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Fragmentation of Bioactive Electrospun PLLA Fibers

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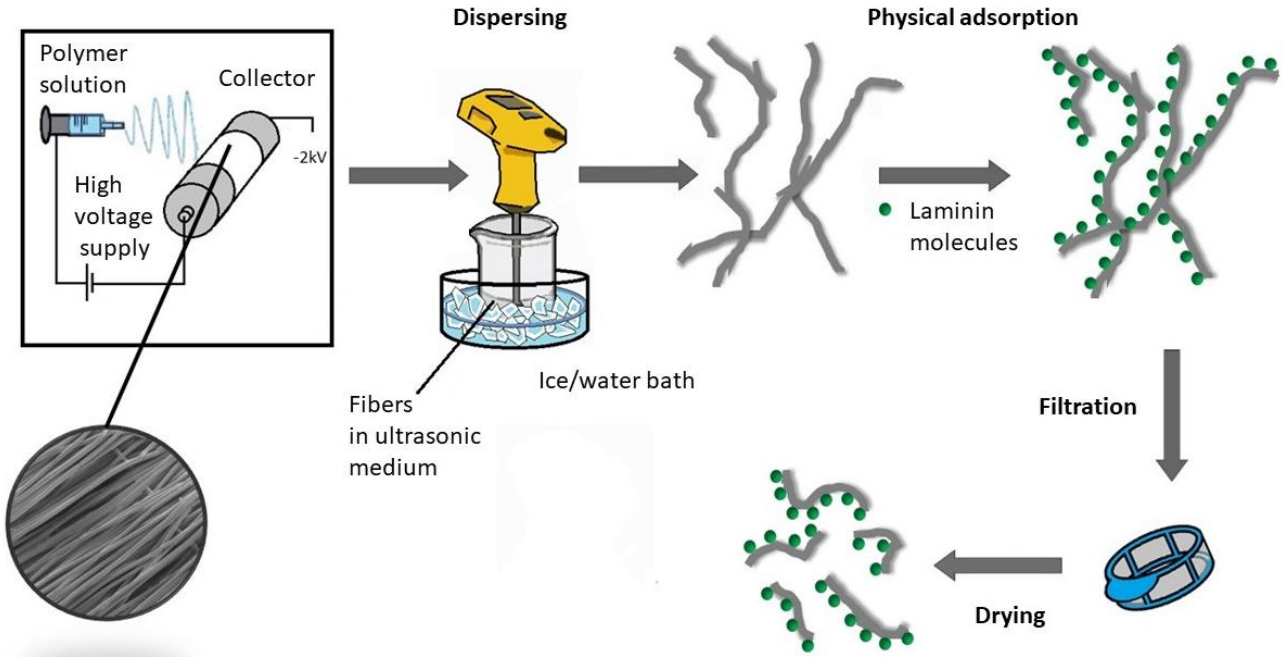
Graphical Abstract



Abstract

The aim of this research was obtaining short electrospun nanofibers which in future studies could serve as a supportive and bioactive component loaded into the hydrogel system. A poly(l-lactide) acid (PLLA) is biocompatible and characterizes relative brittleness, which enables effective mechanical fragmentation [**1**]. In these studies, PLLA nanofibers were fragmented using ultrasonication, which effectiveness was thoroughly studied and optimized. Such parameters as the type of sonication medium, processing time, and various PLLA molecular weights were investigated. A post-fragmentation of short fibers was carried out to narrow down the nanofibers length distribution. Additionally, short PLLA nanofibers were modified with laminin by physical adsorption. That functionalization allowed to overcome hydrophilicity and biochemical inertia of PLLA, providing IKVAV (Ile-Lys-Val-Ala-Val) effective sites for cell adhesion [**2,3,4**]. Laminin, as one of the ECM components, contains protein receptors, which are decent especially from the

perspective of neural cells. In this regards especially important is the IKVAV sequence, responsible for effective neuron differentiation [**2**]. This protein is essential especially for nerve regeneration - it effectively binds Schwann cells and regeneration of axons takes place only in the laminin presence [**2,5**].

In the future studies short bioactive nanofibers might be loaded into the injectable hydrogel for neural tissue engineering applications. Such an approach might provide nanofibers dispersion in the liquid media, injectability of hydrogel system, an appropriate mechanical and biological properties, that mimic native extracellular matrix (ECM).

In these studies scanning electron microscopy (SEM) was used to determine fiber length, and hence the effectiveness of shortening depending on the used sonication medium, the duration of the process as well as PLLA molecular weight. A gel permeation chromatography (GPC) was used to check if ultrasonic treatment decreases PLLA molecular weight. A water contact angle (WCA) was measured to evaluate the surface wettability after physical adsorption. A bicinchoninic acid assay (BCA) was carried out to detect and quantify the amount of laminin adsorbed to the fibers.

The ultrasonic fragmentation was successfully optimized by choosing isopropanol as a sonication medium, relevant fragmentation time of 60 min and selection of appropriate polymeric material with the highest molecular weight, which among others showed the highest level of fragmentation. The post-fragmentation filtration through 40 um filters removed the fraction of long fibers reducing fiber length distribution to c.a. 50 µm. The GPC results showed that the molecular weight distribution of PLLA is not affected by both electrospinning and subsequent fragmentation. The WCA showed an increase of hydrophilicity on modified surface, while BCA assay performed effective laminin immobilization to the PLLA fibers.

These studies show that such classic and simple methods as ultrasonication and physical adsorption effectively provide a macroscopic and bioactive change of PLLA oriented electrospun fibers enabling their further use as fillers for injectable hydrogels for regenerative medicine applications.

Keywords: Electrospinning; ultrasonication; short fibers, polymers; scaffold.

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