STUDY OF PREPARATION AND STRUCTURE OF POLYCAPROLACTONE AND BIOCOMPOSITE NANOFIBERS FROM HYDROXYAPATITE/POLYCAPROLACTONE

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Abstract

The preparation (by centrifugal jet spinning method) and structure of polycaprolactone (PCL) nanofibers and composite nanofibers hydroxyapatite (HAp)/PCL were studied. First, solutions of PCL in chloroform with concentration 5 to 9 wt.% were prepared, then dispersions HAp in PCL solutions with concentration from 0.1 to 0.5 wt.% of HAp in 9 wt.% were prepared. HAp was synthesized by sol-gel the method at room temperature and pressure and pH 12. The nanofibers were spun at the rate from 6 000 to 11 000 rpm. Two sizes of collector were used. The morphology and thickness of the nanofibers in dependence on the properties of solutions or dispersions and conditions of spinning were studied. Finally, bioactivity of nanofibers using simulated body fluid (SBF) solution was also tested. The results could be used for preparation of scaffolds in bone tissue engineering.

Keywords:

Nanofibers, centrifugal jet spinning, polycaprolactone, hydroxyapatite

1. INTRODUCTION

It is important to understand the biomechanical and biological properties of bone for choosing of new materials and methods for bone tissue scaffold preparation. Bone is complex, highly organized and specialized connective tissue. It is physically hard, rigid and strong. Bone microscopically contains relatively few cells with abundant intercellular matrix in the form of 30 wt.% collagenous fibers (mainly Type I collagen) and 70 wt.% inorganic compounds (mainly hydroxyapatite). Bone is hierarchically organized form macro- to nano- scale, where the basic plate-like building blocks of hydroxyapatite nanocrystals are incorporated into collagen nanofibers. The nanometer size of the inorganic component in natural bone is considered to be important for the mechanical properties of the bone [1,2,3]. In the past few decades, nanofibers were prepared mainly by method of electrospinning. Although electrospinning is easy and laboratory effective method, there are several disadvantages like request of high voltage, conductive solutions and long time of spinning. The new methods of nanofibers preparation - centrifugal jet spinning practically do not have these disadvantages. This method use centrifugal force instead of electrostatic force as in the electrospinning [4]. The process of centrifugal jet spinning consisting of a perforated reservoir rotating at high speeds along its axis of symmetry, which propels a liquid, polymeric jet out of the reservoir orifice that stretches, dries, and eventually solidifies to form nanoscale fibers Fig. 1. Key parameters for controlling of nanofibers geometry are I. reservoir centrifugal speed, II. distance of reservoir spinning jet and collector, III. diameter of spinning jet, IV. viscosity of solution, V. room temperature and humidity and VI. evaporation rate of solvent. By this method, we can produce nano scale fibers at high quality [5]. For integration of scaffold in the body is needed to formation of bonds between them. Those bonds are provided by the formation of bonelike hydroxyapatite on the scaffold surface in the body. If is the hydroxyapatite form on the surface, we can call this scaffold bioactive. Bioactivity can be laboratory predict by testing in SBF solution with ion concentrations nearly equal to human blood plasma [6].

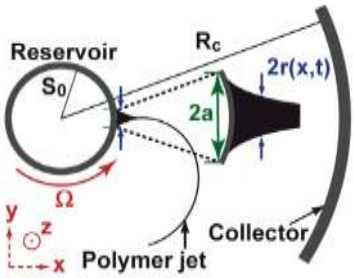

Fig. 1 Top view diagram of a fiber projecting from the reservoir, towards the collector [5]

2. EXPERIMENTAL

2.1 Hydroxyapatite Synthesis

Calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$) from Penta (AR), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) from Lachema (AR) and ammonia wt.25% (NH_3) from LachNer (AR) were used as starting precursors. The schematic presentation of the procedure is given in Fig. 2. First, 0.3 M $Ca(NO_3)_2.4H_2O$ was prepared in deionized water. Ammonia was added and the solution was stirred untill constant pH = 12 was obtained. 0.3 M solution of $(NH_4)_2HPO_4$ in deionized water was prepared. The pH was again adjusted by ammonia to 12. This solution was added dropwise into the first solution, to achieve a Ca/P atomic ratio of 1.67. The solution was kept at constant pH = 12 and it was rigorously stirred for 1 h after mixing the two components and was allowed to age for 24 hr at room temperature. The formed HAp gel was separed by centrifugation and used for preparation of HAp paste in chloroform. Phase composition of the HAp powder was analysed using the X-ray diffraction (XRD) with CoKα. Scanning electron microscope MIRA 3 from TESCAN was used to observe the morphology and particle size. Specific surface area was measured using Chembet – 3000, Quantochrome Instruments.

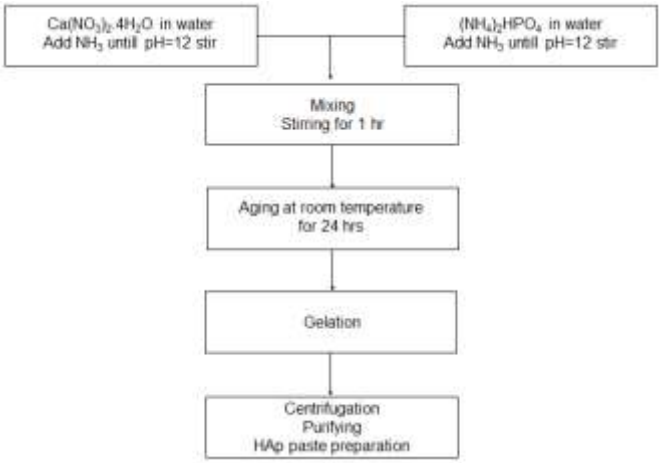

Fig. 2 Flow chart of the hydroxyapatite preparation by the sol–gel route

2.2 Preparation of PCL nanofibers and composite HAp/PCL nanofibers

PCL polymer solution and HAp/PCL dispersion were used for centrifugal spinning. Chemicals were obtained: PCL (M_n 80 000) from Sigma Aldrich, Czech Republic and chloroform from Lach-Ner s.r.o. (AR). Solutions concentration were 5, 6, 7, 8, 9 wt.% PCL in $CHCl_3$. Concentrations of HAp in dispersions were 0.1, 0.2, 0.3, 0.4, 0.5 wt.% in 9 wt.% PCL solution . All of the samples were carefully injected into the spinneret cylindrical reservoir top hole. Centrifugal spinning was carried from 6 000 to 11 000 rpm. The formed fibers were collected on a round collector. Two sizes of collector were used, with diameter 37.5 cm

and 54 cm. The morphology and thickness was observed using scanning electron microscope VEGA TS 5136 XM, Tescan.

3. RESULTS AND DISCUSION

X-ray analysis of the synthesized hydroxyapatite confirmed the presence of the only phase – hydroxyapatite Fig. 3. Crystallite size – 20 to 30 nanometers, was determined using SEM images at Fig. 3. The specific surface area of the nanoparticles determined by Multi light BET analysis was 65.4 m^2/g, which corresponds to the average particle size of 29 nm, assuming spherical particle shape. Therefore the prepared particles were both, in phase and in size, suitable for preparation of composite nanofibers.

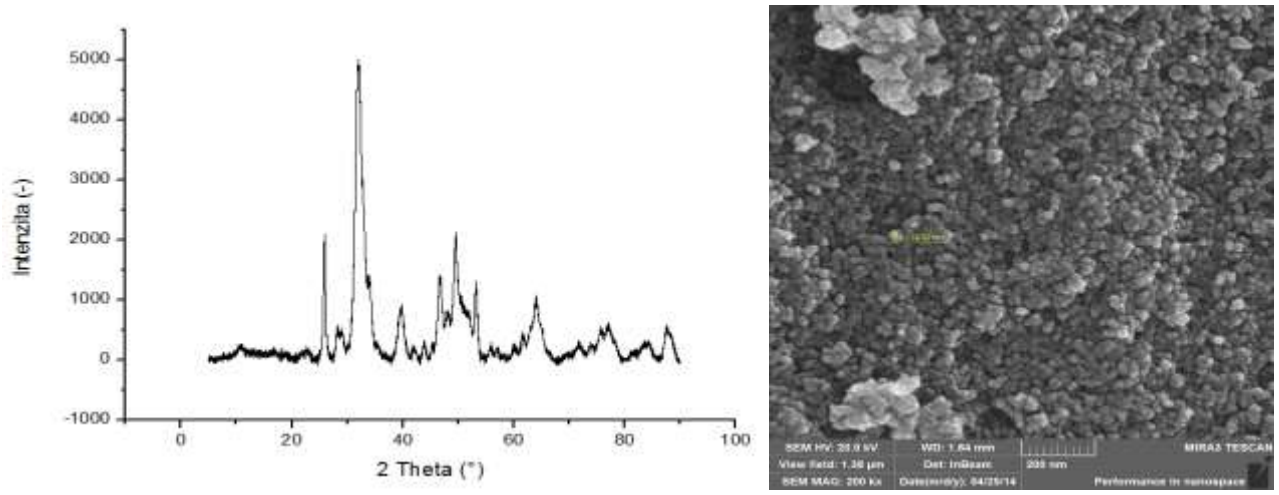

Fig. 3 XRD patterns of HAp powders, SEM image of HAp powders

The morphology and the thickness of nanofibers in dependence on the spinning speed and the size of collector were studied. The solutions with lower viscosity were spun at rather low speed, the higher viscosity solutions were well spinnable even at higher speeds. The same results were found for 54 cm diameter collector on which the solutions with higher viscosity were rather workable. Polycaprolactone nanofibers have a thickness ranging from 100 nm to 9 μm depending on the concentration of solutions. Similar results have been found by [7], who have dealt with centrifugal spinning of 16% solution of polycaprolactone in dichloromethane with nanofibers thickness 220 nm. Although all solutions formed fibers well, the best fibers were formed from 7 wt.% solution. Solutions with lower concentration formed fibers with beads and defects Fig. 4, on the other hand, solutions with higher concentrations formed fibers with the large thickness Fig. 5. The best nanofibers with diameter (540 ± 185) nm were spun from 7 wt.% solution by speed 8 000 rpm on a 37.5 cm collector, Fig. 6.

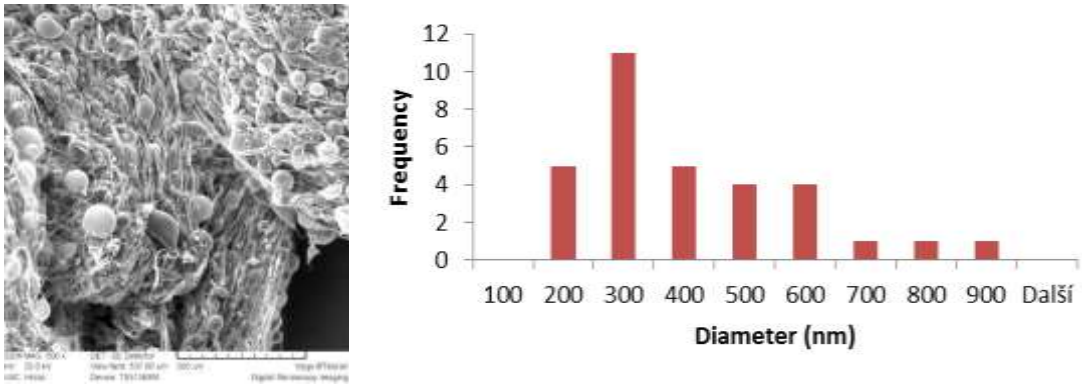

Fig. 4 The morphology and thickness of the nanofiber sample prepared from 5 wt.% PCL spun at a speed of 6000 rpm, collector 37.5 cm

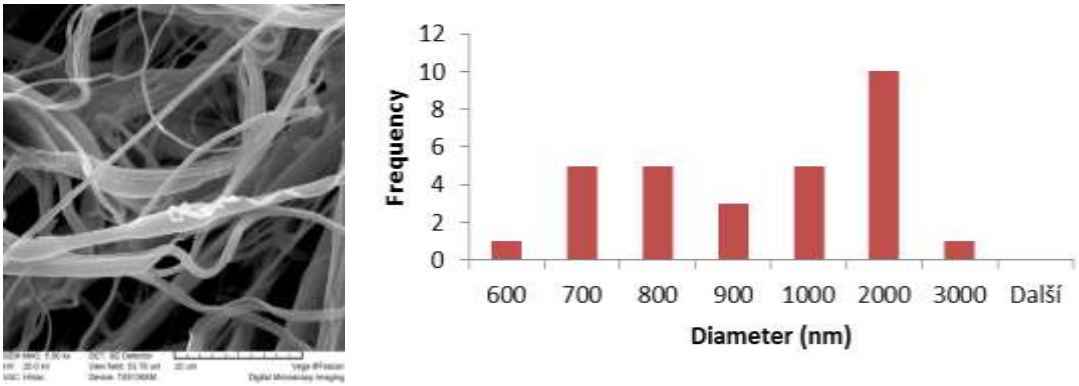

Fig. 5 *The morphology and thickness of the nanofiber sample prepared from 9 wt.% PCL spun at a speed of 9 000 rpm, collector 37.5 cm*

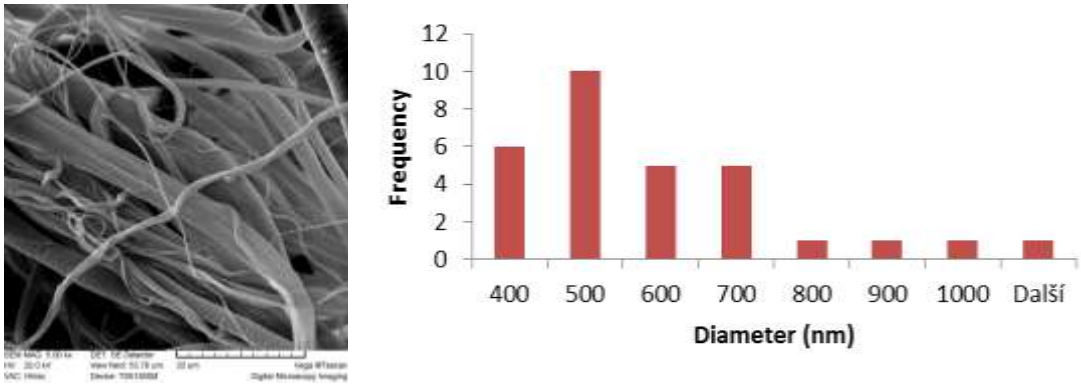

Fig. 6 *The Morphology and thickness of the nanofiber sample prepared from 7 wt.% PCL spun at a speed of 8 000 rpm, collector 37.5 cm*

The fibers spun from 0.5 wt.% dispersion of HAp in PCL solution contained largest amount of inorganic phase. Large agglomerates surrounding the fibers are seen on the Fig. 7 a. The TEM image showed the presence of even small particles in the order of tens of nanometers Fig. 7 b. Similar structure of β-calcium phosphate/polycaprolactone composite nanofibers was presented in [8].

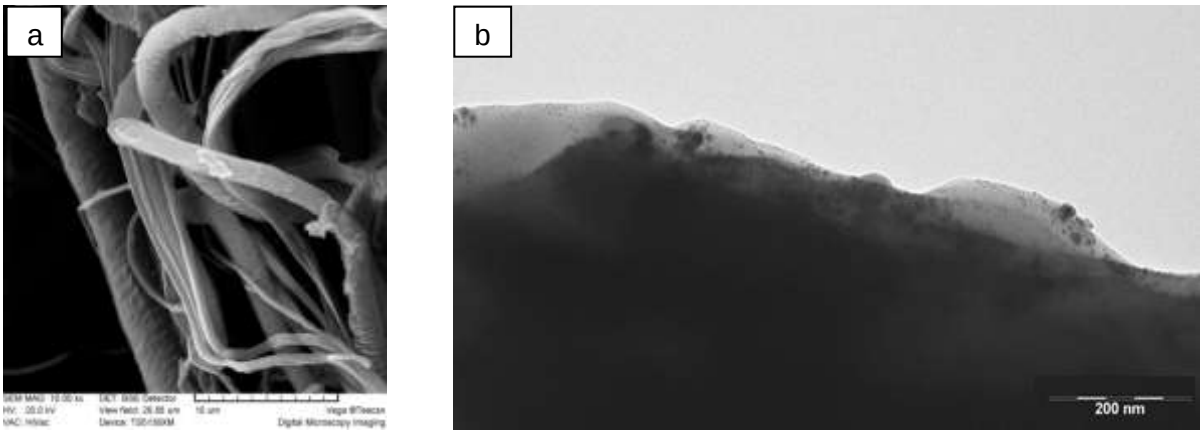

Fig. 7 a) SEM BSE image of PCL/0.5 wt.% HAp fibers b) TEM image of PCL/0.5 wt.% HAp fibers

The best bioactive interaction in SBF solution showed nanofibers prepared from solution of 8 wt.% PCL, 8 000 rpm, collector 37.5 cm, Fig. 8. The surface of these nanofibers was densely covered by HAp crystals after seven days of interaction. The composite nanofibers also contain HAp crystals but it was not possible to say with confidence, if the present HAp crystals was original or formed by SBF solution.

Fig. 8 SEM BSE image 8 wt.% fibers with HAp phases

CONCLUSION

The biopolymer nanofibers of polycaprolactone and inorganic composite nanofibers with hydroxyapatite were prepared by the centrifugal jet spinning method. The best results were achieved by spinning of 7 wt.% PCL solution at speed 8 000 rpm and collector 37.5 cm. The nanofibers diameter was (540 ± 185) nm. The hydroxyapatite/polycaprolactone composite nanofibers with good morphology, structure and distribution of hydroxyapatite were also prepared. The prepared nanofibers were bioactive after 1 week of testing in SBF solution.

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