NEEDLE-PUNCHED FIBROUS POLYCAPROLACTONE SCAFFOLD FOR BONE TISSUE ENGINEERING

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Abstract

In the present work a three dimensional composite scaffolds for bone tissue engineering were created by a carding and needle-punch processes. Highly porous nonwoven fabrics were obtained from PCL and PCL/n-HAp cut fibers without the use of any chemicals during the manufacturing process. The properties of nonwoven scaffolds were examined by SEM, FTIR, DSC and TGA methods. The average pore diameter as well as the pore size distribution of nonwoven fabrics were measured by a capillary flow porometry. The obtained results suggest that needle-punching method can be used to produce highly porous microstructures with an interconnected pore network.

Keywords: *needle-punched nonwovens, scaffold, hydroxyapatite, poly(ε-caprolactone)*

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Introduction

The design of the scaffold plays a significant role for bone tissue engineering, since the matrix provides the cells with a tissue-specific environment and architecture [1]. Ideal scaffold for bone tissue engineering should possess the following characteristics: (1) highly porous microstructure with an interconnected pore network; (2) be biodegradable with a controllable degradation time; (3) have suitable surface chemistry; (4) adequate mechanical properties that match those of the tissue at the site of implantation, and (5) be easily processed to form a variety of shapes and sizes [2]. Poly(ε-caprolactone) (PCL) is a semi-crystalline biodegradable polyester. It is well known that degradation of semicrystalline polymers occurs in two stages. The first stage consists of degradation of the amorphous phase, resulting in an increase in crystallinity of polymer. The second stage starts when majority of the amorphous regions are degraded; subsequently the crystalline phase is degraded [3]. The advantages of fibre based scaffold over other kinds of structures is that they present remarkably increased surface area for cell attachment and a significantly improved interconnected pore architecture that provides easier pathways for diffusion of gases, transportation of nutrients and migration of cells [4]. Using electrospinning it is possible to achieve the biomimetic nonwoven scaffolds that are composed of a large network of interconnected fibres and pores, resembling the topographic features of the natural extracellular matrix (ECM) [5]. However electrospun scaffolds retain several problems such as low stiffness and mechanical stability as well as lack of control of pore diameter and distribution [6]. Needle-punched nonwoven fabrics made of resorbable fibres can meet all criteria which are considered necessary for designing the ideal scaffold structure. The type of needle, number of punches per measured area, number of layers and amount of fibre entanglement within the layers can be designed to create a structure with the necessary properties to facilitate growth of tissue [7]. Moreover it is possible to modify the surface of needle-punched nonwoven fabrics with an electrospinning method. Hydroxyapatite (HAp) is a calcium phosphate-based bioceramics which is frequently used as a bone graft substitute. HAp is bioactive, osteoconductive, non-toxic, nonimmunogenic and its structure is crystallographically similar to that of bone apatite [8]. Our earlier research indicated that it was possible to incorporate the nanohydroxyapatite (n-HAp) particles into poly(ε-caprolactone) matrix during melt spinning process. Presence of HAp particles on the surface of PCL fibres after melt spinning was confirmed by SEM and FTIR studies. The current work was conducted as an extension of previous studies. Our goal was to develop needle-punched nonwoven produced from biodegradable PCL and PCL/n-HAp fibres without the use of chemicals during the manufacturing process. The properties of both nonwoven scaffold after needle punch process were compared and examined.

Materials and Methods

Scaffold production

For this study, polycaprolactone fibres (PCL) containing nanohydroxyapatite (n-HAp), prepared at the Institute of Textile Engineering and Polymer Materials, ATH-University of Bielsko-Biala, were selected. Before the nonwovens were formed, the polymer was extruded by melt-spinning and subsequent stretching to fabricate filaments. Fibres were extruded from the melt with a temperature of 170°C and were spun with a take up velocity of 247 m/min. Nano-hydroxyapatite (5 w/w %) was added to the polymer powder before melting. The receiving of masterbatch of PCL/n-HAp, before the principal process of forming fibres was applied. The other parameters of the forming process were: rotation of the extruder screw 9 rpm and rotation of the passer 10 rpm [9]. Nonwoven scaffolds were prepared from cut PCL and PCL/n-HAp fibres. Initially combed fibres were used to produce a fibrous web by mechanical processing using a laboratory carding machine (FIG. 1a). Then PCL and PCL/n-HAp fibers were bonded together using a needle bed to entangle them (FIG. 1b). For both samples the same amount of needling and needling depth was used. The stitch density (the number of needle penetrations per cm^2) was 180.

Methods

A microscopic observation of obtained nonwoven scaffold was made using scanning electron microscopy (Jeol, JSM-5500). Before the observation, the samples were coated with gold using a sputter coater. A PMI capillary flow porometer was applied to measure the pore size distribution of nonwoven fabrics. The FTIR spectra of the nonwoven scaffolds were determined using the Nicolet spectrophotometer (64 scans, in the range of 500 – 3500 cm^{-1}, resolution of 4 cm^{-1}). Mechanical properties of the nonwoven scaffolds were determined on a Zwick-Roell Z 2.5. universal material testing machine at a constant speed of 10 mm/min. The dimensions of samples (width 15 mm, length 100 mm) were measured using digital micrometer and the thickness was measured by Thickness Tester. The samples were tested under tension until failure. DSC (5100 TA Instruments) and TGA (TA Instruments Q 500 TGA) analyses were performed at the following conditions: heating rate - 10°C/min, and nitrogen gas flow - 40 ml/min.

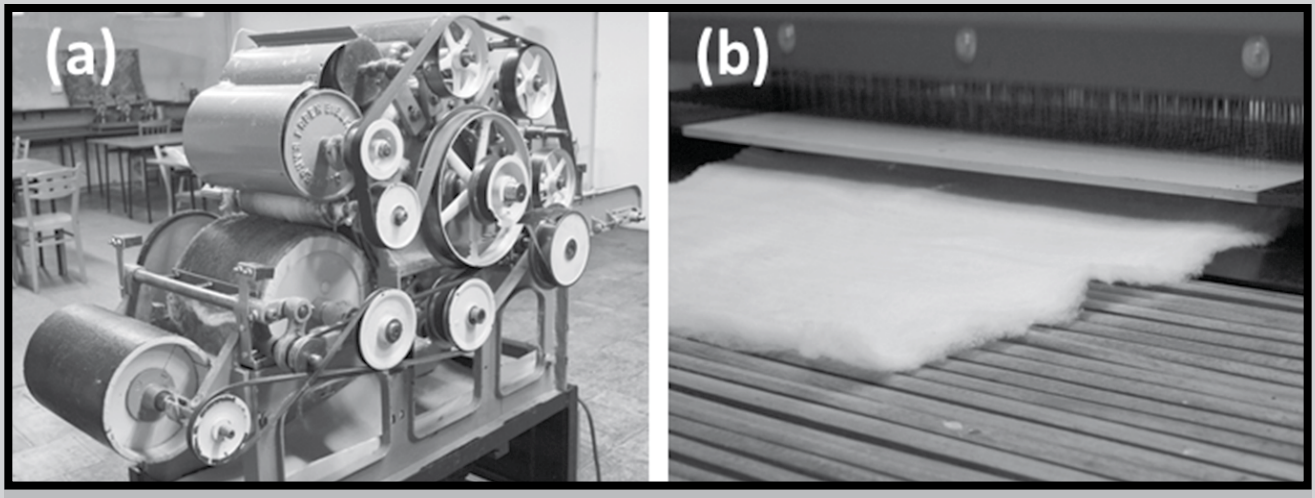

FIG. 1. (a) Laboratory carding machine; (b) Needle-punching nonwoven machine.

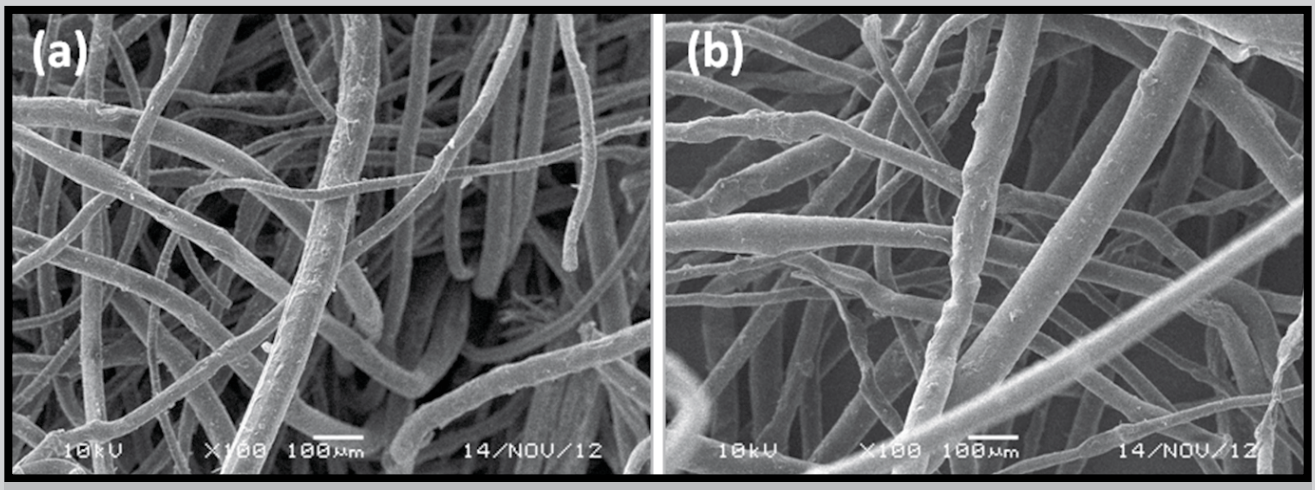

FIG. 2. Microstructure of (a) PCL and (b) PCL/n-HAp nonwovens.

Result and Discussion

The SEM images of the fibrous scaffolds are shown in FIG. 2. The PCL and PCL/n-HAp fibres in the nonwoven scaffold appeared uniformly and randomly distributed without preferential orientation. The microstructure images of nonwoven PCL and PCL/n-HAp demonstrate that they have large surface areas with a high porosity. The results of porosity tests are shown in FIG. 3. The main fraction of pores of unmodified PCL nonwoven fabric is in the range of 160-280 µm. The main fraction of pores of modified PCL/n-HAp nonwoven is centred at 180 µm, however also pores with bigger diameter in the range of 200-400 µm were observed. Fibre diameter distributions before carding process and after carding and needle-punching processes are shown in FIG. 4. The calculated average diameter for polycaprolactone fibre before carding process was 41.8 µm ± 18 µm, whereas after technological processes the average fibre diameter was slightly smaller 37.6 µm ± 18 µm. In the case of composite fibres, the PCL/n-HAp fibre diameter before carding process was 44.4 µm ± 20 µm and after needle punching process fibre diameter was 49.9 µm ± 22 µm. The carding is a process by which fibres are straightened. The fibres diameter distribution presented in FIG. 4 showed that carding process affected the fibres diameter, causing thinning of fibres. The difference in fibre diameter distribution before and after carding process is more pronounced in the case of pure PCL fibres, which are more elastic than modified PCL/n-HAp fibres. FIG. 5 shows FTIR spectra for pure PCL nonwoven fabric and PCL/n-HAp nonwoven fabric.

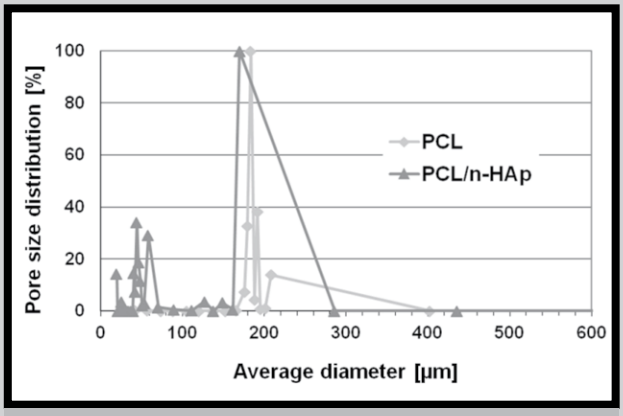

FIG. 3. Pore size distribution of PCL and PCL/n-HAp nonwovens.

The characteristic bands of PCL corresponding to the C=O stretching vibration of the ester carbonyl groups are located at 1727 cm^{-1}. The peaks at 1150-1500 cm^{-1} are related to the asymmetric stretching vibration of –COO– and the stretching vibration of –C–O bonding at the main polymer chain. In the case of PCL/n-HAp nonwoven the FTIR analysis confirmed that HAp particles remain on the surface of composite fibres after carding and needle-punching processes, which was evidenced by the presence of phosphate groups peaks PO_4^{3-} (associated to HAp) at 568 and 600 cm^{-1} [10].

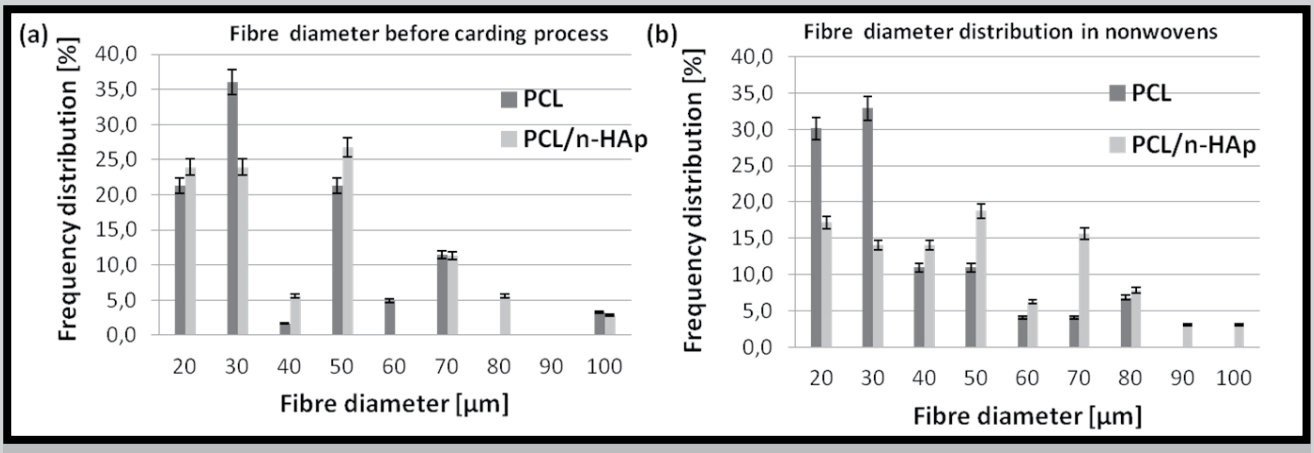

FIG. 4. Fibre diameter distribution: (a) before carding process; (b) after carding and needle-punching process.

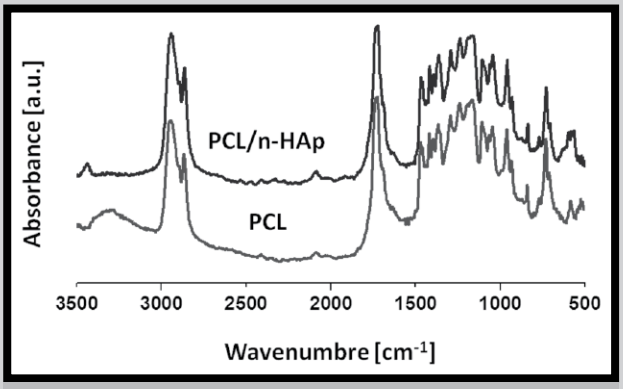

FIG. 5. FTIR spectra of PCL and PCL/n-HAp nonwovens.

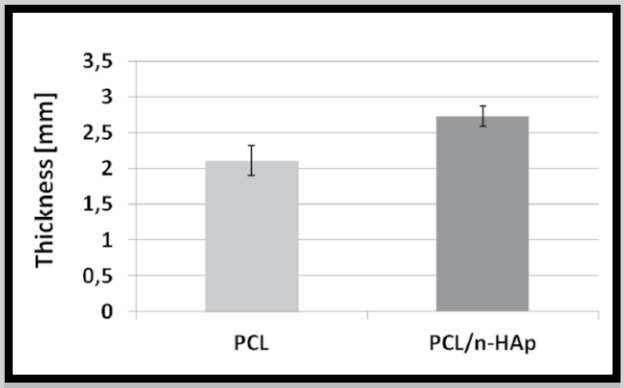

FIG. 6. Thickness of PCL and PCL/n-HAp nonwovens.

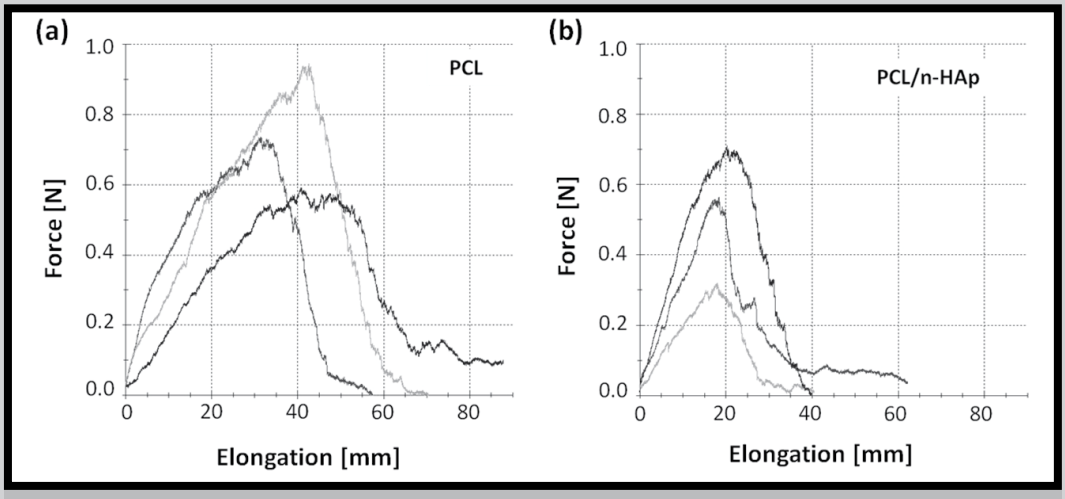

FIG. 7. Force – elongation curves of (a) PCL and (b) PCL/n-HAp nonwovens.

The results of thickness test are shown in FIG. 6 and the typical force-elongation curves for both nonwoven fabric are presented in FIG. 7. The highest tensile force among the samples of 0.95 N and the largest elongation (about 70 mm) was observed in the case of pure polycaprolactone nonwoven fabric. The higher tensile strength was observed for pure PCL nonwovens (8.75 kPa). In the case of composite PCL/n-HAp fibres tensile strength was 5.04 kPa. FIG. 8 shows the TGA analyses of PCL and PCL/n-HAp nonwovens. TGA curve of PCL nonwoven showed a two-step degradation process (FIG. 8a) whereas for PCL/n-HAp nonwoven three stages of degradation were observed (FIG. 8b) probably related to the loss of physically adsorbed water and gradual dehydroxylation of HAp powder. The degradation (or decomposition) of PCL starts at approximately 300°C. Temperatures of a maximum rate of weight loss proceeding during thermal degradation of PCL/n-HAp nonwovens (curve dTG on FIG. 7a-b) were higher than those of pure PCL nonwovens. For both samples, the melting temperature was 59°C, and the DSC curve shows one endothermic peak of melting (FIG. 9), which means that the incorporation of hydroxyapatite nanoparticles into PCL fibres had no influence on the mean size of PCL crystallites.

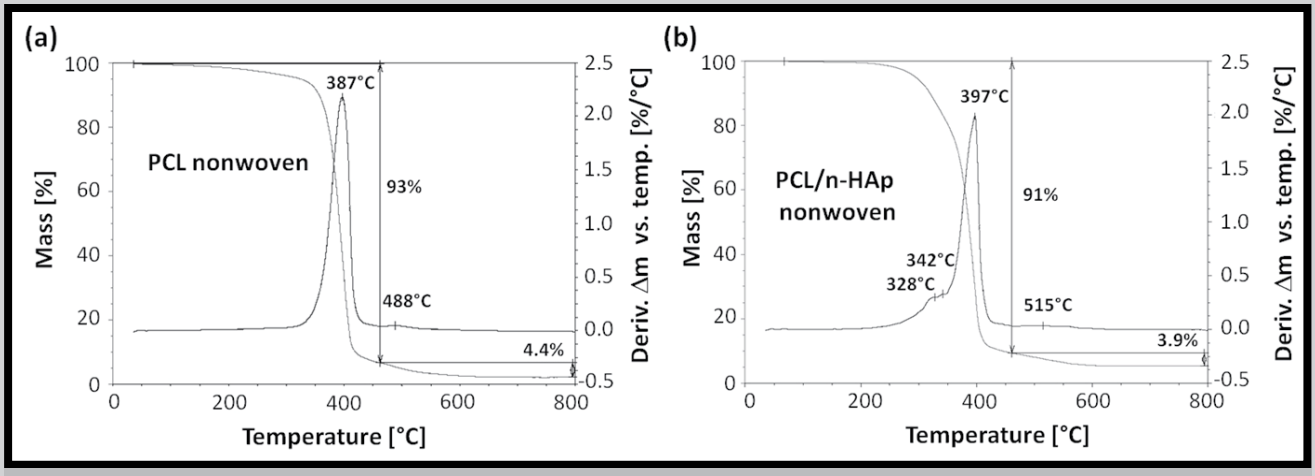

FIG. 8. TGA curves of (a) PCL and (b) PCL/n-HAp nonwovens.

Conclusion

Tissue scaffold is a 3D structure that provides a site for cell attachment, proliferation and differentiation; therefore it must possess desired porosity with an interconnected pore network and be easily processed to form a variety of shapes and sizes. Needle punching is the oldest and best established method of forming nonwoven textile materials. The aim of the presented work was to develop needle-punched nonwoven produced from PCL and PCL/n-HAp fibres without the use of chemicals during the manufacturing process. The obtained results suggest that needle-punching method can be used to produce highly porous polycaprolactone-hydroxyapatite nonwoven scaffolds. Moreover microstructure of nonwoven scaffold obtained by needle-punch process can be easily adjusted according to application. Our future work will focus on production of hybrid scaffold made of nonwoven micro-fabrics (in order to provide sufficient mechanical parameters as well as 3D structure) and of electrospun nanofibers (to ensure proper adhesion, proliferation and growth of cells and tissue).

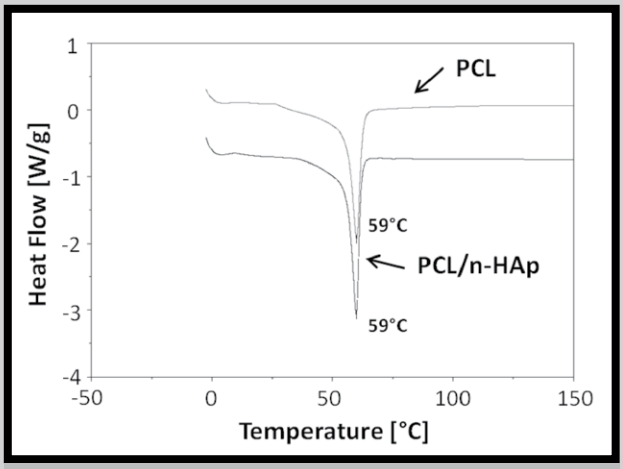

FIG. 9. DSC curves of PCL and PCL/n-HAp nonwovens.

Acknowledgments

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