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Crack-free polydimethylsiloxane–bioactive glass–poly(ethylene glycol) hybrid monoliths with controlled biomineralization activity and mechanical property for bone tissue regeneration

Jing Chen [a], Yuzhang Du [a], Wenxiu Que [a,*], Yonglei Xing [a], Xiaofeng Chen [c], Bo Lei [a,b,c,**]

[a] Electronic Materials Research Laboratory, Key Laboratory of the Ministry of Education, International Center for Dielectric Research, Frontier Institute of Science and Technology, Xi'an Jiaotong University, Xi'an 710049, China
[b] State Key Laboratory for Manufacturing Systems Engineering, Xi'an Jiaotong University, Xi'an 710054, China
[c] National Engineering Research Center for Tissue Restoration and Reconstruction, Guangzhou 510006, China

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ABSTRACT

Crack-free organic–inorganic hybrid monoliths with controlled biomineralization activity and mechanical property have an important role for highly efficient bone tissue regeneration. Here, biomimetic and crack-free polydimethylsiloxane (PDMS)–modified bioactive glass (BG)–poly(ethylene glycol) (PEG) (PDMS–BG–PEG) hybrids monoliths were prepared by a facile sol–gel technique. Results indicate that under the assist of co-solvents, BG sol and PDMS and PEG could be hybridized at a molecular level, and effects of the PEG molecular weight on the structure, biomineralization activity, and mechanical property of the as-prepared hybrid monoliths were also investigated in detail. It is found that an addition of low molecular weight PEG can significantly prevent the formation of cracks and speed up the gelation of the hybrid monoliths, and the surface microstructure of the hybrid monoliths can be changed from the porous to the smooth as the PEG molecular weight increases. Additionally, the hybrid monoliths with low molecular weight PEG show the high formation of the biological apatite layer, while the hybrids with high molecular weight PEG exhibit negligible biomineralization ability in simulated body fluid (SBF). Furthermore, the PDMS–BG–PEG 600 hybrid monolith has significantly high compressive strength (32 ± 3 MPa) and modulus (153 ± 11 MPa), as well as good cell biocompatibility by supporting osteoblast (MC3T3-E1) attachment and proliferation. These results indicate that the as-prepared PDMS–BG–PEG hybrid monoliths may have promising applications for bone tissue regeneration.

1. Introduction

Since development of the bioactive materials by Hench and Polak, a large of bioactive glass, glass-ceramics, hydroxyapatite and calcium phosphate ceramics have been widely studied for potential applications in bone tissue repair and regeneration [1]. Among these biomaterials, bioactive glasses (BG) have been accepted as excellent bone regeneration materials due to their high osteoconductivity and osteoproductivity ability [2,3]. In addition, the bone regeneration ability of BG has been considered to be related with the formation of bone-like apatite layer when placed in simulated body fluid (SBF) [4]. Hence, many studies have focused on developing BG-based biomaterials with good biomineralization activity for enhanced bone tissue regeneration.

Native bone tissues possess excellent mechanical and biological properties due to their representative inorganic–polymer hybrid composition at a molecular scale. However, conventional BG and ceramics usually possess relatively poor mechanical properties, such as brittleness, which limits their wide applications in bone tissue regeneration [5]. Therefore, developing BG–polymer hybrid biomaterials with controlled structure and properties in recent years has been attracted considerable attention in the field of bone tissue regeneration [6–8]. Actually, the hybrid biomaterials should

* Corresponding author. Fax: +86 29 82668794.
** Corresponding author at: Electronic Materials Research Laboratory, Key Laboratory of the Ministry of Education, International Center for Dielectric Research, Frontier Institute of Science and Technology, Xi'an Jiaotong University, Xi'an 710049, China. Fax: +86 29 82668794.
E-mail addresses: wxque@mail.xjtu.edu.cn (W. Que), rayboo@xjtu.edu.cn (B. Lei).

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have a typical inorganic–organic structure in which inorganic phase could be dispersed in organic matrix at a molecular level. The hybrids should also possess a good structure stability and biomineralization activity in SBF. Based on these concepts, the BG sol-based gelatin, chitosan and poly(caprolactone) hybrid biomaterials have been developed and investigated as a potential material for bone tissue engineering applications [9–11]. However, due to the significant difference between silica-based sol and polymer phase, the resulted hybrids had either low mechanical property or poor biomineralization activity [9,10]. Therefore, fabrication of the BG-based polymer hybrid biomaterials with good mechanical property and biomineralization activity is still necessary and important.

Due to the high biocompatibility and good hemocompatibility, the polydimethylsiloxane (PDMS)-based materials have been widely used as the implanted devices *in vitro* and *in vivo* [12–14]. In addition, the PDMS-based materials also showed a good mechanical strength as compared to other polymers. In our previous study, crack-free BG monoliths could be fabricated at mild conditions by adding PDMS with a low molecular weight into BG sol [15]. PDMS phase could be hybridized with BG phase at a molecular level. However, pure PDMS–BG hybrids usually possess a brittle mechanical property and are very easy to be crumble.

Here, the biocompatible poly(ethylene glycol) (PEG) was added into the PDMS–BG hybrid system to improve its mechanical property (toughness) and retain the high biomineralization activity. It is also expected that PEG incorporation can improve the hydration ability, increase the contact area with SBF and then show high biomineralization activity. Furthermore, effects of the PEG incorporation on structural property, mechanical property, biomineralization activity and osteoblast cell biocompatibility of the PDMS–BG–PEG hybrid monoliths were also studied.

2. Experimental

2.1. Fabrication of the PDMS–BG–PEG hybrid monoliths

Tetraethoxysilane (TEOS, $Si(OC_2H_5)_4$), calcium nitrite ($Ca(NO_3)_2{\cdot}4H_2O$) and hydrochloric acid (HCl, 35%) were used as starting inorganic constituents. Polyethylene glycol (weight-average molecular weight includes 300, 600, 1000, 4000 and 20,000) (Guanghua chemical factory, China) and polydimethylsiloxane (PDMS, HO-[$Si(CH_3)_2$-O-]$_n$H, Alfa, USA) with an average molecular weight of 1100 were used as organic components. First, TEOS of 4.166 g was pre-hydrolyzed for 15 min in aqueous solution containing tetrahydrofuran (THF) of 0.684 g and isopropyl alcohol (IPA) of 0.7813 g, 35% HCl of 0.075 g, together with distilled water of 0.36 g. Then, PDMS of 1.788 g was added to the solution until the catalysis and hydrolysis reaction for 2 h. After an additional stirring for 20 h, Ca $(NO_3)_2{\cdot}4H_2O$, H_2O and IPA were mixed with the solution for 1 h, followed by adding predetermined contents of the PEG 300, PEG 600, PEG 1000, PEG 4000 and PEG 20,000 were separately added into the solution. Then, the mixed solution was stirred for 3 h again, aged in a covered plastic container for 24 h at ambient condition. Subsequently, the resulted hybrid gels were kept at 28 °C for 7 days and then dried at 60 °C for 3 days. Finally, the PDMS–BG–PEG hybrid monoliths were heated at 100 °C for 1 day. The calculated weight percent of BG for all samples was about 42 wt% (similar with each other).

2.2. Morphology and structure characterizations

Chemical structure of the specimens was characterized using Fourier transform infrared (FTIR) absorption spectroscopy (FTIR, Vetex70) with KBr tablet method. The crystalline phase composition of the as-prepared samples was examined by X-ray diffractometer (XRD, D/MAX-2400) with Cu Kα radiation, at 40 kV and 30 mA, from 20 to 70° at a scanning rate of 0.02°/s with a step of 0.02°. The surface morphology, microstructure and element composition of the specimens were examined by scanning electron microscopy (SEM, JEOL JSM-6390) equipped with Energy Disperse Spectra (EDS).

2.3. Evaluation of biominerialization evaluation in simulated body fluid (SBF)

The *in vitro* biomineralization activity of the as-prepared hybrid monoliths was evaluated through monitoring the formation of the apatite layer on the surface of the hybrid monoliths in SBF [16]. Briefly, the specimens of 10 mm×10 mm×2 mm in size were polished with a # 1500 abrasive disk, washed with distilled water and dried at 40 °C. The SBF which has a similar composition to human blood plasma (an inorganic ion concentrations in mM: Na^+ 142, K^+ 5.0, Mg^{2+} 1.5, Ca^{2+} 2.5, Cl^- 147.8, HCO_3^- 4.2, HPO_4^{2-} 1.0, SO_4^{2-} 0.5) was prepared by dissolving reagents including NaCl, $NaHCO_3$, KCl, $K_2HPO_3{\cdot}3H_2O$, $MgCl_2{\cdot}6H_2O$, $CaCl_2$ and Na_2SO_4 into distilled water and buffering to a pH value of 7.4 at 36.5 °C by adding $(CH_2OH)_3CNH_2$ and 1 M HCl. The specimens were then soaked in 30 ml SBF at 37 °C for various periods. After the soaking, all samples were washed with distilled water and dried at 40 °C for 1 day. The formed apatite layer on the surface of samples was studied by FTIR, XRD and SEM-EDS. Element composition of the apatite layer was analyzed using the electronic dispersive spectrometer (EDS) equipped on the SEM.

2.4. Compressive mechanical properties measurement

Mechanical properties of the hybrid monoliths were carried out by testing the compression strength using a mechanical testing machine (Roell Z005, Zwick, Germany). Compressive stress-strain curves were recorded by mechanical analysis software at a crosshead speed of 1 mm min^{-1}. All measurements were made at room temperature in air. Six specimens were tested for each composition. The Young's modulus was determined from the slop of the initial linear elastic portion of the load-deflection curve, loading geometry and specimen dimensions on the basis of the simple linear elastic beam analysis. At least five species per sample were measured. The obtained results were represented as average value and standard deviation (SD).

2.5. Cell biocompatibility investigation of the hybrid monoliths

The osteoblast cell line (MC3T3-E1) was used to investigate the cell biocompatibility of the hybrid monoliths. Cell culture was carried out under standard growth medium in a humidified atmosphere with 5% CO_2. Before cell being seeded, the hybrid monoliths were cut into a size of 10 mm × 10 mm and sterilized with ultraviolet (UV) irradiation for 30 min. Cells were seeded on the surfaces of the hybrid monoliths with a density of 5000 cells per well. Cell proliferation was evaluated by using a commercial AlamarBlue™ assay kit (Life Technologies). The final fluorescent intensity of the cell solution after incubation with Alamar Blue kit was recorded by a microplate reader (Molecular Devices, USA) according to the instruction book. At least 5 species per sample were measured to obtain mean value and standard deviation (SD). The adhesion and morphology of the cells were analyzed by using LIVE/DEAD viability kit (Molecular Probes). The staining procedure was according to the manufacture instruction. The cell morphology was observed by using a fluorescence microscope (IX53, Olympus). Tissue culture plate (TCP) was used as a control. At least five species per sample were tested for obtaining the mean and standard deviation.