Composite Nano-Fiber Mats Consisting of Biphasic Calcium Phosphate Loaded Polyvinyl Alcohol – Gelatin for Biomedical Applications

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Abstract: Electrospun blends of biphasic cacium phosphate (BCP) loaded polyvinyl alcohol (PVA)-gelatin (GE) were created with the aim of fabricating biodegradable scaffolds for tissue engineering. The process parameters including the electrical field and tip-to-collector distance (TCD) were investigated. The morphology of these hybrid scaffolds were characterized by scanning electron microscope (SEM). X-ray diffraction (XRD) was used to determine the crystallinity of the membrane. Adhesion of osteoblastic cells (MG-63) onto the BCP loaded PVA/GE composite nano-fiber mat was performed to assess potential of the product as a bone scaffold. This result suggests that the BCP loaded PVA/GE composite nano-fiber mat has a high potential for use in the field of bone regeneration and tissue engineering.

Keywords: Electrospinning, nano-fiber, biphasic calcium phosphate nanoparticles, bone tissue engineering

1 Introduction

Several requirements are necessary in the design of tissue engineering scaffolds, including a high porosity, large surface area, adequate pore size, and uniformly distributed interconnected porous structures throughout the matrix [1]. Biodegradable polymeric fiber structures can provide a large surface area, and a relatively high porosity, which can be optimized for specific applications. Due to the structural similarity between electro-spun polymeric mats and the collagen fiber arrangement in the natural extracellular matrix component of bone, the electrospinning process has drawn a great deal of attention in scaffold processing for tissue engineering [2]. Within the last few years, this technique has proven to be especially suitable for biomedical applications, including tissue engineering of scaffolds, wound dressings, drug delivery, and in other fields such as medical implants [3-6].

Biodegradable polymers have been proposed as a possible alternative to other tissue engineering materials and have garnered a large amount of attention. These polymers can be easily processed into 3-D porous structures with the proper degradation behavior and mechanical strength[7]. Among the biodegradable and biocompatible polymers, polyvinyl alcohol (PVA), gelatin (GE) and their blends have extensive applications as surgical sutures, implant materials, drug carriers, and scaffolds for tissue engineering [3, 8]. PVA is a non-hazardous material and does not cause any injuries to the skin upon contact. Due to their excellent biocompatibility and biodegradability, gelatin fibers are widely used in a variety of biomedical applications, including wound or burn dressings, surgical treatments, and tissue engineering of bone, skin, and cartilage [3, 4, 8]. PVA/GE nano-fibrous membranes with different compositions could have different clinical applications, especially the controlled release of drugs and bone tissue engineering [9, 10].

The most common strategy employed to engineer bone is to use a scaffold combined with osteoblast cells, or cells that can mature/differentiate into osteoblasts and regulatory factors that promote cell attachment, differentiation, and mineralized bone formation [11]. However, the requirements for designing and production of an ideal scaffold for bone regeneration are very complex and not yet fully understood. It is generally agreed that the scaffold must be a biocompatible, porous (more than 90% and pore sizes between 100-350μm), interconnected and permeable in order to permit the ingress of cells and nutrients[12]. Clearly, structures designed with biodegradable fibers can meet all these criteria and may serve as a scaffold for the engineering of bone. Many different fiber-based polymeric matrices have been tested with different cell types to create a bone construct. Recently, biphasic calcium phosphate (BCP) ceramic, which consists of hydroxyapatite (HAp) and β-tri calcium phosphate (TCP), has received attention for applications as bone substitutes and scaffold materials. In addition, BCP ceramics exhibit various mechanical properties and biological responses depending on their compositional ratio of HAp and β-TCP. BCP showed good resorption, osteoconductivity, biodegradable bone, and replacement material.

The fabrication of a composite material from PVA/GE nano-fibers for using in tissue engineering or in guided tissue regeneration, particularly for bone regeneration, has already been reported earlier [8, 13]. In this study, nano-structured scaffolds of BCP nano-particles loaded in PVA/GE nano-fibers were fabricated via electrospinning for biomedical application

2 Materials and Methods

Poly (vinyl alcohol) (PVA, 99+% hydrolyzed) was obtained from Aldrich Chemical Co (USA). Gelatin Type A (Approx. 300 Bloom, Sigma, St.Louis, MO) from porcine skin was obtained in powder form. The MG-63 cell line was obtained from Korean Cell Bank. Dulbecco's Modified Eagle's Medium (DMEM, HyClone, Logan, UT), fetal bovine serum (FBS, Grand Island, NY), penicillin-streptomycin (antibiotics) and trypsin-EDTA were purchased from GIBCO (Carlsbad, CA). BCP nano-powder was synthesized by microwave hydrothermal method using $Ca(OH)_2$ (99.995%, Aldrich Chemical) and H_3PO_4 (85-87%, Dongwoo Fine Chemicals, Korea) as precursors .

For electrospinning (eS-robot, Electrospinning/Spray system), the solutions were placed into a 10 ml syringe fitted to a needle with a tip (diameter of 25 gauges, inner diameter 0.25 mm), a syringe pump (lure-lock type, Korea) for controlling feed rates, and a grounded cylindrical stainless steel mandrel was used to collect the mat. The morphology of the electro-spun fibers was examined by scanning electron microscopy (SEM, JSM-7401F). The crystal structures of the BCP-PVA/GE composite membranes were subjected by X-ray diffraction (XRD) (Rigaku, D/MAX-2500 V).

MG-63 cells were maintained and suspended in a humidified incubator at 37 ºC and a 5% CO_2 atmosphere (incubator, ASTEC, Japan) in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin. MG-63 cells were seeded on the PVA/GE and BCP-PVA/GE scaffolds at a density of 10^4 cells/cm^2. The attachment behaviors of MG-63 were observed after 1 hour of incubation.

3 Results and Discussion

The electrospinning conditions were optimized to produce a uniform PVA/GE and BCP-PVA/GE nano-fiber. The optimized parameters were determined by examining the effect of different electrospinning parameters, including applied voltage and tip to collection distance (TCD) on fiber diameter. The amount of GE added to the PVA solution was up to 80 v/v% as previous report[13] and the amount of BCP loaded PVA/GE as 20, 40 and 50 wt/v%[5]. In this study, the concentration of BCP loaded in PVA/GE was increased up to 60 wt/v%. Figure 1 showed SEM images of the PVA/GE, BCP nanoparticles and BCP-PVA/GE prepared by electrospinning. The applied voltage and TCD were fixed at 22 kV and 10 cm, respectively. As shown in Fig. 1, nano-scale PVA/GE and BCP-PVA/GE blends were obtained with diameters ranging from 300 to 500 nm.

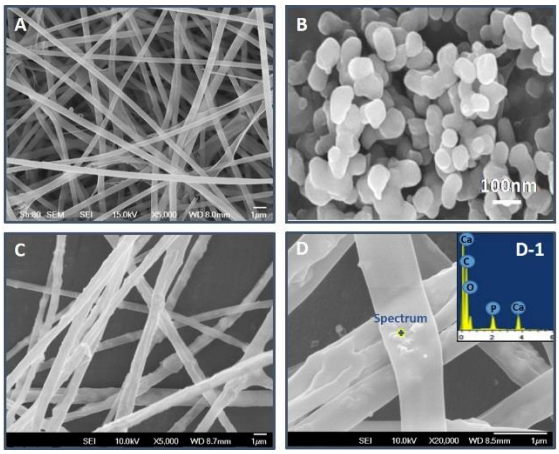

Figure 1. The morphology of nano-fiber PVA/GE (A), BCP nano-particles (B), BCP loaded PVA/GE in low magnification (C) and high magnification (D) and EDS spectrum (D-1).

The diameter of 60%BCP-PVA/GE fibers was found to be larger than PVA/GE fibers. The appearance of Ca, P spectrum in EDS profile confirmed that the BCP nanoparticles were presence in the PVA/GE fibers. BCP nano-particles were well distributed in the PVA-GE nano-fibrous matrix and appeared on the surface of PVA/GE fibers. Due to the nano-size of the BCP-PVA/GE scaffolds, the electrospun fibers had a high, specific surface area, which is beneficial for tissue engineering applications.

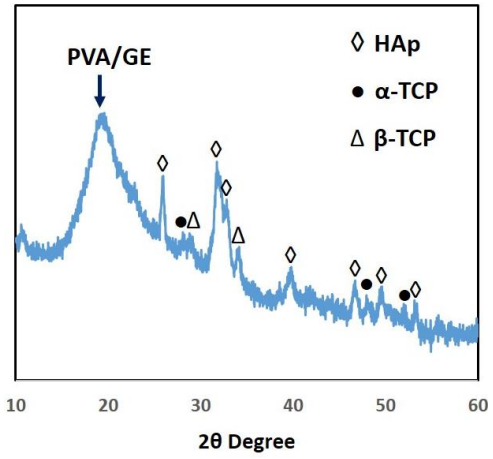

Figure 2. XRD profile of 60% BCP nanoparticles loaded PVA/GE fibers.

XRD profile of BCP-PVA/GE membrane was performed to examine the crystalline structure of the composite as shown in Figure 2. PVA/GE fibers showed a crystalline property with the peak around 20 2θ. The strong peaks of HAp at 26, 31, 32, 40, 47, 49, 53, and few peaks of TCP at 27, 28, 33, 48 and 52 are clearly observed in Figure 2. The results confirmed that BCP nanoparticles were loaded into PVA/GE nano-fibers and retained their crystalline structure during electrospinning.

Pore of various diameters were formed within the nano-fiber structures and the interconnected porous structure provided a large surface area for cell residence and proliferation, and effectively simplified the exchange of nutrients between the scaffold environments. Figure 3 showed that the osteoblast cells were attached on both PVA/GE and BCP-PVA/GE after 1 hour of incubation. The surface topography of nano-fibers plays an important role in cell behavior, including cell adhesion [14]. It is known that human cells can attach and organize themselves well around fibers with diameters smaller than those of the cells [15]. This might explain the behavior of the MG-cells on the combined scaffolds, which had a nano-scale structure.

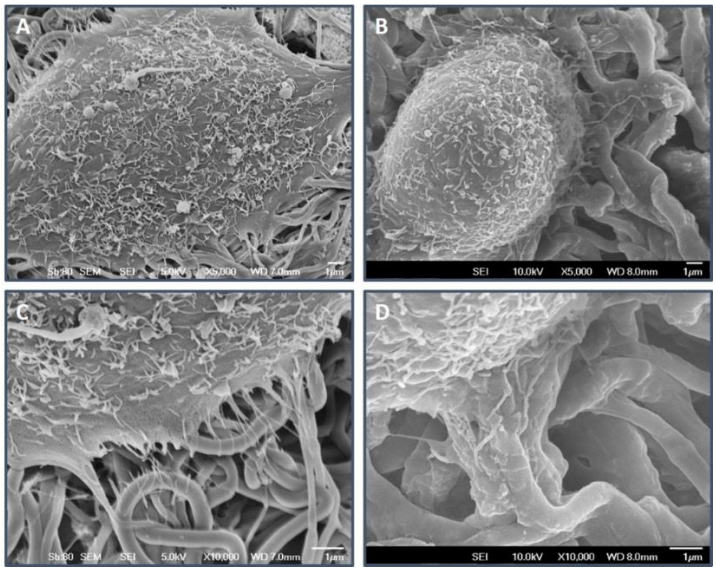

Figure 3. The morphology of MG-63 on PVA/GE scaffolds (A and C) and on BCP-PVA/GE scaffolds (B and D) after 1 hour of seeding.

4 Conclusions

PVA/GE and BCP-PVA/GE fibers were produced using the electrospinning technique. Fibers could also be formed into 3D structures, which have the potential for use as bone tissue engineering scaffolds. BCP nano-particles were well distributed in the PVA-GE nano-fibrous matrix for improving similarity to bone component. We also found that the mesh structures of the scaffold were suitable for cell adhesion containing BCP nano-particles. This was confirmed by the results of the in-vitro cell culture studies. In addition, osteoblasts that grew over the scaffold surfaces exhibited the appropriate morphology and displayed good attachment, suggesting that the developed scaffolds might be used for bone tissue engineering applications.

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