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Investigation of bismuth doped bioglass/graphene oxide nanocomposites for bone tissue engineering

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ABSTRACT

In this study, bismuth doped 45S5 nanobioactive bioglass (nBG) and graphene oxide (GO) nanocomposites were developed and characterized in terms of microstructural, mechanical, bioactivity and biological properties. Bismuth (Bi) - doped nBG was synthesized by sol-gel method and sintered at 600 °C for 2 h. Nanosized GO was homogeneously mixed with Bi doped bioglass at various ratios to prepare nanocomposites. Addition of Bi increased the density of nBG samples while a considerable decrease in density was observed for nanocomposites with GO incorporation. Bi improved the diametral tensile strength of nBG and addition of 2.5% GO to the composite also increased the diametral tensile strength of the nanocomposites. However, addition of more than 2.5% GO had negative effect on the diametral tensile strength of the composites. Bi doping to bioglass and its composite with GO increased the biocompatibility of 45S5 nBG in which 96.5BG1Bi2.5GO (containing 96.5% BG 1% Bi 2.5% GO in weight ratio) showed highest cell viability. Overall, it can be concluded that composites of Bi doped 45S5 nBG with GO hold promise as biomaterial for biomedical applications.

1. Introduction

Composition of BG is similar to that of biological hydroxyapatite (HA) which is composed of calcium and phosphate ions. Their ability to bond to hard tissues makes them good candidates to be used as biomaterials. Among BGs, 45S5-BG is widely used as a dental biomaterial. The main drawback of BG species is their poor mechanical properties limiting their use in load bearing applications. On the other hand, their bioactivity, osteoconductivity and controllable biodegradability make them suitable for their use as precursors in biomaterial production. In vivo studies reported that they accelerated the formation of apatite layer that occludes the dentinal tubules leading to dentin re-mineralization for longer periods. Moreover, BGs accelerate bone growth three times higher than HA when used as a hard tissue implant as reported in the literature. The advantages of BGs over other bioceramics such as HA, tricalcium phosphate and etc. are their ability to bond faster to the bone, osteogenic properties and degradation in the body [1,2]. When inserted into defect area of damaged bone, 45S5-BG helped bone repair and provides strong structural support due to good bonding with bone. Furthermore, 45S5-BG was shown to have high solubility in physiological media and it formed a continuous phase including different cations in the silica structure with type III bonding to bone [3,4]. The previous works reported that 45S5 BG showed good antibacterial activity. It was used in treatment of dentine hypersensitivity, dental enamel erosion and incipient enamel demineralization [3,4]. Moreover, BG was used as an ossicular implant to decrease conductive hearing loss [4].

The first BG developed was 45S5 and it was composed of SiO_2 (45 wt%), CaO (24.5 wt%), Na_2O (24.5 wt%) and P_2O_5 (6 wt%). It had a brittle structure and showed low fracture toughness which limited its application in load bearing areas. Thus, bioglass/polymer composites were used to improve flexibility and crack resistance of BG [5]. Moreover, BGs were widely used as coatings for metal implants such as Mg, Ti and stainless steel. Such coatings have been widely used to facilitate and enhance the fixation and integration of metallic oral and orthopedic implants [6].

Bi is currently used in production of pharmaceutical products and it is less toxic than other heavy metals [7]. Bi salts were used for treatment of gastrointestinal disorders, syphilis and hypertension. Bi based pharmaceutical products were reported for antibacterial and anticancer properties [8,9]. Furthermore, Bi compounds are detectable with X-ray (radiography and computed tomography) and this makes Bi doped materials a good candidate for bone and dental implants for observing implant adhesion and their morphological change over time at the

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implant site.

Graphene has a special 2D structure making it ideal for reinforcement in various composites [10]. Due to its outstanding intrinsic strength and high specific surface area, graphene was extensively employed to toughen a wide variety of bioceramics, such as silicon nitride [11], HA and 58S-BG [12]. Moreover, it was reported that the presence of graphene did not impair in vitro bioactivity and cytocompatibility of graphene coated 45S5-derived scaffolds [13].

In this study, a novel nanocomposite (Bi-BG/GO) of Bi doped 45S5-nBG and GO was produced for the first time. Bi-BGs were synthesized by sol-gel method and mixed with GO to evaluate their microstructural, mechanical, bioactivity and biological characteristics to develop an ideal biomaterial for orthopedic and dental tissues.

2. Materials and methods

2.1. Synthesis of bioglass and graphene oxide

45S5-nBG and doped-BGs were synthesized by sol-gel method. The precursors used for 45S5-BG were orthosilicate (TEOS, Merck, Germany), triethyl phosphate (TEP, Merck, Germany), calcium nitrate tetrahydrate (Merck, Germany), ethanol (Sigma-Aldrich, Germany), nitric acid (Merck, Germany) and distilled water. In the first step, TEOS was dissolved in ethanol/nitric acid solution and stirred for 30 min. Then, calcium nitrate tetrahydrate was added to solution and stirred for 1 h. TEP was added to solution and stirred for 10 min. Finally, ammonia was added to solution to obtain the gel form. The synthesized sample was dried overnight at 60 °C and sintered at 600 °C for 2 h.

In addition to the main precursors used in the synthesis of pure bioglass, Bismuth (III) nitrate pentahydrate (Merck, Germany) was added to the mixture of precursors to synthesize bismuth doped bioglass (Bi-BG) in different molar ratios.

GO was synthesized by the oxidation of graphite powder. Typically, graphite powder and concentrated H_2SO_4 were put into a flask under mechanical stirring in an ice bath followed by addition of $KMnO_4$ slowly to keep the temperature of the suspension lower than 20 °C. After the addition of water, the solution was stirred at 95 °C for another 15 min. Then, it was followed by drop-wise addition of H_2O_2 during which the color of the solution changed from dark brown to yellow. The mixture was filtered and washed with 1:9 HCl aqueous solution to remove metal ions. The resulting solid was dispersed in water to form a nano-sized GO aqueous dispersion which was then added to BG and homogenized for 10 min to obtain nBG/GO nanocomposites. Table 1 shows the compositions of synthesized nanocomposite groups. To characterize various properties of pure nBG, Bi-nBG and Bi-nBG/GO blends, samples were compacted into discs (13 mm diameter, 2 mm thickness) under 250 MPa for 1 min using a cold press (Carver 3887 Hydraulic Press, USA).

2.2. Structural and mechanical characterization

Density of the materials was measured by Archimedes method. The differences between amount of the weight in water and dry weight gave the volume of the discs. Thus, densities of the materials were calculated according to this formula:

Table 1
Composition of the synthetized Bi-BG species.

Annotation	Bioglass (wt%)	Bismuth (wt%)	Graphene Oxide (wt%)
BG	100	0	0
99BG1Bi	99	1	0
97BG3Bi	97	3	0
96.5BG1Bi2.5GO	96.5	1	2.5
94BG1Bi5GO	94	1	5

$$\text{Density} = \frac{\text{Dry weight x density of water}}{(\text{Dry weight} - \text{weight in water})} \quad (1)$$

Furthermore, the percent densities of the materials according to theoretical densities for each composite were determined through the formula (2). Theoretical densities were assumed as 2.7, 9.78 and 1.84 g/cm^3 BG, Bi and GO, respectively [50–52].

$$\rho = \frac{(wt\%_{BG} * \rho_{BG}^{theo}) + (wt\%_{Bi} * \rho_{Bi}^{theo}) + (wt\%_{GO} + \rho_{GO}^{theo})}{(wt\%_{BG} + wt\%_{Bi} + wt\%_{GO})} \quad (2)$$

The presence of the different phases was investigated by XRD (Rikagu DMAX 2200). The samples were scanned from 10° to 90° with 2Θ angles with a scan speed of 2.0°/min. XRD results were compared with Joint Committee on Powder Diffraction Standards (JCPDS) in order to determine the positions of the diffracted planes.

Presence of characteristic bonds in the samples was investigated by FTIR. The samples were compacted into discs with potassium bromide. The spectra records were performed from 4000 cm^{-1} to 400 cm^{-1} using 512 scan by BioRad FTS 175 C.

The diametral tensile strength of samples was determined by universal mechanical testing machine (LS 500; Lloyd Instruments, UK). During the compression, a maximum tensile strength was generated across the flat surface diameters of the discs normal to the loading direction. The tensile strength of the samples was calculated with the following formula:

$$S = \frac{2F}{(\pi \cdot D \cdot t)} \quad (3)$$

where F: failure force; D: sample diameter; t: sample thickness.

Vickers micro-hardness measurements were performed by HMV-2 Vickers micro hardness tester (Shimadzu Co., Kyoto, Japan). Samples were first polished with SiC papers (Buehler Ltd., Lake Bluff, IL, USA) from 600 to 1200 grades and they were then polished with a 1 μm monocrystalline diamond suspension (Buehler Ltd., Lake Bluff, IL, USA). Approximately 10 measurements were performed on each sample with a diamond indenter at 1.961 N force for 20 s. The average μ-hardness was calculated by the formula below:

$$HV = 0.001854(P/d^2) \quad (4)$$

HV: Vickers hardness; P: Applied force (N); d: Average length of two diagonals.

2.3. Bioactivity analysis

The bioactivity of bioglass samples were determined through incubation in simulated body fluid (SBF) having similar ion concentrations with the human blood plasma at various time points (1, 5 and 10 days). Briefly, the samples were rinsed thoroughly with phosphate buffered saline (PBS, 0.1 M, pH 7.4) twice to remove uncombined particles and then sterilized with 70% (v/v) ethanol-dH_2O for 2 h. Finally, air-dried samples in aseptic conditions were placed in 1X SBF which was prepared as reported elsewhere [14]. At the end of each incubation period, samples were removed gently, rinsed with PBS and absolute ethanol respectively to eliminate residual SBF and samples were dried in atmospheric oven at 100 °C for 1 h. Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDX) analyses were conducted to determine morphological and elemental properties of samples after SBF incubation (JEOL JSM-6335F FEG/SEM equipped with analytical EDX attachment, Japan).

2.4. Cell culture studies

Prior to cell culture studies, the discs were rinsed twice with PBS to remove uncombined components after pelleting procedure. All of the discs were sterilized with 70% (v/v) ethanol-dH_2O for 2 h and UV irradiated for 1 h on both sides.