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Mesoporous nano-bioglass designed for the release of imatinib and *in vitro* inhibitory effects on cancer cells

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ABSTRACT

For treating bone cancer, controlled drug delivery is an important strategy. Bioactive scaffolds are widely used biomaterials due to their usefulness in localized drug delivery. The aim of this study was to develop mesoporous bioglass (MBG) with improved bioactivity and controllable drug delivery rate. By using pluronic 123 (P123) as a template, a facile sol-gel route was employed for the synthesis of MBG nanoparticles (NPs). The composition of the prepared sample was estimated by using energy dispersive X-ray spectroscopy (EDX). These nanoparticles demonstrated the specific surface area of 310 m^2/g and pore size of 13 nm as measured by brunauer-emmett-teller (BET) and barrett-joyner-halenda (BJH) method, respectively. The spherical shape of NPs was confirmed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Imatinib (IMT); an anti-cancer drug was loaded with the efficiency of 77.59%. The drug release kinetics were precisely controlled by changing the pH (4.4 to 10.4) as well as drug loading concentration (0.2–1.0 mg/mL). The maximum cumulative drug release of 81% was observed over a time period of 250 h at pH of 4.4. Importantly, significant inhibitory effects on the viability of the MG-63 osteocarcinoma cancer cells at 12.19 μg/mL of IMT-MBG were observed. Furthermore, MBG demonstrated ionic dissolution with the release of Ca, K, Si, Na, and P ions upon immersion in simulated body fluid (SBF), which support the formation of hydroxycarbonate apatite (HCA), as confirmed by wide-angle X-ray diffraction (WAXD) pattern and fourier transform infrared (FTIR) spectroscopy. These features proved that IMT-MBG system is effective for bone tissue regeneration and bone cancer treatment.

1. Introduction

Much attention has been focused recently on curing major bone defects resulting from trauma, infections, genetic malformations and tumors [1–3]. Malignant tumors are the most common causes of death in modern society and surgery, immunotherapy, chemotherapy, and radiotherapy are the most commonly used treatment strategies [4,5]. However, none of these conventional methods have attained clinical satisfaction till now but localized drug delivery to bone formation has posed major contest for clinicians [6]. To resolve these problems, porous bioactive scaffolds are widely explored biomaterials due to their efficacy in improving local drug delivery, biodegradability, bone bonding ability, osteogenic capability and excellent bioactivity [1,6–9]. These scaffolds come in contact with physiological fluids when grafted into the body. They bond directly to the bone tissues very rapidly, without inflammation, toxicity, and foreign body response. The rapid reactions take place on the surface of MBG when it comes in contact with human blood plasma or SBF during *in-vivo* and *in-vitro* investigations, respectively [4,5,10,11]. The quick ionic dissolution leads to the formation of HCA layer on the surface of MBG. The released ions trigger gene expression and stimulate osteoblast proliferation for the rapid formation of bones [12]. Sol-gel derived MBG can be synthesized by using templates such as *N*-cetyl trimethylammonium bromide (CTAB), polyethylene glycol (PEG), P123 and F127 [11,13]. It inherently has a high specific surface area as compared to non-mesoporous bioglass with the same composition and consequently, boosts the bioactivity and provides the options of loading drugs and biomolecules [14–16].

In the present study potassium-doped mesoporous bioglass (MBG) has been synthesized by using P123 as a template. Imatinib (IMT) was selected as an anticancer drug because of the ability to cure bone cancer. It is the first line chemotherapeutic drug approved by FDA and has not been reported in drug release systems of bioglass. So the drug loaded MBG system was fabricated and release kinetics at different pH was studied. Inhibitory effects of IMT-MBG on MG-63 osteocarcinoma cancer cells lines were also evaluated. The results of this study may provide positive hints for advances in the field of regenerative medicines. Thus, MBG having an anti-cancer drug might be used locally as implantable drug delivery device, that can deliver targeted action in a more precise style [17–19].

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2. Materials and method

2.1. Materials

All the chemicals were of analytical grade. Ammonia (NH_3, 25%, Merck), Tetraethyl orthosilicate (TEOS, 99%, Fluka), triethyl phosphate (TEP, ≥99.8%, Sigma), calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$, ≥ 99.0%, Sigma), disodium hydrogen phosphate (Na_2HPO_4, ≥98.5%, Sigma), ammonium dihydrogen phosphate (NH_6PO_4, ≥99%, Sigma), potassium nitrate (KNO_3, ≥99.5%, Sigma), sodium carbonate (Na_2CO_3, ≥99%, Sigma), sodium chloride (NaCl, ≥99.99%, Sigma), potassium chloride (KCl, ≥99.99%, Sigma), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$, ≥98.5%, Sigma), anhydrous sodium sulphate (Na_2SO_4, ≥99%, Sigma), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$, ≥99.99%, Sigma), sodium bicarbonate ($NaHCO_3$, ≥99% Sigma), pluronic 123 (P123, average Mn ~1100, Sigma), nitric acid (HNO_3, 11 M, Sigma), ethanol (C_2H_5OH, ≥99%, Sigma), deionized water, tris(hydroxymethyl)aminomethane ($NH_2C(CH_2OH)_3$, ≥99.8%, Sigma), phosphate buffer saline (PBS, 98%, Fluka), osteocarcinoma cell line MG-63.

2.2. Synthesis of MBG

MBG with composition $51SiO_2 \cdot 20CaO \cdot 20Na_2O \cdot 5K_2O \cdot 4P_2O_5$ mol% was prepared by using sol-gel procedure [18,20]. For this purpose, P123 (4 g, 3.6363 mmol) was dissolved in a mixture of 250 mL deionized water, 25 mL of ethanol and 25 mL of 0.1 M HNO_3. TEOS (17.731 g, 85.11 mmol) was added to it and stirred for 1 h at room temperature. Then TEP (0.769 g, 4.22 mmol), $Ca(NO_3)_2.4H_2O$ (8.428 g, 35.689 mmol), KNO_3 (1.202 g, 11.889 mmol) and Na_2CO_3 (3.41 g, 32.17 mmol) were added to this mixture with the time interval of 0.5 h along with continuous stirring [15]. When a sol was formed by hydrolysis of all these reagents, 25% ammonia solution was added dropwise till the formation of a gel. This gel was poured into teflon molds, kept at room temperature for 24 h, then heated at 200 °C for 4 h and at 700 °C for 24 h in the furnace with the heating rate of 10 °C/min to remove moisture and other organic moieties [21,22].

2.3. Preparation of SBF solution

To prepare SBF solution for the biomimetic process, NaCl (6.5456 g, 83.38 mmol) was dissolved for 5 min in 960 mL of deionized water in a glass beaker kept at 37 °C. Then $NaHCO_3$ (2.2682 g, 27 mmol), KCl (0.373 g, 5 mmol), Na_2HPO_4 (0.1419 g, 0.99 mmol), $MgCl_2.6H_2O$ (0.3049 g, 1.499 mmol), 10 mL of 1 M HCl solution, $CaCl_2 \cdot 2H_2O$ (0.3675 g, 2.5 mmol), Na_2SO_4 (0.071 g, 0.5 mmol) and Tris buffer (6.057 g, 50 mmol) were added, stirred and dissolved. Each of this reagent was added with the time interval of 5 min. Then pH electrode was placed into the solution and 30 mL of 1 M HCl was added in small portions until the pH of the solution became 7.4.

2.4. Drug loading to MBG

IMT solutions with concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL were prepared in phosphate buffer saline (PBS). These solutions were shaken at room temperature with 50 mg of MBG in 5 mL of each of the above-prepared solutions. These drug-loaded NPs were filtered, gently washed with deionized water and dried at room temperature. The concentration of loaded drug was calculated by determining the difference of concentration of the solutions before and after loading with the help of UV–vis spectrophotometer (Shimadzu UV-265). UV–vis was scanned from 200 to 700 nm and concentration of all samples was calculated at $\lambda_{max} = 490$ nm for IMT [23–25].

2.5. In vitro drug release studies

Drug loaded MBG was taken in a glass vial, immersed in 3 mL of PBS solution and kept at 37 °C. After pre-determined intervals, 1 mL of the solution was taken out carefully from this mixture and the same amount of fresh PBS was added to it. The concentration of drug was determined in solutions taken out from the vial by using UV–vis spectrometer. Each experiment was performed thrice and the average value was reported.

2.6. Toxicity screening of the MBG and IMT-MBG complex by MTT assay

Osteocarcinoma cell line MG-63 was used to find out the inhibitory effects of MBG and IMT-MBG complex on the viability of these cells. 10,000 cells of MG-63/well were seeded a day prior to the introduction of different concentrations of MBG and IMT-MBG complex to find out the inhibitory effect of MBG alone and with drug loaded. The graded concentrations for the experiment were 0, 3.125, 6.25, 12.5, 25, 50 and 100 μg/mL [26,27]. After 24, 72 and 120 h of the treatment culture medium was removed and 100 μL of 0.5 mg/mL MTT was added to each well. The absorbance of formazan product was measured by the microplate reader (spectra MAX, USA) at 490 nm and inhibitory effect was calculated by Graph Pad prism 6.0.

2.7. Characterizations

Transmission electron microscope JEM-1400 Plus, scanning electron microscope and energy dispersive X-ray (EDX) Hitachi S3400N was used to the determine size, surface morphology, and composition of MBG NPs. Pore size distribution and the specific surface area was determined by Barrett-Joyner-Halenda (BJH) and Brunauer-Emmett-Teller (BET) nitrogen adsorption-desorption isotherms (Micromeritics ASAP 2405N). The ionic (Na, Si, K, Ca and P) concentrations for MBG, when

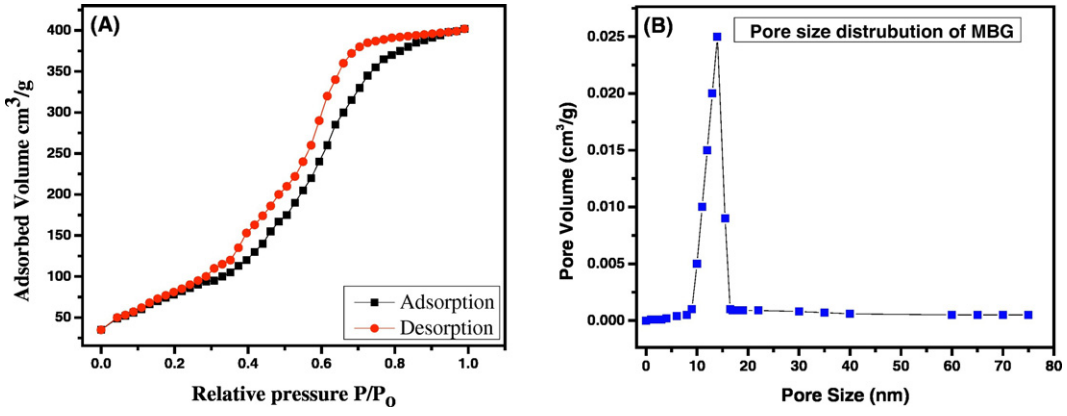

Fig. 1. (A) BET adsorption-desorption curve of MBG (B) pore size distribution of MBG.