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Fabrication, characterization and osteoblast responses of poly (octanediol citrate)/bioglass nanofiber composites

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ABSTRACT

In this study, a poly (octanediol citrate) (POC)/bioglass nanofiber composite was prepared by incorporating electrospun bioglass nanofiber (5, 10, and 15% w/w) into the POC matrix. The bioglass nanofiber interacted with POC via physical adsorption and carboxylate formation, and thus the addition of bioglass nanofiber increased the glass transition temperature, modulus and strength; however, decreased the elongation at break when the amount of bioglass nanofiber was up to 15% w/w. Compared to the pure POC elastomer, the POC/bioglass nanofiber composites exhibited accelerated stimulation to the mouse bone marrow mesenchymal stem cells (MSCs) on the cell growth and osteogenic differentiation. The level of alkaline phosphatase activity and cellular mineralization was higher for the POC/bioglass composites compared to the pure POC and increased with increasing amount of bioglass nanofiber within 14-day culture period. The gene expression of collagen type I and osteocalcin was also proportional to the bioglass nanofiber content of the composite. The results of this study showed that the composite of POC/bioglass nanofiber supported the osteogenic differentiation of MSCs and would be an excellent biomaterial candidate for applications in bone regeneration.

1. Introduction

Developing a new, inexpensive, and widely sourced substrate for bone regeneration is a challenging task, because of increasing number of patients suffering from bone defects in recent years, but the supplements of autograft and allograft are not adequate to the demand [1,2]. Despite a number of reports focusing on biodegradable materials such as absorbable polymers and bioactive ceramics, the low mechanical properties of polymers and inherent brittleness of ceramics inhibit their applications alone in hard tissue engineering [3–5]. Recently, various inorganic nanomaterials, including nanoparticles, nanowires and nanofibers with different morphologies and functions, have appeared continuously and been applied in the field of drug delivery, therapy, sensors, etc. [6–12]. It is a suitable approach to solve the above problem by inducing the inorganic nanomaterials into polymer matrix, because the composites of biodegradable polymers and inorganic phases can combine their merits and address the outages, and similar reports have been published and exhibit excellent effect [13–15].

Over the past years, absorbable polymers have always been widely utilized as a matrix of the composites owing to their special characteristics such as good biocompatibility, controllable biodegradability, and ease of processing [16–20]. In particular, poly (octanediol citrate) (POC) as a biodegradable elastomer has attracted significant attention in tissue engineering [21–24]. Although it is a thermoset polyester, its pre-polymer can be dissolved in normal solvents such as ethanol, making it suitable for various processing methods [22]. It is prepared from the polycondensation reaction of citric acid and 1,8-octanediol, thus it has a non-toxic degradation product. In addition, the cross-linking density of POC can be altered by tuning the initial monomer molar ratio, curing time or temperature, leading to a broad range of mechanical properties and degradation rate [21]. Moreover, the free carboxylic groups from citric acid facilitate the interaction with cells, enhancing the compatibility to various types of cells such as myoblasts, osteoblasts, and articular chondrocytes [25].

However, it is not suitable for hard tissue engineering because of its inferior mechanical properties. Several techniques have been investigated to yield the mechanical integrity, and the composite of POC with ceramic fillers exhibits excellent merits because the fillers such as hydroxyapatite, β-tricalcium phosphate, and bioactive glass possess the similar component to bone and thus can improve the interaction with the cells [26–29]. Particularly, bioactive glasses have special advantages than other fillers in hard tissue engineering. Bioactive glasses have excellent osteoconductive and osteoinductive properties and can bond to both soft and hard tissues and have a positive effect on the cell migration and differentiation. More importantly, the released ions from bioactive glass would stimulate the expression of genes of osteoblastic

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cells and further promote the differentiation of osteoblastic cells and mesenchymal stem cells. Although there are several reports on the composite of POC-bioactive glass, the fillers of bioactive glass were irregular with the size of tens to hundreds of microns [29–31]. The properties of composite are very sensitive to the size of the filler, and the studies reported that nano-filler is more effective for enhancing the mechanical property and biocompatibility in comparison to micro-filler [32]. Bioactive glass nanofiber prepared by electrospinning is favorite filler for various polymers owing to its large surface to volume ratio, high bioactivity, and specific shape. Furthermore, the nanofibrous bioactive glass showed good dispersion in the matrix, whereas nanoparticles always agglomerate within the matrix [33,34].

In this study, POC/bioglass nanofiber composites were fabricated by incorporating the bioglass nanofibers obtained by electrospinning into the POC elastomer, aiming to improve the mechanical and biological properties to match the hard tissue. In particular, bioglass electrospun nanofiber was accurately tailored into short fibers, because of higher dispersion of short fibers in the matrix in comparison to long fibers. Furthermore, MSCs were induced to observe the effect of the composites on osteogenic differentiation of cell in vitro.

2. Materials and methods

2.1. Materials

Poly (vinyl pyrrolidone) (PVP, 1,300,000 Da), citric acid and 1,8-octanediol were purchased from Aladdin Co. Ltd.(China). Calcium nitrate tetrahydrate, tetraethyl orthosilicate and triethylphosphate were purchased from Maya Reagent Company (China). All the other chemicals were of analytical reagent grade.

2.2. Fabrication process

Bioglass ($70SiO_2{\cdot}25CaO{\cdot}5P_2O_5$) precursor solution was prepared by mixing tetraethyl orthosilicate (3.35 g), calcium nitratetetrahydrate (0.79 g), and triethyl phosphate (0.29 g) in 25 mL of ethanol, followed by adding hydrochloric acid (HCl, 1 mol/L) as the catalyst in the solution at a volume ratio of 1:50, and the reaction mixture was stirred continuously for 12 h followed by aging for 48 h at 40 °C. Subsequently, the bioglass precursor solution was mixed with PVP/ethanol solution (0.08 g/mL) at a volume ratio of 1:1 to form a homogeneous electrospinning solution.

The electrospinning apparatus comprised a high voltage power supply, an infusion pump, a rolling drum collector, and a plastic syringe fitted with a stainless-steel blunt needle of 21G size. The solution was injected using a needle at a flow rate of 0.5 mL/h, and 8 kV voltage was applied to form a jet, which was flying to the rolling drum (3000 rpm) and solidified to a oriented fibrous membrane. The fibrous membrane was vacuum dried for 24 h at 40 °C, followed by calcination at 600 °C for 5 h. After cooling to room temperature, the aligned bioglass electrospun nanofibers were taken out and cut into short fibers with a length of ~5 mm. The short fibers were collected and stored in a dry environment.

The POC pre-polymer was synthesized following the literature procedure [21–24]. Briefly, a mixture of 1:1 M ratio of citric acid and 1,8-octanediol was melted at 160 °C for 15 min under nitrogen atmosphere, followed by stirring at 140 °C for 45 min. Subsequently, different amounts of bioglass short nanofibers (5, 10, and 15% w/w) were dispersed in the POC pre-polymer/ethanol solution (20% w/v) by stirring and ultrasonication to obtain a series of composite solutions. In addition, the composite solutions were transferred into Teflon dishes to evaporate the solvent, and then the dishes were post-polymerized at 80 °C for 3 days in an air dry oven, followed by 120 °C for 1 day in a vacuum drying oven. The composite films were peeled from the dishes and stored at 4 °C, prior to use.

2.3. Characterization

The morphology of the electrospun bioglass fiber and composite films were observed by scanning electron microscopy (SEM) (FE-SEM Model JSM-7011F, Japan) at an accelerating voltage of 20 kV, and all the samples were coated with gold to produce a conductive surface prior to characterization. The diameters of resulting fibers were analyzed using software Image J ($n = 5$).

Chemical characteristics of the bioglass nanofibers and composite films were evaluated by an attenuated total reflection Fourier transform infrared (ATR-FT-IR) spectrophotometer (Nicolet iS5, USA). The spectra were obtained in the range of 400–4000 cm^{-1} with a resolution of 4.0 cm^{-1} and 32 scans.

X-ray diffraction analysis was characterized by X-ray diffractometer (Bruker D8 Advance, Germany) equipped with Cu-Kα source and operating at 40 kV and 100 mA. The diffraction patterns were obtained at a scan rate of 1°/min.

The thermal properties of all samples were investigated by the differential scanning calorimetry (DSC) measurements (Perkin-Elmer Company, USA) in a temperature range from − 50 °C–50 °C at a heating rate of 5 °C/min under a nitrogen atmosphere. Samples were loaded in an aluminum crucible under dry condition.

Mechanical properties of the composites were characterized using a uni-axial testing machine (Instron 3365) with a 100 N load cell. The cross-head speed was set at 10 mm/min. All the samples were cut into rectangular shape with two-dimensions of 40 × 5 mm^2 and vertically mounted on two gripping units of the tester. The sample thicknesses were measured with a micrometer having a precision of 1 μm. At least five samples were tested for each sample.

2.4. Cell culture

Green fluorescent protein (GFP) positive mouse bone marrow mesenchymal stem cells (MSCs) (Sidansai Biotechnology Company, China) were cultured in α-modified minimum essential medium (α-MEM) with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C with 5% CO_2 in a humidified incubator. Prior to cell seeded, the composite samples were cut into small disks with the similar diameter to the plate well, and then placed on the bottom of the wells. Subsequently, the plate was transferred to super-clean bench for UV irradiation for 24 h. The cells were trypsinized and counted using a hemocytometer after reaching 80–90% confluence and seeded on the samples (after UV sterilization) with a density of 1 × 10^4 cell/well. The MSCs cultured after 1 and 3 days were observed by fluorescence microscopy (Olympus BX53, Japan).

2.5. MTT assay

At the designed time (1, 3, 5 day) the cells were incubated in MTT (5 mg/mL) for 4 h in 5% CO_2 at 37 °C. Then 100 μL sodium dodecyl sulfate (10% w/w SDS in 0.01 M HCl) was added in each well and incubated for day and night to dissolve the internalized purple formazan crystals. The absorbance was measured at 570 nm with a reference wavelength of 630 nm by microplate reader (Bio-RAD 680, Bio-rad Co., USA).

2.6. Calcium content

The calcium content was measured following the literature report [35]. Prior to the measurement, the standard curve was established by adding ortho-cresolphthalein complexone to a series of calcium solution and measuring the solution at 575 nm using a microplate reader. After that, the cell layer was collected from the sample after culturing for 3, 7, and 14 days, and shaken in the dilute HCl solution for 6 h. Subsequently, the supernatant from centrifugal separation was collected for measurement using a microplate reader (Bio-RAD680), and the calcium