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Acid catalysed synthesis of bioactive glass by evaporation induced self assembly method

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ABSTRACT

Bioactive glass (BG) with uniform spherical morphology was prepared by EISA (Evaporation Induced Self Assembly) process using non-ionic Pluronic F127 as structure directing agent and phosphoric acid as a source of P_2O_5 and compared with conventional precursor triethyl phosphate (TEP). EISA method proceeds through ionic interaction mechanism by protonation of surfactant and silicic species. H_3PO_4 provides H^+ and PO_4^{3-} ions in reaction medium which play critical role in reaction mechanism. Hydrogen ions increase the protonation of H_2O and surfactant, while PO_4^{3-} ions act as bridging molecule between different cations, ensuring incorporation of phosphorus in BG network. TEP participates by proton acceptance mechanism, creating competitive environment. Thus H_3PO_4 facilitates the formation of BG in presence of non-ionic surfactant Pluronic F127. The prepared glasses were characterized by FTIR, SEM-EDX, TGA-DSC and BET surface analyzer. Uniform spherical morphology, improved dispersity, relatively large surface area and better cells focal attachment were observed for BG-H_3PO_4, prepared by using H_3PO_4. SiO_2–CaO–P_2O_5 mol% composition of BG-H_3PO_4 was (66:24:10) close to the theoretical value (65:25:10), while for BG-TEP the actual ratio was (77:20.5:2.5). The surface reactivity, studied by soaking in simulated body fluid, showed rapid growth of hydroxyapatite with Ca/P ratio 1.67 on BG-H_3PO_4. The proliferation of MC3T3 cells on BG-H_3PO_4 was remarkably improved as compared to conventional BG. Thus BG-H_3PO_4 can be considered for biomedical applications in future especially for drug loading and composite application where homogeneous and uniform structure are of utmost importance.

1. Introduction

Bioactive glasses (BGs) are the third-generation biomaterials, developed by Larry Hench (BG) in late 1960, with an ability to activate genes that stimulate regeneration of living tissues [1–3]. They have the ability to form chemical bond with bone. The physical and biological properties of BGs depend on their composition, method of preparation and choice of precursors [4]. Evaporation Induced Self Assembly (EISA) method is a modified sol-gel method, in which different polymers (quaternary amines, block co-polymers etc.) are used as a structure directing agent to produce ordered two-dimensional (2D) or three-dimensional (3D) homogeneous structures [5–8]. These structure directing agents increase the surface area of the materials and also influence the final shape and size of the material. Further, the biological properties of bioactive glass also depend on morphology of glasses [9]. Researchers [10,11] have found that the natural precursor and reaction conditions strongly affect the properties of the bioactive glasses. Siqueira et al. [10] has used two different precursors of phosphorous i.e. triethyl phosphate (TEP) and phosphoric acid (H_3PO_4), as a source of P_2O_5 for synthesis of bioactive glass by sol-gel method. They found that phosphoric acid changes the thermal behavior of BG and also rate of hydroxycarbonate apatite formation. In our previously reported study [12], BG has been prepared by hydrothermal method using phosphoric acid as source of P_2O_5. It has been observed that H_3PO_4 in the presence of HNO_3 increases the rate of hydrolysis and reduces the size of sol particles, thus influencing the surface area and in turn rate of apatite formation of bioactive glasses. However, the effect of these precursors on reaction mechanism of bioactive glass in EISA method has not been

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discussed so far. In present study we have discussed the role of phosphoric acid during the synthesis of BG by ionic interaction mechanism.

Bioactive glass have been prepared by EISA method (using non-ionic surfactant pluronic F127 as structure directing agent), which takes place through ionic interaction mechanism. The effect of two different precursors, triethyl phosphate (TEP) and H_3PO_4 on physical and biological properties of bioactive glass have been discussed. Phosphoric acid leads to formation of bioactive glass particles with uniform spherical morphology, more phosphorous contents and higher surface area as compared to glass prepared by using TEP. The final composition of glass depends on nature of the precursor due to different dissociation constants and reaction mechanism, when prepared under same reaction conditions. The uniform particle size distribution, homogeneity and large surface in mesoporous materials are the properties of utmost importance for drug delivery, coating and composite applications of biomaterials.

2. Materials and methods

All the precursors including Tetraethyl orthosilicate (TEOS, 98%), triethyl phosphate (TEP, 99%), phosphoric acid (85%), nitric acid (HNO_3), calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$, 99%), Pluronic F127 ($EO_{106}PO_{70}EO_{106}$) and ethanol were purchased from Sigma Aldrich (UK) and were of analytical grade.

2.1. Preparation of BG through EISA method

BG with theoretical composition $65SiO_2$–$25CaO$–$10P_2O_5$ (mol%) was synthesized through EISA process. Non-ionic surfactant Pluronic F127 ($EO_{106}PO_{70}EO_{106}$) was used as structure directing agent. 2.88 g of Pluronic F127 was dissolved in ethanol; while marinating pH at 2 using 2 M HNO_3. Then 26.9 mmol of TEOS, 5.4 mmol of phosphoric acid (H_3PO_4) and 11.0 mmol of $Ca(NO_3)_2.4H_2O$ were dissolved in ethanol separately. These solutions were mixed with Pluronic F127 solution slowly, with 4 h stirring interval after each addition. The resulting solution was dried in a petri-dish under ambient conditions to get transparent gel. The resultant transparent membrane was finely grind and calcined at different temperatures (600 °C & 700 °C) for 6 h (at heating rate of 2 °C·min^{-1}). The prepared sample was labelled as BG-H_3PO_4. BG of same composition was also prepared using conventional TEP as source of P_2O_5 and the sample was designated as BG-TEP.

2.2. In vitro bioactivity test

The in vitro bioactivity was studied by immersing the powdered bioactive glass samples (BG-H_3PO_4 and BG-TEP) in Simulated Body Fluid (SBF) solution for different time intervals as reported by [13]. SBF preparation was previously reported by Kokubo and Takadama [14]. Each sample was run in triplicate. The samples were immersed in SBF solution (75 mg of bioactive glass in 50 mL SBF). The samples were incubated at 37 °C for different time points: 0 h, 1 day, 7 days, 14 days and 21 days. At the end of each time period, the samples were removed from the incubator and the powder was collected using centrifuge. The powder was immediately washed with de-ionized water three times and subsequently with acetone. The hydroxyapatite layer formation was investigated by SEM-EDX and FTIR spectroscopy.

2.3. Cell culture

2.3.1. Sample preparation for cell culture

Powdered samples were first sterilized with ethanol followed by washing with PBS solution. DMEM was supplemented with 10% Fetal Calf Serum (FCS, Sigma Aldrich, UK), 2 mM L-glutamine (Sigma Aldrich, UK), 100 µg/mL of penicillin and streptomycin (Sigma Aldrich, UK)). The appropriate dilutions of sample particles were made for the various concentrations of particles in tissue culture medium. These dilutions were added in Transwell® (Corning®, Sigma Aldrich, 6.5 mm transwell) permeable with Millipore filter (8 µm pore polycarbonate membrane) at bottom.

2.3.2. In vitro cell proliferation

Cell proliferation was studied by Alamar blue assays using mouse osteoblast cell line (MC3T3-E1) cells cultured till they were 90% confluent before seeding. Cell lines were used between passage 3 to 4 in DMEM and seeded in 24-well plates. The transwells containing samples were placed in these culture plate wells in triplicate. MC3T3 were seeded into each well at a population of 20,000 cells per well and allowed to attach for 24 h before placing the transwells. The cell proliferation was determined by Alamar Blue™ assay. The cell viability was determined by measuring fluorescence of Alamar Blue after day 1, day 4, day 7 and days 14 of culturing with MC3T3 cells. For each time period, samples seeded with cells were washed with PBS (phosphate buffered saline) tablets. Then Alamar blue® solution (Sigma Aldrich) diluted PBS (1:10) was added into the cell seeded sample and incubated at 37 °C for 4 h. The Fluorescence plate reader (Bio-TEK, NorthStar Scientific Ltd) was used to measure the fluorescence at 570 nm. The control group was the tissue culture plastic (TCP) and the readings from the samples were subtracted from TCP to obtain the exact absorbance of proliferating cells on specimens.

2.4. Statistical analysis

The biological experiment was conducted in triplicates. All presented data referred to mean ± standard deviation (SD). 'One-way ANOVA' followed by Tukey's post-hoc test was performed to check the significant statistical difference in results. Results with p-values of ≤ 0.05 (*) had been taken as statistically significant. The data was analysed using software 'Graphpad Prism 5.0'.

2.5. Characterization techniques

The morphology and elemental composition of bioactive glass samples were done using Scanning electron microscope (SEM) from TESCAN Vega3 LMU with built-in x-act Energy dispersive X-ray detector (EDX). Before imaging, the samples were coated with gold for 90 s using a sputter coater (Quorum Technologies). The EDX analysis was performed on uncoated samples using an acceleration voltage of 20 kV and beam intensity of 10 pA. SEM images were taken at voltage of 15 kV and a beam intensity of 4 pA.

Structural analysis was done by Fourier Transform Infra-Red Spectrometer 'FTIR-Nicolet 6700' with a photo-acoustic cell to identify the changes that may occur before and after calcination of glasses. Both calcined (at 600 °C) and non-calcined samples were analysed by FTIR within 4000–400 cm^{-1} (mid-range IR) at 4 cm^{-1} resolution and 256 number of scans.

Thermal properties of the glasses were analysed by Thermogravimetric Analysis and Differential Scanning Calorimetric (TGA-DSC), using 'Q600 SDT, TA Instruments, US' within a temperature range of 25 °C to 1000 °C (heating rate of 5 °C·min^{-1}) under nitrogen environment.

Surface area was measured with BET nitrogen gas adsorption-desorption method using 'Micromeritics New TriStar II Surface Area and Porosity system'. The samples were first degassed at 300 °C (heating ramp 10 °C/min) under nitrogen flow for 4 h before measurements. BJH method was used for calculations of pore volume and pore size distribution measurements.

3. Results

Surface morphology of bioactive glasses was done at two different temperatures (i.e. 600 °C and 700 °C) to investigate the effect of temperature on morphology due to different thermal behavior (discussed in