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# The effect of a solvent on cellular response to PCL/gelatin and PCL/collagen electrospun nanofibres

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ABSTRACT

Bicomponent polycaprolactone/gelatin and polycaprolactone/collagen fibres were formed by electrospinning using two kinds of solvents: a representative of commonly used solvents with this polymer composition, highly toxic hexafluoroisopropanol (HFIP) and alternative, less harmful one, the mixture of acetic (AA) and formic (FA) acids. Both material types were subjected to investigations of structure and in-vitro cellular activity. Viscosity and Fourier transform infrared spectroscopy (FTIR) measurements shown that the type of solvent used influences the structure of solution and conformation of polymer molecules. In-vitro quantitative tests as well as cell culture morphology observations proved that materials electrospun with the use of 'green' solvents can yield similar results to those obtained by made with toxic ones. Slightly better cellular response to materials electrospun from HFIP can be explained by relatively well dispersed components within the fibre and more expanded conformation of molecules, resulting in better exposition of RGD (Arg-Gly-Asp) binding sites to cells' integrin receptors.

## 1. Introduction

Electrospinning is an effective method for the formation of nano- and submicron fibres from many polymers, which offers much diversity of results depending on a number of variables. Electrospun materials are used for a variety of applications, among which creation of scaffolds for tissue engineering is one of the most important.

It might seem that the main advantage of nanofibrous materials is their morphological similarity to the extracellular matrix, but without mimicking mechanical and chemical characteristics of the tissue they are employed to replace or support we only waste our resources. Choosing a material pair consisting of a synthetic biodegradable polymer and a polypeptide is a good starting point, from where only a systematic and careful optimization can lead to a desired goal.

In the most common case of electrospinning from polymer solutions, a solvent is a component which is as much important in the whole material system as polymer composition is. The choice of a solvent is significant for a few reasons.

The first one is related to the effect of a solvent used in electrospinning on solution spinnability. It is well recognized that the properties of solvents, such as dielectric constant or boiling temperature, can strongly affect both the electrospinning process, as well as material final morphology, fibre roughness and diameter. There is relatively a large number of papers devoted to such effects [1–6].

The second implication, which is much less described in the literature, is related to the effect of a solvent on the viability of cells cultured on scaffolds. Most of the papers indicating such relationship focus directly on the effect of possible solvent residues on cells' behaviour, suggesting supposed cytotoxicity of materials with even ppm traces of some solvents like HFIP [e.g. 7,8]. In the case of bicomponent materials, the understanding of solvent interaction with both polymers' molecules, which may affect cells' response to the resulting scaffold material, is yet to be covered.

The last aspect worth taking into consideration, is that the right choice of a solvent may substantially reduce the cost or/and operators' exposure to harmful, toxic fumes.

The aim of this paper is to both investigate what influence a solvent used for electrospinning of bicomponent fibres has on their biological activity as well as explain the mechanisms that stand behind it. To do so, we compare the results of two material types, blends of polycaprolactone with either gelatin or collagen, one obtained with the use of a conventional perfluorinated alcohol solvent, the other with an alternative, less toxic one. A set of quantitative in-vitro cellular studies, scanning electron microscopy (SEM) morphology observations and fluorescence imaging will provide us with enough information to achieve the first goal. Analysis of viscosity measurements of polymer solutions and with FTIR results will let us understand the origin of the outcome of cellular experiments.

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Our investigation is motivated by the fact that every solvent interacts on a molecular level with polymer molecules determining not only solubility of polymers but also their molecular conformations. This fact can be crucial for biological activity of molecules which contain specific sequences for cell attachment like collagen or gelatin with their Arg-Gly-Asp (RGD) sequences. We suppose that the availability of RGD sequences for integrin ligands from cell membranes will depend strongly on molecular conformation of polypeptides. Additionally, in the case of polypeptides, there is a problem of denaturation with the extent which could depend also on a solvent used.

When dealing with bicomponent fibres situation becomes even more complex because of mutual molecular interactions not only between polymer and a solvent but also between both polymers. It was shown in one of our previous papers [9] that the internal structure of a system depends greatly on a strength of a solvent leading to relatively homogeneous blend in the case of strong solvent (HFIP) and to components separation (emulsion) when relatively weak solvent is used (mixture of acetic and formic acid). This fact has additionally significant implications for the kinetics of polypeptides leaching from fibres what was shown previously [10].

We hope to answer the question of how the replacement of conventionally used solvents with the alternative one in electrospinning of fibres of such material composition affects their possible use in tissue engineering. To resolve this issue, it is necessary to balance not only the raw numerical results, but also convenience, utility and environmental aspect of employment of a particular solvent.

## 2. Materials and methods

### 2.1. Materials

Acids (glacial acetic acid pure 99.5–99.9% (AA), formic acid pure 98–100% (FA)) were purchased from Avantor PM Poland and Chempur, respectively. Collagen Type I lyophilized from calf skin was purchased from Elastin Products Company, Inc. PCL (Mn = 80,000 g/mol), gelatin from porcine skin Type A (gel strength ~ 300 g Bloom), 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), Triton X-100, trypan blue solution, paraformaldehyde, glutaraldehyde, hexamethyldisilazane (HDMS) and Thiazolyl Blue Tetrazolium Bromide (MTT reagent) were purchased from Sigma-Aldrich Co. Dulbecco's Modified Eagle Medium (DMEM), phosphate buffer saline pH 7.4 (PBS, no calcium, no magnesium), penicillin-streptomycin antibiotic (10,000 U/mL), fetal bovine serum, trypsin, NucBlue and ActinGreen (Molecular Probes, fluorescent dyes), CyQuant and PrestoBlue (DNA and cell viability tests) were purchased from Thermo Fisher Scientific. CellTiter-Glo® Luminescent Assay was purchased from Promega.

### 2.2. Samples names

Samples were named as follows:

P stands for polycaprolactone, G for gelatin, C for collagen, H for hexafluoroisopropanol, and A for a mixture of acetic and formic acid (AA/FA) that were used. As an example, PGA indicates a sample consisting of polycaprolactone and gelatin prepared using acids. If necessary, additional information is provided in text or in descriptions of figures.

### 2.3. Solutions preparation

Polymer solutions for electrospinning materials for cell studies were prepared by dissolution at room temperature by stirring for approximately 24 h on magnetic stirrer before electrospinning process. Total polymer concentrations were set on 5% w/w for solutions based on HFIP and 15% w/w for AA/FA mixture as optimized previously [9]. Each set consisted of solutions with PCL to gelatin content ratios 9:1, PCL to collagen 9:1 and pure PCL as a reference material. For FTIR measurements, solutions of pure gelatin and collagen for electrospinning were made with polymer concentrations alike stated above.

### 2.4. Electrospinning

The electrospinning equipment was operated in horizontal mode. It consisted of two syringe pumps (New Era Pump Systems, NE-1000 model and KD Scientific KDS-100-CE model) placed on the opposite sides of a grounded rotating drum collector (4 cm radius and 12 cm length). Two high voltage generators were connected with positive terminal to stainless steel needles to provide doubled efficiency of electrospinning process. Distances between needles and collector were 15 cm, flow rate of solution on both sides was 600 µl/h and inner diameter of needles was 0.34 mm. Except for pure gelatin and collagen, which required higher voltage during electrospinning, around 17 kV, solutions were electrospun with voltage in a range 11–14 kV, which was optimal to obtain uniform fibres and to maintain stable electrospinning process. All materials for cell studies were electrospun at similar temperature (22–24 °C) and humidity (45–55%). Pure gelatin and collagen, being more sensitive to changes in ambient conditions than PCL or its blends with biopolymers, required temperature stabilized at 24 °C and humidity not lower than 55%. Right after electrospinning all materials were placed under a fume hood to ensure no residual solvent remains in fibres before next experimental steps.

### 2.5. Solution viscosity measurements

For viscosity measurements, performed using rotational viscometer Brookfield HADV-III Ultra with cone/plate configuration, a series of solutions were prepared with polymer concentrations 5% and 15% w/w for AA/FA and 5% w/w for HFIP. Polymer ratios ranged from 100% PCL to PCL to gelatin 3:1 with an increment of 5% with an addition of 100% gelatin solution. A detailed information concerning polymer concentrations and PCL to gelatin ratios can be found in Table 1.

Additionally a set of biopolymer solutions with both types of solvents were prepared in four concentrations: 1.6%, 3.3%, 5% and 6.8%. Relatively low concentrations of gelatin and collagen in both acids and HFIP had very low viscosities. To measure it, much higher rotation speeds were required which meant higher shear rates in cone-plate configuration, compared to normally used 40 $s^{-1}$, chosen previously and explained in details in authors earlier publication [9]. That was the reason to use 300 $s^{-1}$ shear rate, which applied to all gelatin and collagen solutions.

In the case of pure collagen and gelatin which are biologically active polymers, we tried to deduce the differences in molecular conformation of both polypeptides in both solvent systems. Such comparison is possible using Mark-Houwink (M-H) equation:

$$[\eta] = KM^a \quad (1)$$

in which $M$ is the polymer molecular mass, $K$ and $a$ are constants dependent on polymer-solvent interactions and temperature, $[\eta]$ is the intrinsic viscosity defined as the limit of the reduced viscosity, $\eta_{red}$ at

**Table 1**
Viscosity of PCL/Gt solutions using AA/FA (5% and 15% w/w) and HFIP (5% w/w) at the shear rate 40/s.

| | Viscosity, Pa s | | |
|---|---|---|---|
| PCL content, % | AA/FA 15% | HFIP 5% | AA/FA 5% |
| 100 | 4.305 | 1.056 | 0.0642 |
| 95 | 4.187 | 1.081 | 0.0606 |
| 90 | 3.492 | 1.073 | 0.0557 |
| 85 | 3.424 | 0.977 | 0.0509 |
| 80 | 3.212 | 0.812 | 0.0472 |
| 75 | 3.131 | 0.91 | 0.0412 |
| 0 | 1.056 | 0.192 | 0.0157 |