PROPERTIES OF BILAYER GELATIN/POLYCAPROLACTONE SCAFFOLDS

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Abstract

In this study, nanofibrous composite scaffolds have been fabricated in order to mimic the physical architecture of native extracellular matrix. Gelatin is a good candidate to mimic the chemical composition of natural collagen. It has many integrin-binding sites for cell adhesion and differentiation, which are found in collagen. However, electrospun scaffold made of gelatin had very poor mechanical properties. Therefore, in this study, bilayer nanofibrous scaffolds made of gelatin and poly(caprolactone) were produced by sequential electrospinning. The microscopic morphology, mechanical properties and porosity of electrospun bilayer gelatin/polycaprolactone scaffold were investigated.

[Engineering of Biomaterials, 104, (2011), 2-4]

Introduction

In tissue engineering, scaffold are designed to serve as a temporary, artificial extracellular matrix (ECM) in order to provide an optimal environment for cells adhesion, proliferation and differentiation. Moreover the matrix should provide mechanical support and regulate cell activities. Gelatin as well as gelatin/synthetic polymers, have been gaining interest as a tissue engineering scaffold [1-5]. Gelatin exhibits similar properties to collagen, excellent biodegrability, non-antigenicity and cost efficiency [6]. Beside gelatin can promote cell adhesion, migration, differentiation and proliferation [7]. However, poor mechanical properties and water solubility have restricted gelatin's applications as nanofibrous scaffold in tissue engineering field. Recent studies have shown that combination of natural origin polymers (such as gelatin) within synthetic polymers would optimize the physico-chemical and biological properties [8-9]. Polycaprolactone (PCL) is a bioresorbable polymer with excellent mechanical properties [10]. 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 intensive [11-12].

In order to produce bilayer gelatin/polycaprolactone scaffold, an electrospinning technique has been applied. Electrospinning is an easy and effective method that has been used to produce nanofibrous scaffolds out of wide range of materials. A number of processing parameters such as: applied voltage, polymer flow rate, and capillary-collector distance can greatly influence the properties of the generated fibres. By combining mentioned above parameters we can tailor the final microstructure of scaffolds [13-14].

In this study electrospinning of bilayer gelatin/polycaprolactone was found to be an efficient technique to modify PCL scaffolds. The incorporation of gelatin improved the hydrophilicity of gelatin/PCL nanofibrous scaffold and PCL provided a mechanical support.

Materials and methods

Gelatin (type A, from porcine skin) was purchased from Sigma-Aldrich. To prepare spinning solutions, 3 g of gelatin were dissolved in 30 ml of trifluoroethanol. Polycaprolactone (PCL) was purchased from Sigma-Aldrich (Mn= 70 000 - 90 000 g/mol). Chloroform and methanol 1:1 (POCH, Poland) were used as solvents for this polymer.

Scaffold fabrication

The electrospinning system (made by Institute of Textile Engineering and Polymers Materials, ATH, Bielsko-Biała) consisted of a high-voltage power supply, an infusion pump, a stainless-steel blunt-ended needle, a 10 ml plastic syringe and a custom-made rotating collecting drum. For electrospinning each sample of the prepared solutions was stocked in a 10 ml plastic syringe with a needle whose inner diameter was 0.7 mm and the filled syringe was set up in the electrospinning apparatus. Both solutions were electrospun at a fixed voltage of 30 kV and distance (15 cm) between needle tip and collector. Baking paper sheet wrapped on a rotating metal drum was used as the collecting device. In order to obtain bilayer gelatin/PCL scaffold, first PCL solution was electrospun and then gelatin was e-spun over PCL nanofibrous substrate (FIG. 1). In addition two other scaffolds made of PCL and gelatin were obtained as reference materials.

Characterization of scaffolds

The surface morphology of the composites scaffolds was examined using scanning electron microscopy (SEM, Jeol JSM 5500). Pore size distribution was determined using PMI capillary flow porometer [15]. Mechanical properties of the electrospun gelatin, gelatin/PCL and PCL scaffolds were determined using uniaxial testing machine (Zwick-Roell Z 2.5.) under a cross-head speed of 1.0 mm/min. All samples were cut into strips of 20 x 100 mm (weight x length). At least three samples were tested for each of electrospun fibrous scaffold. The thickness of samples was measured with a Thickness Tester (TILMET 73). A pressure of 2 kPa was applied for all of the thickness measurements.

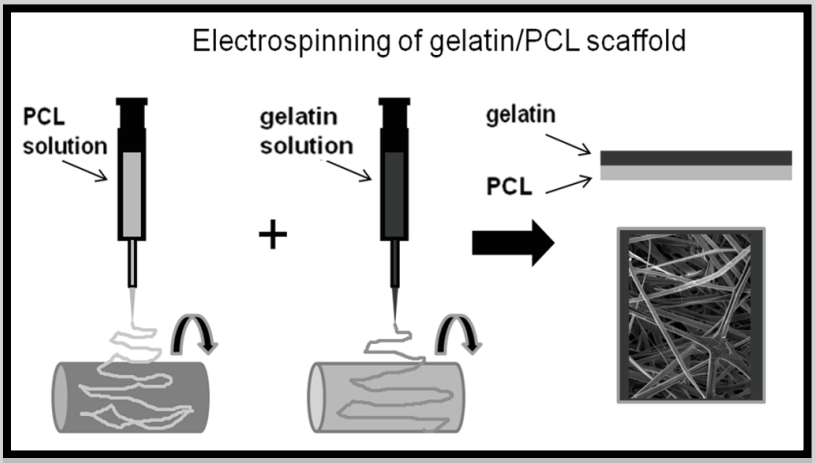

FIG. 1. The electrospinning process of bilayer samples.

Results and Discussion

In order to mimic the physical architecture of natural extracellular matrix, a method to fabricate nanofibrous gelatin/PCL composite scaffold was developed in this study. FIG. 2 shows the SEM micrographs of composite bilayer gelatin/PCL scaffolds (both sides: FIG. 2c-d), as well as pure gelatin (FIG. 2a) and pure PCL (FIG. 2b) as reference samples. The microstructures of both sides of composite scaffold were similar to those of proper reference materials. The ribbon shaped fibers of gelatin nonwoven scaffold had a thickness of 200-700 nm and a width up to 5 μm. Rapid solvent removal from the surface of the jet tended to form a skin on the jet as dried. As the evaporation progressed, the skin remained as a hollow tube, which collapsed into flat ribbon. Small branches between fibers were observed in the case of gelatin fibers as well as on the gelatin side of composite scaffold. The SEM images of PCL and PCL side of composite scaffold showed smooth and bead free surfaces of the nanofibers. The diameter of electrospun PCL fibers ranges from 300 nm to 1.2 μm. The pore size and interconnectivity between pores are also important parameters of the scaffolds. Pore size of the pure PCL scaffold were measured to be in the range of 1-2 μm whereas for pure gelatin scaffolds 2-16 μm (FIG. 3). Addition of gelatin onto PCL scaffold increased the average pore diameter of nanofibrous composite scaffold. Fiber structure, geometrical arrangement of the fibers, individual fiber properties and interaction between fibers greatly influence the mechanical properties of nanofibrous scaffold. Representative stress-strain curves for gelatin, PCL and gelatin/PCL scaffolds are shown in FIG. 4. Compared with gelatin/PCL and pure PCL electrospun scaffolds, the gelatin nanofibrous sample showed relatively low mechanical properties. The addition of PCL greatly increased the strength and elastic behavior of the composite fibrous scaffold. The enhanced properties of finer diameter fibers (PCL) are attributed to the gradual ordering of the molecular chains and modest increase in the crystallinity of the fibers.

Since the gelation temperature of gelatin is very close to cell culture temperature (37°C) the gelatin scaffold must be crosslinked to improve its thermal and mechanical stabilities prior to its tissue engineering applications [12]. Further study will be focus on physical and chemical crosslinking methods for gelatin and on the incorporation of bioactive inorganic nanoparticles within the gelatin/PCL phase reaping up the combinatory roles of bone–bioactivity and rigidity of inorganic phase, degrability and hydrophilicity of gelatin and optimal mechanical properties of PCL.

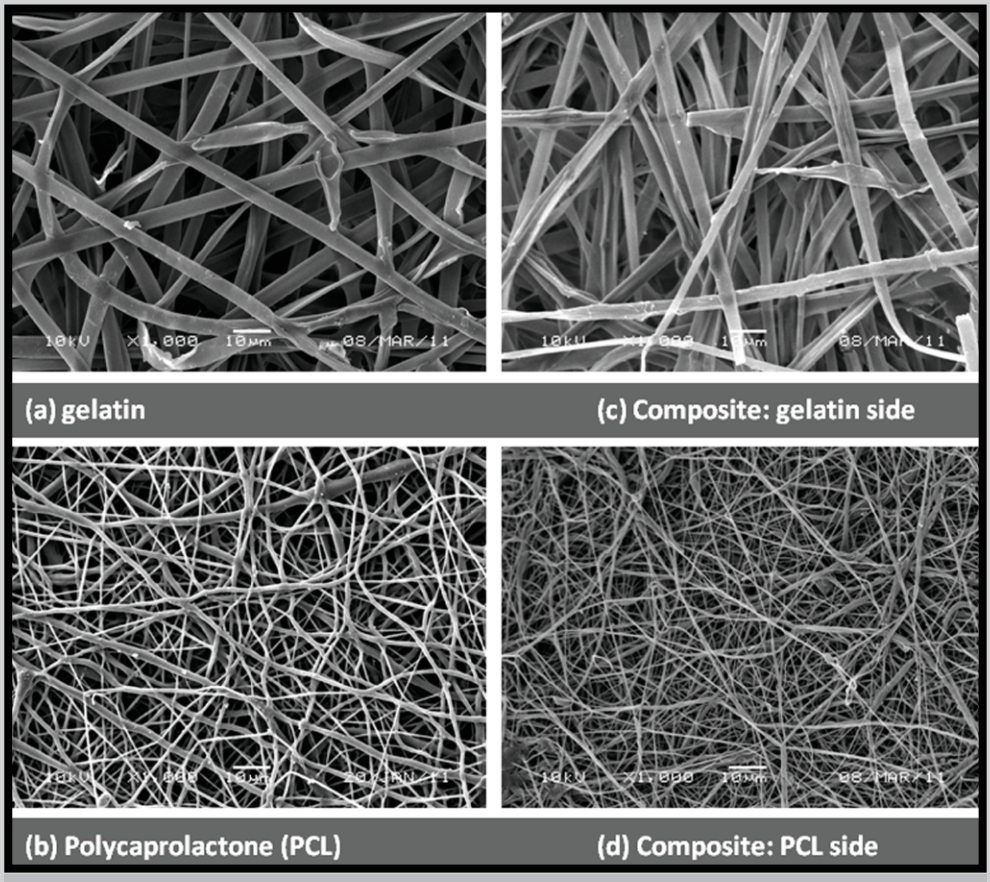

FIG. 2. SEM micrograph of (a) gelatin nanofibers, (b) PCL nanofibers, (c-d) bilayed gelatin/PCL scaffold.

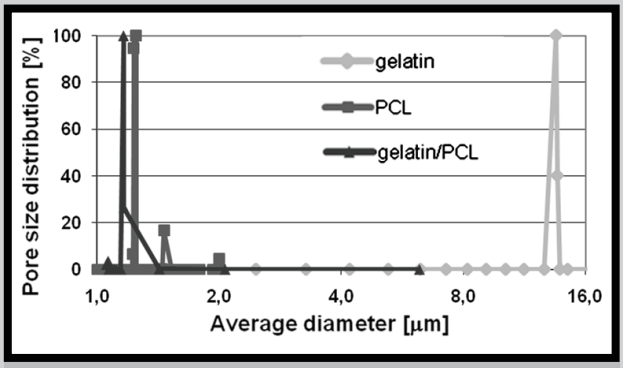

FIG. 3. Pore size distribution of electrospun gelatin, PCL and gelatin/PCL scaffolds.

Conclusions

A significant amount of research has been directed to electrospinning nanofibrous scaffolds targeted for bone tissue regeneration. Moreover there is increasing research on the surface modifications in order to regulate cell functions from the initial cell adhesion to osteogenic stimulation of cells. In this work nanofibrous gelatin/PCL scaffolds have been successfully fabricated by electrospinning technique. Obtained results clearly showed that electrospun bilayer gelatin/polycaprolactone composite had better mechanical properties and pore size distribution than pure gelatin scaffold. The enhanced strength and porosity of obtained electrospun material would be very beneficial for tissue engineering applications. The cross-linking study of the composite and analysis of their in vitro behavior are in progress in our laboratory.

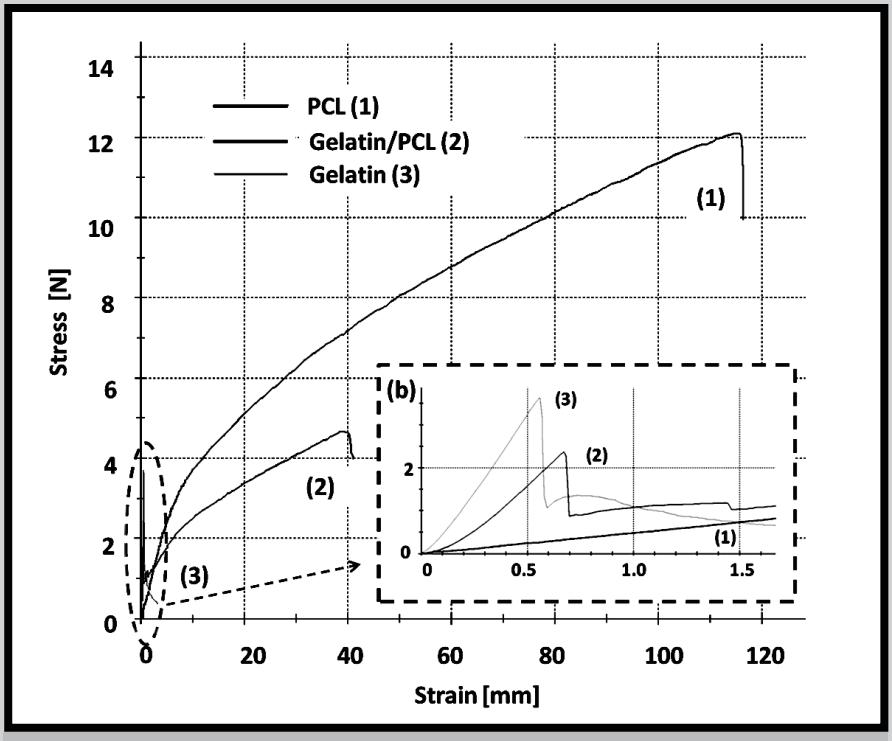

FIG. 4. Stress-strain curves obtained from tensile tests performed on electrospun samples (1) PCL, (2) gelatin/PCL, (3) gelatin.

Acknowledgements

This work was supported by Polish Ministry of Science and Higher Education (Iuventus Plus, project number: IP2010034270).

References

[1] Z.X. Meng, Y.S. Wang, C. Ma, W. Zheng, L. Li, Y.F. Zheng. Electrospinning of PLGA/gelatin randomly-oriented and aligned nanofibers as potential scaffold in tissue engineering. Materials Science and Engineering C 30 (2010) 1204-1210.
[2] Z.M. Huang, Y.Z. Zhang, S. Ramakrishna, C.T. Lim. Electrospinning and mechanical characterization of gelatin nanofibers. Polymer 45 (2004) 5361-5368.
[3] L. Ghasemi-Mobarakeh, M.P. Prabhakaran, M. Morshed, M.H. Nasr-Esfahani, S. Ramakrishna. Electrospun poly(ε-caprolactone)/gelatine nanofibrous scaffold for nerve tissue engineering. Biomaterials 29 (2008) 4532-4539.
[4] X. Liu, P.X. Ma. Phase separation, pore structure and properties of nanofibrous gelatin scaffolds. Biomaterials 30 (2009) 4094-4103.
[5] A. Kejing, L. Haiying, G. Shidong, D.N.T. Kumar, W. Qingqing. Preparation of fish gelatin/poly(L-lactide) nanofibers by electrospinning. International Journal of Biological Macromolecules 47 (2010) 380-388.
[6] S.A. Sell, M.J. MacClure, K. Garg, P.S. Wolfe, G.L. Bowlin. Electrospinning of collagen/biopolymers for regenerative medicine and cardiovascular tissue engineering. Advanced Drug Delivery Reviews 61 (2009) 1007-1019.
[7] J.P. Chen, C.H. Su. Surface modification of electrospun PLLA nanofibers by plasma treatment and cationized gelatin immobilization for cartilage tisse engineering. Acta Biomaterialia 7 (2011) 234-243.
[8] J. Lee, G. Tae, Y.H. Kim, I.S. Park, S.H. Kim, S.H. Kim. The effect of gelatin incorporation into electrospun poly(L-lactide-co-ε-caprolactone) fibers on mechanical properties and cytocompatibility. Biomaterials 29 (2008) 1872-1879.
[9] S. Panzavolta, M. Gioffre, M.L. Focarete, C. Gualandi, L. Foroni, A. Bigi. Electrospun gelatin nanofibers: optimization of genipin crosslinking to preserve fiber morphology after exposure to water. Acta Biomaterialia 7 (2010) 1702-1709.
[10] L. Shor, S. Guceri, R. Chang, J. Gordon, Q. Kang, L. Hartsock, Y. An, W. Sun. Precision extruding deposition (PED) fabrication of polycaprolactone (PCL) scaffolds for bone tissue engineering. Biofabrication 1 (2009) 1-9.
[11] P. Fabbri, F. Bondioli, M. Messori, C. Bartoli, D. Dinucci, F. Chiellini. Porous scaffolds of polycaprolactone reinforced with in situ generated hydroxyapatite for bone tissue engineering. Journal of Materials Science: Materials in Medicine 21 (2010) 343-351.
[12] X. Liu, L.A. Smith, J. Hu, P.X. Ma. Biomimetic nanofibrous gelatin/apatite composite scaffolds for bone tissue engineering. Biomaterials 30 (2009) 2252-2258.
[13] N. Bhardwaj, S.C. Kundu. Electrospinning: A fascinating fibre fabrication technique. Biotechnology Advances 28 (2010) 325-347.
[14] S. Agarwal, J.H. Wendorff, A. Greiner. Use of electrospinning technique for biomedical applications. Polymer 49 (2008) 5603-5621.
[15] I. Rajzer, W. Chrzanowski, W. Binias, E. Sarna, J. Janicki. Biomimetic fibrous composite membranes for bone tissue engineering. Engineering of Biomaterials 93 (2010) 2-5.