DRAWING FIBROUS SCAFFOLDS WITH PRECISE STRUCTURE FOR TISSUE ENGINEERING

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Abstract

This work is focused on evaluation of scaffolds made by drawing technique for the cell adhesion, viability and proliferation. With drawing we are able to produce very precise fibres with specified orientation from different polymers (PCL, PVA, PVB and others). This characteristic could be an advantage in tissues with ordered pattern. In the primary study the handmade fibres were tested for the cell growth and the results showed the potential of the cells to overgrow the gaps between the fibres. On account of these results further tests were necessary to be done with the precise machine-made fibrous scaffolds. The machine-made fibres are thinner, the gaps between the fibres are smaller and the whole structure is denser. In this study we focused on a planar scaffolds with fibres ordered either in one, in two or in three directions. Mice fibroblasts (3T3 Swiss Albino) were used for the *in vitro* experiment. The scaffolds were tested at day 1, 3, 7, 14, 21 and 28 after cell seeding. MTT assay, fluorescent microscopy and SEM analysis were used for the evaluation of cell adhesion, viability and proliferation. The results from microscopy analysis as well as from the MTT assay show, that the machine-made scaffolds are capable of supporting cellular attachment and proliferation during our *in vitro* tests. The scaffolds with crossed fibres show better results compared to fibres ordered in one direction. Nevertheless the one-direction fibres have a great potential in many tissue engineering applications, e.g. for muscles or neural tissue.

Keywords:

nanofibres, drawing, scaffolds, tissue engineering

1. INTRODUCTION

Sincein the human body many tissues are with oriented manner, it might be advantageousto have specialized scaffold with fibresordered in defined direction. Specific structure of scaffold should allow and support the growth of cells in designated pattern. For this purpose the technology of mechanical pulling of individual fibres (fibre drawing) was used. It is a process proceeded without directly affecting electrical field [1 - 3]. That is why drawing is relatively simple method and it is possible to do it even by hand [4, 5]. But to make more precise scaffolds with defined order of fibres, specific “Micromanipulator” (Fig. 1) was investigated by engineers from our university in cooperation with the Department of Applied Cybernetics and Department of Nonwovens and Nanofibrous Materials. The Micromanipulator pulls a single fibre out of the polymer solution droplet with a diameter from 300 nm to 5 μm. It can make individual or aggregated micro / nano fibres and place them precisely in the direction we need. This phenomenon is advantageous in the case of tissues with oriented structure, e.g. muscle tissue, nerve tissue in some cases and others. This suggests the usage of such scaffolds in reparative medicine – spinal lesions, hernia meshes etc.

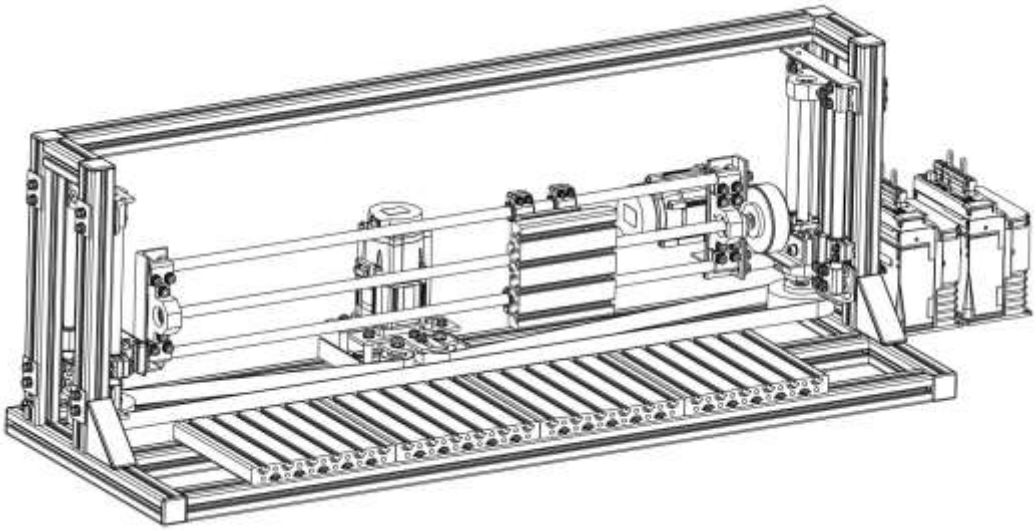

Fig. 1: Primary lay-out of Micromanipulator [2].

2. MATERIALS AND METHOD

2.1 Materials

A 12% solution of poly – Ɛ – caprolactone (PCL, Mw 70.000 – 90.000, Sigma-Aldrich) in 100% chloroform was used for drawing.

2.2 Drawing

The mechanical drawing was used to prepare the scaffolds for biological experiments. The motion program of Micromanipulator consisted of parabolic interpolation with S – curve acceleration and deceleration profile (500ms and 1500 ms). Top speed of drawing element was 0,08 m/s with 100 fibres / 1 mm and it took approximately 2 hours to make 9 scaffolds for *in vitro* testing. In the first step the droplet of a PCL solution is placed on an underlay. A movable element, in this case a needle, touches the droplet anderodes its surface. In the next step, the needle starts moving away and due to a surface tension of the polymer, it forms a fibre (Fig. 2). The PCL solution solidifies as a result of the evaporation of the solvent, so finally the length of the fibre is extended partially by using the core still-liquid material and partially by stretching the surface [4, 5]. The process ends when the needle lays the fibre down on the underlay in the defined distance.

Fig. 2: Drawing technique [6].

2.3 Sample evaluation

The diameter of the fibres within all three types of scaffolds (str I, str II, str III) was measured from pictures made by scanning electron microscope (Tescan, VEGA3 SB easyprobe) using NIS Elements programme.

2.4 Sample preparation

Three types of scaffolds were prepared varying in number of layers and orientation of fibres. Fibres were ordered either in one, in two or in three directions (str I, str II, str III) (Fig. 3). Fibres have different diameter, mainly in units of micrometers and thinner. Scaffolds are made as single fibres fixed within the supporting ring (Fig. 3). The size of the ring is designed to fit in 24-well cultivation plate and allows better manipulationwith the fibres as well as it keeps the scaffold in the designed pattern. The ring was made by machining from PMMA (Titan Multiplast). Immobilized scaffolds were sterilized in 70% ethanol for 30 minutes and washed several times in phosphate buffer saline (PBS, pH 7,4) prior to cell seeding.

Fig.3: One direction fibres fixed within the supporting ring [6].

2.5 Cell culture

3T3 mice fibroblasts (3T3 Swiss Albino, ATCC) were maintained in Dubelco's Modified Eagle's Medium (DMEM, Lonza) with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin / streptomycin / amphotericin B (Lonza). Cells were cultured in an incubator (37°C, 5% CO_2). Medium was changed three times a week and the second passage was used for the in-vitro experiments.

2.6 Cell viability and proliferation

Concentration of cells seeded in particular wells of 24-well plate was $1*10^5$. Cell viability and proliferation was measured by MTT assay on day 1, 3, 7, 14, 21 and 28 after cell seeding. 50 µl of MTT solution and 150 µl of DMEM was added to all the samples and was incubated for three hours at 37°C, 5% CO_2. The formazane crystals were dissolved in acidic isopropyl alcohol and the absorbance was measured at 570 nm with the reference at 650 nm.The samples were diluted prior to themeasurement when needed. The final absorbance is related to the dilution.

2.7 Microscopy analysis of cell proliferation

After day 1, 3, 7, 14, 21 and 28 of cell seeding all scaffolds were processed for a microscopy analysis. All the samples were washed with PBS prior to fixation to remove unattached cells. The scaffolds processed for SEM and fluorescent microscopy were fixed with 2,5% glutaraldehyde or ice-cold methanol, respectively. After the fixation the samples for SEM were dried up with upgrading concentration of ethanol (60%, 70%, 80%, 90%, 95% and 100%). Afterthe drying, thesamples were sputtered with gold and were analysed by scanning electron microscope (Tescan, VEGA3 SB easy probe). For a fluorescent microscopy, the samples were rinsed with PBS after thefixation and incubated for 15 minutes with DAPI at room temperature in the dark. After incubation period, the samples were rinsed with PBS and analysed by the fluorescent microscope (NICON Eclipse Ti-e).

3. RESULTS AN DISCUSSION

In our experiment we tested planar scaffolds with fibres ordered either in one, in two or in three directions (str I, str II, str III) (Fig. 5). The scaffolds were tested for cell adhesion and proliferation on day 1, 3, 7, 14, 21 and 28 after cell seeding by MTT assay, fluorescent and scanning electron microscopy. The results from the microscopy analysis as well as from the MTT assay show, that the machine-made scaffolds are capable of supporting cellular attachment and the proliferation during our *in vitro* tests (Fig. 4, 6, 7). The data from

the MTT assay and the fluorescent microscopy reveal similar rates of cell adhesion (day 1) in all the three types of scaffolds. However, the 14th day after cell seeding the difference in the proliferation rate between the samples can be observed by the MTT assay (Fig. 4).This result can be caused by the inhomogeneity of the samples (Fig. 5). The results from the MTT assay as well as from the fluorescent microscopy also show that the cells after day 14 of our experiment have covered almost the whole surface of the scaffold and they do not proliferate any more (Fig. 4, 6). The results from our experiment from fluorescent microscopy also show very important phenomenon that the cells prefer the growth in the direction of fibres, which could be beneficial for biomedical applications (spinal lesions, hernia meshes) (Fig. 6). The pictures from the fluorescent microscopy as well as from the SEM also show, that the gaps between the fibres are still too big in some cases to be overgrown (Fig 6, 7). The pictures from the SEM show the big difference in sample homogeneity. The fibres form rather fascicles than to be separated next to each other (Fig. 5, 7). This feature can be advantageous in some cases (muscular tissue and others), but for another applications the single fibre nets with smaller distance of fibres could be more useful (hernia meshes). To achieve more uniform structure of the scaffold, fully automated Micromanipulator is needed.

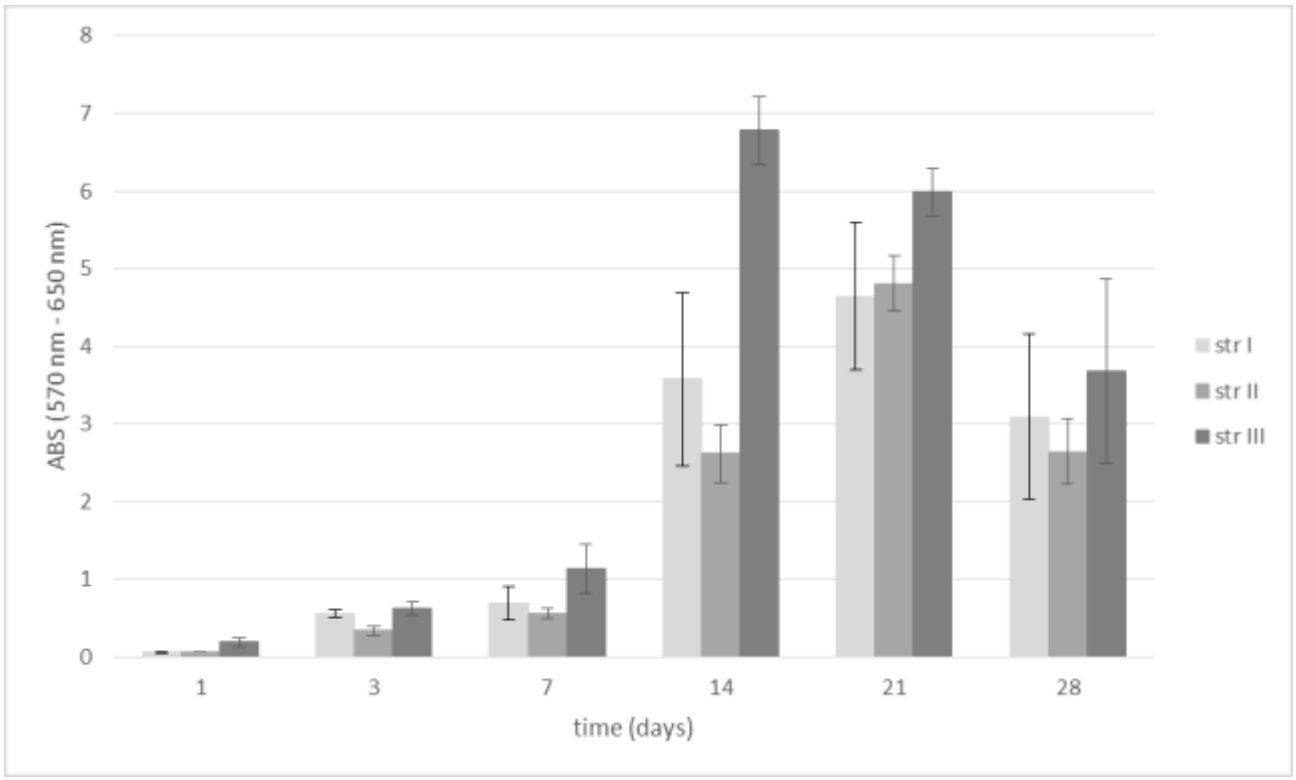

Fig. 4: The MTT assay of all thethree types of scaffolds tested.

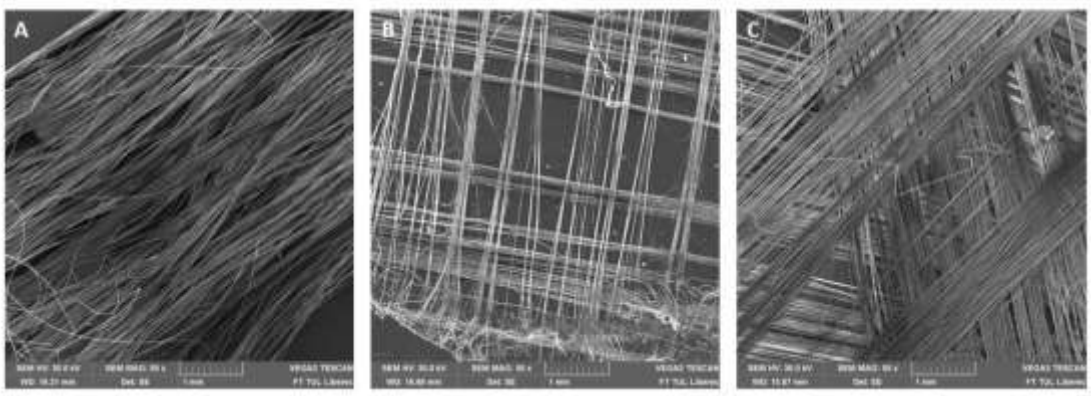

Fig. 5: SEM of three types of scaffolds – one (A), two (B) and three (C) direction (Tescan, VEGA3 SB easy probe, magnification 50x).

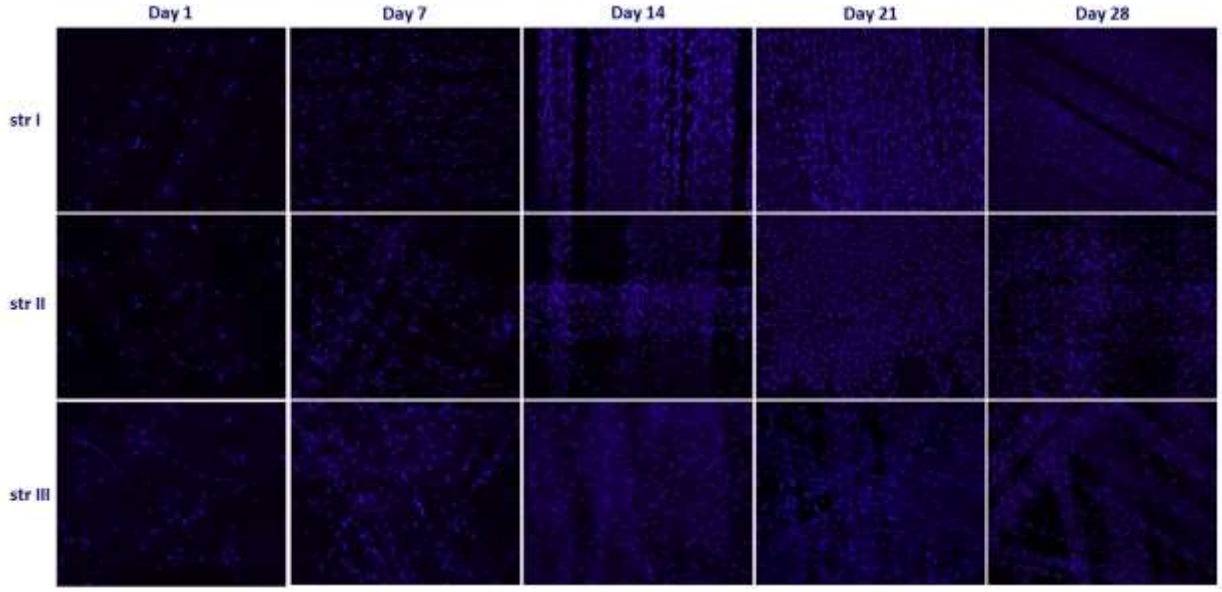

Fig. 6: Fluorescent microscopy of all three types of scaffolds within testing days (DAPI staining, magnification 100x)

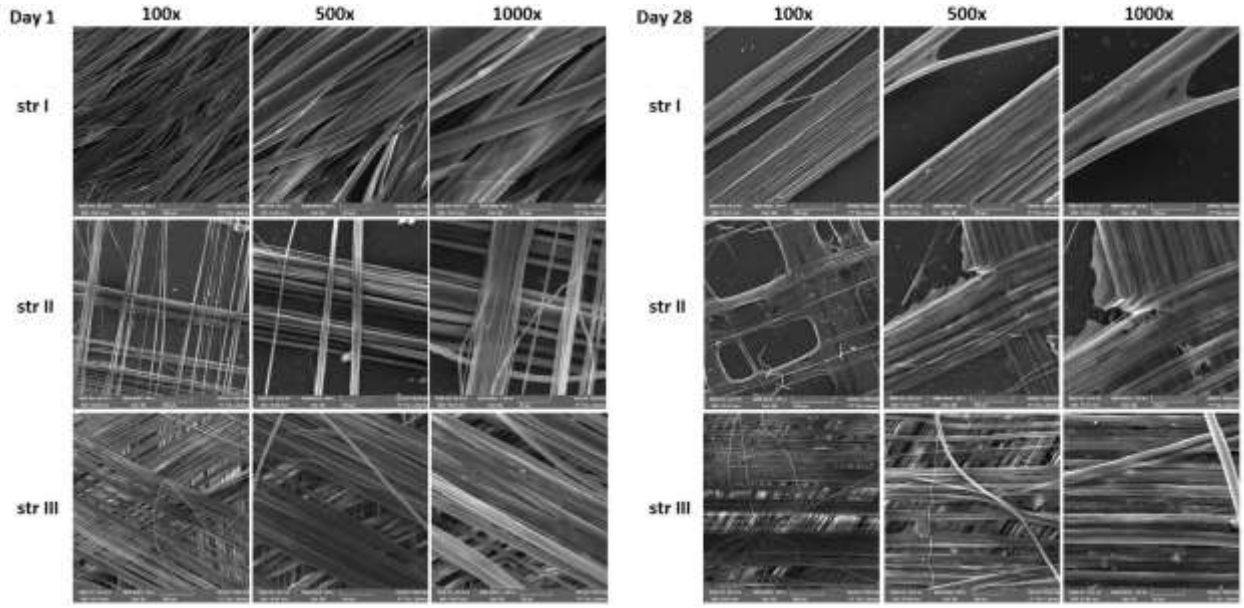

Fig. 7: The SEM of all three types of scaffolds seeded with cells at day 1 and 28 after cell seeding (magnification 100x, 500x, 1000x).

CONCLUSIONS

For our experiment, we prepared three types of scaffold, differing in the number of direction of the fibres (str I, str II, str III). The microscopy analyses as well as the MTT assay show that all thesamples are capable of supporting cell adhesion and proliferation during our experiment. Also, the results confirm that the cells prefer the growth in the direction of fibres. The three direction scaffolds (str III) show higher viability (MTT assay) within all testing days. For further studies, more homogenous samples are needed to confirm our results. To achieve this, Micromanipulator needs to be supplied by fully automated control.

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