Synthesis and electrospinning of star-shaped poly(L-lactide) with different arm lengths

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Abstract. Star-shaped biodegradable poly(L-lactide)s are perspective materials with tailored properties and degradation rates which can be controlled by number and length of the arms. Star-shaped 6-arm poly(L-lactide)s with arm lengths from 10 to 50 units were synthesized by ring-opening polymerization and studied by NMR, GPC and DSC. The effect of arm length on electrospinning of the star-shaped polymers was demonstrated. Structure of non-woven materials based on star-shaped PLLA and its mixtures with linear high molecular weight PLLA and PEO was studied.

1. Introduction
Poly(L-lactide) (PLLA) and PLLA-based copolymers are widely used for various biomedical and pharmaceutical applications [1-4]. Still there are some obstacles in the development of scaffolds for tissue engineering based on polylactide, such as high hydrophobicity, slow degradation and poor potential for modification due to the lack of functional groups. To overcome these problems polylactides with star-shaped architecture and low molecular weight can be used. Branched PLLAs are characterized by higher water sorption due to the presence of additional hydroxyl groups leading to a faster degradation [5–7]. In order to improve the biocompatibility and cell adhesion properties the end-groups can be modified by biologically active compounds. Thus, the use of star-shaped PLLA allows the development of new types of materials with predetermined properties. In addition, solutions of low molecular weight branched polymers are characterized by low viscosity. This important advantage will allow to create materials based on linear and branched polymers with a high content of the latter while maintaining the required level of mechanical properties. It is favorable to use electrospinning as a method for fabrication of scaffolds for tissue engineering. Unlike other manufacturing methods, electrospun fiber-based matrices are more resistant to stretching, have a high degree of porosity and structure similar to native extracellular matrix of tissues [8–10]. The goal of our research was to synthesize 6-arm star-shaped PLLAs with well-defined microstructure and to determine optimal parameters for electrospinning of the polymers with different arm lengths.
2. Experimental

2.1. Materials
L-lactide (LA) (purchased from Sigma-Aldrich) was recrystallized one time from dry butyl acetate and dried in vacuum at 50 °C for 6 h before use. Tin(II) 2-ethylhexanoate (stannous octoate, Sn(Oct)2) and dipentaerythritol were purchased from Sigma-Aldrich and used as received. Poly(L-lactide) 4032D grade with $M_w$ of 200 kDa and PDI of 1.6 was purchased from NatureWorks LLC (United States).

2.2. Synthesis of star-shaped 6-arm poly(L-lactide)
Star-shaped 6-arm poly(L-lactide) (6-arm PLLA$_n$) with different arm lengths was synthesized by bulk ring-opening polymerization of L-lactide, using dipentaerythritol as a multifunctional initiator and Sn(Oct)$_2$ as a catalyst. Degree of polymerization of PLLA arms was adjusted by varying the feed molar ratios of LA and initiator ([M]/[I]). The typical synthesis procedure of 6-arm poly(L-lactide) with theoretical composition 6-arm PLLA$_{50}$ was as follows: L-lactide (10 g, 69 mmol), dipentaerythritol (0.117 g, 0.46 mmol) and stannous octoate (0.005 g, 0.012 mmol) were placed in a dried glass reaction flask equipped with a magnetic stirrer. The polymerization was carried out in bulk at 170 °C for 5 h under argon atmosphere. After the reaction, the resulting product was cooled to room temperature and dissolved in tetrahydrofuran and then poured into excess of hexane to precipitate the polymerized product. Six-arm star-shaped poly(L-lactide) (6-arm PLLA$_{50}$) was obtained after filtering and drying in vacuum at 80°C for 24 h.

2.3. Electrospinning
Solutions of star-shaped polylactides were prepared in chloroform (CF) or its mixture with ethanol (EA) in the ratio of 90:10. To increase the fiber-forming properties of the solutions, polyethylene oxide (PEO $M_w = 2000$ kDa, Sigma-Aldrich) or linear poly(L-lactide) 4032D were added.

2.4. Methods

2.4.1. Gel permeation chromatography. Molecular weight of polymers was determined by GPC using Knauer liquid chromatograph equipped with RI detector. Tetrahydrofuran (THF) was used as an eluent with a flow rate of 1 ml/min. Polymer solutions in THF were filtered through 0.45 µm membrane filters before the injection. Experiments were performed at 40 °C using Agilent PLgel column (particle size 5 µm, pore size 10$^3$ Å).

2.4.2. Nuclear magnetic resonance. Conversion and molecular structure of synthesized star-shaped polymers were studied by $^1$H NMR. Spectra were recorded at room temperature on a Bruker DPX-300 spectrometer. Dried deuterated chloroform was used as a solvent.

2.4.3. Differential scanning calorimetry. Thermophysical properties of the polymers were studied using a Perkin Elmer DSC8500 calorimeter in the temperature range of 35 – 210 °C and heating rate of 10 °C min$^{-1}$; the sample weight was about 15 mg. The degree of crystallinity $X_c$ was calculated according to equation 1:

$$X_c = \frac{\Delta H_m - \Delta H_c}{93.6} \times 100\% , \quad (1)$$

where $\Delta H_m$ is the melting enthalpy, $\Delta H_c$ is the crystallization enthalpy and 93.6 J/g is theoretical value of melting enthalpy of 100% crystalline PLLA [11].

2.4.4. Microscopy. For investigation of electrospinning process the obtained materials were collected on a glass plate and assessed by optical microscopy on Zeiss Axio Imager 2 microscope. Microstructure and morphology of fibrous materials were studied by scanning electron microscopy (SEM) using Versa 3D DualBeam apparatus (FEI, USA) in high vacuum mode with the accelerating voltage of 1 kV.
3. Results and discussion

3.1. Synthesis and characterization of star-shaped 6-arm poly(L-lactide)

The polymerizations of L-lactide were carried out with dipentaerythritol as multifunctional initiator and Sn(Oct)$_2$ as catalyst in bulk at 170 °C to produce 6-arm star-shaped poly(L-lactide) with different arm lengths. The reaction scheme for 6-arm PLLA is shown in figure 1.

![Synthesis scheme of 6-arm PLLA$_n$.](image)

As known, in the presence of stannous octoate hydroxyl-containing compounds act as co-initiators of ring-opening polymerization [12]. Dipentaerythritol was expected to initiate the polymerization of L-lactide at each hydroxyl unit and form the star-shaped structure with six arms. The reaction time for star-shaped PLLA with different compositions varied depending on the [M]/[I] molar ratio, since the time required to reach equilibrium conversions was different. The synthesis conditions as well as the conversion values that were achieved are displayed in table 1.

| Sample       | [M]/[I] | T, °C | t, h | Conversion, % |
|--------------|---------|------|-----|---------------|
| 6-arm PLLA$_{10}$ | 30      | 170  | 24  | 96            |
| 6-arm PLLA$_{25}$ | 75      | 150  | 8   | 96            |
| 6-arm PLLA$_{50}$ | 150     |      | 5   | 97            |

Figure 2 shows the typical $^1$H-NMR of a synthesized 6-arm PLLA. The successful polymerization of L-lactide using dipentaerythritol was evidenced by the disappearance of the peak of -CH$_2$-OH groups in the initiator molecule and by the appearance of -CH$_2$-O-R (2) (4.12 ppm) peak. Thus, all of the hydroxyl groups of dipentaerythritol initiated the polymerization. The presence of terminal hydroxyl groups in the molecules of star-shaped PLLA was confirmed by the occurrence of the signal of -OH (5) (4.35 ppm) groups. In addition, the formation of star-shaped PLLA was confirmed by the appearance of peaks of methine (3) (1.56 ppm) and methyl (4) (5.58 ppm) protons of the PLLA arms. The average degree of polymerization and molecular weight of the samples were determined based on the ratio of the integrated intensity of the methine groups of PLLA and the methylene groups of the initiator.
The molecular characteristics, melting temperature and degree of crystallinity of synthesized 6-arm PLLAs are presented in table 2. Optimal polymerization conditions allowed to obtain a well-defined molecular structure and almost equal values of calculated and experimental degree of polymerization (DP) in each arm. Samples had a narrow polydispersity index (PDI) varying from 1.11 to 1.14. Star-shaped PLLA with 10 monomer units in the arm was amorphous. Increase in the arm length from 25 to 50 units have led to an increase of melting temperature from 127 to 146 °C and degree of crystallinity ($X_c$) from 39 to 55%.

### Table 2. Molecular characteristics, melting temperature ($T_m$) and enthalpy ($\Delta H_m$), degree of crystallinity ($X_c$) of synthesized 6-arm PLLA$_n$ with different molecular weight.

| Sample         | $M_n$, g/mol | $M_w$, g/mol | PDI | $M_n$, g/mol | DP of PLLA in each arm | $T_m$, °C | $\Delta H_m$, J/g | $X_c$, % |
|----------------|--------------|--------------|-----|--------------|-----------------------|----------|-------------------|---------|
| 6-arm PLLA$_{10}$ | 5 800        | 6 700        | 1.14| 4 600        | 10                    |          | -                 | 0       |
| 6-arm PLLA$_{25}$ | 16 300       | 18 100       | 1.11| 11 050       | 25                    | 127      | 36                | 39      |
| 6-arm PLLA$_{50}$ | 33 300       | 37 100       | 1.11| 21 850       | 50                    | 146      | 51                | 55      |

3.2. Electrospinning of star-shaped 6-arm poly(L-lactide)

For fabrication of scaffolds for tissue engineering it is crucial to determine the optimal compositions of spinning solutions as well as parameters of electrospinning process. Due to low molecular weight star-shaped PLLAs with 10 and 25 units in the arm did not have a fiber-forming properties. To improve them high-molecular weight polymers (PEO and/or linear high-molecular weight PLLA) were introduced into spinning solutions. Chloroform (CF) or its mixtures with ethyl alcohol (EA) were used as solvents. Compositions of the spinning solutions and parameters of the process are presented in table 3.

### Table 3. Compositions of the spinning solutions and parameters of the electrospinning process.
Sample | Composition of spinning solutions (weight fractions) | Voltage, kV | Distance, cm | Flow rate, ml/h
--- | --- | --- | --- | ---
6-arm a | 25% 6-arm PLLA₁₀ + 75% CF | 20 | 31 | 1
PLLA₁₀ b | 10% 6-arm PLLA₁₀ + 0,5% PEO + 89,5% CF/EA | 25 | 34 | 2
6-arm c | 6% 6-arm PLLA₁₀ + 3,6% linear PLLA + 0,3% PEO + 90,1% CF/EA | 20 | 31 | 4
PLLA₂₅ d | 25% 6-arm PLLA₂₅ + 75% CF | 20 | 31 | 10
6-arm e | 36% 6-arm PLLA₂₅ + 64% CF | 15 | 22 | 4
PLLA₅₀ f | 10% 6-arm PLLA₂₅ + 0,5% PEO + 89,5% CF/EA | 25 | 31 | 2
6-arm g | 27% 6-arm PLLA₅₀ + 73% CF | 20 | 31 | 2
PLLA₅₀ h | 25% 6-arm PLLA₅₀ + 75% CF/EA | 15 | 22 | 4

The results of the process of electrospinning from prepared solutions (table 3) are presented in figure 3. Firstly, it was important to optimize the composition of spinning solution and process parameters for fabrication of fibers.

As can be seen, due to the low molecular weight of the 6-arm PLLA₁₀ (Mₙ = 4 600 Da), the regime of electrospinning was replaced by an electrospray process. As a result, spherical particles with a diameter of 50 to 70 μm were detected on a glass slide (figure 3a). After addition of a fiber-forming additive (PEO) to the solution, its viscosity significantly increased. After dilution of the solution, it was possible to fabricate fibers with a diameter of 4 to 30 μm (figure 3b). The resulting fibers had a wavy surface, probably due to the residual solvent. Low strength of the produced fibrous materials have led

Figure 3. Particles and fibers obtained by electrospinning of solutions a-h described in table 3.
to its destruction while removing from the receiving electrode. Therefore, the spinning solution was
diluted again and linear PLLA was added. As a result (figure 3c), the formed fibers demonstrated a
smooth surface and their diameter decreased down to 3-8 μm. At the same time, the increased strength
of the manufactured fibrous material was sufficient enough to remove it from the receiving electrode.
Figures 3d and 3e demonstrate the results of the spinning of a 6-arm PLLA_{25} solution (M_n = 11 050 Da).
It can be seen that due to the poor fiber-forming properties of the solution, production of defect-free
fibers was not possible. After adding PEO, fibers with a diameter of 4 to 20 μm were obtained. These
process parameters allowed to produce fibrous material with satisfactory mechanical characteristics.
Star-shaped polymer with highest molecular weight – 6-arm PLLA_{50} (M_n = 21 850 Da) demonstrated
good fiber-forming properties (figure 3g and 3h). It was possible to obtain fibers with a diameter of 2 to
14 μm from its solutions without any additive. SEM images and fiber diameter distributions of produced
non-woven materials are presented in figure 4.

![SEM images and calculated distributions of fiber diameters of non-woven materials based on 6-arm PLLA_{10} (a), 6-arm PLLA_{25} (b) and 6-arm PLLA_{50} (c).](image)

Figure 4. SEM images and calculated distributions of fiber diameters of non-woven materials based on 6-arm PLLA_{10} (a), 6-arm PLLA_{25} (b) and 6-arm PLLA_{50} (c).

Fractions of the fibers can be seen on the microphotographs, which can be caused by low strength of
materials and their damaging during removing from the electrode. All the produced fibrous materials
were characterized by a rather wide, but still unimodal distribution of fiber diameters. Microporous
structure of produced materials is favorable for cell cultivation ensuring the penetration of the cells in
the whole volume of the scaffold. In order to increase the strength of materials star-shaped PLLAs with
higher molecular weight as well as their mixtures with linear high molecular weight PLLA will be investigated.

4. Conclusions
6-arm PLLAs with narrow PDI and controlled arm length of 10, 25 and 50 units were synthesized and characterized. DSC analysis showed fully amorphous structure for polymer with 10 units in the arm, while 6-arms PLLAs were semi-crystalline with melting temperature of 127 and 146 °C, crystallinity of 39 and 55% respectively. Star-shaped PLLA with arm length of 10 and 25 units did not demonstrate fiber-forming properties. Fibrous materials based on these polymers were produced only in combination with linear high molecular weight PLLA or PEO. At the same time, electrospinning of 6-arm PLLA50 was performed without any additives. The obtained fibrous material was characterized by fiber diameters of 2-14 μm.

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