ELECTROSPINNING AND BIOCOMPATIBILITY OF POLYMER-CERAMIC NANOFIBERS FOR TISSUE ENGINEERING

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Abstract

Polycaprolactone is known as biocompatible material for long time and it is considered as possible material for bioscaffolds. Since natural structure of hard tissues (bones) and most of soft tissues (skin, neural tissue) is fibrous, there are attempts to mimic such a structure. Polycaprolactone (PCL) nanofibers with diameter of approximately 180 nm were prepared by electrospinning method. For better biocompatibility and bioactivity, a composite fibrous structure was prepared. Hydroxyapatite nanoparticles were synthetized via precipitation reaction and further hydrothermal treatment. The ceramic particles were added to modified polycaprolactone precursor and the solution was electrospun. The prepared fibers and as well as dried original precursor were in-vitro tested for cytotoxicity by direct contact test. Mouse fibroblast from L929 line were used for cell cultivation. It was found out that fibrous structure and presence of ceramic particles have a positive influence on cell activity and proliferation - the growth rate of the cells was significantly higher compared to bulk polymer precursor.

Keywords: Nanofibers, electrospinning, biocompatibility, polycaprolactone

1. INTRODUCTION

Regenerative medicine requires new materials and structures for faster and gentler treating of large wounds. Nowadays, allo- or autografts are the most common way how to cure extensive skin injuries and large bone injuries are usually treated with bioinert metallic (e.g. titanium) or ceramic (alumina or zirconia) implants. More convenient method would be implantation of a scaffold that would support natural cell activity on the damaged cells and that would be replaced by new tissue after some time without any residues in the patient's body. There are several attempts to prepare such a scaffold - bulk implants, porous materials and fibrous structures. Considering natural structure of the human tissues, fibrous scaffolds are very promising for regenerative medicine. [1,2,3]

There are several ways of preparation of fibrous structures, e. g. electrospinning, self-assembling and phase separation. [4] The electrospinning is a versatile technique suitable for preparation of polymer, ceramic and composite fibers with range of the diameter from 100 nm to 100 µm. The final morphology of the fibers is influenced by electrospinning parameters (mainly by accelerating voltage) and composition of the precursor. Ceramic fibers can be also modified by subsequent heat treatment. The versatility of the process opens wide field for research in biomaterials - modification of prepared structure with aim to influence the activity of the cells is still an open chapter. [2,3,5]

2. EXPERIMENTAL

2.1. Materials and methods

Polycaprolactone with molecular weight of 40 000 g/mol (Sigma Aldrich, Germany) was used as initial material for preparation of the nanofibers. Polycaprolactone was dissolved in mixture of acetic and formic acid in ratio 3:1 to obtan 10 wt% polycaprolactone solution. To prepare ceramic-polymer composite structure, hydroxyapatite nanoparticles were added to the precursor. The hydroxyapatite ultrafine powder was prepared according to precipitation reaction (1). The product was then hydrothermally treated in a reactor (Ultraclave

Milestone, Italy) at the temperature of 250 °C for 5 hours. The powder was added to 6 wt% solution of polycaprolactone in mixture of acetic acid and formic acid in ratio 3:1. The concentration of the hydroxyapatite in the solution was 4.5 wt%. To obtain a homogenous precursor, the ceramic particles were dispersed by ultrasound probe for 3 minutes.

$$Ca(NO_3)_2 + (NH_4)_2HPO_4 \longrightarrow Ca_5PO_4)_3OH + NH_4NO_3 \quad (1)$$

The precursors were electrospun by 4SPIN (Contipro, The Czech Republic). Parameters of electrospinning were the same for both solution and they are listed in **Table 1**. For better morphology and uniformity of the fibers, warm air (35 °C) was blown at rate of 24 $l.s^{-1}$ around the emitter.

Table 1 Electrospinning parameters of polycaprolactone based precursors

Accelerating voltage (kV)	Emitter-collector distance (mm)	Feeding rate ($ml.hod^{-1}$)	Size of the emitter needle (ga)
25	100	28	19

The as-spun fibers were analyzed by scanning electron microscope (Verios, FEI, USA) - structural was done. After structural analysis, cytotoxicity tests were provided on the both type of fibers. The fibers were deposited on a circle slide glass in a thin layer to guarantee transparency of the samples that is crucial for cytotoxicity tests. The test was done on five samples of each type always for three days. Mouse fibroblasts from L929 line were used for the testing. A photo was taken every 30 s on seven fields of vision. The results were compared with data obtained from the baseline sample (blank sample) and samples with continuous layer of the precursors.

3. RESULTS

3.1. Preparation of the ceramic nanopowder

Structure analysis of the hydroxyapatite powder was done before and after the hydrothermal treatment. As-synthetized ceramics was in the form of agglomerated flake-like particles. The hydrothermal treatment resulted in change of morphology of the particles - the shape was changed in needle-like one. The needles had diameter of approximately 30 nm and they were about 600 nm long which was promising for their further incorporation into the nanofibers. The effect of the hydrothermal treatment is visible in **Figure 1**.

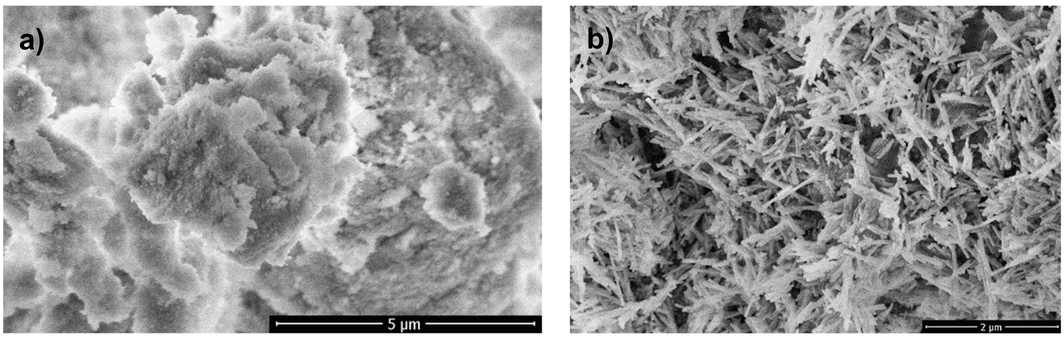

Figure 1 a) Highly agglomerated hydroxyapatite powder before the hydrothermal treatment, b) the nanoparticles with needle-like shape after the hydrothermal treatment

3.2. Preparation of the fibers

Uniform and smooth nanofibers with diameter of approximately 180 nm were prepared by electrospinning in hot air of the polycaprolactone precursor (see **Figure 2a**). Fibers with incorporated hydroxyapatite particles were not as uniform as pore polycaprolactone ones. The ceramic-polymer fibers had visible defects in the structure and the ceramic particles penetrated through the polymer out of the fibers as can be seen in **Figure 2b**. These spots were identified as the places that would enhance proliferation of the cells.

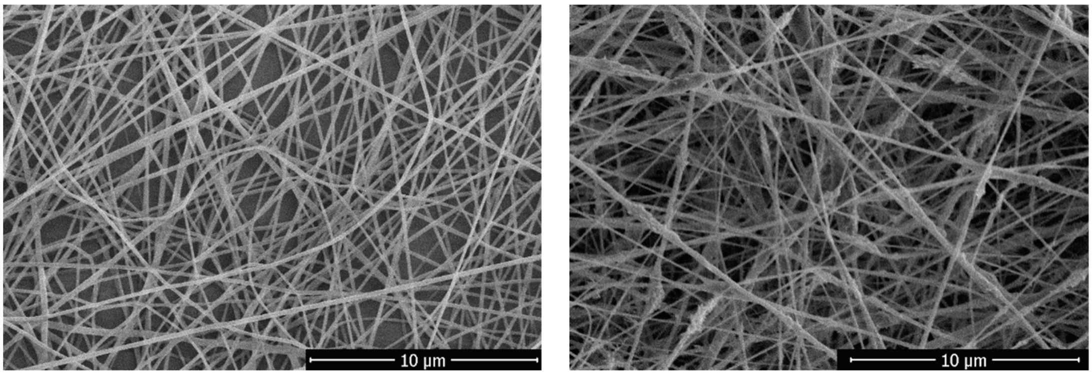

Figure 2 a) As-spun polycaprolactone nanofibers,
b) as-spun composite nanofibers with hydroxyapatite particles

3.3. Cytotoxicity tests

Test of the cells proliferation confirmed beneficial effect of the fibrous structure and presence of the ceramic compound on the cells activity. As can be seen in **Figure 3**, polycaprolactone as bulk material is biocompatible but it is not bioactive. Addition of the ceramic particles enhanced the biological properties, but the proliferation was still slower than on the baseline sample. However, on the electrospun ceramic-polymer nanofibers the proliferation was the fastest in comparison to all tested samples. Specific growth rate was calculated by measuring the numbers of the cells attached to the sample (see **Figure 4**). With exception of layered samples, the specific growth rate increased until 48 hours. The highest specific growth rate after 48 hours had polymer-ceramic fibrous sample. The specific growth rate then decreased that could be caused by formation of cells monolayer on the top of the sample (see **Figure 5**), so the cells did not have enough space for further proliferation. The high specific growth rate of the ceramic-polymer sample after 48 hours of cultivation shows accelerated cells activity on the fibrous samples at the beginning of the cells cultivation that indicates good bioactivity properties.

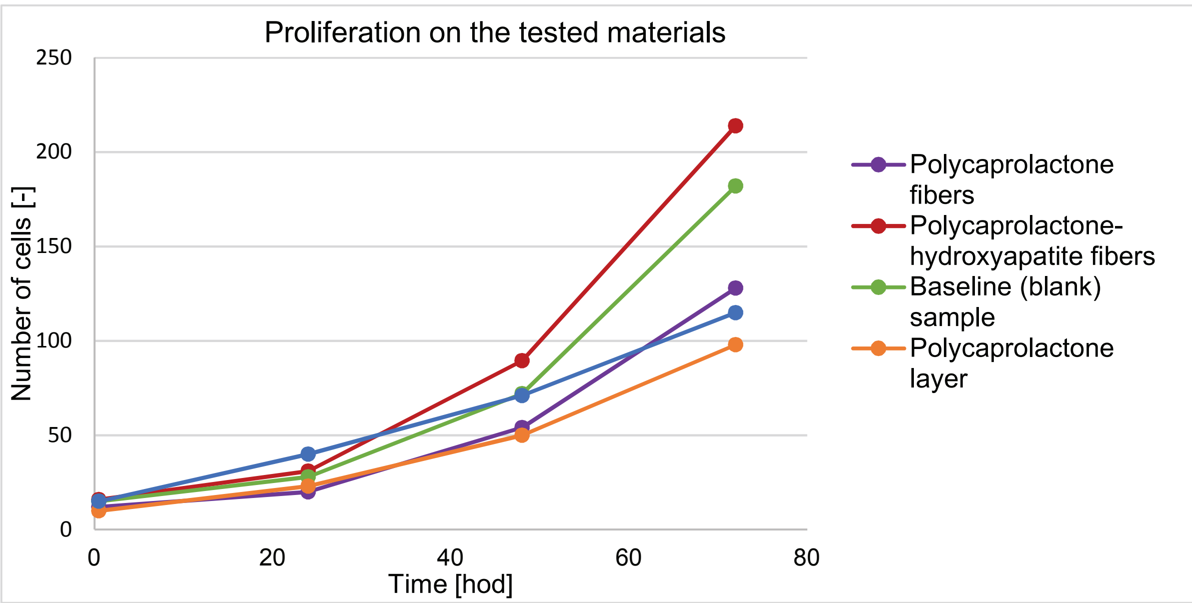

Figure 3 Proliferation of the cells on the tested materials

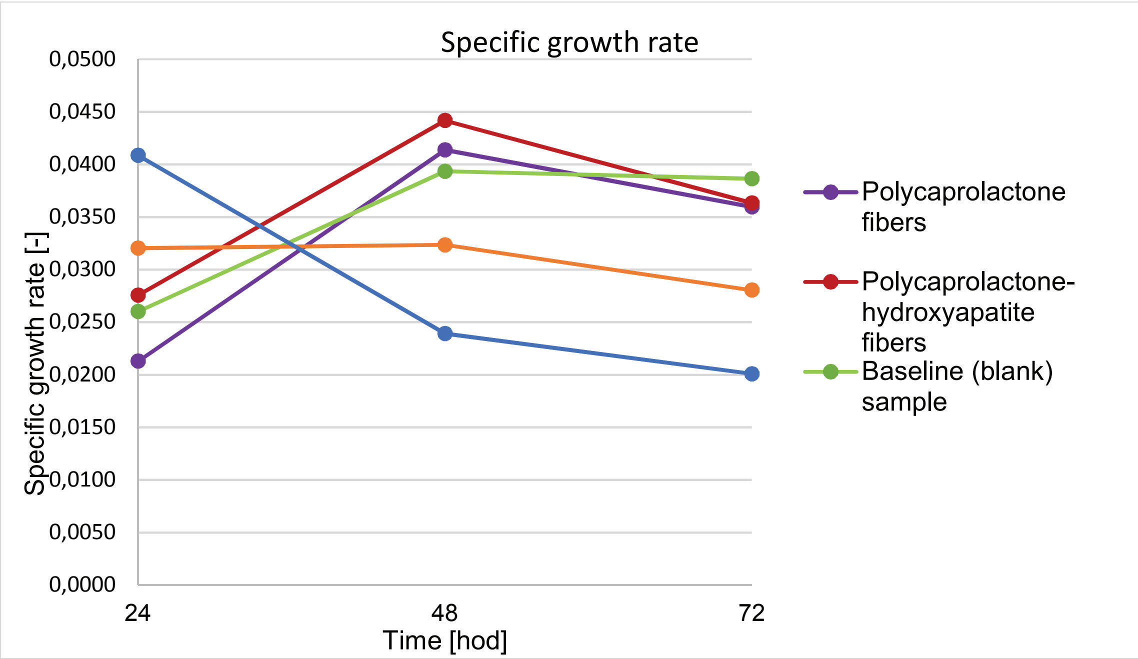

Figure 4 Specific growth rate of the cells on the tested materials

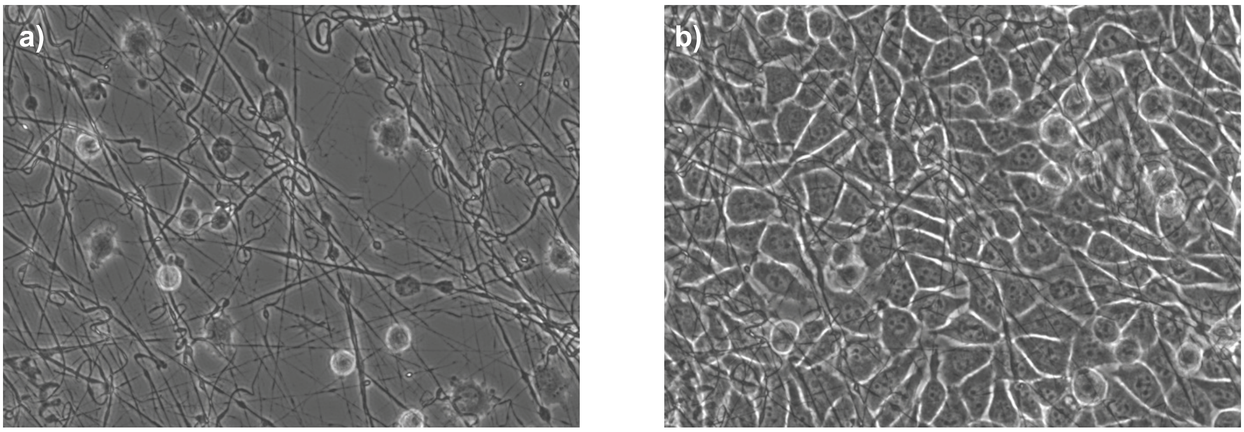

Figure 5 Polycaprolactone-hydroxyapatite fibrous structure with cell culture a) at the beginning of the test, b) after 3 days of testing

4. CONCLUSION

The experiments proved positive effect of the fibrous structure and ceramic phase in the composite on activity of the cells. While ceramic particles enhance proliferation of the cell culture, the polymer compound provides mechanical properties important for manipulation with the material which is beneficial compared to usual ceramic scaffolds. The future research will concern to better surface characteristics (e.g. wettability) of the composite fibers to reach even faster proliferation of the cells.

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