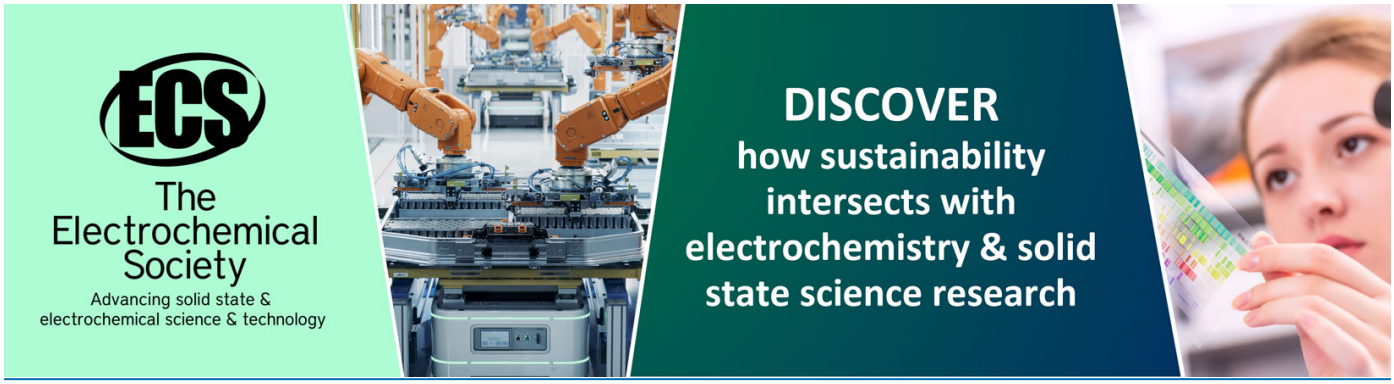
Fabrication and characterization of polycaprolactone cross- linked and highly-aligned 3-D artificial scaffolds for bone tissue regeneration via electrospinning technology



Fabrication and characterization of polycaprolactone cross-linked and highly-aligned 3-D artificial scaffolds for bone tissue regeneration via electrospinning technology

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Abstract. Novel technologies allowed the scientific community to develop scaffolds for regeneration of bone tissue. A successful scaffold should possess specific macroscopic geometry and internal architecture to perform biological and biophysical functions. In this study the process of polycaprolactone microfibrous development with either cross-linked or highly-aligned three-dimensional artificial mats via electrospinning technology for potential application in tissue engineering is described. The morphology and size of electrospun fibers were assessed systematically by varying the rotation speed of grounded collector. It was found that the diameter of the fibers decreased by increasing the rotation speed of collector. The morphology of the fibers changed from cross-linked to highly-aligned at appr. 1000-1100 rpm.

1. Introduction

With the development of the modern bone regenerative medicine, more and more novel materials based on the concept of the nature are used [1]. These materials possess bioinspired, multiple bone-like structures with unique properties close to that of native human skeletal system.

Nowadays, the development of biomaterials for substitution of bone tissue defects with predetermined architectonics and individual functional optimization as alternative for available prostheses and implants is one of the most important challenges [2].

The three-dimensional (3-D) scaffolds define as porous solid 3-D structures to perform various functions, including promotion of the cells adhesion and formation of the extracellular matrix (ECM). Several characteristics, such as morphology, porosity, biodegradation rate, acceptable biocompatibility are required for the fabrication of 3-D scaffolds. The physical characteristics as pores geometry of the scaffold play a crucial role in the formation of the natural ECM by providing support for the cells and vascularization. Insufficient vascularization can lead to inaccurate cell integration and proliferation, resulting in cell death in the constructs [3].

Presently, there are three methods accessible for the synthesis of 3-D fiber structure, using different physical principles: phase separation [4], self-assembly [5] and electrospinning [6]. Electrospinning is the most broadly used technique and present the most promising results for tissue engineering applications, when compared with other methods. The reason of success is that this technique allows to fabricate fibers with the diameter from 3 nm to 5 μm or even more [7].

After the development of the elecrospinning process by Zeleny [8] in 1914 it was patented in 1934 by Formhals [9]. During electrospinning an electric field is applied to convert polymer solution into the form of micro- or nano-fibers. Schematic diagram of the electrospinning process for manufacturing of fiber-structured mats is shown in Figure 1. When an external electric field is applied between a pendant droplet of a polymer solution and a metal collector which serves as a counter electrode, the solution gets charged and electrostatic repulsion affects the surface tension of the droplet and it is deformed into a conical shape (Taylor cone).

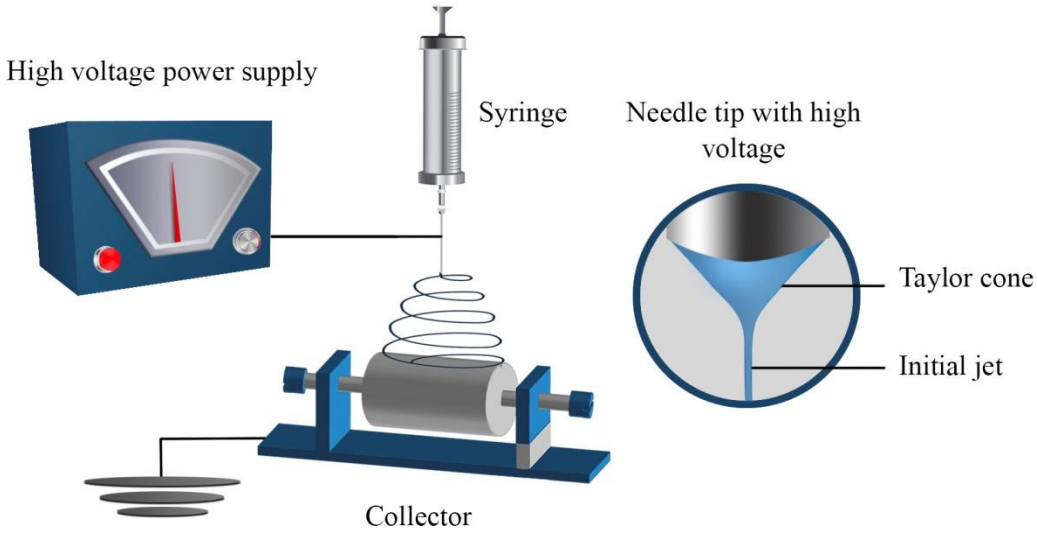

Figure 1. Schematic representation of the electrospinning system

When the electric field strength approaches a critical value, which is sufficient to overcome the surface tension of the droplet, a charged, continuous jet of a polymer solution is ejected from the needle towards a collector. The fluid jet of a solution accelerates towards the collector, undergoes significant bending instability and whipping.

As the polymer is moved from the needle towards the grounded collector, the solvent evaporates quickly. On the surface of the collector, a mat of solid nanofibers in nonwoven form is deposited. A variation of the rotation speed results in increase of the mechanical properties of scaffolds [10].

The method of electrospinning is well suited to produce natural and synthetic biocompatible polymers for diverse biomedical applications. These materials can be potentially used as wound dressings, artificial blood vessels and bone tissue engineering scaffolds [11].

Polymers are organic materials which consist of large macromolecules represented by covalently bonded chains of atoms. Unless they are cross-linked, the macromolecules interact with one another via weak secondary bonds (hydrogen and van der Waals bonds). The mechanical, thermal and other properties of the polymers are influenced by several factors, including the composition of backbone, side groups, structure of chains, and the molecular weight of the molecules [12]. Polycaprolactone (PCL) is biodegradable aliphatic polyester with desirable mechanical properties and slow degradation rate, which makes it possible to be used for hard and soft tissue repair. This study aimed to develop PCL electrospun scaffolds for bone tissue engineering with enhanced biocompatibility and vascularization. This polymer was used in the study due to higher resistivity to degradation rate in biofluids compared with other bioresorbable polymers [13].

In the present study, the feasibility of electrospinning process of polymers for bone tissue engineering applications was demonstrated. The effect of the rotation speed of the collector on the scaffolds fibers orientation and morphology was studied.

2. Materials and methods

2.1 Polymer

The PCL polymer with an average molecular weight of 80 kDa and density of 1.145 g/mL at 25 °C and chloroform ($CHCl_3$) were obtained from Sigma-Aldrich (St. Louis, MO, USA) Electrospinning solutions were prepared by dissolving PCL in chloroform with a stirrer and ultrasonic homogenizer to a concentration of 9% (w/v). Viscosity of the polymer is a crucial characteristic to prepare the uniform and homogeneous structure of the scaffolds [14]. It was measured with Rotational Rheometer: RheolabQC (Anton Paar GmbH, Austria) and the value of the viscosity for 9% (w/v) PCL solution was 1.47 kg/(s·m).

2.2 Electrospinning

A custom-made electrospinning apparatus with a high-voltage generator (Weil am Rhein, Germany) was designed. A syringe pump was used to feed the solutions through extension tube capped with blunted 24-gauge needles (inner diameter of 0.55 mm). The polymer solution was delivered at a constant flow rate of 2 ml/h. The cylindrical stainless steel collector rotating with the speed ranging from 200 to 1100 rotation per minute (rpm) was used. The fixation of the syringe was made in a horizontal plane of the infusion pump. Delivery of the polymer solution to the needle was done through a plastic tube, which was fixed vertically at a distance of 5 cm above the collector. A high voltage potential (7 kV) was applied between the tip of the needle and the collector. The polymer solution was forced going through the needle and collected as fibers on the rotating cylinder. The well-aligned and cross-linked fibrous membranes were separated from the collector and used for further analysis.

2.3 Surface characterization

A Fourier transform infrared (FT-IR) spectrometer Bruker Equinox 55 (Saarbrücken, Germany) was used for ATR–FT-IR measurements. Topographic images of the fibrous scaffolds were examined using a Keyence VHX Portable Digital Optical Microscope (Neu-Isenburg, Germany), which allows performing microscopic examination of the samples.

3. Results and discussion

To study the effect of the rotating collector speed on the morphology and structure of the microfiber mats, the rotation speed of the collector was varied. Optical micrographs of electrospun PCL scaffolds are shown in Figure 2. The thickness of the scaffolds is ~ 0.2 mm.

It was revealed that with the rotation speed of collector in the range from 200 rpm to 800 rpm randomly oriented structures and smooth morphology of the electrospun PCL microfibers were obtained. An average interconnected pores size of three-dimensional fibrous mesh is ~10-50 μm, which are composed of randomly oriented microfibers with diameter ranging from 4 μm to 6 μm (Figure 3: a-d). Was noted that the by increasing the collectors speed to 1000 and 1100 rpm, the fibers diameter ranged from 4 μm to 2 μm (Figure 3: e, f). The morphology of the scaffolds has become oriented along the longitudinal axis which resulted in an aligned structure of the scaffold. When the rotation speed of the collector increases, the number of pores decreases.

This controlled alignment in two- and three-dimensional architecture of the fibers can positively affect cell differentiation and proliferation, since ECM found in living tissues has regular and defined structure [15]. It is reported that the aligned structure of the scaffold can serve as a base for creation *in-vivo*-like morphology and mechanical deformations can be uniformly delivered to all cells in the cultures [16].

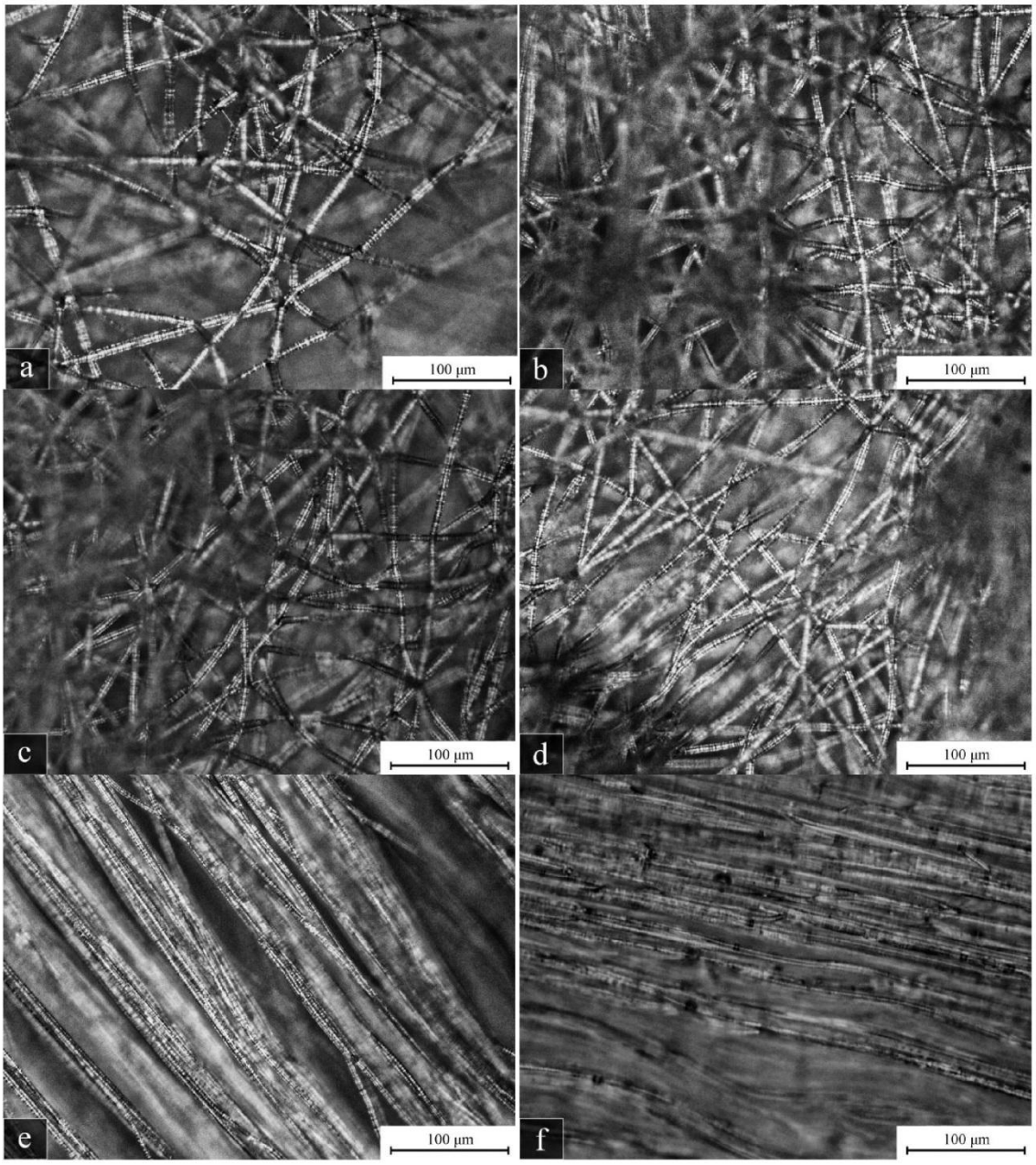

Figure 2. Optical micrographs of microfibrous PCL scaffolds: (a) 200 rpm, (b) 400 rpm, (c) 600 rpm, (d) 800 rpm, (e) 1000 rpm, (f) 1100 rpm.

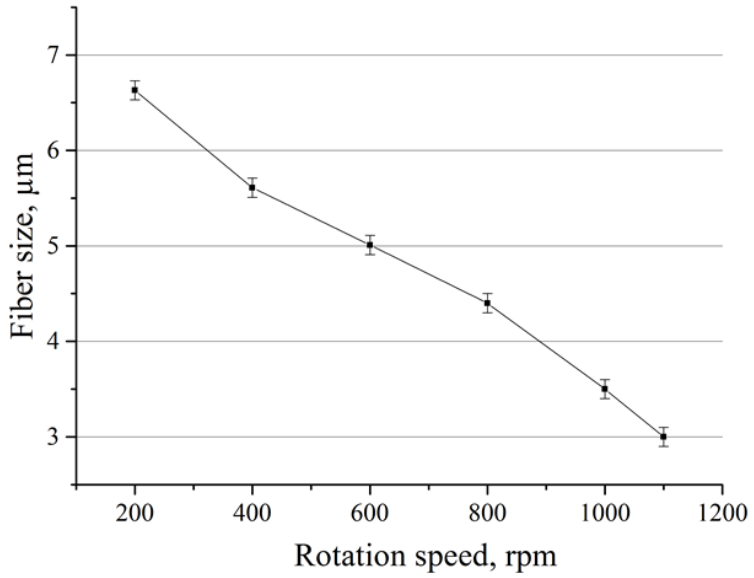

Figure 3. Dependence of the PCL fiber size from rotation speed of the collector

In Figure 4 a FT-IR spectrum of PCL in the wavelength range of 3000-600 cm^{-1} is shown. From these data carbonyl stretching mode around 1727 cm^{-1} can be observed. The most intensive bands which correspond to PCL are presented in Table 1. It is known that the band at 1293 cm^{-1} relates to the backbone C–C and C–O stretching modes of the crystalline phase in the PCL (see Table 1), which also characterize the structure of the scaffold [17]. So, after different manipulations with the polymer, the composition of the PCL was not changed.

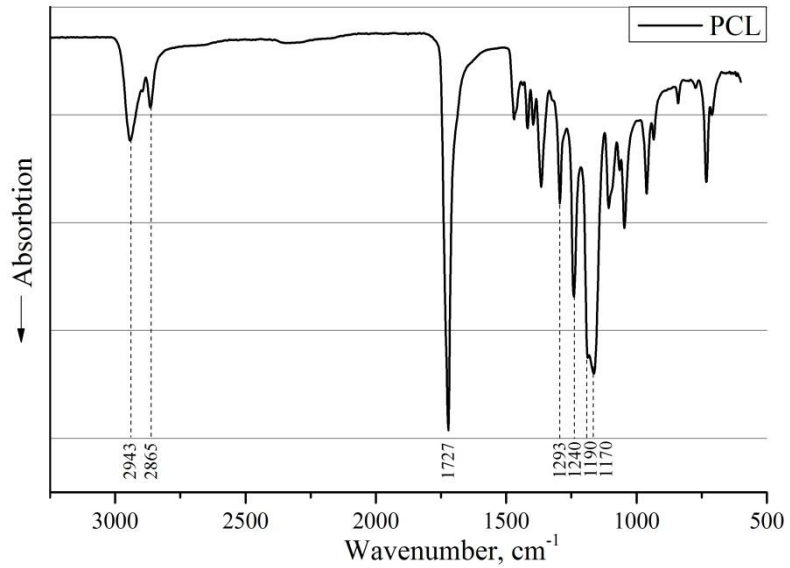

Figure 4. FT-IR spectrum of PCL mats in the region 3000-600 cm^{-1}.

Table 1. Characteristic infrared bands of PCL [17], [18]

Value [cm^{-1}]	Vibration	Abbreviation
2943	Asymmetric CH_2 stretching	ν_{as} (CH_2)
2865	Symmetric CH_2 stretching	ν_s (CH_2)
1727	Carbonyl stretching	ν (C=O)
1293	C–O and C–O and C–C stretching in the crystalline phase	ν_{cr}
1240	Asymmetric COC stretching	ν_{as} (COC)
1190	OC–O stretching	ν (OC–O)
1170	Symmetric COC stretching	ν_s (COC)

4. Conclusion

In this study, electrospun microfiber PCL scaffolds were developed and the influence of the rotation speed of the collector during electrospinning process on the structure of the polymer fibers was investigated. The results revealed that by increasing the speed rotation, the fibers diameter decreases, presenting a structure preferentially oriented in one particular direction, as confirmed by optical microscope analyses. This study serves as a basis for future development of electrospun 3-D polymer PCL scaffolds for repair of bone defects.

Acknowledgements

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