STUDY OF THE INTERACTION OF MOUSE FIBROBLASTS WITH BIODEGRADABLE POLYESTERS MICRO/NANOFIBROUS MATERIALS USING BY LIVE CELL IMAGING

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Abstract

Tissue engineering and regenerative medicine are ever-growing multidisciplinary fields. Along with the development of tissue carriers, methods for testing the biocompatibility of these materials are also being developed. This work deals with research, development, and optimisation of the preparation of a fibre system intended for monitoring the direct interaction of the 3T3 mouse fibroblast cell line with polymeric materials in real-time (live cell imaging). The fabricated microfiber model (grid) was seeded with the 3T3 mouse fibroblast cell line, and the interaction between the materials and the cells was monitored for three days by optical microscope. The interaction between the materials and the cells was observed during a picture of cells on the fibres. The advantage of this method is the absence of other chemicals like chemicals for the visualisation of cells (fluorescence staining) or chemicals for measuring cell viability because cells adhering to fibres are visible in transmitted light.

Keywords: Microfibers, drawing, grid model system, cell interaction

1. INTRODUCTION

In recent decades, tissue engineering has been a highly developed multidisciplinary field combining material science, engineering, biology, medicine, etc. The goal of tissue engineering is to create tissue carriers that support, regenerate and restore the function of damaged tissue due to acute or chronic injury [1,2].

With the development of materials intended for tissue engineering and regenerative medicine, methods for testing the biocompatibility of these materials are also being developed. However, monitoring the interaction of cell lines with tissue carriers still has limits. Laboratory methods commonly used in practice are based on monitoring the metabolic activity of cells, or DNA quantification, during the experiment. For morphological analysis, cells adhered to the surface and in the material's structure are fixed and subsequently observed using fluorescence and electron microscopy [3].

One of the methods developed in the last decade is monitoring living cells in real-time using a time-lapse recording. When live cells are observed using optical microscopy in real-time, this method is used with the help of phase contrast to keep the cell population adhered to the bottom of the culture wells and to monitor the influence of different drugs, chemicals or nanoparticles on the cells [4]. However, cells adhered to the surface of the tissue carriers are not detectable using phase contrast. Therefore, cells are used that are affected by fluorescent labelling, which is bound to the cell structure. The cells can thus be observed at specific wavelengths in the emission spectrum using fluorescent microscopes [5]. However, the disadvantage remains the necessity of chemically affecting the cell line, which can cause different behaviour, whether in metabolic activity, adhesion or cell proliferation, compared to an unaffected line.

The geometrically oriented microfiber system prepared by the drawing method allows monitoring of the cellular interaction with polymeric materials over time without using contrast marking. This method could bring a new understanding of cellular interaction with the tested material. Previous studies demonstrated that on oriented fibres prepared by the drawing method, cells which adhered to the fibres were preferentially oriented in the direction of the fibres [6].

2. MATERIALS AND METHODS

2.1. Materials

For fibre preparation was used, poly-ε-caprolactone (PCL, Mw 70000 – 90000, Sigma-Aldrich) was in different solvent systems like chloroform ($CHCl_3$), acetic acid (CH_3COOH) and their combination in ratio 8:2 ($CHCl_3$:CH_3COOH).

2.2. Preparation of fibres using the drawing method

The preparation of fibres by the drawing method uses mechanical forces. A drop of polymer solution is pushed from the tip of the needle onto the pad. Then the hand is moved away from the fall; thanks to the expansive solvent system and the viscoelastic properties of the polymer, fibre is formed (**Figure 1**). A laboratory equipment manipulator based on the robotic arm "Uarm swift pro" (**Figure 1A**) was used for fibre preparation. For handling of polymer solutions was designed as a special extruder for the robotic arm, based on the positive extrusion pipette (Microman pipette) from the Gilson company [7]. The device was constructed by Ing. Andrii Shynkarenko, PhD from the Department of Manufacturing systems and Automation at the Technical University of Liberec (KSA, TUL).

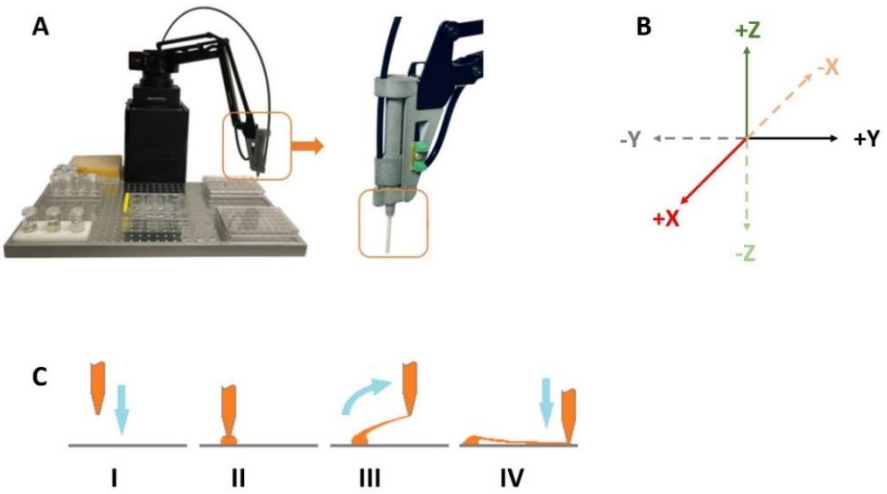


Figure 1 Robotic arm „Uarm swift pro" and detail of extrude with tips for drawing fibres (A), the coordinate system for the movement of the robotic arm (B) and the schematic principle of removing the fibre using the robotic arm (C) [7].

2.3. Optimization of fibre preparation using the drawing method

Optimisation of the fibre preparation by the robotic arm drawing method (Uarm swift pro) based on different solvent systems and PCL polymer solution concentrations. The solutions shown in **Table 1** were used to prepare the fibres. A Tescan Vega 3 scanning electron microscope (Tescan, Czech Republic) was used to evaluate the morphology of the fibres composed of the individual solutions. The morphology of the fibres was assessed from the point of view of fibre diameters. Each polymer solution was prepared with 150 fibres and 100 fibre diameter were measured because some fibre broke during the drawing process.

Table 1 Used concentration of polymer PCL80 and solvent system for drawing fibres

Sample	The concentration of PCL80 (wt%)	Solvent systems
A	10	$CHCl_3$
B	12	$CHCl_3$
C	14	$CHCl_3$
D	10	$CHCl_3$:CH_3COOH (8:2)
E	12	$CHCl_3$:CH_3COOH (8:2)
F	15	$CHCl_3$:CH_3COOH (8:2)
G	10	CH_3COOH
H	15	CH_3COOH
I	20	CH_3COOH

2.4. Microfibre model system

A geometrically oriented microfiber system was prepared to monitor cells' interaction with the polymer material. The microfibers were made from 12% PCL in pure chloroform (sample B, **Table 1**) and arranged perpendicular to each other (grid) (**Figure 2A**). The robotic arm feed speed was 20000 mm/min, the displacement path 100 mm, the arm stroke height 10 mm and the distance between the fibres 0.1 mm. For each direction of the grid, was draw 200 fibres. The resulting grid was fixed in PMMA conical carriers (**Figure 2B**). The carriers were made using injection moulding. These carriers were subsequently sterilised using UV-C for 40 minutes in a sterile flow box; after sterilisation, samples were inserted into a 24-well plate where the microfibers grid was incubated with cells.

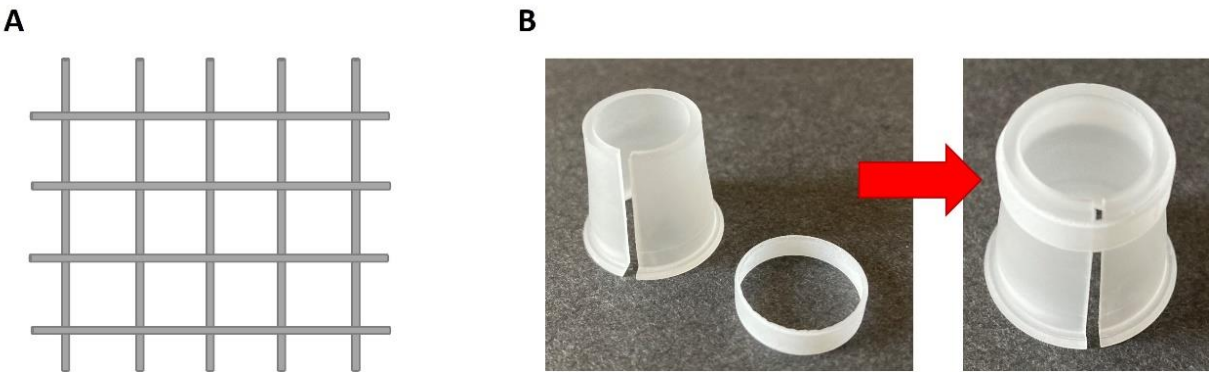


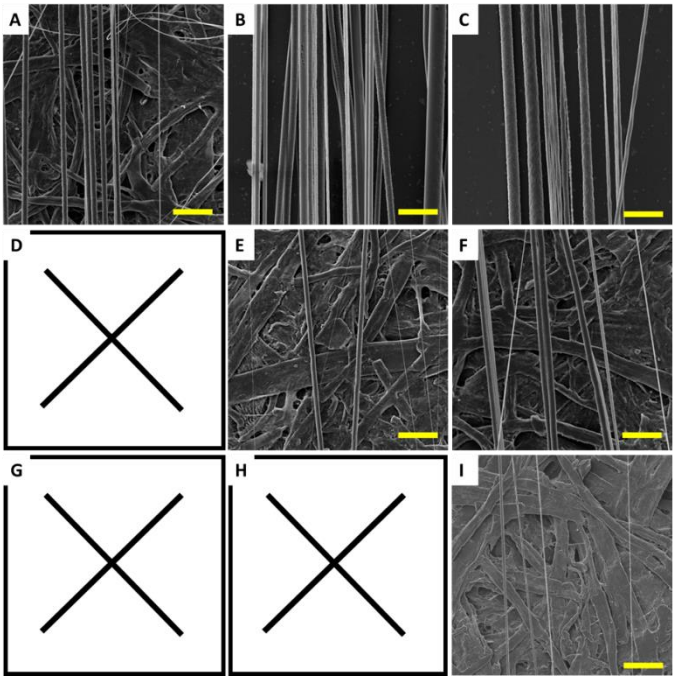
Figure 2 Schema of microfiber grid model system (A) and PMMA conical carrier with fixation ring (B).

2.5. Cell interaction with fibre model system

The cell line for testing was 3T3 mouse fibroblasts. Medium for incubation was DMEM High Glucose (Dulbecco's Modified Engles Medium, Merck, Czech Republic) with 10% fetal bovine serum (Biosera, Czech Republic), 1% glutamine (Biosera, Czech Republic) and 1% antibiotic - Pen / Strep Amphotericin B (Lonza, Czech Republic). Incubation PMMA conical carriers with PCL grid were in 24-well plates. The concentration of cells seeded in the well used was $1.5*10^4$. Samples were incubated with cells for 4 hours in a CO_2 incubator at 37 °C (Heracell Vios 160i, Thermo Fisher Scientific, Czech Republic). Then the PMMA conical carriers with grid and adhered cells on the fibres were removed into the pure well, and add complete medium. The well plate was incubated in a CO_2 incubator at 37 °C (XL incubation model system, Zeiss, Germany). The incubator is part of the Axio Vision optical microscope (Zeiss, Germany). Every 10 minutes, take an image of the fibre grid with cells. Cells were imaged in transmitted light using phase contrast.

3. RESULTS AND DISCUSSIONS

Different concentrations of PCL polymer in other solvent systems were used to optimise the preparation of fibres by the drawing method using the "Uarm" laboratory equipment (**Table 1**). Fibres did not form from solutions D, G, and H. Microfibres' very stable process formation was observed for solutions A, B, C and I. For solutions E and F, fibres frequently had visible breakage during the drawing of the fibre, and the fibres were not evenly arranged. The produced microfibers were morphologically evaluated using scanning electron microscopy (**Figure 3**). For each type of solution, fibre diameters were measured from the images taken and statistically evaluated using a box plot (**Figure 3**).



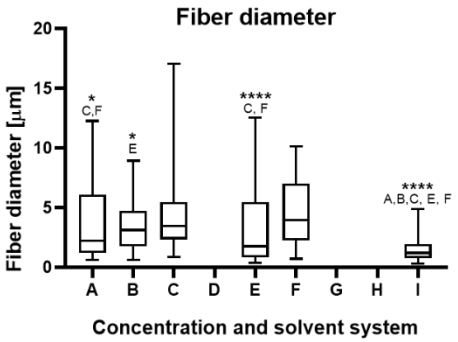


Table with statistic evaluation of fiber diameter		
Sample	**Mean [µm]**	**Standard devition [µm]**
A_10% PCL in $CHCl_3$	3.63	2,85
B_12% PCL in $CHCl_3$	3.46	1,97
C_14% PCL in $CHCl_3$	4.70	3.50
E_12% PCL in $CHCl_3$:CH_3COOH 8:2	3.05	2.68
F_14% PCL in $CHCl_3$:CH_3COOH 8:2	4.94	2.50
I_20% PCL in CH_3COOH	1.44	0.91

Figure 3 Scanning electron microscope images of fibres prepared by drawing method (left, A-I), boxplot of fibre diameters for individual tested solutions (top right), and table of fibre diameter values and standard deviations (bottom left). The scale bar is 50 µm.

The diameter of fibres produced by the drawing method from PCL was in the order of micrometres. The weakest fibres were prepared from the solution I (20% PCL in pure acetic acid). With this solution, approx. 70 % of the diameters of the produced fibres were around 1 µm. The mean value of the fibre diameter is 1.44 µm, and solution I (PCL) showed the lowest fibre variability (standard deviation). Fibres formed from solution B showed the second lowest variability of measured fibre diameters, and the mean value of the fibre diameter is 3.5 µm. The highest fibre diameter was measured for solution F (4.94 µm). Solution C showed the most significant fibre variability.

Based on the results, one selected polymer solution was to prepare a geometrically oriented fibre model (grid), which was subsequently used to monitor the interaction of living cells over time. However, weaker fibres may not be suitable for cell adhesion. There is a reason why was chosen a polymer solution from which formed homogeneous fibres with a diameter of around 3.5 µm, and the variability of fibre diameters was lowest than another solution. Solution B (12% PCL in pure chloroform) was used to prepare the microfibers grid. The created microfiber grid was fixed in a PMMA conical carrier (**Figures 4A and B**) and seeded with mouse fibroblast cells. During 72 hours, cell interaction was monitored by optical microscope Axio Vision (**Figure 4C and D**).

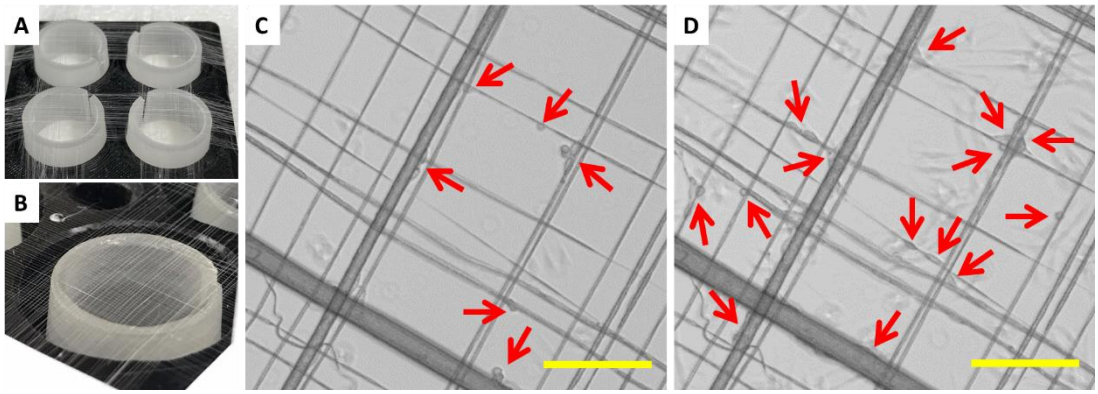


Figure 4 Preparation of a microfiber grid for cell interaction testing (A), detail of the microfiber grid before fixation on to the PMMA conical carrier (B), a picture of cells adhered to the fibres of the model system (grid) after 5 hours from seeded (C) and a picture of cells attached to the fibres after 72 hours from seeded (D). Red arrows indicate adhered cells on fibres. The scale bar is 200 µm.

Figure 4C shows cells that adhered to the PCL fibres of the model system. The first image of the fibre grid with cells was taken approx. 5 hours after deployment. The cells are visibly still round. **Figure 4D** shows cells after 72 hours of interaction with PCL fibres. The number of cells has visibly increased. The shape of the cells here is somewhat elongated, and the cells surround the fibres of the grid.

4. CONCLUSION

As part of the experiment, the production of drawing fibres was optimised. The fibres were prepared on the laboratory equipment "Uarm swift pro" designed by Andrii Shynkarenko (KSA TUL). Different concentrations of PCL (Mn 80000) were tested in other solvent systems. A solution of 12% PCL in pure chloroform (solution B) was selected from tested solutions. The microfibers produced from this solution were homogeneous compared to the other solutions used, and the fibre drawing process was very stable. The diameter of the fibres was around 3.46 µm. This solution subsequently prepared a fibre grid as a model system for monitoring the interaction of living cells over time. The fibrous grid was seeded with 3T3 mouse fibroblasts, and cell interaction was observed for 72 hours. From the images in Figure 4D, it can be seen that after 72 hours, the cells adhered to the material (they have an elongated shape), and their number has visibly increased since seeded.

This method is innovative precisely because it is unnecessary to monitor the cell interaction by affecting the cells with any fluorescent or other chemical for its visualisation. Cells are observed on polymer fibres only in transmitted light using phase contrast. The microfiber model system appears suitable for monitoring the interaction of living cells with polymeric microfibers over time. The model system can monitor the movement of cells along fibres. When using a higher concentration of cells and a more prolonged incubation with the materials, the model could be suitable for evaluating the time required for the growth of defined significant defects (distance of individual fibres to each other).

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