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Influence of the Rheological Behaviour in Electrospun PCL Nanofibres Production for Tissue Engineering Applications

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A strategy to obtain functional tissues engineering with desired biomechanical properties was used to develop scaffolds with morphologies mimicking the native environment to guide tissue regeneration. Non-woven scaffolds, with fibre dimensions at a nanometre scale, can mimic the physical structure of natural extracellular matrices (ECM). Though its clinical application is yet limited, nano/micro fibrous scaffolds produced by electrospinning gains more and more interest in different Tissue Engineering fields. The electrospinning technique is controlled by several parameters, such as polymer solution and processing ambient, being one of the most important parameters the solution viscosity, which allows defining the minimum viscosity needed to obtain fibres.
This research work investigates the rheological behaviour of PCL solutions to produce nanoscale fibre meshes for cartilage application. Poly (ε-caprolactone) (PCL) solutions were prepared using glacial acetic acid (AA) and glacial acetic acid with triethylamine (AA/TEA) at different concentrations. It was necessary to double the value of the critical concentration (c*), that is 10 wt% for PCL/AA and 9,6wt% for PCL/AA/TEA, to prepare suitable fibres. Results also show that a more homogenous mesh can be produced by adding TEA.

1. Introduction

Tissue engineering is a multidisciplinary field, combining efforts of biologists, engineers and clinicians towards the development of biological substitutes to maintain, restore or improve tissue and organ function. Tissue engineering comprises three main strategies: i) cell-based strategies, which involves the direct in vivo implantation of isolated cells or cel scaffold that provides a substrate for implanted cells and a physical support to organize the formation of the new tissue (Meyer *et al*, 2009, Ringeisen *et al*, 2008).
A variety of techniques have been proposed to produce scaffolds from biomaterials being one of these the electrospinning (Yang *et al*, 2008). Electrospinning is a technique to create submicron to nanometre scale fibres from a polymer solution or melt with some common characteristics to electrospraying and the traditional fibre drawing process (Nukavarapu *et al*, 2008). The sub-micron range spun fibres produced by this process offer several advantages like high surface area to volume ratio, harmonious porosity and the ability to manipulate nanofibre composition in order to get desired properties and function (Bhardwaj *et al*, 2010).
The electrospun meshes by this technique have fine filaments that appear to promote the communication between the matrix and the cells which leads to increased cellular adhesion and proliferation due to a greater number of anchor points (Kuo *et al*, 1991, Saetone *et al*, 1994, Moore *et al*, 1995) . However, it is clear from the literature that the physical and mechanical properties of the electrospun polymer meshes are very dependent on the solvent used in the electrospinning process. For instance, Kanani et al. (2011), studied the effect of different solvents (glacial acetic acid, 90% acetic acid, methylene chloride/dimethyl formamide, glacial formic acid and formic acid/acetone) on the morphology of PCL nanofibrous meshes (Kanani *et al*, 2011). They found that the use of glacial acetic acid as solvent led to the formation of fibers with nonuniform distribution of fibre diameters. Scheren et al. (2011) have also evaluated the use of solvents such as chloroform, formic acid, acetic acid, methanol and ethanol in the preparation of PCL Scaffolds (Scheren *et al*, 2011). They emphasised the major potential of the solvent mixture formic acid/

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acetic acid for the electrospinning of PCL. In this work we propose to understand the dependence of the type of solvent, as well as of the polymer concentration, on the type of PCL fibers and the suitability of such fibres for their use in tissue engineering. This was carried out by dissolving different concentrations of PCL in solvents with different polarities and observe the fiber formation. Our physicochemical interpretation underlines the importance of having PCL solutions above the polymer overlap concentration to get fibers.

2. Materials and Methods

2.1 Materials

For nanofibres meshes preparation was used PCL (Mw 50000[g/mol]), by Perstorp Company (CAPA 6500), dissolved AA (Mw 60.05 [g/mol]), by Labsolve company and AA added with 2wt% of TEA (Mw 101.19 [g/mol]), obtained from Sigma-Aldrich. After solutions preparation with different concentrations (1.5wt%, 3wt%, 6wt%, 9wt% and 11wt %) were placed in a shaking incubator during 24 hour, and heated between 25ºC and 65ºC, with a heating rate of 5ºC/min.

2.2 Methods

2.2.1 Rheology

The rheological analysis, allow us to estimate the molecular organization of the polymeric solutions and also to predict its dynamic properties, i.e. the viscosity. Solutions were analyzed using Reologica StressTech Rheometer at different temperatures: 15ºC, 20ºC and 25ºC.

2.2.2 Electrospinning

Electrospun meshes were produced using the homemade system shown in Figure 4. The following processing conditions were considered: syringe volume – 2.5 mL; needle diameter – 0. 6 mm; applied voltage – 10 kV; distance between the needle and the collector – 10 cm and flow rate - 0.72 mL/h.

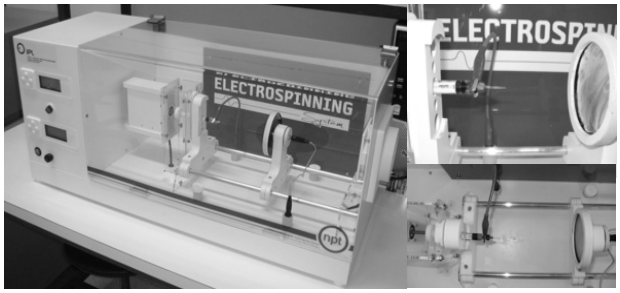


Figure 1. Equipment of Electrospinning process.

2.2.3 Scanning Electron Microscopy (SEM)

Each mesh of electrospun fibres was examined by scanning electron microscopy (model: FEI Quanta 600F) using a Cambridge Instruments S360 SEM. The samples were coated with gold prior to examination. The SEM images were used to evaluate the morphology of each mesh and the diameter distribution within each mesh.

3. Results and Discussion

3.1 Rheology

Homogenous solutions were readily prepared up to a concentration of PCL of 11wt% for systems based on AA and those prepared with AA and 2% of TEA.

The Figure 2a shows the variation of the zero-shear viscosity of the PCL solutions as a function of the polymer concentration with acetic acid as the solvent. The viscosity increases with the concentration of PCL until a cross-over point at c ~ 5.0 for solutions dissolved in AA and c ~ 4.8 for solutions dissolved in AA/TEA, where the rate of increase of the viscosity with concentration markedly increases. This happens because of the transition from dilute to semi-dilute regime where the polymer chains form entanglements. Figure 2b shows the equivalent data for the solutions containing TEA. Shenoy *et al.* (2005) have argued

that the critical concentration for the formation of fibres by electrospinning is twice of the cross-over concentration (Shenoy et al, 2005).

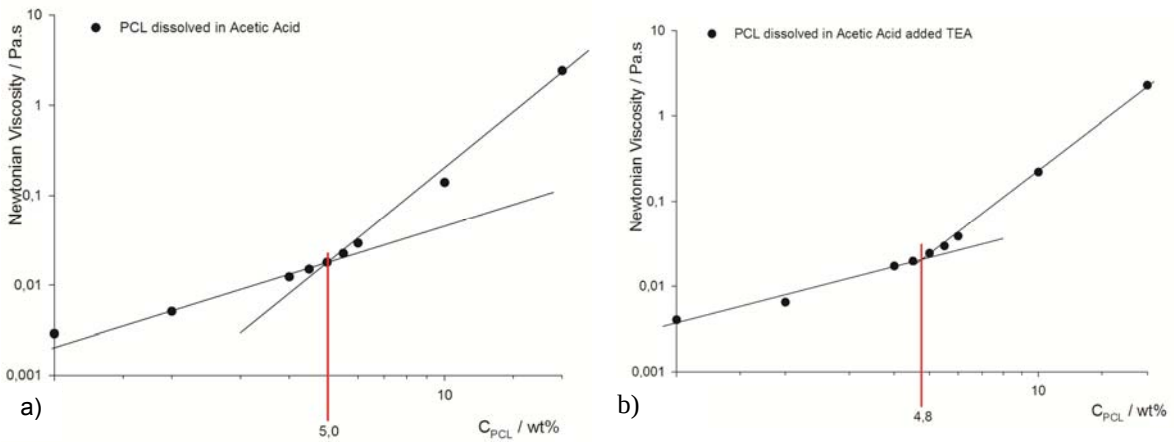


Figure 2. Plots of the zero shear viscosity against polymer concentration for solutions of (a) AA and (b) AA with 2% of TEA.

According the Table 1 it is possible to observe the influence of the temperature and the viscosity of the solutions. With increasing of the temperature the viscosity decreases, because of the higher polymer dynamics and increase the concentration, consequently the viscosity increase too, because there are more polymer chains. With rheological measurements was possible observed that the solutions presents a Newtonian behaviour, that is, the viscosity is independent of the shear rate applied.

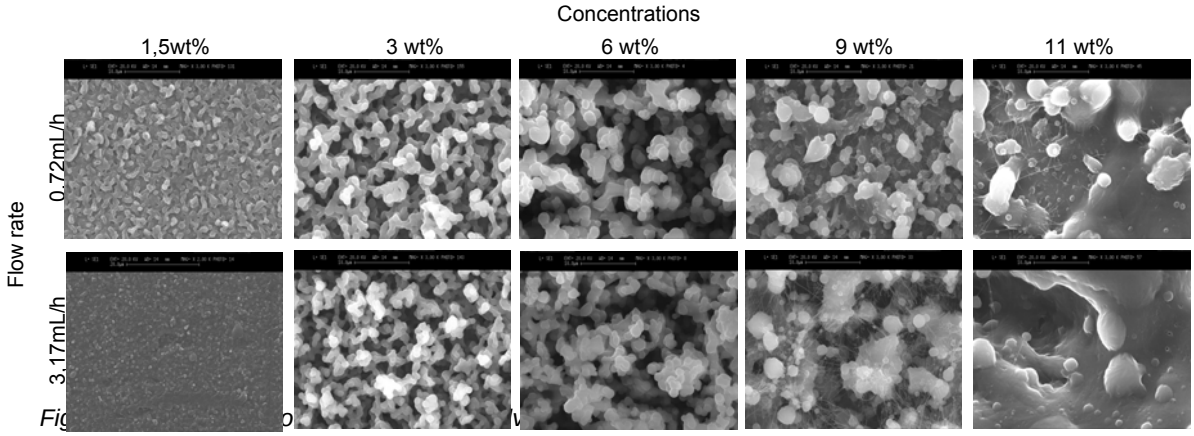
Table 1: Viscosity measurements with temperature influence

	Concentration (wt%)	η15º (Pa.s)	η20º(Pa.s)	η25º(Pa.s)
PCL/AA	11	1.582E-01	1.383E-01	1.208E-01
	9	7.164E-02	6.363E-02	5.582E-02
	6	2.454E-02	2.153E-02	1.950E-02
	3	8.987E-03	8.237E-03	7.599E-03
	1.5	5.337E-03	5.000E-03	4.752E-03
PCL/AA/TEA	11	1.511E-01	1.328E-01	1.145E-01
	9	6.427E-02	5.653E-02	4.958E-02
	6	2.710E-02	2.408E-02	2.114E-02
	3	8.093E-03	7.023E-03	6.470E-03
	1.5	5.356E-03	4.145E-03	3.959E-03

3.2 SEM

The morphologies of the electrospun mats produced from acetic acid based solutions of PCL for the two different syringe flow rates are shows in Figure 3. For solution concentrations below 9 wt% only irregular shaped particles are observed of ~ 1μm in size. The morphology appears to arise from largely spherical droplets merging to form larger aggregates. For solutions contain 9wt% PCL and with the higher flow rate produce a small number of fibres (diameter ≈ 38.5 nm) amongst a high level of particles. For the highest concentration both feed rates produce a more continuous morphology.

Figure 4 shows the morphologies of the electrospun mats produced from PCL solutions based on acetic acid and triethylamine. In contrast to the solutions of acetic acid only, electrospraying only happens for 1.5wt% and 3wt% concentration when TEA is added with an increase in concentration to 6 wt%, there are already some fibres but widely dispersed especially with the higher flow rate. For a 9 wt% concentration, we can observe the presence of many fibres although there are also highly interconnected particles; owing to the charges these particles have which attract the fibres for those places. The highest concentration



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solutions (11 wt %), produce meshes of filaments with excellent quality and with little beading especially for the higher flow rate. This suggests that we are close to the flow rate and processing conditions appropriate for this solution.

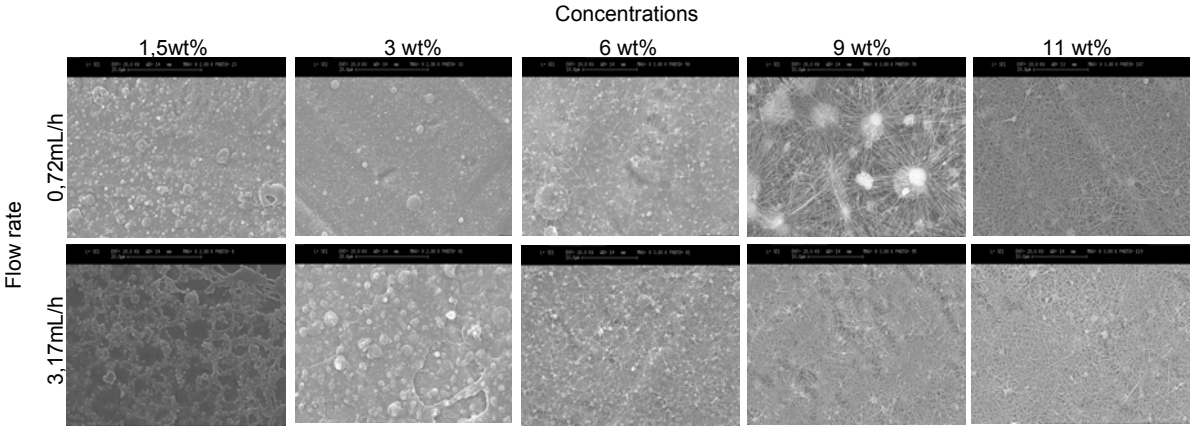

Figure 4. Morphology of PCL meshes dissolved in AA/TEA.

Figure 5 shows a plot of the average fibre diameter against the zero shear viscosity for fibres prepared from PCL solutions in Acetic acid and TEA. In calculating the average we have not included any beading or other aggregates. The trend can observe, such as in the bibliography, the diameter average increase when the viscosity increase too (Bhardwaj *et al*, 2010, Homayoni *et al*, 2009).

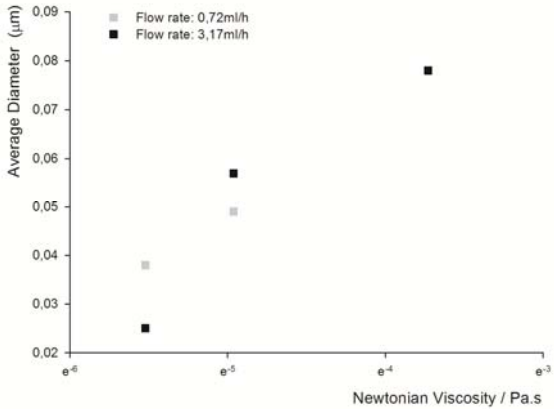

Figure 5. Newtonian viscosity vs Average diameter.

The viscosity of the solution has an effect on the electrospinning and the resultant fibre morphology. In general, the viscosity of the solution is connected to the extent of the entanglement of the polymer molecule chains within the solution. When the viscosity of the solution is too low, electrospraying may occur and polymer particles are formed instead of fibres. At a lower viscosity, where usually the polymer chain entanglements are lower, there is a higher similar that beaded fibres are obtained instead of smooth fibres. Therefore, factors that affect the viscosity of the solution will also affect the electrospinning process and the resultant fibres.

From the electrospinning data, the minimum concentration to obtain fibres is 10wt% for the Acetic Acid – PCL system and 9.6wt% for the Acetic Acid + TEA – PCL system. These values are c.a. twice of the values obtained for the overlap concentration, c* (intersection between the two lines in Figure 2). This critical concentration refers to the onset of the chain overlapping and to a transition from a dilute to semi-dilute regime. This comparison clearly indicates that the polymer system needs to be well above the overlap concentration to avoid problems as spraying or heterogeneous beads. When the polymer concentration is twice the overlap concentration, the network is established and the overlapping rejects any kind of spraying.

This work main objective was to propose a mesh of nanofibres for tissue engineering, so we need a mesh to put in to a scaffold to increase the adhesion surface, and consequently increase the area for cellular adhesion. This will lead to a faster regeneration of the tissue. We also need that the nanofibres present adequate mechanical properties to achieve the desired behaviour.

Through rheology (Figure 2 a) and b)), we can observe that for an increase in concentration the viscosity increases too, as there are more chains of polymers and the arrangements of molecules in the solution are minor, the solution became more viscous. Consequently the fibre diameter is higher as the solution has more chain of polymers.

The polymer concentration determines the spinnability of a solution, whether a fibre forms or not. The solution to occur must have a high enough polymer concentration for chain entanglement. Though, the solution cannot be either too dilute or too concentrated. The polymer concentration influences both the viscosity and the surface tension of the solution, and consequently the diameter of fibres. If the solution is too dilute then the polymer fibres will break up into droplets before reaching the collector, due to the effects of surface tension. However, if the solution is too concentrated, then fibres cannot be formed due to the high viscosity, which makes it difficult to control the solution flow rate through the capillary. With the c* determination we defined the minimum viscosity needed for fibres production when uses solution of PCL dissolved in Acetic acid and acetic acid with TEA.

Through the rheologic analyse we can define three different zones: the first corresponding to the solutions that only allow produce beads, the second zone we can produce a mix of beads and fibres and, finally, the third zone we can produce only fibres. The importance of this knowledge is known the c*, critical concentration, through this we can guarantee the production of fibres using the minimum viscosity in any kind of equipment for solutions with PCL.

Rheological studies were carried out to determine the viscosity of all solutions. For the acetic acid solutions the results shows a Newtonian behaviour, i.e., a linear relation between stress and shear rate. Additionally it was verify, as expected, a decrease of the viscosity with temperature and increase with solution concentration. Those relationships are important not only to optimise the process but also for simulation propose. The viscosity of the acetic acid solution without TEA is higher than the viscosity of the solution with TEA, as this amine is also a solvent diluting the solution.

The characteristics of the meshes depend on the solution viscosity as shown by this work. If the viscosity is too low it is not possible to produce any kind of meshes, occurring an electrospraying phenomenon (spray of small droplets). This was observed with the solution of acetic acid without TEA, where the viscosity was too low for the flow rates used.

From this work it is possible to conclude that the use of PCL dissolved in acetic acid with TEA is adequate to produce meshes for tissue engineering applications. TEA can also be used to control the viscosity of the solutions.

4. Conclusions

The addition of small quantities of triethylamine to solutions of PCL/acetic acid transforms the process from electrospraying to electrospinning leading to the formation of nanoscale fibres suitable for tissue regeneration applications. Through this work, is possible to known that for this solvent system is necessary have to 9.6wt% of PCL to produce electrospun meshes without beads.

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