PREPARATION AND CHARACTERIZATION OF NANOFIBROUS MATERIALS WITH A SHISH-KEBAB STRUCTURE

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Abstract

Biodegradable nanofiber materials are widely used for biomedical applications such as tissue engineering. These materials are characterized by submicron fibre diameter, small pore size, and large specific surface area (surface to volume ratio), and this structure is very similar to the natural extracellular matrix and is a favorable environment for the growth of eukaryotic cells. These days research focuses on other modifications of nanofiber structures for upgrades these structures or to create specific properties e.g. antimicrobial character. Fibre morphology and structure are able modified during their preparation, a suitable method for that is electrospinning technology and post-process modification. Electrospun materials with nanofiber backbone decorated with protruding periodic pattern of polymer crystals are termed nanofiber shish-kebabs. The shish-kebab structure can be formed by post-process recrystallization in suitable liquids (dilute polymer solution, partial solvent, or a mixture of solvent and non-solvent, solvent or partial solvent, or dilute polymer solution evaporation. The crystal period can be controlled to be a few hundreds of nanometres. Partial enzyme-catalyzed degradation of electrospun nanofibers is another method to prepare a similar structure. In this case, amorphous parts of polymer nanofibers are preferentially degraded, and the remaining macromolecules form new structures, mainly crystal structures resembling nanofiber shish-kebab. The aim of this study was preparation, description, and characterization of mentioned structure.

Keywords: Electrospinning, shish-kebab structure, post-process modification, recrystallization, polycaprolactone, enzymatic-degradation

1. INTRODUCTION

Biocompatible polymers are widely used materials for biomedical applications. [1,2] One of the main representatives is the biodegradable polyester polycaprolactone. Mainly due to its properties (biodegradability, cytocompatibility, low glass transition temperature and a melting point of 60 °C, availability, solubility, processability, etc.), it is applied in the areas of tissue engineering in the form of various types of scaffolds. [3] Much attention is focused on nanofibrous scaffolds. Technology suitable for the preparation of nanofibrous scaffolds is electrospinning technology. [4]

Currently, research is focused on modifying the nanofibrous structure. By changing or modifying the structure of nanofibers, new properties can be achieved. For example, the creation of a more attractive environment for the restoration of damaged tissues or the antibacterial character of the material and many others. [5] Modification of fibre structure can be achieved in many ways. [6,7] One of them is post-process recrystallization in a suitable liquid (dilute polymer solution, partial solvent, or a mixture of solvent and non-solvent, solvent or partial solvent, or dilute polymer solution evaporation) Electrostatically spun nanofibers, decorated with periodically protruding patterns of polymer crystals they are called shish-kebab nanofibers. The shish-kebab structure, respectively the distance of the crystalline phases (kebabs) can be controlled. [8,9] Another way to create a shish-kebab structure is the application of partial enzymatic degradation. Short-term exposure of nanofibrous polyester materials to enzymatic action causes hydrolytic cleavage of polymer chains and the restructuring of fibres. [8]

2. MATERIAL AND METHODS

2.1. Materials

Polycaprolactone PCL 80 (Mn 80 kDa; Merck, Germany) was used for the preparation of nanofibrous layers. A mixed solvent was used consisting of chloroform and dimethylformamide (Penta, Czech Republic) in a ratio of 6:4 by weight. For post-process modification of nanofibrous materials was used a solution of Polycaprolactone PCL 45 (Mn 45 kDa; Merck, Germany) dissolved in chloroform (Penta, Czech Republic) and distilled water. Partial enzymatic degradation was performed by Lipase from *Pseudomonas cepacia* (specific activity 30 ≥ $U \cdot mg^{-1}$; Merck, Germany). Sodium azide (NaN_3) was obtained from Merck (Germany).

2.2. Electrospinning

The polymer PCL 80 was added to the mixed solvent and stirred at room temperature until the polymer was completely dissolved to generate 12 wt% solutions. The Nanofibrous layer was prepared using electrospinning technology (Nanospider™ 1WS500U, Elmarco, Czech Republic). The prepared polymer solutions were electrospun at a humidity of 40% and temperature of 22 °C. The applied voltage was -10 kV (collector) and +30 kV (spinning string). The electrode distance was 180 mm, a carriage was 1 s, and the rewinding speed was 10 mm/min. The diameter of the string was 0.4 mm, and the size of the hole was 0.7 mm. After that, material was stored at room temperature until modification.

2.3. Nanofiber modification

Post-process recrystallization and partial enzymatic degradation were used for nanofibrous materials modification and preparation of the shish-kebab structure.

Post-process recrystallization method (PPR)

Nanofibrous material was cut to the samples (100x100 mm). Diluted PCL 45 solution was used for forming a shish-kebab structure on nanofibers. A mixture of good solvent and non-solvent created a suitable condition for forming a shish-kebab crystal structure. 1.5 wt% solution was prepared by dissolving polymer pellets in acetic acid as a good solvent for PCL on a magnetic stirrer at 70 °C. Distilled water as non-solvent for PCL was added in the next step. Solvents were in the ratio of 77:23 by weight. After that, the solution was cooled down at room temperature and then stored in the fridge at 4°C until use. A prepared solution was applied to nanofibrous sheets without fixation by a wash bottle (spray). After that, were modified materials were placed on silicon paper and dried at room temperature in a laboratory digester. As the solvent evaporated, the shish-kebab crystal structures formed.

Partial enzymatic degradation method (PED)

Nanofibrous material was cut into small samples (the weight of each sample was 50 ± 5 mg) and placed into 5 ml vials. Enzymatic catalyzed degradation was carried out at 37 °C for 24 hours. As a medium was used phosphate buffer solution (PBS with 0.02 % NaN_3, pH 7.4) with enzyme Lipase (0.5 $U \cdot ml^{-1}$) and non-modified material was incubated only in PBS. After degradation were materials washed twice with distilled water and dried at 25 °C in the laboratory dryer before further investigation.

2.4. Morphological analysis

Morphological analysis was performed by scanning electron microscopy (Vega Tescan 3, Tescan, Czech Republic) after sputter coating 12 nm of gold (Quorum Q50ES, Quorum Technologies, Great Britain). Samples were analyzed at an accelerating voltage of 15 kV. The-as taken images were processed by using of software Image J. The average diameter and the diameter distribution were evaluated from 300 randomly measured values from 4-5 images. Shish-kebab structure was evaluated by a measure of the periodic size of kebabs, size of kebabs, and shish diameters as defined in the schematic image in **Figure 1**. Respectively periodic size distances of kebabs were measured in 1-10 µm sections, and then divided by the number of pitches.

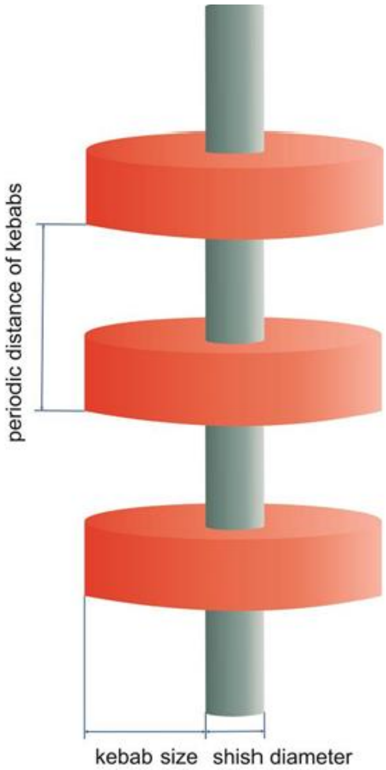

Figure 1 A schematic structure of shish-kebab

3. RESULT AND DISCUSSION

Post-process modification and creation of protruding periodic pattern of polymer crystal called shish-kebab at nanofibers were studied. Post-process recrystallization by diluted solution and partial enzymatic degradation were used as two methods of modifying PCL 80 fibres.

Figure 2 represents the results of SEM analysis for PCL 80 without modification and for PCL80 after individual modifications. It can be seen from the images that the formation of a shish-kebab structure occurs in both types of post-process treatment of nanofibers compared to the original structure of PCL80 nanofibers. However, the PPR method achieves a very regular shish-kebab structure, especially for fibres with a diameter of around 1µm. Compared to the PPR method, the PED method does not achieve such results. The shish-kebab structure is very irregular and indented; it is observed mainly in fibres with a larger diameter. For fibres with smaller diameters, we observe fibre restructuring, defects and even complete fibre degradation.

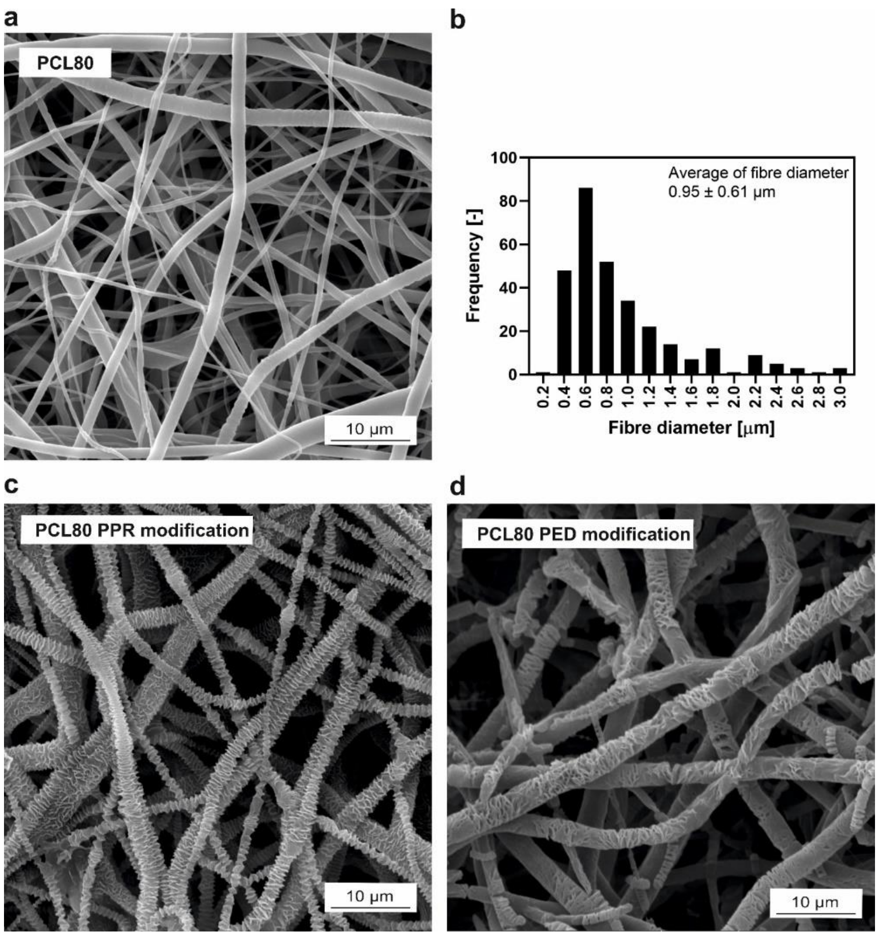

Figure 2 SEM images of PCL 80 (without modification) (a), PCL80 after post-process recrystallization by the diluted solution of PCL45 in AA/dH_2O (77:23, w:w) (c) and PCL80 after partial enzymatic degradation (24 hours) (d). Scale bare 10 µm. The image of PCL80 is appended by the histogram of fibre diameter (b).

The average periodic distance of kebabs for PCL80 after PPR modification was determined by measurement to be 0.46 ± 0.1 µm, and the height of the kebab (kebab size) was determined to be 0.29 ± 0.09 µm. The regularity of the structure observable by SEM analysis can also be supported by histograms of the analyzed characteristics (distances of individual kebabs and kebab size), as shown in **Figure 3**. The average periodic distance of kebabs for PCL80 after PED modification was determined by measurement to be 0.56 ± 0.15 µm. The height of the kebab was not due to the irregularity and fragmentation of the shish-kebab structure measured. An analysis of the diameter of the fibres in terms of the effect of degradation on the formation of the shish-kebab structure or the fragmentation and disintegration of the fibres was attached here. We observe (**Figure 4**) that a shish-kebab structure is formed for fibres of higher diameters (around 3.00 µm ± 0.89 µm), while for fibres around 1.12 ± 0.32 µm, fibre restructuring, fragmentation and fibre degradation already occur. Finer fibres (below 0.9 µm) are observed sporadically. They were probably completely eliminated due to degradation.

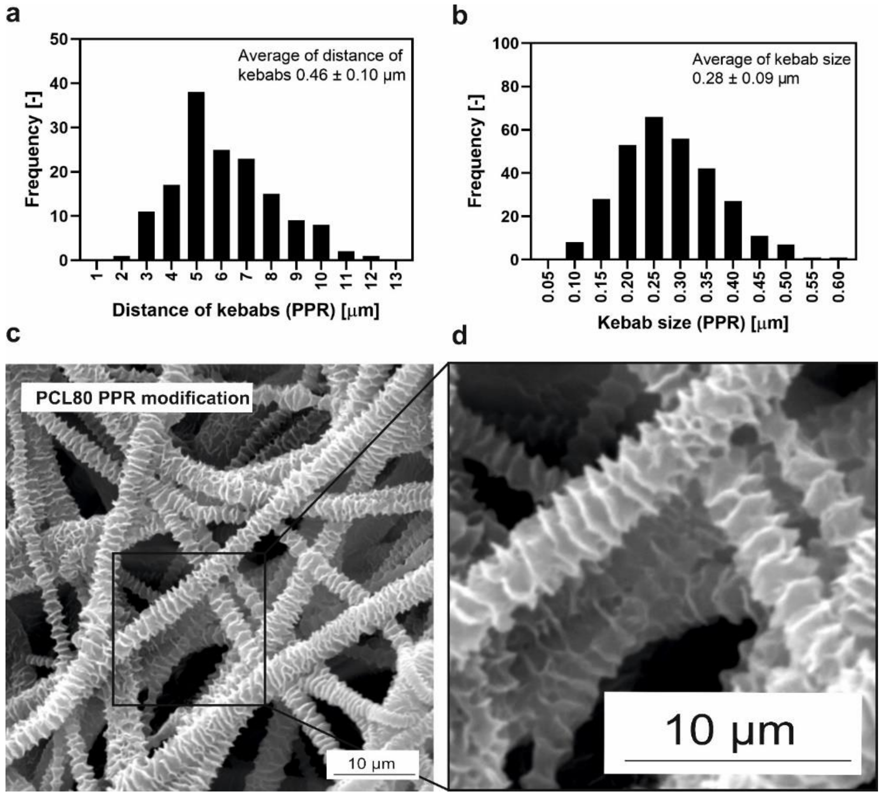

Figure 3 SEM image of the shish-kebab structure of PCL 80 nanofibres after post-process recrystallization (scale bare 10 µm) (c-d); a histogram of observed structure characteristic: a periodic distance of kebabs (a) and size of kebabs (b).

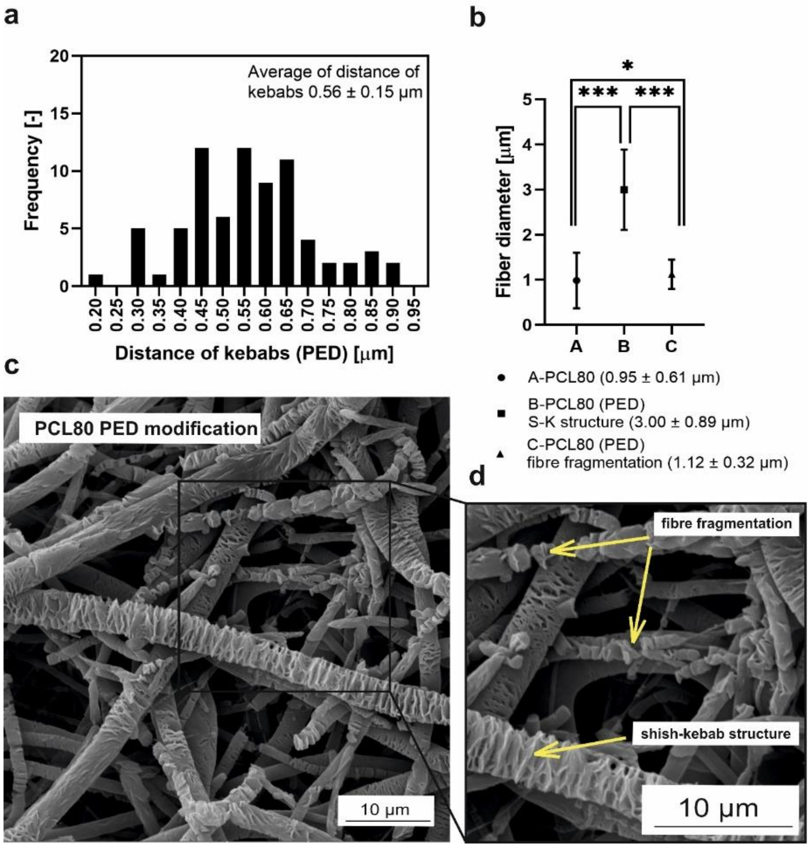

Figure 4 SEM image of the shish-kebab structure of PCL 80 nanofibres after partial enzymatic degradation (c-d). Scale bare 10 µm. A histogram of the periodic distance of kebabs (a) and graph of degradation influence to creation of shish-kebab structure or fibre fragmentation (b).

4. CONCLUSION

In the presented study, two methods of modification of PCL nanofibers were applied with the aim of creating a shish-kebab structure; the post-process recrystallization (PPR) method under the influence of a diluted solution of PCL45 in AA:dH2O (77:23, w:w) and partial enzymatic degradation (PED)) due to lipase (incubation 24 hours). Two main characteristics of the shish-kebab structure of the modified materials, the periodic distance of the kebabs and the height of the kebabs (kebab size), were analyzed. Through morphological analysis, it was observed that a more regular shish-kebab structure was achieved by the PPR method. While the PED method creates a shish-kebab structure irregularly. Probably caused by a different rearrangement of the fibre structure. In PPR, there is a gradual formulation of kebab lamellas in the nuclei of crystalline areas in the fibres. [9], [10] While with the PED method, we observe the hydrolytic cleavage of chains primarily in amorphous regions, then the crystalline phases (remaining macromolecules) form a new structure - individual kebabs on nanofibres. Fibres of finer diameters can be fragmented or even degraded due to hydrolytic cleavage, and no shish-kebab structure is formed. [8]

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