoparticles, was found to have good biocompatibility and induce excellent bone cellular proliferation [40]. Liu et al. exemplified that the graphene, dopamine and hydroxyapatite based hybrid materials exhibits no cytotoxic effect on L929 fibroblast cells [41].

4. Conclusions

In conclusion, a facile approach for effective *in-situ* deposition of HA over GR nanosheets has been reported. This technique offers the production of well exfoliated single-layer GR nanosheets and replaces the utilization of toxic reducing agents with biocompatible EDA. The strong adherence of HA on GR nanosheets was achieved without ruining the native structure of GR nanosheets. In addition to its simplicity, the reported method is rapid and economic, which can be upgradable to mass production. Furthermore, it may be of great potential to promote the applications of *f*-GR in biomedical engineering, especially in orthopedic, dental and maxillofacial fields.

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Highlights

- It is a facile and effective method for deposition of HA on GR nanosheets.
- It avoids the use of harmful reducing agents like hydrazine, NaBH_4 etc.
- GR nanosheets were produced using bio-compatible, ethylenediamine.
- The graphitic structure of synthesized GR nanosheets was high ordered.
- The ratio of Ca to P in HA was 1.64, which is close to ratio in natural bone.

Fig. 1. ATR-IR spectra of (a) graphene oxide, (b) graphene nanosheets, (c) functionalized graphene nanosheets (f-GR) and (d) hydroxyapatite.

Fig. 2. XRD patterns of (a) graphene oxide, (b) graphene nanosheets and (c) functionalized graphene nanosheets (f-GR).

Fig. 3. FESEM images of (a–c) graphene oxide and (d–f) functionalized graphene nanosheets (f-GR).

Fig. 4.
TEM images of functionalized graphene nanosheets (f-GR).

Fig. 5.
EDS of graphene nanosheets (*f*-GR).

Fig. 6.
Raman spectra of (a) graphene nanosheets and (b) graphene nanosheets (*f*-GR).

Fig. 7. TGA profiles of (a) graphene oxide, (b) graphene nanosheets and (c) functionalized graphene nanosheets (f-GR).