



A new composite scaffold of bioactive glass nanoparticles/graphene: Synchronous improvements of cytocompatibility and mechanical property



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ABSTRACT

This study presents a simple method of synthesizing bioactive glass nanoparticles/graphene nanosheets composite (BGs/GNS) scaffolds using the sol-gel and mold-compressing strategies. Characterizations of BGs/GNS scaffold revealed that BGs with an average diameter of 28.75 nm were densely anchored onto both sides of GNS. When the mass ratio of BGs to graphene oxide was set as 10, this scaffold showed better cytocompatibility and higher osseointegration ability with surrounding tissues than the other scaffolds. The introduction of GNS also significantly enhanced the hardness and Young's modulus of BGs. Given the excellent performance of this scaffold, it has potential applications in bone regeneration and implantation.

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1. Introduction

Over the past decades, bioactive glasses (BGs) have been widely investigated in the field of tissue engineering because of their resorbable properties and tendency to form a bioactive hydroxyapatite (HA) layer, which ensures strong interfacial bonding between host bone tissue and implant [1–3]. Therefore, BGs have considerable potential for bone tissue repair and regeneration [4]. However, BGs do not have the required mechanical strength to replace bone tissue [5], and improving their mechanical properties has become primary focus to ensure the successful clinical application of this material.

Thus far, two basic methods have been used to enhance the mechanical properties of BGs by incorporating second phase reinforcements, which include polymers and inorganic materials [6]. For some typical polymers, poly(ϵ -caprolactone) [7], poly-L-lactide-co-glycolide [8], chitosan [9], and PVA [10] are utilized as BGs reinforcements, that improve the mechanical properties to a certain extent. However, the BGs entrapped in polymers tend to aggregate into larger-sized clusters owing to their high sur-

face energy and hydrophilic surface. In addition, these polymers can weaken the mineralization capability of BGs, resulting in poor osseointegration ability. Nevertheless, the existing drawbacks of the polymers utilized as reinforcements of BGs can also be overcome. Compared with polymers, some inorganic materials such as Ti alloy [11], carbon fibers [12] and carbon nanotubes [13] have higher mechanical strength. The mechanical properties of BGs can be greatly improved upon the incorporation of these inorganic materials, making them suitable for the mechanical demand of high-load implants. Therefore, the mechanical properties during the reinforcing phases are the key factors that control the counterparts of BGs, and searching for a new reinforcing material has become the focus of present research.

Graphene nanosheets (GNS) are one of the thinnest and strongest materials, that have recently gained considerable attention as reinforcing materials [14–18]. The theoretical tensile strength (150 GPa) and Young's modulus (1.0 TPa) of GNS make it viable either as reinforcement or as filling material that can improve the mechanical properties of BGs. GNS are far superior to other known reinforcements in terms of transferring their mechanical properties to host materials [19]. In addition, no obvious inflammatory response happened when GNS was incorporated into the microenvironment in vivo, confirming their excellent biocompatibility [20–22]. Therefore, the integration of GNS and BGs will

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produce a new composite material with good mechanical properties and excellent biocompatibility. However, insufficient research has focused on the synthesis of BGs on the GNS surface.

Based on the reasons above, this work is the first to successfully synthesize BGs/GNS composite scaffold using sol-gel and mold-compressing strategies. Results show that the cytocompatibility and mechanical properties of BGs have been enhanced significantly.

2. Experimental section

2.1. Materials

The chemicals of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (CA), tetraethyl orthosilicate (TEOS), graphite powders, and ammonium hydroxide solution (28%) were purchased from Sinopharm Chemical Reagent Co., Ltd. Dimethylsulfoxide (DMSO) and Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco. Alkaline phosphatase (ALP) assay kit was purchased from Nanjing Jiancheng Bioengineering Institute. Trypsin-EDTA solution and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were supplied by Sigma. Fetal bovine serum (FBS) was purchased from Hangzhou Sijiqing Biological Engineering Materials Co., Ltd. Osteoblast cells (MC3T3-E1) were kindly provided by the Fourth Military Medical University. All the other chemicals were analytical grade and used without purification. Ultrapure water ($>18 \text{ M}\Omega \text{ cm}$) was used for rinsing and as the solvent as well.

2.2. Synthesis of BGs/GNS composites

Graphene oxide (GO) colloid solution was prepared from graphite according to the modified Hummers method [23]. First, graphite powder (3 g) was put into a mixed solution of concentrated H_2SO_4 (12 mL), P_2O_5 (2.5 g) and $\text{K}_2\text{S}_2\text{O}_8$ (2.5 g). The mixture was stirred for 4.5 h keeping the temperature stable at 80°C . Then, the mixture was cooled down to room temperature and diluted with 0.5 L ultrapure water and left overnight. Successively, the above mixture was filtered and washed with ultrapure water using microporous membrane to remove the residual acid. After being dried under ambient condition, the product was put into the cold concentrated H_2SO_4 (0°C , 120 mL). Then, KMnO_4 (15 g) was added gradually under stirring and the temperature of the mixture was kept to be below 20°C by cooling. Subsequently, the mixture was

Table 1

The reaction conditions for synthesis of BGs/GNS composites scaffold.

BGs/GNS	TEOS	GO	EtOH	HNO_3	H_2O	EtOH	$\text{NH}_4 \cdot \text{H}_2\text{O}$	CA
	g	mg/mL	mL	μL	mL	mL	mL	g
	Solution A				Solution B			
BG10GO1	2.08	12	13.8	22.5	8.46	32	1.94	0.35
BG5GO1	1.04	12	6.9	11.25	4.23	16	0.97	0.175
BG1GO1	2.08	6	13.8	22.5	8.46	32	1.94	0.35

stirred at 35°C for 2 h, and then diluted with ultrapure water (250 mL) keeping the temperature below 50°C . After being stirred for 2 h, additional 0.7 L ultrapure water was added. Then 20 mL 30% H_2O_2 was added to the mixture, and the color of mixture changed into brilliant yellow along with bubbling. The mixture was filtered and washed with 1:10 HCl aqueous solution (1 L) to remove metal ions followed by 1 L ultrapure water to remove the acid. The resulting solid was dried in air and diluted to make a GO dispersion. Finally, it was purified by dialysis for several weeks to remove the remaining metal species. BGs/GNS composite with BG/GO (wt/wt) = 10:1 (abbreviated as BG10GO1) was synthesized according to the following steps: 2.08 g of TEOS was added into 13.8 mL of ethanol, subsequently 5.66 mL of GO with a concentration of 12 mg/mL was slowly added into the solution to form the solution A, and its pH was adjusted to 2 with $22.5 \mu\text{L}$ HNO_3 . To prepare the solution B, 8.46 mL of water was added into 32 mL of ethanol, and then the pH was adjusted to 11.5 with 1.94 mL of $\text{NH}_4 \cdot \text{H}_2\text{O}$. After 1 h of stirring, the solution A was slowly injected into the solution B under vigorous stirring, then the solution of 0.35 g of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ dissolved in 4 mL water was added and the reaction was continued for the next 8 h. After reaction, the composite can be obtained by vacuum filtration of the resulting reaction solution through a $0.44 \mu\text{m}$ of micropore filter membrane. The obtained composite was processed into a columned scaffold with 8 mm diameter and 9 mm height through a mold-compressing method (at 20 MPa pressure) and high temperature calcination (at 1300°C under Ar atmosphere protection) (Fig. 1). Likewise, columned scaffolds of BG1GO1 [BG/GO (wt/wt) = 1:1] and BG5GO1 [BG/GO (wt/wt) = 5:1] were also synthesized according to the reactive condition listed in Table 1.

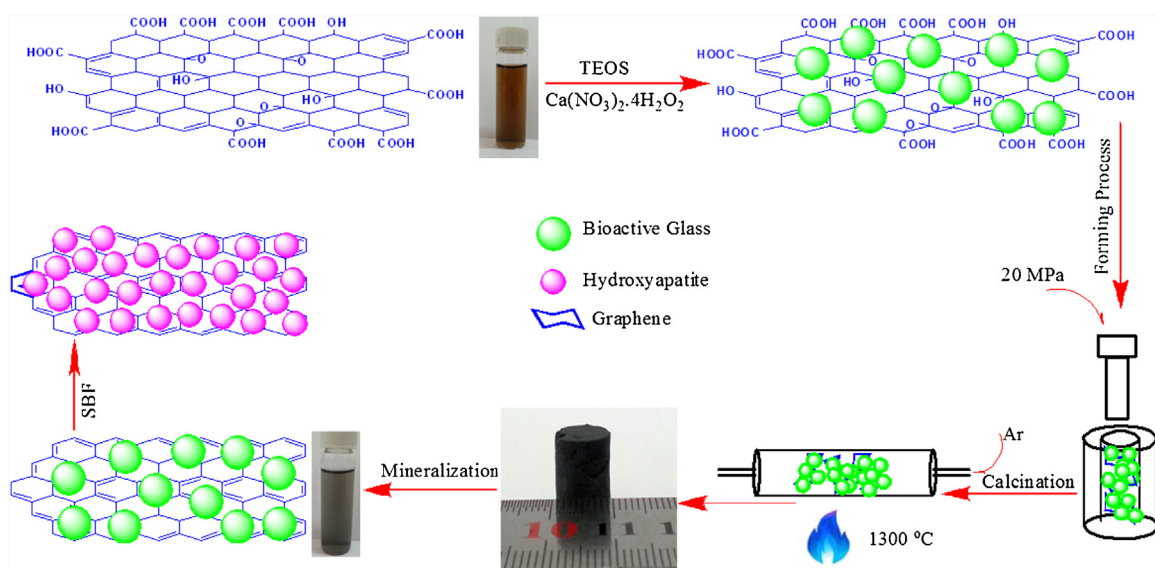


Fig. 1. Illustrations for the preparation of composite scaffold and mineralization.

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