EVALUATION OF PCL AND PCL/HAP SCAFFOLDS PROCESSED BY ELECTROSPINNING AND POROGEN LEACHING TECHNIQUES

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Abstract

In order to improve the pore size of the polycaprolactone (PCL) and polycaprolactone/hydroxyapatite (PCL/HAp) nanofibrous scaffolds, salt-leaching technique together with electrospinning method were applied. Salt particles were incorporated within the polymer nanofibrous matrix and then were leached out to generate some macropores. Microstructure, pore size distribution and average fibre diameter of the scaffold were investigated by scanning electron microscopy and PMI capillary flow porometer. Mechanical properties were determined by means of tensile test. Presence of hydroxyapatite and chemical characterization of the scaffold were done by FTIR analysis.

[Engineering of Biomaterials, 103, (2011), 4-7]

Introduction

Although the electrospun nanofibrous structure generates a network of open-pores, limited pore size restricts its use in tissue engineering [1-2]. Pore size plays an important role in the ability of a cell to migrate and infiltrate the electrospun scaffolds. In this study electrospinning was combined with salt leaching technique in order to improve the pore size of electrospun nanofibrous scaffold.

Poly(caprolactone) (PCL) is biocompatible, biodegradable and non-toxic polyester. The good solubility of PCL, its low melting point (59-64°C), long-term degradation properties (>24 months to lose total mass) and exceptional blend-compatibility has stimulated extensive research into its potential application in the biomedical field [3]. The advantage in using PCL scaffolds in tissue engineering deals with its desirable biostability [4]. PCL exhibits more prolonged mechanical strength than other bioresorbable polymeric materials and degrades at a rate compatible with the bone regeneration [5]. However its poor hydrophilicity caused a reduction of cell adhesion, proliferation and differentiation. Electrospinning of PCL along with nanoparticles of hydroxyapatite can improve the cell attachment kinetics. Hydroxyapatite (HAp) particles due to its structural and compositional similarity to minerals of natural bone have been widely utilized in the fabrication of bone tissue engineering scaffold [6-9]. PCL/HAp composite scaffolds have an advantage of a more hydrophilic character than pure PCL scaffold, thus a better interaction with the biological environment can be achieved [3]. Beside the existence of bone-bioactive inorganic components within polymer biomaterials generally favors calcium phosphate mineralization followed by an osteogenic differentiation process [10].

This study describes the development and characterization of PCL and PCL/HAp fibrous meshes aimed at application in bone tissue engineering. The scaffolds were prepared by combining electrospinning method and a porogen-leaching technique. Using porogen particles (such as water-soluble inorganic salts) that will dissolve generating a porous structure with pore size depending on the size of porogen particles, an attempt to improve the scaffold porosity was made. The microstructure, porosity and mechanical properties of obtained scaffold were examined.

Materials

Polycaprolactone (PCL) was purchased from Sigma-Aldrich (Mn= 70 000-90 000 g/mol). Chloroform and methanol (POCH, Poland) were used as solvents. Hydroxyapatite was produced at the AGH-UST (Cracow, Poland). An average size of the HAp particles was 23 nm. Fine sodium chloride (POCH, Poland; particle size: 2.5-4.5 μm) was used as porogen.

Scaffold processing

In order to prepare PCL and PCL/HAp composite scaffolds, two types of manufacturing methods were applied: (1) electrospinning (for pure PCL and PCL/HAp composite scaffold) and (2) electrospining mixed with porogen-leaching technique. The manufacturing began with dissolving 2.5 g of PCL in 40 ml of 1:1 chloroform/methanol solvent mixture at 50°C. PCL solution was mixed with 20 wt% of HAp using sonicator. The solutions were fed into 10 ml plastic syringe fitted with a stainless-steel blunt needle of 0.7 mm in diameter and an injection rate of 1.5 ml/h using an infusion pump. A high voltage of 30 kV was applied. The fibres were collected on a flat collector wrapped with a piece of baking paper sheet which was kept at a distance of 15 cm from the needle tip. In order to obtain PCL-salt (PCL/P) or PCL-salt-HAp (PCL/P/HAp) samples, salt was added over collector during electrospinning. After electrospinning the specimens were immersed in water for salt extraction, and after the salt extraction the samples were dried at 37°C.

Methods

For SEM analysis, the electrospun fibers were sputter-coated with gold (Jeol, JFC 1200 sputer) and observed with SEM (JSM 5500, JEOL). The mean fiber diameters were calculated by selecting 100 fibers randomly observed in the SEM images. Chemical characteristics of the electrospun nanofibrous scaffolds were evaluated by FTIR method using spectrophotometer Nicolet 6700. The spectra were recorded using fotoacustic reflectance device (MTEC Photoacoustics 300 Thermo Nicolet) at the range of 400-4000 cm^{-1} using at least 64 scans and 4 cm^{-1} resolution. The mechanical properties of electrospun samples were measured by uniaxial testing machine (Zwick-Roell Z 2.5.) under the cross-head speed of 1 mm/min (n=3). From the stress-strain curves tensile strength was obtained. Pore size distribution was measured using PMI capillary flow porometer.

Result and Discussion

Porosity is a key factor in designing scaffold for tissue engineering since proper morphology is needed to promote tissue growth, cell infiltration and transportation of nutrients into the scaffold [5]. Electrospinning method allows to obtain a fibrous scaffolds with a high porosity due to the high ratio of surface area to volume.

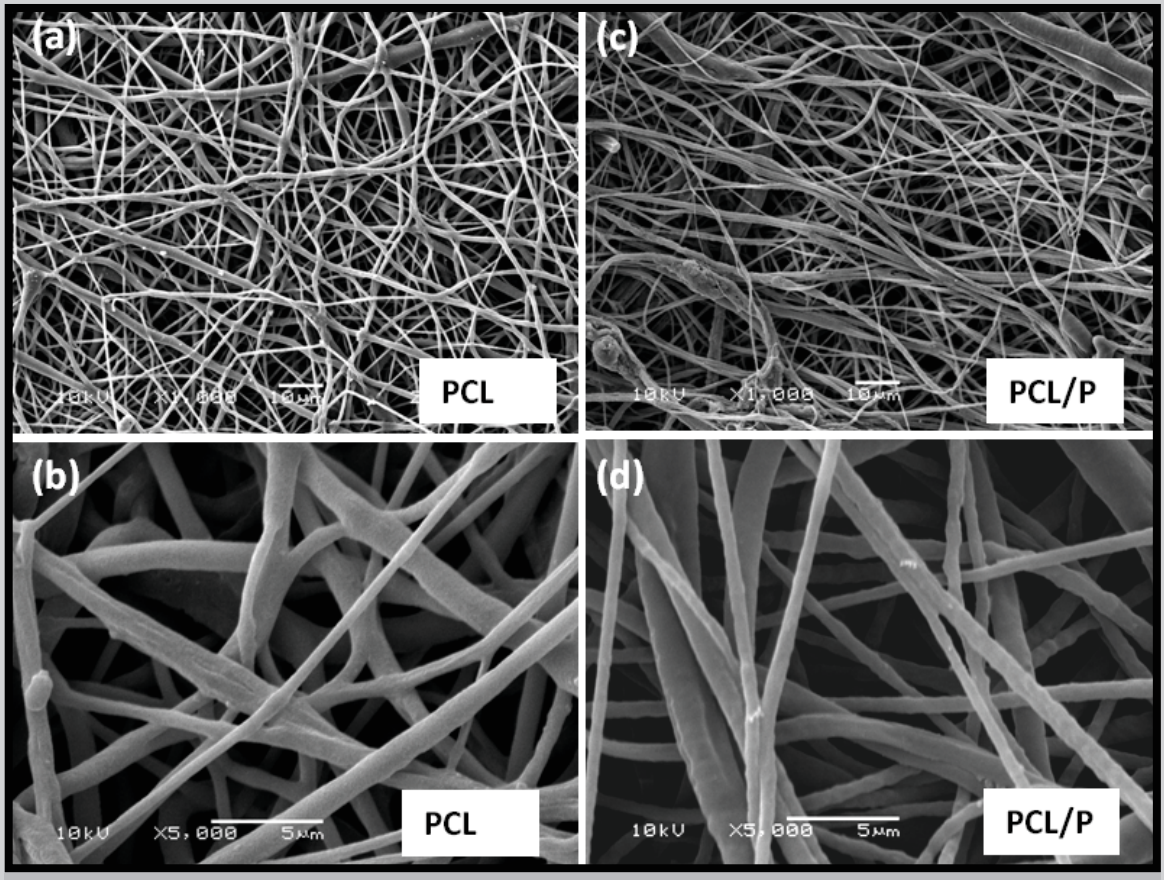

FIG. 1. SEM images of electrospun PCL scaffolds (a-b) and PCL/P scaffolds prepared by mixed electrospinning/particle leaching process (c-d).

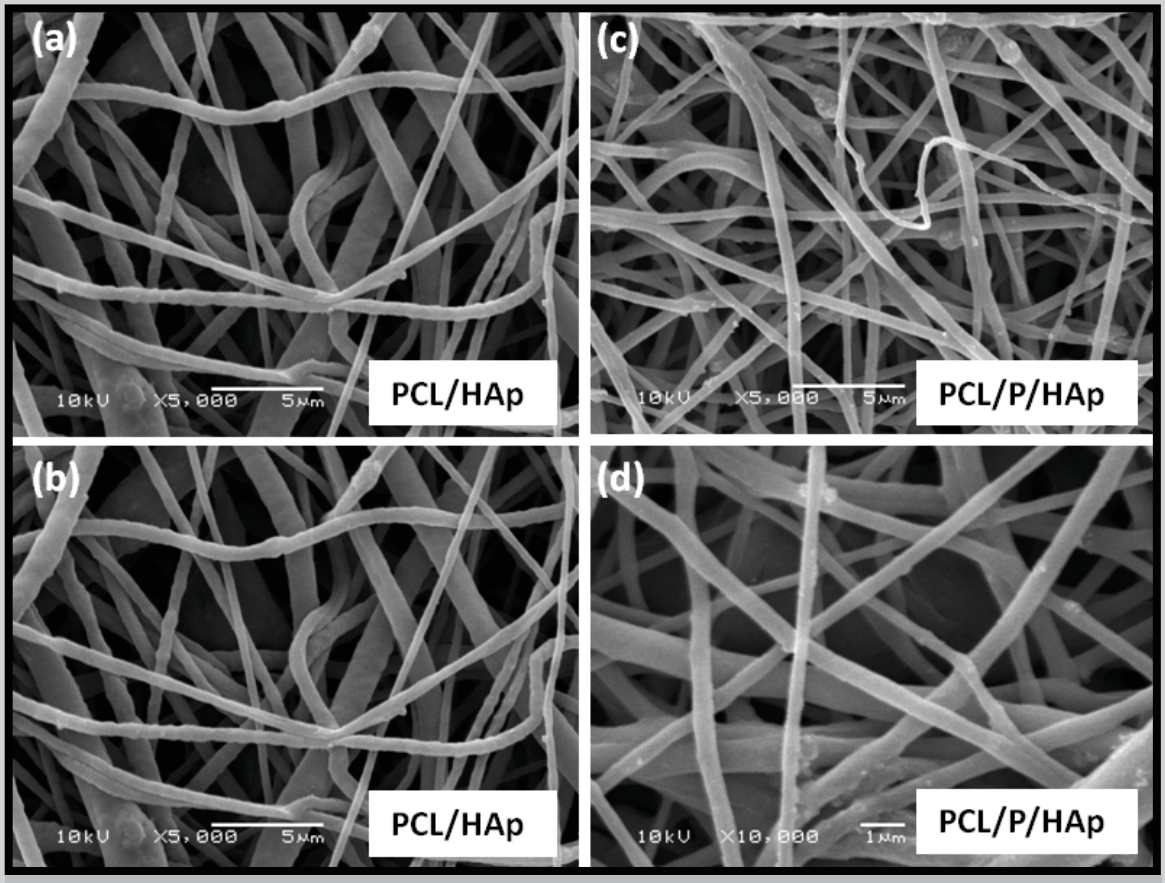

FIG. 2. SEM images of electrospun composite PCL/HAp scaffolds (a-b) and PCL/P/HAp scaffolds prepared by mixed electrospinning/particle leaching process (c-d).

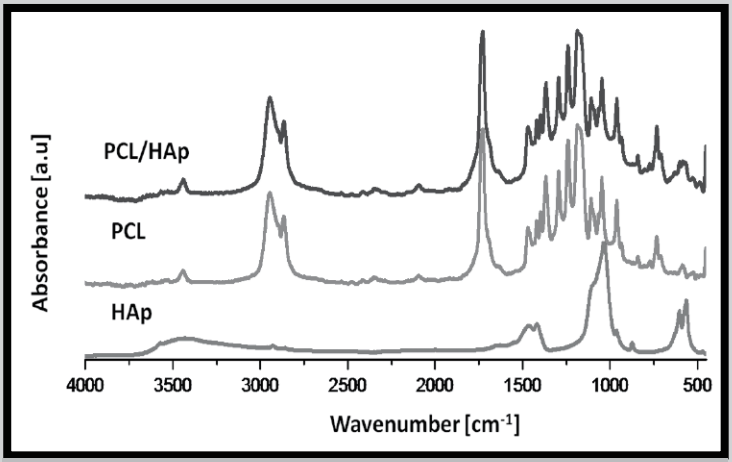

FIG. 3. FTIR spectra of PCL, PCL/HAp and HAp samples.

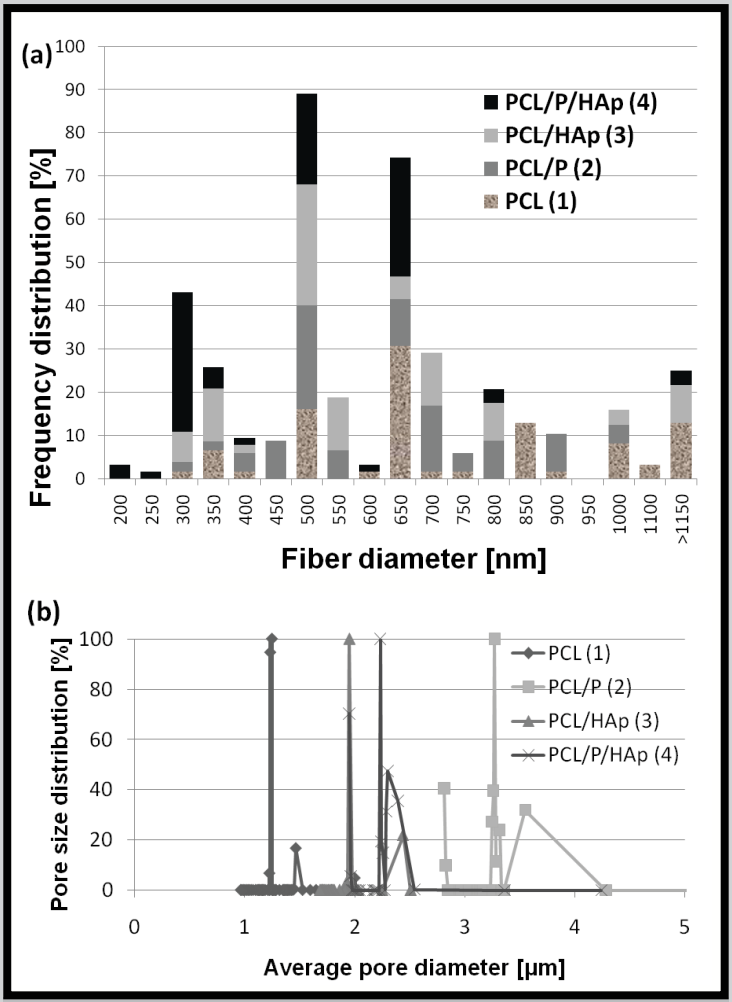

FIG. 4. Fibres diameter distribution for PCL nonwovens (a); Pore size distribution for PCL nonwovens (b).

Depending on the polymer/solvent used and various processing parameters a number of different pore size can be obtained, however the average pore size in scaffold obtained using electrospinning is insufficient.

FIGs 1-2 show the representative morphologies of nanofibers obtained from electrospinning of pure PCL solution (FIG. 1a-b), HAp modified solution (FIG. 2a-b) and samples prepared from the same solutions but obtained by mixed electrospinning/porogen leaching techniques. Pure PCL sample consisted of randomly oriented, smooth, uniform and bead-free nanofibers. The morphology of PCL/HAp sample has not been affected much by the addition of HAp. The FTIR analysis (FIG. 3) confirmed successful incorporation of HAp particles into PCL nonwoven, which was evidenced by the presence of phosphate groups peaks (associated with HAp). The average fiber diameter was about 770 ± 340 nm for pure PCL and 560 nm ± 170 for modified PCL/HAp sample (FIG. 4a). It was found that addition of hydroxyapatite particles into PCL solution reduces the fiber diameter. Probably the addition of HAp into PCL solution can cause an increase in net charge density during electrospinning and hence a larger force of electrostatic repulsion. The massive increase in electrostatic repulsion tends to increase the surface area and leads to a decrease of fiber diameter. Therefore the average diameter of pure PCL fibers was higher than for PCL/HAp fibers. On the other hand HAp particles agglomerate easily therefore fibers' diameter distribution for both samples is in the range of 300-1150 nm. Porosity of nanofibrous scaffold depends of randomly oriented nature of the nanofibers. In the case of PCL/P and PCL/P/HAp samples, morphology was also slightly influenced by pore-forming agent. Addition of salt during electrospinning resulted in increase of pore size (FIG. 4b). The average diameter of the PCL/P and PCL/P/HAp fibers was about 590 nm ± 170 and 560 nm ± 290, respectively. All samples show bimodal distribution of pores size. Pure PCL sample exhibited a very narrow distribution of pore size centered at 1.25 µm and 1.5 µm whereas for HAp modified sample the main pore fraction was centered at 1.9 µm (FIG. 4b). PCL/P sample shows a bimodal distribution of pore size diameter with a significant fraction of porosity in the range of 3.3 µm and 3.5 µm, which is twice higher than for electrospun PCL. In the case of PCL/P/HAp sample the main pore size diameter is in the range of 2.2-2.5 µm. Stress-strain curves and tensile strength of electrospun nonwovens are shown in FIG. 5a-b, respectively. It should be considered that higher porosity of PCL/P and PCL/P/HAp nanofibrous scaffold are responsible for their lower mechanical properties as compared with pure PCL and PCL/HAp samples. Unfortunately the mechanical properties were also affected by the incorporation of HAp nanoparticles. Inorganic nanoparticles generally agglomerate easily and cannot be intermixed well. The addition of HAp serves as weak links in PCL. These weak links become stress concentrators in continuum matrix and thus reduce the tensile strength of the composite nanofibers. Therefore the tensile strength of pure PCL was higher than of PCL/HAp sample. Probably the use of dispersant in a further study will considerably reduce aggregation.The tensile strength decreased from 2.22 MPa for pure PCL nonwoven to 1.48 MPa for material obtained by mixed electrospinning/porogen-leaching technique and from 1.33 (PCL/HAp) to 0.92 (PCL/P/HAp).

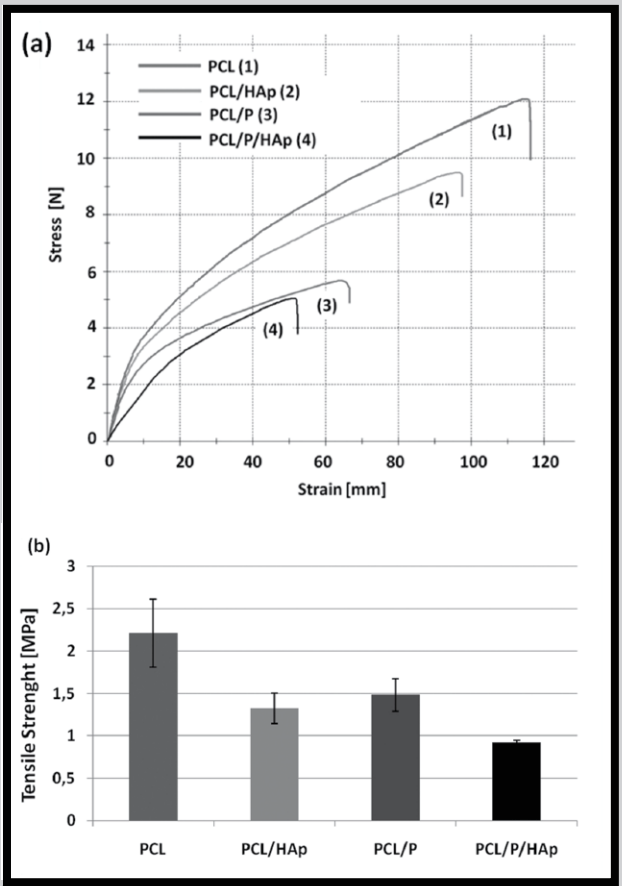

FIG. 5. Stress-strain curves obtained from tensile tests performed on electrospun samples (a). Tensile strength of electrospun nonwovens (b).

Conclusions

In order to mimic both the physical architecture and chemical composition of natural bone, a new method to fabricate porous PCL/HAp composite scaffold was developed in this study. Electrospinning technique has been widely used to fabricate nanofibrous scaffold that mimic native extracellular matrix (ECM), however the average pore size in scaffold obtained using electrospinning is insufficient. It was found in this study that the combined use of two techniques namely electrospinning and porogen-leaching, could be beneficial to fabricate desired pore size and final structure of scaffolds by varying the salt particle size. Pore size ranges of the scaffold could be controlled and the resulting nanofibrous scaffolds could have a predesigned microstructure. The success of a scaffold depends on its ability to provide a functional balance between mechanical strength, porosity and pore size. Therefore future study with the use of different size of porogen particles are needed to be carried out in order to optimize the process parameters.

Acknowledgements

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