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Electrospun gelatin/poly(ε-caprolactone) fibrous scaffold modified with calcium phosphate for bone tissue engineering

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ABSTRACT

In this study gelatin (Gel) modified with calcium phosphate nanoparticles (SG5) and polycaprolactone (PCL) were used to prepare a 3D bi-layer scaffold by collecting electrospun PCL and gelatin/SG5 fibers separately in the same collector. The objective of this study was to combine the desired properties of PCL and Gel/SG5 in the same scaffold in order to enhance mineralization, thus improving the ability of the scaffold to bond to the bone tissue. The scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and the wide angle X-ray diffraction (WAXD) measurements confirmed that SG5 nanoparticles were successfully incorporated into the fibrous gelatin matrix. The composite Gel/SG5/PCL scaffold exhibited more enhanced mechanical properties than individual Gel and Gel/SG5 scaffolds. The presence of SG5 nanoparticles accelerated the nucleation and growth of apatite crystals on the surface of the composite Gel/SG5/PCL scaffold in simulated body fluid (SBF). The osteoblast response in vitro to developed electrospun scaffolds (PCL and Gel/SG5/PCL) was investigated by using normal human primary NHOst cell lines. NHOst cell culture studies showed that higher alkaline phosphatase (ALP) activity and better mineralization were obtained in the case of composite materials than in pure PCL scaffolds. The mechanically strong PCL scaffold served as a skeleton, while the Gel/SG5 fibers facilitated cell spreading and mineralization of the scaffold.

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1. Introduction

Fibrous scaffolds produced by an electrospinning process have attracted considerable interest in bone tissue engineering [1]. Randomly electrospun fibrous scaffolds possess a characteristic microstructure with a high specific surface area, high porosity and high interconnectivity of pores. Moreover, the mechanical and biological properties, as well as the degradability kinetics of the electrospun scaffold, can be manipulated by changing the composition of the polymer solution and processing parameters [2,3]. Using electrospinning, it is possible to produce scaffolds which are similar to the natural extracellular matrix (ECM), which can thus enhance the adhesion, proliferation and growth of cells [4,5]. In order to more accurately mimic the natural ECM, the electrospinning of natural materials, such as gelatin, has gained much attention during the past few years [6–8]. Gelatin has been widely examined as a tissue engineering scaffold because it has a high biocompatibility, and biodegradability, identical composition and almost the same biological properties as collagen [9,10]. Gelatin has also many integrin-binding sites for cell adhesion and differentiation [11,12]. However, the main disadvantage of using gelatin as the main structure component in a scaffold is its rapid degradation rate and weak mechanical properties [13].

The bone ECM is a type of organic–inorganic nanocomposite. Specifically, 70% of the bone matrix is composed of nanocrystalline hydroxyapatite, which is deposited in an orderly manner within a nanofibrous collagen matrix [14,15]. Recent research efforts have been focused on the incorporation of bioactive inorganic particles within the polymeric matrix in order to mimic both the physical architecture and chemical composition of natural bone ECM [16–18]. Many fibrous constructs involve the use of hydroxyapatite nanoparticles or silicon-based glasses with silicon contents over 40–45% (Bioglass®). However, their degradation is poor and their functionality is based on the stable precipitation of a hydroxyapatite layer on their surfaces in order to be linked to the bone. In this work, titanium based nanoparticles with a low content of titanium were developed in order to favor degradation and, thus, ion release competing with hydroxyapatite precipitation. Cells are able to directly detect this extracellular ion concentration such as calcium, which is able to act as an osteoinduction promoter through the regulation of a calcium-sensing receptor (CaSR) [19,20]. Calcium also acts as a cell

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homing recruiting cells from the bone marrow suggesting a CaSR-mediated chemotaxis and a further bone resorption [21,22].

Polycaprolactone (PCL) is a biocompatible and biodegradable polyester with excellent mechanical properties [23]. PCL exhibits more prolonged mechanical strength than other bioresorbable polymeric materials, and degrades at a rate compatible with bone regeneration [24,25]. However, PCL has an intrinsic hydrophobic chemical nature, and its poor surface wetting and poor interaction with biological fluids make cell adhesion and proliferation less efficient.

The ideal scaffold for bone tissue engineering should have good cell affinity, bioactive properties and enough mechanical strength to serve as an initial support. Scaffold inspired by natural bone ECM that combines a natural origin polymer (such as gelatin, which contains nanoparticles of a bioactive calcium phosphate), with a synthetic polymer (such as poly(ε-caprolactone)), could provide optimal physicochemical and biological properties. Applications of such materials are multiple. However, guided bone healing through the regeneration of the periosteum membrane would be an option, especially for osteoporotic bone fractures. To date, numerous studies confirmed that the existence of a relatively high amount of stem cells located in the periosteum make this option as a very promising and potential approach for the initial bone ingrowth [26–28].

The aim of the present work was to prepare electrospun bi-layer fibrous scaffolds, including both the poly(ε-caprolactone) for the backbone of the scaffold and the gelatin modified with calcium phosphate for better mineralization. A hierarchically designed composite material is presented, using a sol-gel method to prepare a bioactive phase as an ion release agent, and electrospinning to mimic the extracellular matrix (ECM), adapting their features to biological requirements. The properties of the composite Gelatin/Calcium phosphate/Polycaprolactone scaffold obtained were characterized by SEM, FTIR, WAXD, TGA and DSC methods. Scaffold bioactivity was examined in SBF as well as in cell culture.

2. Materials and methods

2.1. Materials

Gelatin (from porcine skin, type A) and polycaprolactone (PCL) with a molecular mass of 70,000–90,000 g/mol were purchased from Sigma-Aldrich. 2,2,2-Trifluoroethanol (TFE, ARCOS, Poland) for gelatin and a mixture of chloroform and methanol 1:1 (POCH, Poland) for PCL were used as solvents. Calcium phosphate nanoparticles (SG5) were produced at the Institute of Bioengineering of Catalonia (IBEC, Spain) by the sol-gel method, which was described previously [29]. Briefly, it consists in the controlled mix in a molar ratio: 44.5 P_2O_5–44.5 CaO–5 TiO_2–6 Na_2O of calcium and sodium 2-methoxyethoxide solutions, phosphorus pentoxide ethanolic solution and titanium tetraisopropoxide solution previously prepared in the laboratory under nitrogen atmosphere. Solutions were aged for one week at 70 °C in vigorous stirring. Finally, nanoparticles were collected by centrifugation. To prepare the spinning solutions, 3 g of gelatin was dissolved in 30 ml of 2,2,2-trifluoroethanol (TFE) and 2.5 g of PCL in 40 ml of chloroform/methanol (1:1) mixture. 0.2 g of calcium phosphate (SG5) powder was added into the gelatin solution. Stable dispersion of SG5 powder was achieved by sonicating the slurry. Three solutions were prepared: (1) gelatin/TFE; (2) gelatin/SG5/TFE; and (3) PCL/chloroform/methanol.

2.2. Scaffold fabrication by electrospinning

Scaffolds were produced by electrospinning, whose set-up consisted of a high voltage supply, an infusion pump and a collector (rotary drum). For scaffold fabrication, each sample of prepared slurries was placed in a syringe (10 ml) topped with a needle whose diameter was 0.7 mm and then connected to 30 kV voltage. The solution flow rate was 1.5 ml/h, and the distance between the tip and the collector was 15 cm. The rotary drum was wrapped in silica-coated paper. In order to produce a hybrid nonwoven fabric (two-layer composite), gelatin/SG5 fibers were spun onto a nanofibrous PCL substrate. Four electrospun scaffolds were prepared: (1) gelatin (Gel); (2) gelatin modified with calcium phosphate (Gel/SG5); (3) polycaprolactone (PCL) and (4) composite Gelatin/Calcium phosphate/Polycaprolactone (Gel/SG5/PCL).

2.3. Scaffold characterization

The electrospun scaffold samples were covered by a sputtered gold coating and analyzed by a scanning electron microscope (JEOL JSM 5500). Fiber diameters were determined based on SEM images. The average diameter of the fibers was determined by performing measurements on 100 fibers.

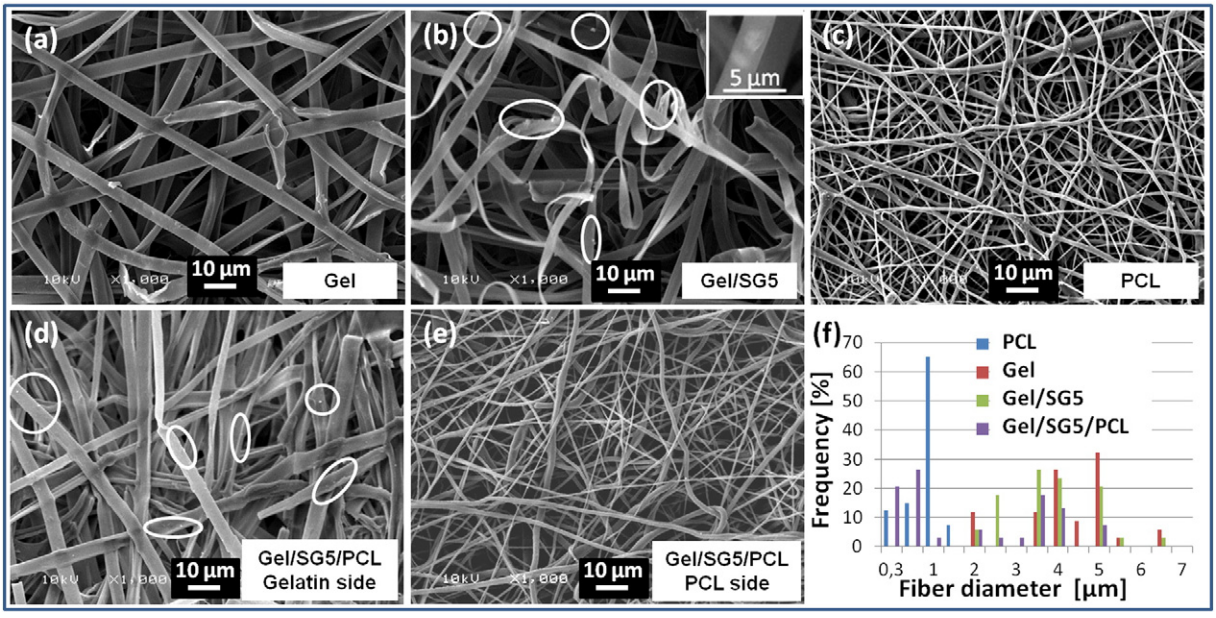

Fig. 1. SEM images of the fibrous scaffold: (a) gelatin (Gel); (b) calcium phosphate-modified gelatin (Gel/SG5); (c) polycaprolactone (PCL); (d–e) composite Gel/SG5/PCL; and (f) fiber diameter distribution in electrospun scaffolds.

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