Materials Science and Engineering C 61 (2016) 251–256

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Synthesis and osteo-compatibility of novel reduced graphene oxide–aminosilica hybrid nanosheets

Song Chen [a,b,*], Xinxin Du [a], Lan Jia [a], Haixin Chang [c], Toshiyuki Ikoma [d], Nobutaka Hanagata [b,**]

[a] College of Materials Science and Engineering, Taiyuan University of Technology, Taiyuan 030024, PR China
[b] Biomaterials Unit, National Institute for Materials Science, 1-2-1 Sengen, Tsukuba 3050047, Japan
[c] Center for Joining and Electronic Packaging, State Key Laboratory of Material Processing and Die & Mold Technology, School of Materials Science and Engineering, Huazhong University of Science and Technology (HUST), Wuhan 430074, China
[d] Graduate School of Science & Engineering, Tokyo Institute of Technology, O-okayama 2-12-1, Meguro-ku, Tokyo 152–8550, Japan

ARTICLE INFO

Article history:
Received 29 September 2015
Received in revised form 1 December 2015
Accepted 25 December 2015
Available online 28 December 2015

Keywords:
Silica
Graphene oxides
Hybrids
Osteo-compatibility

ABSTRACT

Combination of silica component with other materials is one of the current strategies to design bone regenerative materials. In this study, novel reduced graphene oxide (RGO)–aminosilica hybrid nanosheets with enhanced osteo-compatibility were synthesized from a mixture of 3-aminopropyltriethoxysilane (APTES), graphene oxides (GO) and water. The presence of APTES in the mixture not only caused the conversion of GO to RGO, but also led to the hydrolysis and condensation of itself. It was for the first time reported the reducing role of APTES in the conversion of GO to RGO. It was found that the silicon (IV) ions were released from the hybrid nanosheets in a sustained way. The in vitro osteo-compatibility was evaluated by incubating the hybrid nanosheets with osteoblast MC3T3-E1 cells. A water soluble tetrazolium salt assay quantitatively indicated that the hybrid nanosheets had no significant toxicity and exhibited good biocompatibility. An alkaline phosphatase assay quantitatively indicated that the hybrid nanosheets enhanced the osteoblast differentiation compared to the GO nanosheets. An immunochemical assay further qualitatively indicated that the hybrid nanosheets stimulated the production of osteopontin as typical marker for osteoblast differentiation. Thus, the resultant hybrids nanosheets had a potential application in the bone regeneration.

1. Introduction

Since the discovery of Bioglass® consisting of SiO_2, Na_2O, CaO and P_2O_5, silica-derived materials are currently one type of the most widely applied bone regenerative materials [1]. It is generally accepted that silica is the key component in Bioglass® as its silanol groups (Si–OH) as well as hydrated silicate networks (Si–O–Si) can serve as catalytic sites for supporting nucleation and growth of biological apatite for osteo-integration [2]. Numerous studies have demonstrated that both Si–OH groups and Si–O–Si skeletons are hydrophilic and well biocompatible. Combing silica component with other materials is one of the current strategies to design bone regenerative materials [3–4].

Graphene is constructed by sp. [2]-bonded carbon atoms and has become one novel 2D nanomaterial with a thickness of a single atom [5]. By virtue of such structural feature, graphene and its derivatives exhibit extraordinary electronic and mechanical property and have attracted considerable attention in a variety of applications such as novel electronic and energetic materials [6–7], biosensors [8], anti-cancer drug and DNA delivery systems [9–10]. Moreover, the combination of other inorganic component with graphene component would be helpful to design novel hybrids with enhanced physicochemical properties. For example, the rod gold nanoparticles decorated reduced graphene oxides exhibited an enhanced electrochemical performance and have potential as ultrahigh capacity anode materials for lithium ion battery [11], while the molecular imprinted electrochemical sensor based gold nanoparticles decorating polyoxometalate/reduced graphene oxide exhibited high selectivity and sensitivity and can be used for determination of trace triclosan in waste water [12] and for selective determination of tyrosine in milk [13]. Considering the excellent biological performances of silica component in bone regenerative field, it is highly expected that the combination of silica component with graphene component would yield novel hybrids for bone regeneration.

Several studies have reported on the synthesis of the hybrids from silica and graphene components. Kou et al. synthesized GO–silica hybrids as general building blocks for large-area super-hydrophilic coatings through deposition of ~50 nm silica nanoparticles on the surface of GO [14]. Zhang et al. prepared silica–GO hybrid composite particles with enhanced thermal stability through deposition of ~500 nm silica spheres on the surface of GO [15]. Guardia et al. synthesized graphene–mesoporous silica nanoparticle hybrids by selective growth

* Correspondence to: S. Chen, College of Materials Science and Engineering, Taiyuan University of Technology, Taiyuan 030024, PR China.
** Corresponding author.
E-mail addresses: chensong2009@126.com (S. Chen), HANAGATA.Nobutaka@nims.go.jp (N. Hanagata).

http://dx.doi.org/10.1016/j.msec.2015.12.056
0928-4931/

of ~50 nm silica nanoparticles on graphene oxide sheets using a cationic surfactant-templated method [16]. However, in the above studies, graphene component and silica component were mixed via a mainly physical interaction and graphene component was completely embedded in the silica matrix.

Recent researches have indicated that silane coupling agents could be utilized as silica source to produce silica-derived bone regenerative materials as their utilization would not lead to the formation of large silica spheres. Their functional groups such as epoxy and amino groups would be useful to synthesis of silica hybrids. In our previous studies [17–18], glycidoxypropyltrimethoxysilane (GPTMS) was used as both silica source and cross-linker to synthesize the collagen–silica hybrid nanofibrils through the opening ring reaction between epoxy groups of GPTMS and amino groups of collagen. The resultant hybrids exhibited good osteocompatibility and well supported the proliferation and differentiation of osteoblast MC3T3-E1 cells. 3-aminopropyltriethoxysilane (APTES) is the most popular functional reagents and has widely utilized for surface functionalization of various materials such as silica nanoparticles [19], ferric nanoparticles [20], and carbon materials [21]. APTES also exhibited a strong self-catalytic ability and self-catalyzed itself to yield silicate networks. In addition, it has been demonstrated that amine groups might show a strong reducing ability for conversion of GO to RGO, including hydrazine [22], BSA [23], PEI [24], and so on. APTES would be highly expected to serve as both reducing agent and self-catalyst to in situ produce silica–graphene hybrid nanosheets.

Thus, in this study, the silica–graphene hybrid materials were synthesized using APTES and GO as starting materials and their osteocompatibility was evaluated.

2. Experimental

2.1. Synthesis of silica–graphene hybrids

The silica–graphene hybrid materials were synthesized from a mixture of GO, water, and APTES. GO was successfully exfoliated from graphite oxides according to the method described in our previous study [25] and suspended in water at the concentration of 0.2 mg/mL. APTES (0, 0.58, and 1.16 mL) was directly added to 10 mL of GO aqueous solution which was held in a 50-mL flask and the mixture was kept stirring at room temperature for 24 h to yield the silica–graphene hybrids. After centrifugation at 10,000 rpm for 5 min, the resultant silica–graphene hybrids were collected, washed with ethanol, water, and finally freeze-dried at −20 °C. According to the amount of APTES, the resultant hybrids were coded as GOAP0 (APTES = 0, i.e. GO), GOAP058 (APTES = 0.58 mL), and GOAP116 (APTES = 1.16 mL), respectively.

2.2. Characterizations

Surface morphology of the specimens was observed under a scanning electron microscope (SEM; JEOL-6500F, JEOL, Tokyo, Japan). Infrared spectra were collected using a Fourier transform infrared spectrometer (FTIR; Perkin Elmer Spectrum GX FTIR, USA). X-ray photoelectronic spectroscopy (XPS) spectra were carried out on a JPS-9010TR machine (JEOL, Tokyo, Japan). The concentration of Si(IV) released from the specimens was measured with inductively coupled plasma atomic emission spectroscopy (ICPS-8100, Shimadzu, Kyoto, Japan).

2.3. Cell culture

The osteoblast-MC3T3-E1 cells were used to evaluate the in vitro biocompatibility of GOAP058 and cultured in α-Minimum Essential Medium (α-MEM) containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. GOAP0 and GOAP058 were dissolved in the culture medium at the concentration of 0.00625–0.025 mg/mL.

2.4. WST assay

Cell viability was quantitatively evaluated via a water soluble tetrazolium salt (WST) assay. The cells were trypsinized and 50 μL of cell suspension was seeded at the density of 10,000 cells/cm^2 in the 96-well culture plate and incubated at 37 °C overnight. The culture medium was removed and 200 μL of both suspensions of GOAP0 and GOAP058 were separately added to each well and incubated with the cells. At the pre-determined day, the culture medium was removed and 200 μL of WST/culture medium (1:10) mixture was added to each well. After another 4 h of incubation at 37 °C, 50 μL of liquid was taken out and transferred to a new 96-well culture plate. The absorbance of the liquid at 450 nm was measured using a Microplate Reader (MTP-880, Corona Electric Co. Ltd. Japan). Cell viability was further qualified via a Live/Dead assay by which the viable cells were stained with calcium-AM and excited green, while the dead cells were stained with ethidium homodimer-1 and excited red.

2.5. ALP assay

Alkaline phosphatase (ALP) is a typical marker during the early stage of bone cell differentiation and ALP activity in the cells incubated with the specimens was quantified via an ALP assay. The cells were trypsinized and 100 μL of cell suspension was seeded at the density of 10,000 cells/cm^2 in the 24-well culture plate and incubated at 37 °C overnight. The culture medium was replaced with 500 μL of suspension of GOAP0 and GOAP058 at the concentration of 0.025 mg/mL. To induce osteoblast differentiation, after 3 days, the culture medium was changed to differentiation culture medium containing 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM β-glycerophosphate and 50 μg/mL sodium ascorbate.

To analyze the ALP activity, the culture medium was removed from each well and the cells were washed twice with 0.9% NaCl. Subsequently, 300 μL of 0.1% Triton in 0.9% NaCl solution was added to each well. After 10 min, 25 μL of the cell lysis solution were taken out, transferred to a 96-well plate and mixed with 50 μL of ALP working solution at 37 °C for 15 min. Then, 25 μL of NaOH solution were added to stop the reaction. The absorbance of the solution at 415 nm was read using the above Microplate reader.

2.6. Immunochemical assay

Osteopontin (OPN) is one of the typical marker proteins during the late stage of osteoblast differentiation. The immunochemical assay was performed to check the secretion of OPN by the cells. The culture medium was removed from each well and the cells were washed with PBS twice. They were fixed in 3.7% paraformaldehyde for 10 min at 37 °C and permeabilized in 0.1% Triton X-100 for 3 min. After blocked in 3% BSA solution, cells were separately incubated with primary antibody solution (OPN, sc-21,742 antibody, Santa Cruz Biotechnology) and secondary antibody (goat anti-mouse Alexa Fluor 594).

2.7. Statistic analysis

All results in triplicate were expressed as mean standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA) with a significance level of $p < 0.05$.

3. Results and discussion

Raman spectroscopy spectra of GO before and after treated by APTES were shown in Fig. 1. A similar spectrum was observed for all the specimens. Two typical peaks at 1334 cm^{-1} and 1578 cm^{-1} were attributed to D and G bands, respectively. The D band was due to the defects associated with vacancies, grain boundaries and amorphous carbon species, while the G band was due to the in-plane vibration of sp. [2] carbon