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Fabrication of bioactive glass containing nanocomposite fiber mats for bone tissue engineering applications

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ABSTRACT

This study mainly focuses on fabricating nanocomposite fibrous mats for bone tissue engineering. For this purpose, strontium or copper doped bioactive glass particles were successfully incorporated into gelatin/poly(ε-caprolactone) (Gt/PCL) nanofibers through electrospinning process. As the content of bioactive glass increased, the average diameter of the as-spun nanocomposite fiber mats rised. It was further observed that the in vitro bioactivity of the fiber mats enhanced with the inclusion of BG particles into the polymeric matrix. In addition, the release of therapeutic ions were determined as a function of immersion time in SBF, which was in the range of 5.4–10.1 mg/g scaffold and 0.34–1.87 mg/g scaffold for strontium and copper ions, respectively. Although the results were promising, the amount of SrO and CuO in the composition of bioactive glasses can be increased to improve the osteogenic, angiogenic, and antibacterial potential of the nanocomposite fiber mats. Hence, this study provides an insight for future researchers who aim to create nanocomposite materials as multifunctional scaffolds for bone tissue engineering applications.

1. Introduction

The treatment of bone defects resulting from trauma, malignancy, infections, tumors or congenital diseases is a major challenge. Therefore, bone tissue engineering has emerged with the intension to repair, replace or regenerate these bone defects with the aid of biodegradable scaffolds, which serve as a temporary framework for providing a suitable environment that allows cells to synthesize their own extracellular matrix (ECM) and to degrade upon neogenesis of ECM [1–7]. Among existing methods for the fabrication of scaffolds, electrospinning has received much attention as a simple, cost-effective, and versatile technique to prepare non-woven mats consisting of fibers with diameters ranging from microns down to a few nanometers [6–9]. Using electrospinning, it is possible to create scaffolds that mimic the native architecture of the bone ECM owing to its high porosity, high aspect ratio, and large surface area. The large specific surface area of the electrospun scaffolds makes more surfaces suitable for cellular attachment, while the high porosity and the high interconnectivity of pores provide enough space for vascularization required to nourish new bone and to enable the exchange of nutrient and metabolic waste between the scaffold and environment [9].

To date, a variety of natural and synthetic polymers have been investigated for the fabrication of nanofibrous scaffolds. However, natural and synthetic polymers alone cannot meet all the requirements of an ideal scaffold. To overcome the shortcomings of synthetic and natural polymers, blends of two or more types of polymers have been devised by researchers that combines the advantages of both synthetic and natural materials, potentially improving cell affinity while offering ideal mechanical properties for tissue engineering applications. Within this respect, electrospun Gt/PCL nanofibers had been widely studied for engineering diversified tissues, including nerve [10–12], muscle [13], dental [14], cardiac [15], cardiovascular [16], bone [17,18], and cartilage [19,20]. It was reported that blending PCL with gelatin resulted in a new biomaterial with improved mechanical, physical, chemical, and biological properties [8,10,21,22].

Recent research efforts have been focused upon the development of composite materials comprising the biodegradable polymer matrix combined with inorganic components, such as hydroxyapatite [14,23], tricalcium phosphate [24], and bioactive glasses [6,7,25–31]. The reason lying behind that was to mimic both the physical architecture and chemical composition of natural bone ECM since it has a highly complex and well-harmonized

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composite structure that consists of type I collagen fibrils (50–500 nm in diameter) mineralized with a thin, highly crystalline carbonated hydroxyapatite layer [7,31,32].

Among inorganic components, bioactive glasses are a group of inorganic bioactive materials that have been extensively used in the treatment of bone defects, due to their ability to stimulate bone regeneration via dissolution, followed by the formation of a surface layer of hydroxycarbonate apatite upon exposure to physiological fluids [33,34]. This surface layer resembles the chemical composition and structure of bone mineral and thus, plays a key role in forming a bond with the surrounding bone tissues. Since their development, a large variety of bioactive glasses based upon derivations of the 45S5 composition have been developed and applied in bone tissue engineering owing to their good bioactivity, osteoconductivity, osseointegration, and biodegradability [9,35–41]. However, the main drawback of bioactive glasses is their internal stiffness, brittleness and low mechanical properties that make them difficult to use in load-bearing applications [42,43]. Within this respect, in an effort to make use of the intrinsic properties of polymers and bioactive glasses, other researchers attempted to incorporate bioactive glass particles into biodegradable polymers as fillers to form composite nanofibers. It was determined that the addition of the bioactive glass into the polymeric matrix greatly enhanced the mechanical and biological properties [6,7,27–31]. Hence, this study concentrated on loading bioactive glass particles into the Gt/PCL nanofibers by using the electrospinning method to develop a composite scaffold with improved bioactivity, biodegradability, osteoconductivity, and mechanical stability for bone tissue engineering.

In addition, the structural integrity of a scaffold is an important aspect for the determination of the proliferation, differentiation, and long term-survival of the anchorage depended cells in the scaffolds [17]. Since gelatin is water soluble, the electrospun fibers can partially dissolve and lose its fibrous form upon exposure to a high humidity ambient (i.e., 80–90%) for a certain period of time [44,45]. In the literature, several physical (i.e., dehydrothermal treatment, UV irradiation, and plasma treatment) and chemical methods (e.g. chemically modifying gelatin with the use of cross-linking agents, including glutaraldehyde, 1-ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride, and genipin) have been reported for cross-linking of the gelatin [44–51]. Among them, the use of glutaraldehyde is by far the most widely used cross-linking treatment, due to its high efficiency, ease of availability, and inexpensiveness [44]. Therefore, a cross-linking treatment with glutaraldehyde was also performed to preserve the fibrous morphology of the as-prepared mats.

On the other hand, a variety of studies have recently focused on enhancing the biological performance of bioactive glasses by doping them with therapeutic metal ions, including strontium [2–4,33] and copper [1,32]. Upon the dissolution of these bioactive glasses, the controlled release of therapeutic metal ions brings about additional functionalities, such as osteogenesis, angiogenesis, and antibacterial effects. Taken together, we hypothesized that combining polymers with bioactive glasses doped with strontium or copper will enable to develop nanocomposite fiber mats that have a potential to be used as multifunctional scaffolds in bone tissue engineering applications. In this context, emphasis has been placed on investigating the in vitro degradation behavior and bioactivity of the as-prepared nanocomposite fiber mats. To the best of our knowledge, this study is the first report that employs strontium or copper substituted bioactive glass particles to develop a nanocomposite material as a multifunctional scaffold by using electrospinning technique. In this context, strontium and copper containing bioactive glasses and gelatin/PCL blends were used to fabricate nanocomposite scaffolds. The structural, bioactive and thermal behavior of the scaffolds were investigated.

2. Materials and methods

2.1. Materials

Gelatin (Gt, type A, from porcine skin), poly(ε-caprolactone) (PCL, Mn = 70,000–90,000), silicon dioxide (SiO_2, Sigma Aldrich), and copper(II) nitrate trihydrate ($Cu(NO_3)_2 \cdot 3H_2O$) were obtained from Sigma–Aldrich Chemicals. Glacial acetic acid (AcOH), formic acid, glutaraldehyde (GTA), di-sodium hydrogen phosphate anhydrous (Na_2HPO_4), calcium carbonate ($CaCO_3$), and sodium carbonate (Na_2CO_3) were purchased from Merck. Strontium nitrate ($Sr(NO_3)_2$) was supplied from Riedel-de-Haen. All chemicals were used as provided without further purification.

2.2. Preparation of bioactive glass particles

Two modified versions of 45S5 were prepared using a classical melting method in the present study. For this purpose, ca. 8.2 wt% of CaO in Bioglass® composition was replaced with SrO or CuO in order to produce strontium or copper substituted bioactive glass particles (Sr-BG or Cu-BG) with the composition of SiO_2:CaO:P_2O_5:Na_2O:XO (45:22.5:6:24.5:2 wt%, X = Sr or Cu). To prepare bioactive glass (BG) particles, appropriate amounts of precursor chemicals were first placed in a platinum crucible. After that, they were melted at 1350 °C for 2 h and rapidly quenched into deionized water to form frits. As-prepared frits were then ground and placed in the platinum crucible for repeating the melting and the quenching steps in order to obtain a homogeneous structure. Finally, the obtained BG frits were ground (⩽45 μm) to yield the BG particles.

2.3. Preparation of electrospinning solutions

BG particles were first dispersed in a co-solvent of acetic acid and formic acid (1:1 in volume) at room temperature for 1 h. Then, polymers were separately added into the BG containing solvents and stirred at room temperature for 3 h in order to obtain homogenous solutions with BG contents varying from 0% to 7.5% (w/v). Afterwards, 20% (w/v) Gt solution and 15% (w/v) PCL solution were mixed in a Gt/PCL ratio of 7/3 (w/w) at room temperature for 2 h.

2.4. Electrospinning

The as-prepared solutions were transferred to a plastic syringe equipped with a flat stainless steel needle, which was connected to a high-voltage supply. Voltage applied to the needle tip was 22.5 kV. The flow rate was set as 3 ml/h by a syringe pump. Nonwoven electrospun fibers were deposited onto an aluminum foil wrapped around a grounded collector placed at a distance of 10 cm perpendicular to the needle tip. Electrospinning procedure was performed under ambient conditions. The resultant nanocomposite fiber mats were dried at 37 °C for a couple of days to remove residual solvent and then transferred to a desiccator prior to further investigations.

2.5. Cross-linking treatment

Cross-linking process was carried out by placing samples of the as-prepared nanocomposite fiber mats in a sealed desiccator containing 25% (v/v) glutaraldehyde solution in a Petri dish. After 4 days, samples were removed from the desiccator and kept in the fume hood for 2 h, followed by a post treatment at 110 °C for 1 h to remove residual glutaraldehyde and to partially enhance the cross-linking. The success of cross-linking was determined by testing the dissolubility of the cross-linked mats immersed in simulated body fluid (SBF, pH 7.4) at 37 °C for different time points (up to 28 days).