PRODUCTION OF SCAFFOLDS FOR ARTIFICAL CARTILAGE BY CENTRIFUGAL SPINNING

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Abstract

Centrifugal spinning was used for production of biodegradable scaffold for tissue engineering. Polycaprolactone solution was spun on industrial Forcespinning™ machine Fiber Engine 1.1. Forcespun material was tested in-vitro with different cells - 3T3 mouse fibroblasts, PLT human trombocytes and combination of 3T3 with PLT. Growth and proliferation of different cells was monitored using several methods such as scanning electron microscopy (SEM), fluorescent microscopy and MTT tests.

Keywords: Centrifugal spinning, forcespinning, polycaprolactone, scaffold, in-vitro testing

1. INTRODUCTION

Centrifugal spinning, Forcespinning™ or Rotary Jet Spinning, all these terms refer to the technology which allows to produce fibers from polymer solutions or melts due to centrifugal forces. Centrifugal spinning as well as electrospinning, drawing, phase separation etc. enables nanofiber production. Nanofibers are potential materials for several applications including filtration, energy storage, electronics, composite materials, medicine or tissue engineering.

Centrifugal spinning is very simple technology based on the centrifugal forces which create due to rotation of the spinneret unit. Centrifugal forces are necessary for drawing polymeric jet and fiber formation.This technology is able to make fibers from wider range of materials because of no requirements imposed on materials with low dielectric constants as in the case of electrospinning [1]. It is possible to add inorganic particles to polymeric liquid and produce inorganic nanofibers. Several materials were succesfully forcespun such as polyethyleneoxide [1, 2], polycaprolactone [3, 4, 5], polylactic acid [2], polyacrylonitrile [6, 7], polypropylene [8, 9], polyamide [10] or polystyrene [11] to mention some.

Fiber morphology is influenced by processing and material´s parametres such as spinneret angular velocity, orifice radius, size of spinneret, distance of spineret orifice from collector, molecular mass of polymer, concentration of polymeric solution (viscosity, surface tension) or temperature should be included [1, 2, 3, 6].

Generally, for centrifugal spinning is typical wide range of fiber diameters from several nanometers to several micrometers depending on the used material. Fiber stucture is quite fluffy with large numbers of different sized pores. These material properties are suitable for good adhesion and proliferation of cells. This work will focused on the development of scaffolds for artificial cartilage by centrifugal spinning.

2. EXPERIMENT

2.1. Materials

Poly-ε-caprolactone (Sigma Aldrich, Mn = 80,000) dissolved in chloroform (Penta) and ethanol (Penta) in ratio 9 to 1 and in concentration 12 wt. % was prepared by mixing on magnetic stirrer. NIH 3T3 mouse fibroblasts (Sigma Aldrich), human trombocytes PLT (Sigma Aldrich) and combination of 3T3 with PLT were

used for in-vitro testing. Scaffolds were sterilized by 70% ethanol and after they were washed by PBS (isotonic phosphate buffer).

2.2. Methods and evaluation

Polycaprolactone solution was spun on Fiber Engine 1.1 (producer FibeRio Technology Corporation, USA) in Pardam Ltd. (Czech Republic). Fiber layer from centrifugal spinning was used as a scaffold for in-vitro tests with different types of cells (3T3, PLT, 3T3 + PLT). MTT tests and microscopy technique as scanning electrone microscopy (Tescan) and fluorescence microscopy were used for monitoring cells growth and proliferation through fiber scaffolds. Basic statistics were done for evaluation of results.

3. RESULTS AND DISCUSSION

3.1. Centrifugal spinning

Centrifugal spinning of polycaprolactone solution was carried out on Fiber Engine 1.1 at 11,000 rpm. Fiber Engine 1.1, SEM picture and photo of fiber layer from centrifugal spinning on Fiber Engine 1.1 are shown in Figure 1 (a), 1 (b) and 1 (c).

(a) (b) (c)

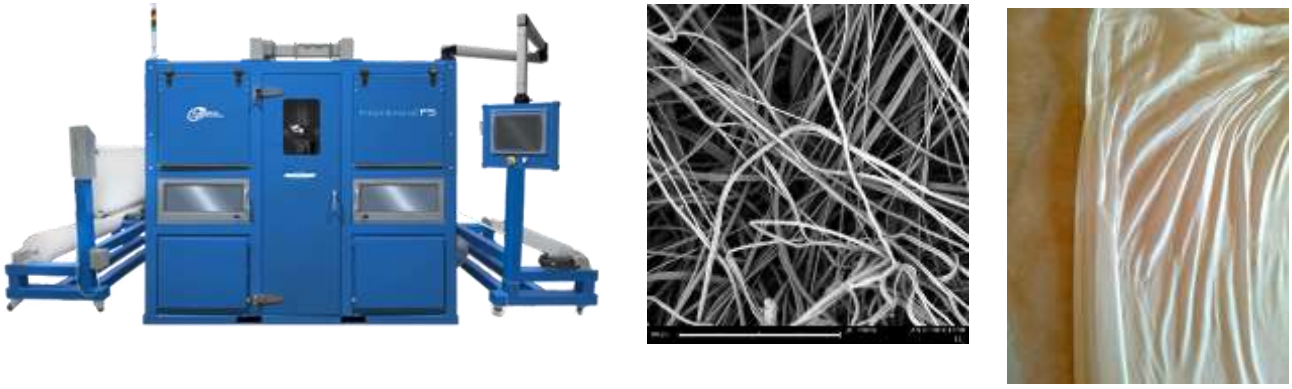

Fig. 1 Centrifugal spinning of polycaprolactone solution. 1 (a) Fiber Engine 1.1 taken from [12]. 1 (b) SEM image of polycaprolactone fibers (scale bar represents 60 μm), 1 (c) fiber layer from centrifugal spinning on Fiber Engine 1.1

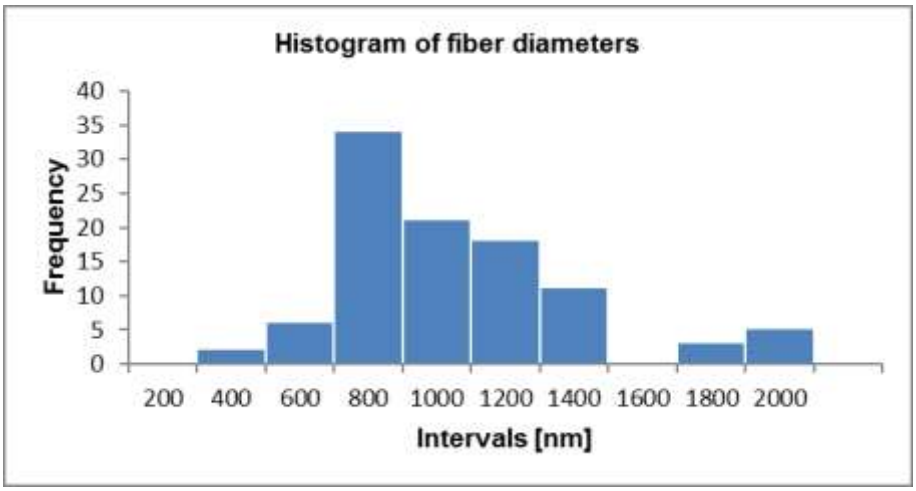

Fig. 2 – Histogram of fiber diameters from centrifugal spinning of polycaprolactone on Fiber Engine 1.1 at 11,000 rpm

For centrifugal spinning is typical fluffy structure and large amount of different sized porous. This is confirmed by value of porosity of forcespun material. A volume of air in the fiber structure is higher than 96,5 % of the total mass of fiber scaffold. Fibers have a wide distribution of fiber diameters – it is seen from the Figure 2 where histogram of polycaprolactone forcespun fiber diameter is shown. A mean of polycaprolactone forcespun fibres was aproximatly 942 nanometres and standard deviation was 347 nanometres.

3.2. Preparation of fiber scaffolds and in – vitro tests

Fibrous scaffolds were prepared by cutting fibers layer from centrifugal spinning. Size of each fiber sample was aproximatly 6 mm in diameter. Fiber scaffolds were sterilized by 70% ethanol for 30 minutes and after washed 3 times in PBS (pH 7.4). Prepared scaffolds were placed into 96-well tissue culture plates, and in each culture plates, there were 10^4 cells. NIH 3T3 fibroblasts, PLT human trombocytes and combination of 3T3 with PLT were tested in-vitro for 14 days. Medium was changed each 3 days and testing days were 1st, 3rd, 7th and 14th days. SEM images of fiber scaffolds with different cells in different testing days are shown in the Figure 3. Cells were able grown through fiber scaffolds.

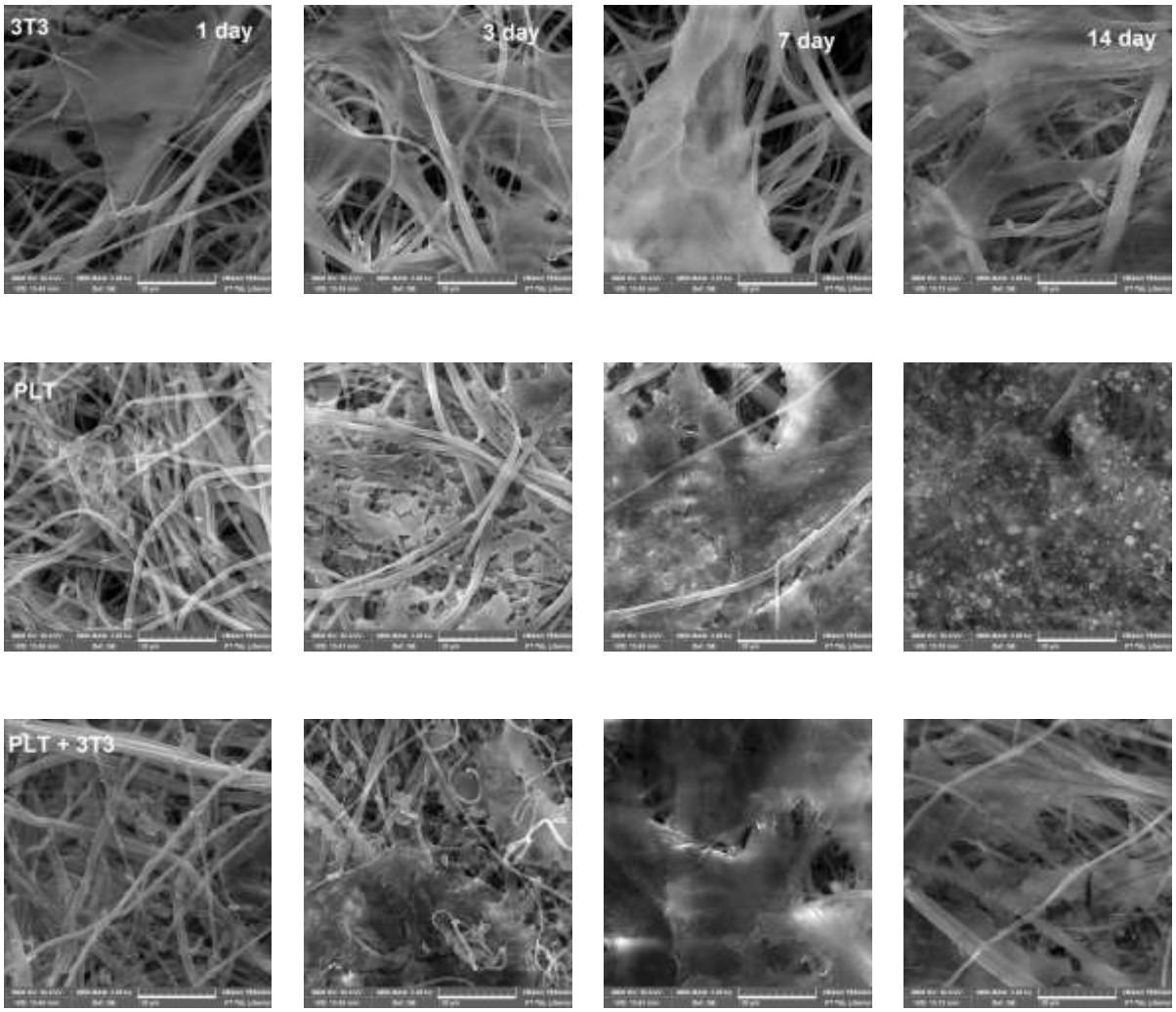

Fig. 3 SEM pictures of fibrous scafflolds with different cells – 3T3 (mouse fibroblasts), PLT (human trombocytes) and combination 3T3 with PLT from 1st, 3rd, 7th and 14th in-vitro testing day. Scale bar represents 20 μm.

Fiber scaffolds were monitorized by fluorescence microscopy at 1st, 3rd, 7th and 14th testing day. In the Figure 4, there are shown composite images consisting of 100 different layers of fiber samples. The brightest

color marks cells situated at the toppest layer. Cells were able to proliferate through all fiber scaffolds prepared by centrifugal spinning. From the Figure 4 it is obvious, that cells are able to grow and proliferate in fibrous forcespun structure.

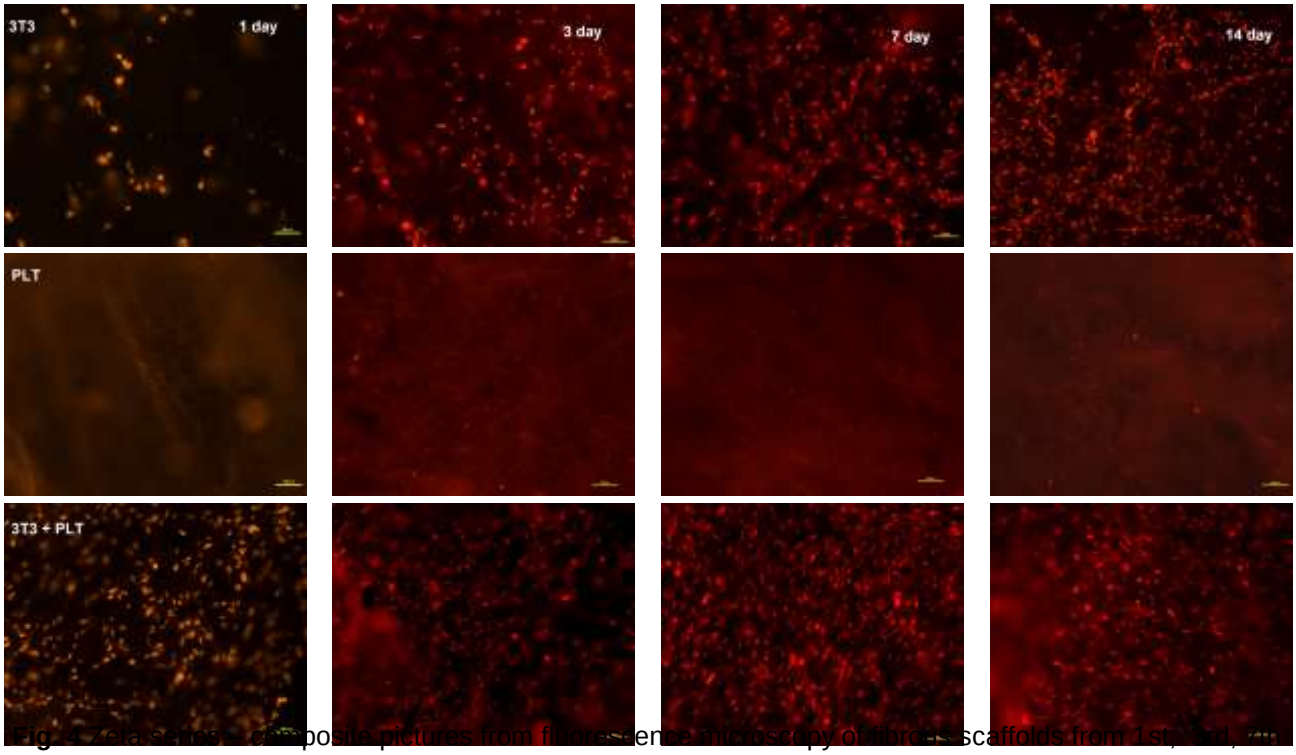

Fig. 4 Zeta series – composite pictures from fluorescence microscopy of fibrous scaffolds from 1st, 3rd, 7th and 14th testing day with different cells - 3T3 (mouse fibroblasts), PLT (human trombocytes) and combination 3T3 with PLT. Scale bar represents 100 μm.

In the Figure 5, there is shown chart of MTT test for 3T3 cells (green color), PLT cells (blue color) and combination 3T3 with PLT (red color).

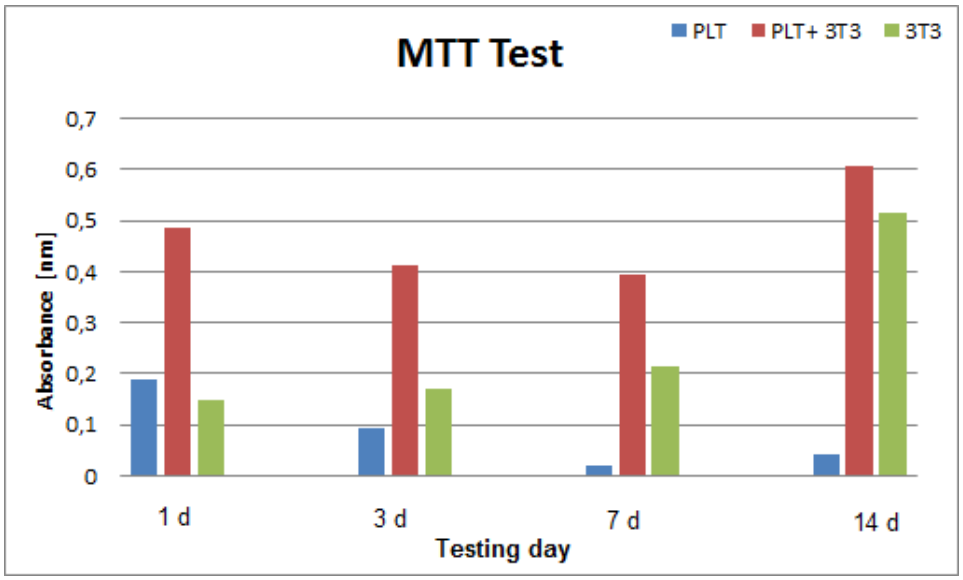

Fig. 5 – MTT test of polycaprolactone forcespun on Fiber Engine 1.1 for 1st, 3rd, 7th and 14th in-vitro testing day. Blue color represents PLT (human trombocytes), red color represents combination of 3T3 with PLT and green color represents 3T3

In the Figure 5, there is shown that 3T3 fibroblasts are able to grow and proliferate good on fiber layer from centrifugal spinning. This is also obvious from fluorescent microscopy in Figure 4. PLT human trombocytes didn´t have good conditions for growth and proliferation and their amount reduced during 14 days. The amount of cells of combination 3T3 with PLT decreased till 7th day but after, maybe due to 3T3, cells started to grow and proliferate more.

4. CONCLUSION

This work focused on preparation of fiber scaffolds by centrifugal spinning. Fibres had different diameters from 350 to 1980 nanometers and fibrous structure was fluffy. Proliferation of NIH 3T3 fibroblasts, PLT (human trombocytes) and combination of NIH 3T3 with PLT was tested on fibrous material spun at 11,000 rpm. It was confirmed, that forcespun fibrous material is suitable for tissue engineering as scaffolds. Cells were able to proliferate through all forcespun fiber structure and therefore it was possible to observe cells on both sides of fiber scaffolds. Further experiments will be performed to verify this assumption.

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