FORMATION AND PROPERTIES OF DBC/PLA MICROFIBRES

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Abstract

In this study, a solution of dibutyrylchitin (DBC)/polylactide (PLA) blend micro and nanofibres were successfully fabricated using blends of 2,2,2-trifluoroethanol (TFE) as solvents. Fibres were produced from the solutions by electrospinning. The DBC/PLA blend solutions in various ratios were studied for electrospinning into micro/nanofibres.
The morphology of the micro and nanofibres was observed by scanning electron microscope (SEM). The biggest diameters of DBC/PLA fibres were obtained for the blended microfibres in ratios of 10/90 and 25/75. The smallest diameter was observed for pure polymers. The antibacterial properties were examined for materials obtained by electrospinning. In the experiments, materials with antibacterial properties were made.
It is likely that the electrospun micro and nanofibres will be used in the native extracellular matrix for tissue engineering.

Key words: *electrospinning, dibutyrylchitin, PLA, microfibres, antibacterial activity*

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1. Introduction

Electrospinning is an important method to produce materials from both synthetic and natural polymers or blends. Electrospinning is a method in which materials in solution or melted are formed into nano or micro sized continuous fibres. The process was patented by Formhals in 1934 [1].

Many researchers have verified that electrospinning parameters, such as including molecular weight of the polymer and solvent, polymer concentration, voltage and collector distance, as well as ambient parameters, influence the morphology of porous nanofibres [2–7].

Polylactide (PLA) is a biodegradable and bioresorbable polymer used in medicine in many applications [8,9]. PLA nanofibres have been formed using the electrospinning method, and the solvents generally used are chloroform, chloromethane and N,N-dimethyl formamide. The effect of different solvent ratios on PLA nanofibres morphology and diameter has also been investigated [10,11].

Dibutyrylchitin (DBC) was obtained from native krill chitin by its esterification with butyric anhydride [12]. Dibutyrylchitin is soluble in many popular solvents and thus its processing is much easier; it is therefore possible to obtain films, fibres and microspheres, etc. DBC nanofibres are successfully prepared by the method of electrospinning [13].

Nanofibres of PLA materials have wide applicability in various fields, such as filtration products, biomedical applications and tissue engineering to produce artificial blood vessels, non-woven fabrics, fuel cells or fibre mats [14].

2. Materials and methods

2.1. Materials

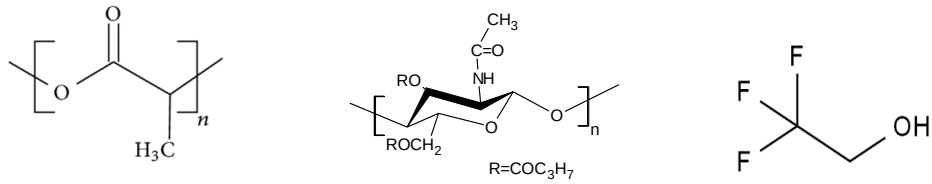
Dibutyrylchitin (DBC) ($C_{14}H_{27}O_7N$)n was synthesised in the Department of Physical Chemistry of Polymers, Technical University of Łódź, of Mw = 160 kDa. DBC is obtained in by esterification process of krill chitin using butyric anhydride ($(CH_3CH_2CH_2CO)_2O$) (Aldrich), and perchloric acid (70% $HCLO_4$) (Merck, Germany) as the catalyst of the reaction.

Dibutyrylchitin was characterised by a degree of acetylation of 0.978 and an intrinsic viscosity value determined for its solution in acetone at 25°C. The intrinsic viscosity values were equal to 1.28 dl/g and 1.37 dl/g.

PLA (Ingeo biopolymer 3052D) was supplied by Nature Works LLC. Designed for injection moulding applications, melt flow index (MFI) was used at 14 g/10 min at 210°C and weight of 2.16 kg.

2,2,2-trifluoroethanol (TFE) was purchased from Sigma–Aldrich.

In the figure 1 a structural formulas of the reagents used in the study are presented.



a) b) c)

Figure 1. Chemical structure of the polymers used for fibre spinning: a) polylactide b) dibutyrylchitin and c) 2,2,2-trifluoroethanol

2.2. Methods

2.2.1. Fibre formation

Fibres were produced from solutions by electrospinning. Figure 2 shows a schematic illustration of the basic setup for electrospinning. It consists of three major components: a high-voltage source, a syringe pump and a collector screen. An electrode is positioned in the needle and the other is attached with a collector. The flow of polymer solution, contained in the syringe, is generated by plunging the solution through the metering pump. A high voltage electrostatic force is generated between the electrodes. Transformation from a drop to a stream in an electric field is known as a Taylor cone. At the point in time when the droplet overcomes the surface tension, it moves towards the collector screen in the form of a fluid jet. On its way to the collector screen, the solvent evaporates and fibres of micro diameter are formed. These fibres accumulate in the collector screen.

In this study, a solution of 10 wt% dibutyrylchitin (DBC) solution in TFE was blended with 10 wt% PLA solution in TFE. Electrospinning was carried out using blended polymer solutions. The spinning solutions with different ratios by weight (DBC/PLA: 0/100, 10/90, 25/75, 50/50, 75/25 and 100/0 w/w) were prepared. At room temperature, the polymer solution was placed into a 5 ml syringe with a tip of inner diameter of 0.7 mm. The applied voltage and the distance between the tip of the spinneret and the collector were maintained at 25 kV and 15 cm, respectively. The fibres were collected as an aluminium foil. The shell flow rate was set to 2.0 ml/h. All the electrospinning processes were carried out at around 60% humidity. The electrospinning was performed at room temperature.

2.2.2. Scanning electron microscopy (SEM)

The morphology and structure of the micro and nanofibres were observed by scanning electron microscopy (SEM) (JSM 5500 LV JEOL scanning microscope) at an accelerating voltage of 10 kV. Before SEM observation, the samples were sputter-coated with gold.

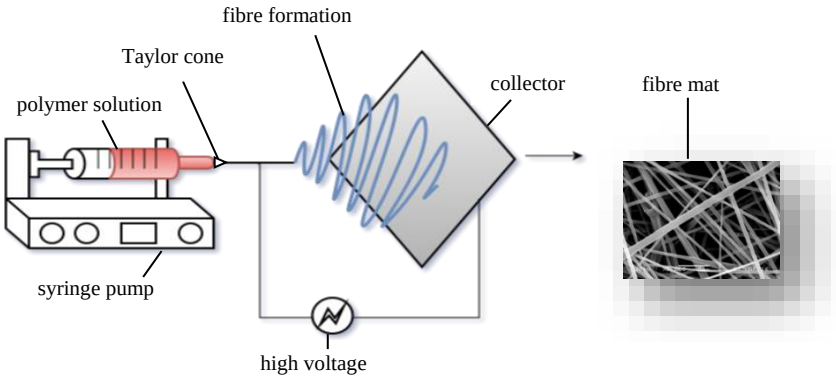


Figure 2. Schematic diagram showing the process of electrospinning.

2.2.3. Optical microscopy and determination of fibre diameters

The microorganisms tested in the zone of fibre contact was observed using a DIAPAN optical microscope (Reichert) coupled with a CCD camera ARTCAM

(Olympus). The fibre diameters of the flat fibrous structure designed was determined using MOTIC IMAGES PLUS 2.0 software.

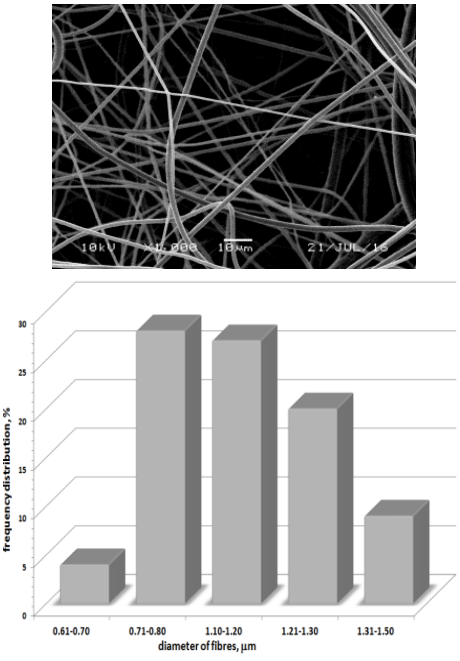
2.2.4. Microbial analysis

The antibacterial properties were examined for materials obtained by electrospinning. They were exposed to microorganisms that can cause nosocomial infections; these were the Gram-positive *Staphylococcus aureus* ATCC No. 25923, Gram-negative *Escherichia coli* ATCC No. 25922, *Pseudomonas aeruginosa* ATCC No. 27853 and fungi *Candida albicans* ATCC No. 10231. Microorganisms were purchased from ATCC. The following media for cultivation of microorganisms were used: Chapman agar, MacConkey agar, Cetrymide agar and Candida agar. Microorganisms were incubated at 37ºC for 24 h. Sterile physiological salt (2 cm^3) was poured into cultured bacteria and fungi. Grown cultures were washed with 1 ml of physiological salt solution, and added to the sterile selective agar. Nanofibre samples (2 cm^2) were applied to the inoculated agars. Samples were incubated at 37ºC, for 24–48 h. After incubation, zones of inhibition were read.

3. Results and Discussion

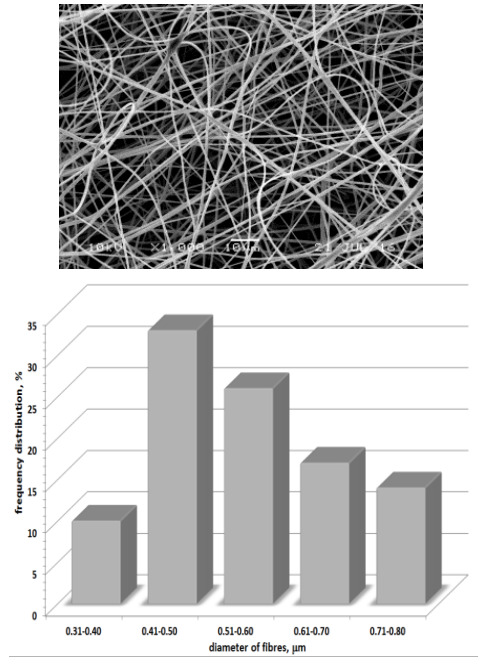
Figure 3a–f shows SEM images of the DBC/PLA blend microfibres with diameter distribution. The non-woven mat of the DBC/PLA blend microfibres had smooth surfaces. A higher content of DBC (Figure 3c (50/50), 3d (75/25) and 3e (100/0)) in the microfibre blends prevented the formation of smooth and thin microfibres due to the cationic properties of DBC.

For each experiment, the average fibre diameter and distribution were determined from about 100 random measurements using micrographs representative of fibre morphology.



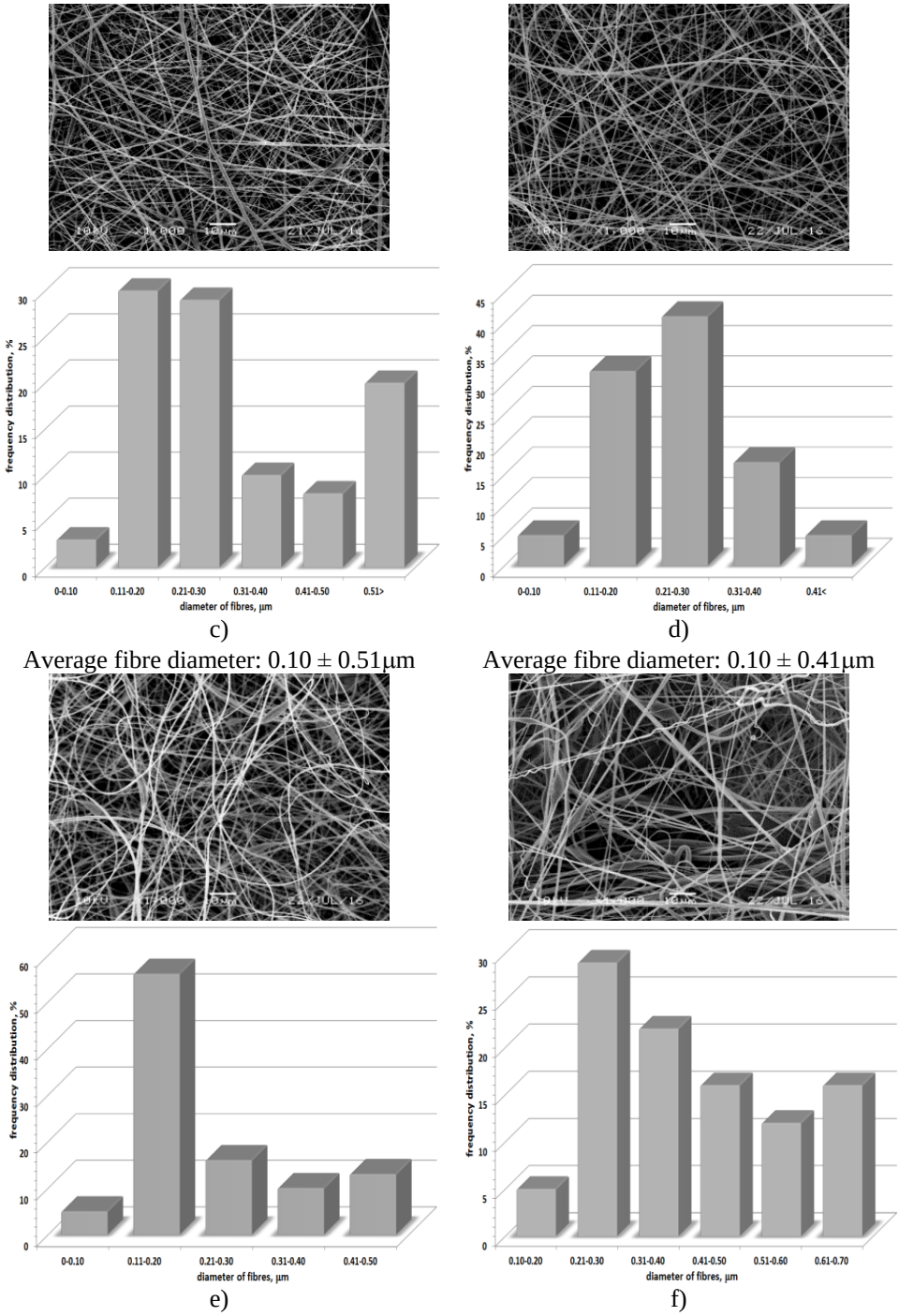
a)

Average fibre diameter: 0.61 ± 1.50 µm



b)

Average fibre diameter: 0.31 ± 0.80 µm



c) Average fibre diameter: 0.10 ± 0.51µm

d) Average fibre diameter: 0.10 ± 0.41µm

e) Average fibre diameter: 0.10 ± 0.50 µm

f) Average fibre diameter: 0.10 ± 0.70 µm

Figure 3 a–f. SEM images and diameter distribution of the DBC/PLA blend microfibres in different weight ratios: a) 10/90; b) 25/75; c) 50/50; d) 75/25; e) 0/100 (pure PLA); f) 100/0 (pure DBC); magnification 1000×.

Figure 4 shows the distribution of the electrospun fibre diameters of all obtained samples.

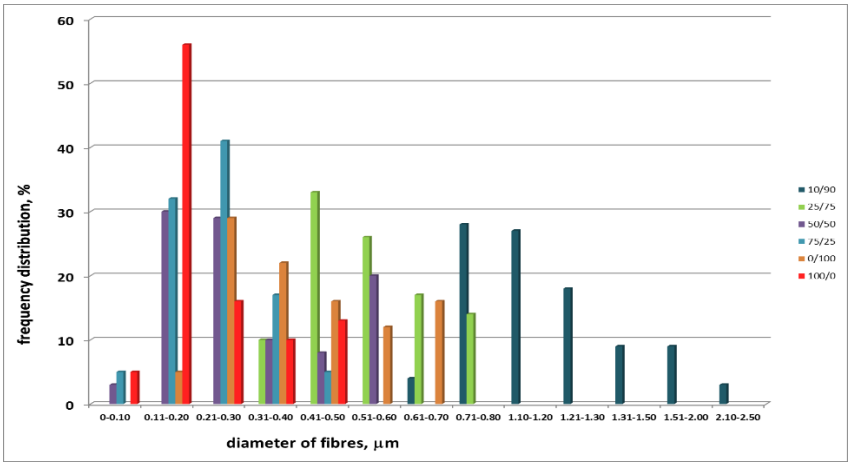


Figure 4. Fibre diameter distribution of DBC/PLA nano and microfibres at different solution concentrations.

The analysis of the data shows that decrease of the PLA concentration in TFE from 50 to 10 wt.% causes a reduction of the fibres from approximately 210 µm to approximately 0.10 µm.

The assessment of antibacterial activity consists of placing a sample on an agar substrate containing bacterial culture and observing its growth under and around the sample.

The results presented here highlight that the nano and microfibres from the polymer blend are characterised by bioactivity (Table 1).

Table 1. Resistance of microorganisms.

Sample	Microorganisms			
	Escherichia coli	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Candida albicans***
0/100 (pure PLA)	-	-	-	-
10/90	+	+	+	+
25/75	+	+	+	+
50/50	+	+	+	+
75/25	+	+	+	+
100/0 (pure DBC)	+	+	+	+

Note: "-" Lack of inhibition growth zone; "+" Zone of the growth inhibition about 1 mm.

Microbiological studies showed that sample 1 (pure PLA) was not biologically active with respect to *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* or *Candida albicans.* There were no inhibition zones in samples of 0/100 (pure PLA).

The remaining samples of the nanofibres showed antimicrobial activity against the tested microorganisms (Table 1). The antimicrobial activity of the sample tested is shown in the form of the so-called inhibition zone of microbial growth, measured in mm. For confirmation of the obtained results (Table 1), photographs (Figure 4 a–m) show the inhibition zones (marked with a red line).

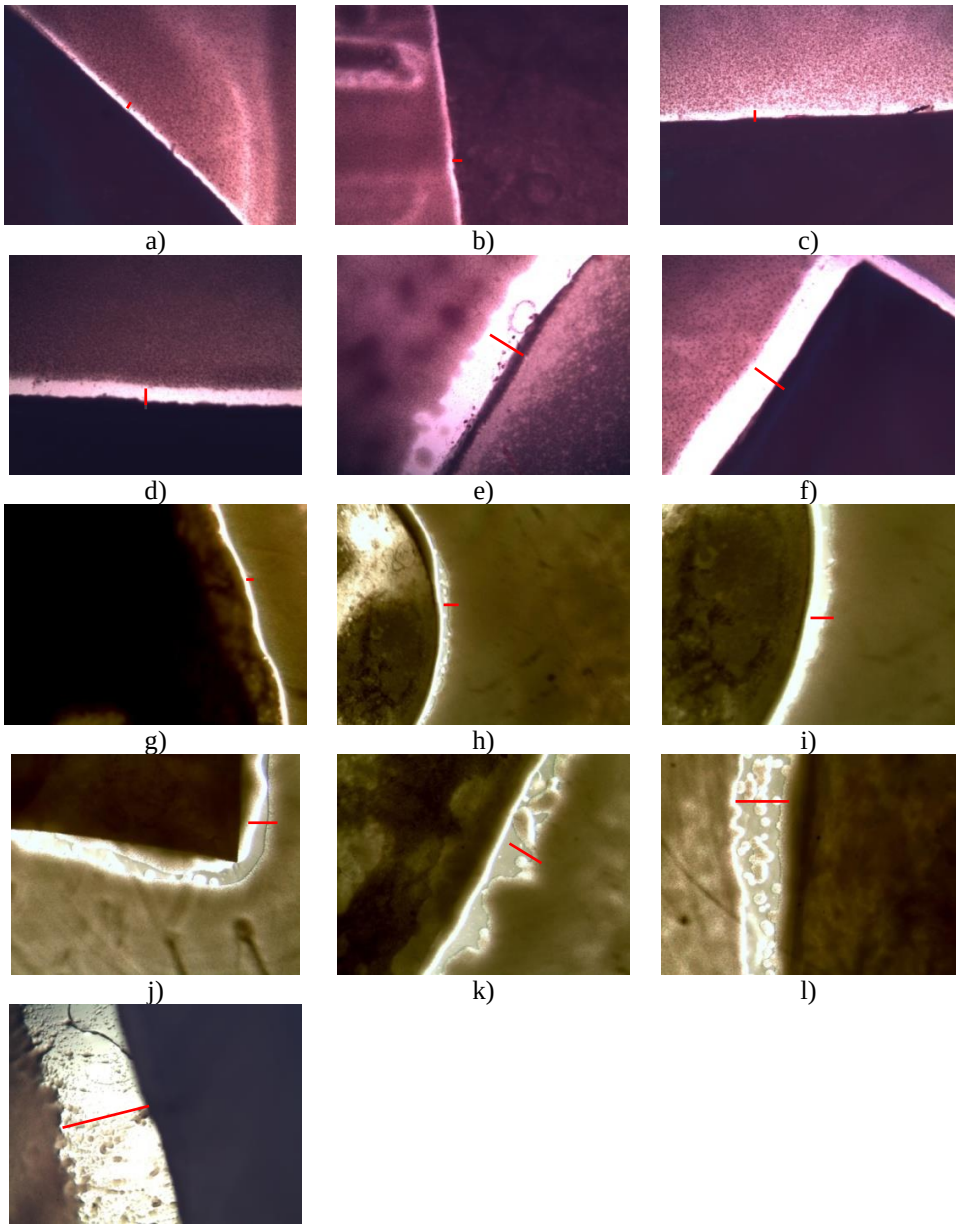


Figure 5 a–m. Antimicrobial properties of DBC/PLA microfibre-treated *Escherichia coli (a–f), Pseudomonas aeruginosa (g–i), Staphylococcus aureus (j–l)* and *Candida albicans (m).* The inhibition zones are marked with a red line.

In the figures, it can be observed that the inhibition zones of bacteria increased with the contents of DBC.

4. Conclusions

In this study, DBC/PLA nano and microfibre blends were prepared by the electrospinning process. We found that 2,2,2-trifluoroethanol is a suitable solvent for the electrospinning of DBC/PLA blended nano and microfibres. The morphology of fibres was more homogeneous in diameter, without defects.

The prepared DBC/PLA nano and microfibres showed a very high bacteriostatic and bacteriocidal activity against the Gram negative bacteria *Escherichia coli* and the Gram positive bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as well as antifungal action against the fungus *Candida albicans*.

5. References

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