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Potassium-doped mesoporous bioactive glass: Synthesis, characterization and evaluation of biomedical properties

Muhammad Shoaib [a], Aamer Saeed [a], Javeed Akhtar [b], Muhammad Saif Ur Rahman [c], Aman Ullah [d], Klaus Jurkschat [e], Muhammad Moazzam Naseer [a,*]

[a] Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan
[b] Department of Physics, COMSATS Institute of Information Technology, Islamabad 45550, Pakistan
[c] Clinical Research Center, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang 310009, People's Republic of China
[d] Department of Agricultural, Food and Nutritional Science, University of Alberta, Canada.
[e] Lehrstuhl für Anorganische Chemie II der Universität Dortmund, D-44221 Dortmund, Germany

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ABSTRACT

A bifunctional mesoporous bioactive glass (MBG) with composition ($49SiO_2 \cdot 20CaO \cdot 20Na_2O \cdot 7K_2O \cdot 4P_2O_5$ mol%) was synthesized by a facile sol-gel method, using polyethylene glycol (PEG 6000) as a soft template. The structure, morphology (spherical with approximate size 1 μm) and composition of MBG were determined by fourier transform infrared spectroscopy, the scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX), respectively. The surface area (189.53 $m^2 g^{-1}$ with the pore size of 21 nm) of MBG was measured by Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH) analysis. The formation of hydroxyapatite (HAp) layer on the glass surface upon immersion in simulated body fluid (SBF) was monitored through X-ray diffraction (XRD) which indicates enhanced bioactivity as compared to previous studies. The animals study, protein adsorption ability, and cytotoxicity investigations show no tissue damage, good biomedical properties and no encumbrance with cell cycle (even at a concentration of 80 μg/mL). Moreover, the cell proliferation analysis reveals the non-toxic property of MBG at a concentration of 20 μg/mL. Notably, a cumulative drug (ciprofloxacin, an antibiotic) release of 75% was observed for first 48 h and the further release of 90% was observed over a period of two weeks. The synthesized MBG also shows osteoblast activity and bone mineralization as revealed by alkaline phosphatase activity (ALP) and osteocalcin formation.

1. Introduction

The treatment of musculoskeletal injuries, particularly the complications associated with infirmities of the bones resulting from infections, trauma, tumors, or genetic malformations, still remains a major challenge [1–3]. Furthermore, these problems are expected to increase in future due to continuous increase in the world population. To resolve these problems, various bioactive porous scaffolds including biopolymers, biometals, bioactive glass/ceramics, and their composites have been studied in recent years [4–12]; however, the ability of such bioactive scaffolds in bone regeneration and bone healing is still limited and needs further investigations.

Metallic implants are considered as the first generation of biomaterials having use in orthopedics [13]. Nonetheless, these implants have to be coated with some bioactive material like hydroxyapatite or biodegradable tri-calcium phosphate ceramics due to their biocompatibility issues [14]. Furthermore, these materials are 5–6 folds stiffer than the natural bones and hence cause stress shielding that results in loosening of the implant and sometimes have to be surgically removed from the body. Later, a new biomaterial, namely bioactive glass having osteoconductive and osteoinductive properties was developed by Hench et al. in the late 1960s [15]. Since then, bioactive glasses have attracted considerable attention and are used in regenerative medicines and bone tissue engineering [16]. Bioactive glass not only bonds with living tissues directly when implanted in the body, but also has the ability to enhance cell responses such as cell proliferation, cell adhesion, calcium deposition and alkaline phosphatase activity [13,14]. A variety of methods such as melt-derived [17], sonication [14,18], sol-gel [19] and flame synthesis [20] have been used to prepare bioactive glass. Due to better homogeneity, low cost, porosity, simple processing, high purity and greater surface area, sol-gel is considered to be the superior method [21–24]. More recently, by employing a similar strategy and using a co-block polymer as a soft template, mesoporous bioactive glass (MBG) have been prepared having enhanced biocompatibility due to the greater specific surface area and more pore volume as compared to non-mesoporous bioactive glass [25]. It is highly ordered, porous and multifunctional biomaterial having drug delivery properties in addition to

* Corresponding author.
E-mail address: moazzam@qau.edu.pk (M.M. Naseer).

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their potential in bone regeneration [26,27]. Although, a considerable progress has been made in the last few years, but these new biomaterials still need further exploration to get optimum results.

The rate of in vivo degradation and level of bioactivity of these materials is strongly dependent on the composition: as the rate of resorption can be controlled by changing the composition [28]. In recent years, the doping of bioactive glasses with Mg, Zn, Cu, Sr, Ag and some other elements have been found beneficial, greatly affecting the biological turnover and the process of bones formation [29–34]. As the potassium is one of the important minor components of hard tissues, helps to reduce the chances of osteoporosis in elderly ages and is involved in apatite biomineralization [35], therefore its doping can be an important consideration in improving the biomedical properties. With this in mind, the present study was aimed to improve the biomedical properties, bioactivity and sustained drug release ability of mesoporous bioactive glass. Few modifications in composition were carried out as compared to the 45S5 bioglass. The amount of silica has been increased and the sodium oxide has been replaced partially with potassium oxide (K2O) in order to evaluate any beneficial or anabolic effects of potassium in bone formation. Importantly, K2O has previously been found as a network modifier, increases the surface to volume ratio, helps in fast hydroxyapatite formation and is also an essential component of some commercially available bioactive glasses such as 13–93 and 6P53B [36–38]. The ciprofloxacin which is an extensively used antibiotic for the treatment of osteomyelitis and infections of joints and bones has been used as a model drug for drug release studies. The results of this new K_2O-doped formulation having increased amount of silica (49%) are promising [39,40] and may provide constructive hints for further developments in this research area.

2. Experimental

2.1. Reagents and chemicals

MBG was synthesized by sol-gel protocol and the following precursors were used [41]. Tetraethyl orthosilicate (TEOS), calcium nitrate tetrahydrate $Ca(NO_3)_2 \cdot 4H_2O$, triethyl phosphate (TEP), potassium nitrate KNO_3, ammonium dihydrogen phosphate $NH_4H_2PO_4$, sodium carbonate Na_2CO_3, absolute ethanol C_2H_5OH, polyethylene glycol (PEG6000) (supplied by Sigma-Aldrich Co (USA)). For biochemical and in vivo studies, balb c mice, albumin, fibrinogen, and globulin (Biotechnology grade) were obtained as lyophilized powders from AMRESCO (Ohio, USA). Dulbecco's Modified Eagle Medium (DMEM), trypsin-EDTA (Gibco), Fetal bovine serum (FBS) (Biological Industries), propidium iodide (BD pharmingen), CCK-8 (Dojindo laboratories, Japan) were used. Deionized water, sodium chloride NaCl, sodium bicarbonate $NaHCO_3$, potassium chloride KCl, disodium hydrogen phosphate Na_2HPO_4, magnesium chloride hexahydrate $MgCl_2.6H_2O$, hydrochloric acid HCl, calcium chloride dihydrate $CaCl_2 \cdot 2H_2O$, anhydrous sodium sulphate Na_2SO_4, Tris $[(CH_2OH)_3CNH_2]$ were purchased from Sigma-Aldrich Co (USA) and utilized to prepare SBF.

2.2. Synthesis

MBG with composition ($49SiO_2 \cdot 20CaO \cdot 20Na_2O \cdot 7K_2O \cdot 4P_2O_5$ mol%) was prepared by using polyethylene glycol (PEG 6000) as a soft template. PEG was dissolved in a mixture of water, ethanol, and nitric acid at pH = 2. TEOS was hydrolyzed and stirred in the same solution. The required amounts of all other reagents were added with 40 minute time interval for each one of them and stirred for 1 h at room temperature. This mixture was converted into a sol, which subsequently turned into a gel at pH 11–12, by adding ammonia dropwise. This gel was stirred for 4 h, aged at room temperature for 24 h, at 70 °C for 12 h and 100 °C for further 12 h to remove complete moisture. For removal of organic moieties, this sample was calcined at 700 °C for 4 h. For drug loading purpose 1 g of synthesized MBG was stirred with 50 mL (25 mg/mL) of drug solution for 24 h, followed by filtration. The residue was dried at room temperature and was weighed to find out the percentage loading capacity of MBG. The concentration of drug was calculated in the filtrate to find the percentage of loaded drug and loading efficiency.

$$\text{Drug loading efficiency} = [(C_i - C_f)/C_i] \times 100$$

Where C_i is the initial concentration of drug and C_f is the concentration of drug in filtrate.

3. Characterization of MBG

The specific surface area and pore size distribution of MBG were measured by N_2 adsorption–desorption isotherms using BET Micromeritics Instrument Corp, Gemini V2.0, and BJH (ASAP 2010) analyses. For this purpose, 0.07 g of the sample was degassed for 2 h at 300 °C and the analysis was performed with N_2 adsorption measurement temperature (−196 °C). The low temperature was used to avoid any thermally induced changes. The structural nature of MBG and the formation of HAp layer on its surface was characterized by XRD analysis, using an X-ray diffractometer (PANalytical, X' Pert Pro, Almelo, Netherlands); Cu Kα being used as the radiation source. It was operated at 40 kV with 2θ value varying from 10 to 70. FTIR measurements were performed at Nicolet iS10 FTIR spectrometer. Each sample was analyzed in wave number range of 4000–500 cm^{-1} using FTIR spectrophotometer at room temperature. Particle size, morphology and elemental composition of the samples was analyzed using SEM, coupled with EDX analysis (Hitachi S3400N). Optical microscopy of tissues was performed by laboratory microscope (MEIJI ML 2100) at 40×. The UV/Visible spectrophotometer (Shimadzu UV-265) was used for measuring the concentration of drug solution.

3.1. In vivo testing of bioactive glass

3.1.1. Animal model

Balb c mice were obtained from National Institute of Health (Islamabad). Before the start of experimental dose, the mice were acclimatized to laboratory conditions for three weeks. The mice were fed with crumbled feed and no mortality was observed during this time period. All experiments were performed in compliance with the relevant laws and institutional guidelines.

3.1.2. Experimental design for short term MBG exposure toxicity

The MBG exposure test was performed by injecting the MBG in *mice* for 7 days. Only male mice were selected for the experimental purpose. The animal grouping and dose concentrations for 4 different concentrations of MBG are shown in Table 1. The experimental groups were exposed to MBG for 7 successive days. After the completion of trials, animals were dissected for skin tissue histopathology. The buffer used for this purpose was phosphate buffer saline (PBS).

3.1.3. Histological analysis

The skin tissues of the treated animals were used for histological evaluations. The tissues were placed in formalin overnight,

Table 1
Animal grouping and their dose concentration.

Group no.	No. of animals	Dose concentration	Vehicle
1	10	Control	PBS
2	10	5 mg/kg of body wt.	PBS
3	10	10 mg/kg of body wt.	PBS
4	10	20 mg/kg of body wt.	PBS
5	10	40 mg/kg of body wt.	PBS