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Physico-chemical and biological studies on three-dimensional porous silk/spray-dried mesoporous bioactive glass scaffolds

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ABSTRACT

The incorporation of a bioactive inorganic phase in polymeric scaffolds is a good strategy for the improvement of the bioactivity and the mechanical properties, which represent crucial features in the field of bone tissue engineering. In this study, spray-dried mesoporous bioactive glass particles (SD-MBG), belonging to the binary system of SiO_2-CaO (80:20 mol%), were used to prepare composite scaffolds by freeze-drying technique, using a silk fibroin matrix. The physico-chemical and biological properties of the scaffolds were extensively studied. The scaffolds showed a highly interconnected porosity with a mean pore size in the range of 150 μm for both pure silk and silk/SD-MBG scaffolds. The elastic moduli of the silk and silk/SD-MBG scaffolds were 1.1 ± 0.2 MPa and 6.9 ± 1.0 MPa and compressive strength were 0.5 ± 0.05 MPa and 0.9 ± 0.2 MPa, respectively, showing a noticeable increase of the mechanical properties of the composite scaffolds compared to the silk ones. The contact angle value decreased from 105.3° to 71.2° with the incorporation of SD-MBG particles. Moreover, the SD-MBG incorporation countered the lack of bioactivity of the silk scaffolds inducing the precipitation of hydroxyapatite layer on their surface already after 1 day of incubation in simulated body fluid. The composite scaffolds showed good biocompatibility and a good alkaline phosphatase activity toward human mesenchymal stromal cells, showing the ability for their use as three-dimensional constructs for bone tissue engineering.

1. Introduction

Bone is a connective tissue with high mechanical resistance derived by its unique design, in which cells are encased in a composite matrix essentially formed by inorganic apatite mineral phase and the organic phase of collagen [1]. The field of bone tissue engineering relies on biomaterial constructs that can offer a valid alternative for the current gold standard treatment i.e. autologous grafts. Scaffolds act as an engineered three dimensional (3D) porous structure that mediates cellular behavior and function, stimulating the replacement and regeneration of injured tissue and delivering biological molecules to the site of interest [2]. The design of bioartificial scaffolds which can combine the properties of organic and inorganic materials, is the aim of the biomimetic strategy in the field of bone tissue engineering [3].

Bombyx mori silk is also termed as mulberry silk. It is made up of globular glue like hydrophilic sericin coated over silk fibroin fibers. The sericin is a highly hydrophilic protein, which covers the silk fibroin fibers. It consists of 18 amino acids and has a molecular weight ranging from 24 to 400 kDa. The high solubility of sericin in aqueous solution limits its application in biomedical field [4,5]. Biopolymers have a low mechanical stability, hence improving the mechanical properties of the biopolymers is essential. Silk fibroin from *B. mori* is a natural biopolymer made up of 45.9% glycine, 30.3% alanine, 12.1% serine, 5.3% tyrosine, 1.8% valine, and only 4.7% of the other 15 amino acid types [6]. The recognized ability of silk fibroin for the fabrication of scaffolds, both by aqueous and organic solvents, made it a special material in tissue engineering as it is one of the few FDA approved biomaterial [7]. Silk fibroin is

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widely used in biomedical applications both in the form of films [8] and 3D constructs [9], for different applications such as micropatterning [10] and microfluidics [11], implants [12] and drug delivery vehicles [13]. The ß-sheet content and morphology of silk fibroin can be controlled by different post processing techniques such as ethanol treatment /autoclaving and the duration of these treatments allows the modulation of the crystal composition, structure, mechanical properties and the degradation rate. [14] However, the lack of bioactivity limited its use for the production of device for bone repair.

To overcome the lack of bioactivity, silk can be combined with inorganic bioactive materials such as calcium phosphate, hydroxyapatite and bioglass. Silk/nano calcium phosphate based composite scaffolds has shown good *in vitro* biocompatibility [15] and no degree of inflammation after 3 weeks of implantation *in vivo* with bone cells growing directly on the surface of the scaffolds [16]. Silk/hydroxyapatite composite scaffolds acted as a platform for the formation of new bone tissue thanks to the osteoconductivity of the material that resulted in increased bone matrix production and secondly, by providing nucleation sites for the newly formed minerals [17,18]. On the other hand mesoporous bioactive glasses (MBGs) has been proposed for the preparation of silk-based composite scaffolds [19–22].

MBGs, developed in 2004 by Yan et al. [23], are glasses with composition similar to the conventional sol-gel glasses (SiO_2-CaO-P_2O_5 or SiO_2-CaO) that present ordered mesoporous channels (pore size ranging from 5 to 20 nm). Due to their high surface area and high pore volume MBGs induce accelerated bioactive responses and show the possibility to incorporate drug molecules, that can be subsequently released [20,21]. In the last decade, the role of mesoporous materials became significant in biomedical field and they were utilized as an effective drug delivery system [24] and as bone fillers [25]. Low mechanical strength, high brittleness and fast degradation rate are the major limiting factors that affect their employment in bone repair. MBGs have been conventionally produced by the combination of sol-gel technique, surfactant templating and evaporation induced self assembly (EISA), requiring a final step of grinding and sieving to obtain them in form of irregular shaped powders [19,21,22,26].

Wu et al. coated MBG scaffolds using silk, by incubating the MBG (molar ratio: Si/Ca/P=80/15/5) scaffolds in silk solution, showing improved attachment, proliferation and differentiation of Bone Mesenchymal Stem Cells (BMSC). The stable ß-sheet structure of silk fibroin improved the relatively unstable MBG interface to support BMSC [21]. The *in vivo* osteogenic capacity of the same composite silk/MBG scaffolds was excellent in calvarial defects of mice [20]. A similar scaffold was utilized in calvarial defects to investigate drug delivery showing enhanced drug loading efficiency and release rate *in vitro* and promoted bone formation *in vivo* [27].

There is vast research interest in exploiting high surface area inorganic materials to improve the mechanical and biological property of composite materials. The chemical composition and shape of the inorganic phase play an important role on the final composite scaffold properties. The needle shaped calcium phosphate minerals improved the bioactivity and mechanical property of polymer based scaffolds [28]. Likewise, nanofiber morphology of bioglass showed to have greater bioactivity and mechanical stability compared with conventional bioglass [29]. Interestingly, spherical shaped bioactive glass microspheres showed to improve the mechanical and bioactivity of polymeric 2D structures [30]. Very recently aerosol-assisted methods have been proposed for the synthesis of mesoporous bioactive glass particles in the form of highly reproducible spherical shaped micron sized particles. The mean size and size distribution can be tuned acting on the composition and process parameters, in contrast to MBGs obtained by conventional EISA method, which are obtained with more time-consuming processes and result in glass powders with irregular shape and larger, not homogeneous size after grinding [31]. The prepared spray dried mesoporous bioactive glass particles (80:20 mol% SiO_2:CaO) have proved to improve the mechanical strength and the bioactivity of an injectable composite cement based on a calcium sulphate matrix [32].

In the present work, spray-dried mesoporous bioactive glass spherical particles with size in the range of few microns with narrow size distribution, prepared by combining sol-gel process and aerosol assisted spray-drying technique [25] (SD-MBG, 80:20 mol% of SiO_2:CaO), were used for the first time to prepare composite silk-based scaffolds by freeze-drying. The spherical SD-MBG particles were used as the reinforcing inorganic phase in the polymer matrix. The possibility to control the particle morphology (i.e. size and shape) and its mesostructure is important for the development of more reproducible materials to be used as drug delivery system or as reinforcing phase in composite materials. The silk/SD-MBG composite scaffolds 10:1 (weight/weight) (Silk/SD-MBG) were characterized for their physico-chemical and mechanical properties. The bioactivity and biocompatibility of the composite scaffolds were also studied to evaluate the efficacy of the construct for bone tissue engineering.

2. Materials and methods

2.1. Materials

Silk cocoons of *B. mori* were obtained from the Department of Sericulture, University of Mysore, Karnataka. Sodium carbonate (Na_2CO_3, $\geq$99.5%), calcium chloride ($CaCl_2$, $\geq$95%), ethanol (C_2H_5OH, $\geq$99.8%) and dialysis tubing cellulose membrane (MWCO - 14,000) were purchased from Sigma-Aldrich. All chemicals used for SD-MBG synthesis were purchased from Sigma-Aldrich. All materials and chemicals were used as received, without any additional purification.

2.2. Methods

2.2.1. Preparation of the regenerated silk fibroin (RSF) solution

5 g of *B. mori* cocoons were cut into small pieces and boiled in 2 L of 0.02 M sodium carbonate solution for 40 min. The fibroin threads obtained, devoid of sericin, was thoroughly washed with deionized water and dried overnight. The dried fibroin was dissolved with 9.3 M lithium bromide solution at 70 °C for 4 h. The viscous sol obtained after dissolution was dialyzed against deionized water for 48 h to remove the salts. 0.5 mL of the obtained regenerated silk fibroin (RSF) solution was placed in a weighing boat and allowed to dry overnight under hood at room temperature to estimate the concentration of the solution, and, if needed, adjusted to be 6% (w/v) by diluting with deionized water [33].

2.2.2. Synthesis of spray-dried mesoporous bioactive glass (SD-MBG)

SD-MBG was synthesized by combining the sol–gel method with the aerosol-assisted spray drying technique using the protocol reported elsewhere [32]. The sol was prepared by dissolving 2.2 g of Pluronic P123 in 8.0 g of ethanol while 10.4 g of TEOS (tetraethyl orthosilicate) were pre-hydrolyzed for 20 min with 5.4 g of diluted hydrochloric acid (pH 2) and 12.0 g of ethanol. The two solutions were mixed together and stirred for 20 min, while 2.95 g of calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$, CaNT) was pre-dissolved in 3.4 g of ethanol. Finally, the CaNT solution was poured into the prepared sol. This final solution was mixed for at least 20 min before spraying, using the Mini Spray-Dryer B-290, equipped with the Inert-Loop B-295 (both from Büchi), needed