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Electrospun polymer scaffolds modified with drugs for tissue engineering

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ABSTRACT

The purpose of this paper was to fabricate nanofibrous scaffolds containing ossein-hydroxyapatite complex (osteogenon) to mimic the native bone extracellular matrix. Polylactide (PLDL) and polycaprolactone (PCL) were used to prepare scaffolds using electrospinning. Unfortunately, both of these biodegradable polymers have poor cell recognition sites leading to poor cell affinity and adhesion, therefore, based on our previous experience, osteogenon-drug was used at the stage of fibers forming by electrospinning. We have compare the physicochemical parameters and mechanical properties of PLDL/osteo and PCL/osteo scaffolds as well as an osteogenon-drug influence on the microstructure of electrospun materials produced for potential application in bone tissue engineering. We have investigated the effect of the microstructure and the chemical composition of electrospun materials on adhesion, proliferation and morphology as well as on the process of differentiation of bone cells. The use of osteogenon improved mineralization, cell adhesion and the rate of cell differentiation.

1. Introduction

Composite fibrous scaffolds have been widely investigated for repair and regeneration of bone and cartilage tissues [1]. The need of better low-cost substitutes in tissue engineering to make easier the transfer to the market in terms of cost-efficiency and regulation has made many groups to look forward alternative strategies to the current ones based on the use of growth factors and cell therapy, which have also many difficulties related to standardization, storage and side effects [2]. The current trend to introduce the proper signaling on biomaterial scaffolds to trigger cell the desired response and control their fate is the mimicking of the extracellular matrix (ECM) [3–6]. Nanofibers of biodegradable polyesters, already approved by medical agencies in many device [7], provide a promising alternative to reach the requirements to transfer lab prototypes into market in a short medium term. Among the variety of polymeric materials which have been used for fabrication of composite fibrous scaffolds [8], poly(ε-caprolactone) (PCL) has been widely considered due to its biocompatibility, suitable mechanical properties, easy-processing ability, and non-toxic degradation products [9–11]. Also a partially amorphous copolymer of 70% L-lactide and 30% DL-lactide (PLDL) is often chosen as biocompatible scaffold because it combine high strength properties and an intermediate degradation rate [12–13]. An ideal scaffold should possess hydrophilic surface and high surface area to support cell attachment [14]. Unfortunately PCL and PLDL as well, have poor cell recognition sites leading to poor cell affinity and adhesion.

Electrospinning method has demonstrated to be the most cost-efficient process technique to mimic ECM with reliable results [15–16]. Nanofibers play an important role in tissue engineering and drug delivery because of the ease with which drugs can be introduced into nanofibrous form of scaffold [17]. For the production of drug-loaded fibrous membrane, the drug and the polymer should be dissolved together and the resulting mixture should be electrospun *via* electrospinning [18–20].

Osteogenon is a drug that affects bone mineralization. Contains the components required for the synthesis of bone. It has twofold effect on bone metabolism: inhibits osteoclasts and stimulates osteoblasts. Osteogenon is an ossein-hydroxyapatite complex (OHC) also containing osteocalcin and type I collagen, which has been applied in tablets for its analgesic effect and reduction of the time fracture consolidation in patients with secondary osteoporosis. Previous results suggested that OHC better stabilizes bone mass in postmenopausal women and in different conditions related to bone loss compared to calcium supplements [21]. OHC also seems to act as analgesic and consolidator enhancer in patients with osteopenia and osteoporosis. The ossein-hydroxyapatite complex is significantly more effective in preventing bone loss than other calcium salts.

In this work, we propose the fabrication and validation of biodegradable polymeric nanofibrous mats loaded with osteogenon for the

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parenteral application in bone regeneration. It is a promising low-cost alternative in terms of simplicity, price and efficiency.

2. Materials and methods

2.1. Preparation of the samples

Electrospinning method was used to produce the samples. Two types of polymers were applied in this study: *co*-polymer of L-lactide and DL-lactide 70:30 (Corbion PLDL 7038, inherent viscosity midpoint 3.8 dl/g, molecular mass ≈ 850,000 Da) and polycaprolactone (PCL, Mw = 80,000, Sigma Aldrich). A mixture of chloroform and methanol (POCH, Poland) for PCL and acetone (Avantor, Poland) for PLDL were used as solvents. To prepare the spinning solutions, 5 g of PCL was dissolved in 90 mL of chloroform/methanol (1:1) mixture and 1 g of PLDL in 50 mL of acetone. Both solutions were mixed with 5% (w/v) of Osteogenon (Osteo, PIERRE FABRE). Stable dispersion was achieved by sonicating the slurry. Polymers were electrospun using a TIC 1092012 electrospinning machine (Bielsko-Biala, Poland), allowing control of voltage in the range of 0–50 kV. 30 mL syringes with a stainless steel needle (inner diameter 0.7 mm were filled with the resulting suspensions). Then, the electrospinning process was carried out at room temperature with a constant feeding rate of 1.5 mL/h. The rotary drum, wrapped in silicone coated paper, was placed at 20 cm distance from the needle tip. The needle was connected to the high voltage supply (20–25 kV). Two types of polymeric nanofibrous mats loaded with osteogenon were formed: PLDL/osteo and PCL/osteo.

2.2. Scaffold characterization

Morphologies of scaffolds were observed with scanning electron microscopy NOVA NANO SEM 200 (FEI EUROPE COMPANY) equipped with EDS. Before SEM examination, all the samples were coated with gold by RF sputtering. The fiber mean diameters were calculated by selecting 100 fibers randomly observed on SEM images. Scaffold pore size measurement was done using Image J software on several SEM images taken from each side of scaffold samples. Surface area of pores in each image was separated from the whole area of the picture and reported as an average value for each scaffold.

The thickness of electrospun scaffolds was measured by the thickness gauge TILMET 73. The thickness test was performed on eight samples of each type of fibrous mats. The values of the average thickness and standard deviation were determined. To assess the mechanical properties tensile tests were performed using Zwick-Roell Z 2.5 testing machine at 10 mm/min crosshead tension speed. Five samples (20 mm width × 100 mm length) of each type of electrospun mat were tested. Tensile strength was determined from the stress-strain curves.

The differential scanning calorimetry measurements (DSC) and the thermal gravimetric analysis (TGA) were performed on NETZSCH STA 449 F3, DSC 2010 TA INSTRUMENTS and SDT 2960 TA INSTRUMENTS, at a heating rate of 10 °C/min in an inert gas atmosphere (nitrogen, flow rate: 40 ml/min).

A Fourier-transform infrared (FTIR) spectrophotometer (FTS Digilab 60 BioRad) was used to compare the chemical structure of osteogenon, PCL, PLDL and the PCL/osteo and PLDL/osteo composite scaffolds. Spectra were recorded using ATR technique, in the 400–4000 cm^{-1} range using at least 64 scans and 4 cm^{-1} resolution.

Mineralization process of PLDL/osteo and the PCL/osteo mats was performed in 1.5 × concentrated SBF. Samples (2.0 × 2.0 cm) were immersed in 14 mL of 1.5 × SBF and incubated at 37 °C for 14 days. The solution was renewed every two days to ensure sufficient ion concentration. After 1, 3, 7 and 14 days of incubation, samples were washed with deionized water. SEM images of the samples were taken upon complete drying process of the washed scaffolds.

Prior to cell culture studies the nanofibrous scaffolds were cut into disks (diameter 14 mm) and sterilized by exposure to ultraviolet (UV) light for half an hour each side. Before cell seeding materials were placed in a 24 well culture plate filled with 0.5 mL of culture medium.

2.3. Cell study

Normal human osteoblasts (NHOst, Lonza, USA) were cultured in OGM culture medium, supplemented with 10% FBS, ascorbic acid and 5% solution of gentamicin and amphotericin-B (OGM BulletKit, Lonza, USA) in an atmosphere of 5% CO_2 at 37 °C. The tests were conducted on cells from passages 4. The cell suspension was obtained by addition of 5% trypsin with EDTA (Lonza, USA). After flushing and centrifugation, the cells were concentrated to 3×10^4 cells/ml in OGM medium supplemented with Differentiation SingleQuots (Lonza, USA). Next, cell were seeded on sterilized electrospun mats: PCL/osteo and PLDL/osteo. Bottom of the well plate (TCPS) was used as a positive control. Cells were cultivated up to 21 days; cell viability/cytotoxicity tests (ViaLight and ToxiLight, Lonza USA) were conducted at day 3 and 7, while cell mineralization (OsteoImage test, Lonza, USA) and ALP activity (4-MUP) was assessed at days: 7, 14 and 21. All tests were performed as described elsewhere [13]. All results were obtained by performing three independent replicates of each experiment. The results are expressed as means ± standard deviations. Statistical analysis was carried out using Tukey's *t*-test. Values of $p < 0.05$ were considered statistically significant. Cell morphology was evaluated using optical fluorescence microscope (Olympus, Japan). Cells were stained for 0.5 min with fluorochrome – acridine orange (AO), and then rinsed with phosphate buffered saline (PBS).

3. Results and discussion

In this work the electrospinning technique was chosen to fabricate two different kinds of fibrous scaffolds using the same electrospinning processing conditions. We attempted to fabricate polymeric scaffolds modified with commercially available drug – osteogenon. The microstructure and chemical composition of osteogenon- drug before grinding down process is shown in Fig. 1(e–f). The SEM micrographs of the PCL/osteo and PLDL/osteo scaffolds are shown in Fig. 1(a–d). The SEM images demonstrate that the scaffolds exhibited morphology with randomly orientated regular and continuous fibers. Additionally as seen in the images, the osteogenon component was homogenously distributed in the case of PLDL/osteo scaffolds and some calcium agglomerates can be observed on the surface of PCL/osteo samples. The average diameter of PCL/osteo and PLDL/osteo fibers were 1.39 ± 0,63 μm and 0.85 ± 0.22 μm respectively (Fig. 1g). The fibers diameters were in the micron size and sub-micron size, respectively. The different fibers diameters resulted from the different viscosity and conductivity of the solutions. The porosity of obtained electrospun scaffolds (Fig. 1h) was about 52% for PLDL/osteo and 53% for PCL/osteo. Also small difference (about 3%) in scaffold thickness was observed. With similar porosity values, the membrane consisting of thinner nanofibers might have smaller pore size [22]. The decrease of PLDL/osteo diameter is leading to the reduction of pore sizes of electrospun nanofibrous scaffold (Fig. 1b).

Tensile strength of nanofibrous mat is an important characteristic for being used as scaffold for tissue engineering, as it should possess adequate mechanical strength for growth of cellular functions. The tensile behavior of electrospun mat depends on the nature of polymer, diameter of the nanofiber and orientation of fibers in the mat [23]. The average tensile strength is illustrated in Fig. 1(i). It could be seen from these results that the osteogenon-drug modified scaffolds showed lower tensile strength than unmodified polymer mats. Osteogenon is an osseinhydroxyapatite complex (OHC) also containing hydroxyapatite and type I collagen. The characteristic FTIR bands of PCL, PLDL, osteogenon and PCL/osteo, PLDL/osteo are presented in Fig. 2. The FTIR spectra of osteogenon-drug (osteo), PLDL and PLDL/osteo composite scaffolds are presented in Fig. 2a. Characteristic PLDL bands are observed (C-O, stretching at 1760 cm^{-1}; CH_3 bending at 1449 cm^{-1}; C-O-C-vibration