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Design of copolymer PLA-PCL electrospun matrix for biomedical applications

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ABSTRACT

Electrospinning is a quite consolidated technique to produce polymer nanofibrous matrices whose nanostructured morphology received great interest for application in the biomedical field i.e. in manufacturing matrices for tissue regeneration.

Purpose of the work to design electrospun matrices made of Poly-L-lactide-*co*-poly-ε-caprolactone (PLA-PCL) 70:30 M ratio and their thoroughly physico-chemical and functional characterization. The ultimate goal of the research work is to obtain electrospun matrices suitable for circular substitution of esophageal defects. However the paper deals with very preliminary investigation mainly on electrospinning process and physical-chemical characterization of the electrospun matrices. The investigation on electrospinning process conditions involves polymer starting solution concentration between 20% w/v and 25% w/v, viscosity and surface tension, but also process parameters of electrospinning apparatus in order to optimize them at achieving homogeneous and reproducible nanofibers.

The optimized electrospun matrices are characterized for their cytocompatibility, morphology (SEM analysis), polymer molecular structure in the solid state (FT-IR analysis) and thermal behavior (DSC analysis) and mechanical properties. In vitro degradation test is performed to evaluate electrospun matrix biodegradability.

Results show good cytocompatibility for all polymer concentrations and electrospinning times. Moreover, the electrospun matrices biodegradation is slower with respect to matrices made by solvent casting method. The behavior is related to modifications in the polymer solid state and to polymer chains structure.

1. Introduction

Electrospinning is a progressive method which produces fibers ranging from the submicron level to several nanometers in diameter, in a high voltage electrostatic field [1].

Briefly, fibers formation steps are: Taylor's cone formation, jet stretch, solvent evaporation and fibers deposition.

A typical electrospinning setup usually includes a reservoir of polymer solution with a metallic capillary connected to high voltage and a metallic collector.

When an electric field is applied between a needle and a collector, surface charge is induced on the polymer fluid deforming a spherical pendant droplet to a conical shape (Taylor's cone). As the electric force overcomes the solution surface tension, a polymeric jet is generated from the surface tension of the droplet and travels towards the collector. The solvent evaporates from the jet in the gap between the needle and collector, and consequently nanofibers are collected [2,3]. Morphology and diameter of the resultant nanofibers depend on many parameters: solution properties, process parameters and environmental conditions. Optimization of these parameters is significant in order to obtain continuous nanofibers with specific morphology and well defined physical and mechanical properties, depending on the type of application.

Solution properties influence fibers formation, for this reason it is very important to select suitable parameters such as: solvents type and ratios, polymer or copolymer concentration, solution viscosity, conductivity and surface tension.

Process parameters define fibers morphology. The most important parameters to set up are: voltage, flow-rate and needle-collector distance. Other important parameters connected with the process steps are: collector types, needle diameter, temperature and humidity inside the electrospinning chamber. In these years electrospinning process has been widely studied, and several authors investigated how process parameters affect fiber formation and morphology [4–7]. Literature

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shows that the process parameters need to be studied and set up depending on type of use forecasted for the electrospun matrix and type of polymer and/or polymers blends used. On the other hand type of polymer and/or polymer blend should be selected depending on the matrix forecasted use.

Some parameters are interconnected and should be evaluated together in order to set up electrospinning conditions. They can be classified as polymer parameter and process parameters. The first are those parameters directly depending on type of polymer and polymer solution, the second depend on electrospinning apparatus.

In example viscosity of the polymer solution affects fiber diameter, and it depends on polymer concentration, and the selected polymer solvent, but also on polymer Mw. Therefore the polymer concentration is a parameter to be fixed as a function of polymer Mw and its solvent.

Polymer solvent in its turn affects conductivity and surface tension of the polymer solution.

Conductivity is an utmost important parameter since it permits Taylor cone formation. Surface tension should be lower than the electric force in order to get Taylor cone. Therefore viscosity of the polymer solution and its surface tension are interconnected. On experimental basis the two parameters can be sized for different percentage composition of solvent blend and fixed polymer concentrations, and results reported in a graph: y and y_1 values at the cross point of the two curves (polymer solution viscosity and the corresponding surface tension) represent, on a theoretical basis, the suitable values of viscosity and surface tension of the polymer solution to be electrospun (it will be experimentally introduced and used in the developed study).

Chain entanglement depends on polymer concentration in the starting solution, for fixed solvent blends, since lower number of chains per unit volume should directly translate to small fiber diameters due to large chain extensibility. As long as process parameters is concerned, fiber diameter decreases when applied voltage raises in suitable ranges (too high voltage causes an excessive jet acceleration and its instability). Fibers diameter decreases when applied flow-rate decreases; high flow-rates values make irregular and melt structures because do not permit suitable solvent evaporation. Needle-collector distance, temperature and humidity inside the electrospinning chamber correlate to polymer solvent evaporation, thus depending on the solvent or solvent mixture selected. Generally speaking formation of regular and not fused together fibers happens when solvent evaporates simultaneously to polymer solution deposition on collector. Moreover fibers diameters decrease when temperature increases. Humidity inside electrospinning chamber affects solvent evaporation rate: low humidity values facilitate solvent evaporation.

Electrospun nanofibers have shown significant potential in diverse fields for a number of applications such as filtration systems, chemical and optical sensors, scaffolds for tissue regeneration including wound healing, drug delivery systems (e.g. to increase apparent solubility of low soluble drugs). Advantages are mainly exerted by electrospun nanofibers unique structure such as the high surface area to volume ratio, small pore size and high porosity [8]. The present research develops in the area of tissue regeneration, with the goal to develop polymeric and/or biological engineered tissue substitutes. Nanofibrous materials seem to be desirable, more than other types of structures, due to their architectural analogy to natural extracellular matrix (ECM) [6,7]. Biodegradable polymers such as poly-L-lactide and poly-ε-caprolactone, thanks to their demonstrated good biocompatibility and safety for the human body, are considered good candidates for designing temporary scaffolds for tissue regeneration. Recent and plentiful literature on electrospinning of these polymers demonstrates the interest on the topic [8,11–21].

The ultimate goal of the research work is to obtain electrospun matrices made of Poly-L-lactide-*co*-poly-ε-caprolactone (PLA-PCL) 70:30 copolymer and suitable for circular substitution of esophageal defects. However the present paper deals with very preliminary investigation mainly reporting about electrospinning process and physical-chemical characterization of the electrospun matrices.

In particular this preliminary study involves setting up electrospinning conditions and polymer solution parameters in order to achieve an electrospun matrix prototype to be in vivo implanted. PLA-PCL 70:30 copolymer was selected for the study. The copolymer composition shows PLA prevalence in order to get suitable degradation time, that in vivo should be completed in 8 weeks. PCL component gives plasticity to the copolymer. The electrospun matrices are thoroughly characterized in vitro for their physical-chemical properties and biological properties. Scientific motivation is to provide detailed physical-chemical characterization of polylactide-*co*-polyε-caprolactone electrospun matrices gathering information and data preparatory to their manufacturing. Electrospinning process has been investigated by several authors and papers available in the literature demonstrate that electrospinning process affects polymer and electrospun matrices properties. There is no general rule, and cases should be investigated one by one. For this reason the authors think important to add their contribution to the literature on the topic.

2. Experimentals

2.1. Materials

Copolymer poly-L-lactide-poly-ε-caprolactone (PLA-PCL) 70:30 M ratio (Resomer LC 703 S – Mw 160.000 Da) was obtained from Evonik Industries (Evonik Nutrition & Care GmbH, 64275 Damstadt). Methylene chloride (MC) and *N*,*N*-dimethylformamide (DMF) analytical grade were supplied by Carlo Erba and used without further purification.

For cellular assays adult fibroblast cells (passage 13) from primary culture fibroblasts Normal Human Dermal Fibroblasts (NHDF) adult donor from PromoCell GmbH (PromoCell GmbH, Sickingenstrabe 63/65, D-69126 Heidelberg, Germany) were used, Dulbecco Modified Eagle's Medium (DMEM) supplemented with 4.5 g/L Glucose and L-Glutamide from Lonza (B-4800 Verviers, Belgium) were used.

2.2. Methods

2.2.1. Theory section: preliminary mathematical approach to set up copolymer solution properties

A theoretical mathematical approach was applied in order to set up suitable process conditions able to obtain nanofibers by electrospinning. Polymer concentration and solvent type affect fiber formation: Berry's number, which is a product of the intrinsic viscosity and polymer solution concentration as described in Eq. (1), was calculated in order to obtain suitable value able to give fibers [22].

$$Be = [\eta]C \tag{1}$$

where Be is Berry number, $C^* = 1/\eta$ is defined as critical polymer concentration $[\eta]$ is the intrinsic viscosity value as obtained from Mark Houwink equation $[\eta] = KM^a$, K and a are the Mark Houwink constants and they are related to the solvent and polymer system [23]; C corresponds to polymer concentration in the selected solvent system.

$[\eta]$ calculated on the basis of PLA-PCL Mw 160.000 Da resulted to be $[\eta] = 59.5$ mL/g in MC:DMF 50:50 v/v mixture.

Depending on Berry's number value, the model reports the polymer solution system to be in electrospraying or electrospinning region. The different cases are reported here below.

$Be < 1$ ($C < C^*$) electric field is too weak to overcome solution surface tension, intermolecular entanglement is significantly weak resulting in electrospraying;

$1 < Be < 4$ ($C^* < C < C_e$) electric field overcomes solution surface tension giving not stable polymer solution jet: irregular fibers form with necklace shape;

$Be > 4$ ($C > C_e$) starting from this value regular fibers form, most regular fiber are obtained for $Be \geq 6$;