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Electrospun poly-L-lactic acid/nanohydroxyapatite nanofibrous composite as a potential bone tissue replacement material

Introduction

Hydroxyapatite (HA) is one of the most widely used bone substitute material among currently investigated biomaterials [Valei-Regi and González-Calbet, 2003]. Chemical properties of HA resemble chemical properties of mineral part of human bone [Drouet et al., 2009]. In spite of its advantageous physical and chemical properties, HA cannot be used in load bearing applications [Bose and Saha, 2003], which limits area of uses for this biomaterial.

For load bearing application polymers, e.g. in form of nanofibers were proposed [Jang and Castano, 2009]. Mechanical properties of polymeric nanofibrous material and its biocompatibility and, in some cases biodegradability, make it highly preferable material for bone substitution applications. However, the chemical composition of most of the commonly used polymers does not promote bone mineralization. To overcome this obstacle, the fibrous nanocomposite structure of polymeric/nanohydroxyapatite materials was proposed [James et al., 2011]. Various polymers with nanohydroxyapatite particles (nanoHAP) were tested in order to produce suitable bone substitute material, and examples include gelatin, collagen, chitosan, polycaprolactone, poly-L-lactic acid, siloxane [Jang and Castano, 2009].

In present study, the nanoparticles of hydroxyapatite (nanoHAP) were synthesized by wet chemical precipitation technique in presence of lecithin as a dispersing agent. Therefore, produced nanoHAP was used to prepare nanofibrous composite of poly-L-lactic acid/hydroxyapatite material by electrospinning. The morphological properties, like average particle size of nanoHAP, structure of nanoparticles, nanofibers average diameter, structure of nanofibrous materials were investigated. Additionally, the chemical composition of all materials was investigated.

Experiments

Polymer solution

Poly-L-lactic acid (PLLA, *Biomer L9000*, Mw > 200 kDa, *Polysciences Inc.*, USA) was dissolved in mixture of dichloromethane: N,N-dimethylformamide (9:1 v/v) (*Carlo Erba*, Spain). Concentrations of PLLA in the solutions are listed in the Tab. 1.

Tab. 1. Nanofibers preparation solutions and average size of fibers

Solution	PLLA concentration [%w/w]	nanoHAP concentration [%w/w]	Solution volume [ml]	Fiber average size ± SD [nm]
PLLA	8	-	20	534±12
PLLA/nanoHAP	4	4	20	202±6

Nanohydroxyapatite dispersion

Nanohydroxyapatite (nanoHAP) was prepared by wet chemical precipitation of nanocrystals. The preparation process is shown in details in flowchart in Fig. 1. Solutions of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $(\text{NH}_4)_2\text{HPO}_4$ (*Chempur*, Poland) and lecithin (*Serva*, Germany) were prepared in deionized water. Values of pH were adjusted by addition of ammonia water (*Chempur*, Poland) and measured by *SevenMulti* (*Mettler Toledo*, Switzerland). Hexane (*Chempur*, Poland), used for purification purpose, was used as shipped. Yellowish color of the resulting powder is caused by traces of lecithin present in the final product.

Resulting nanoHAP particles were suspended in mixture of dichloromethane: N,N-dimethylformamide (9:1 v/v) in concentration listed in Tab. 1. The dispersion was achieved by applying ultrasounds (*Ultrasonic*

Processor UP100H, *Hielsher Ultrasonics*, Germany) for 10 minutes before mixing with polymer solution.

Electrospinning of nanofibers

Polymer solution of PLLA was used to prepare PLLA nanofibers. Mixture (1:1 v/v) of polymer solutions and nanoHAP solutions was used to prepare nanofibrous composite materials. Concentration of each components of solution is listed in Tab. 1.

In order to prepare PLLA nanofibers and composite PLLA/nanoHAP nanofibers electrospinning technique was applied. The electrospinning setup was described in details elsewhere [Wojasiński and Ciach, 2014]. Briefly, the setup consists a syringe pump to supply polymer solution, with feeding rate 2 ml/h, to a nozzle – positively charged electrode of the setup. Between the nozzle and a grounded collecting plate, a high voltage electrostatic field of 17 kV was applied. Polymer solution extruded through the needle was stretched in the high electrostatic field to the form of nanofibers. Nanofibers were collected on the collecting plate.

Nanofibers were produced in room temperature with relative humidity (RH) in range from 40 to 52%.

Characterization of nanoHAP

NanoHAP nanoparticles size were analyzed using *NanoSight* system (*NanoSight Limited*, UK) with NTA 2.3 software. The measurement by *NanoSight* is based on nanoparticle *Brownian* movement tracking analysis in the suspension using light scattering effect. The analysis was performed 3 times after 10 minutes of particles suspending in the solvents with ultrasounds.

Fourier transform infrared spectroscopy (FTIR, *NicoletTM 6700* spectroscope, *Thermo Scientific*, USA) was applied for analysis of chemical composition of nanoHAP particles and identification of traces of lecithin in the particles.

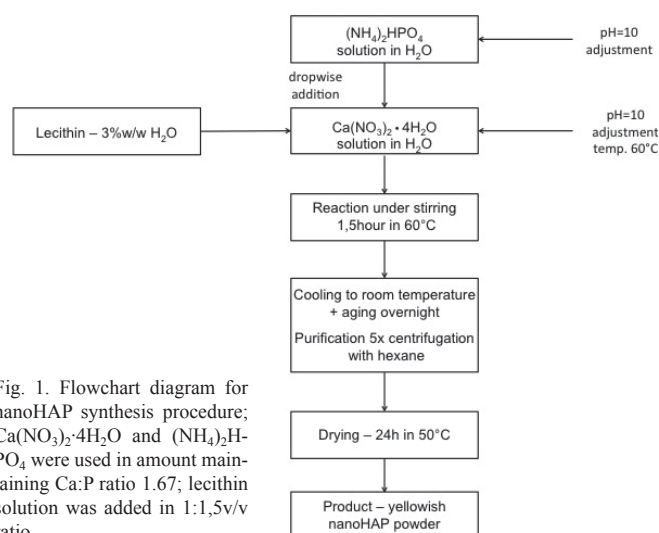


Fig. 1. Flowchart diagram for nanoHAP synthesis procedure; $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $(\text{NH}_4)_2\text{HPO}_4$ were used in amount maintaining Ca:P ratio 1.67; lecithin solution was added in 1:1,5v/v ratio

Characterization of polymer and composite nanofibers

Morphological properties of resulting PLLA nanofibers and PLLA/nanoHAP composite nanofibers were analyzed based on scanning electron microscope (SEM) images (*Phenom*, *FEI*, USA). Prior SEM investigations, samples were covered with 15 nm layer of gold in gold sputter (*550K Emitech*, USA) for improvement of conducting properties of samples. SEM images were analyzed using *Fiji* software (*ImageJ 1.49a*, *NIH*, USA).

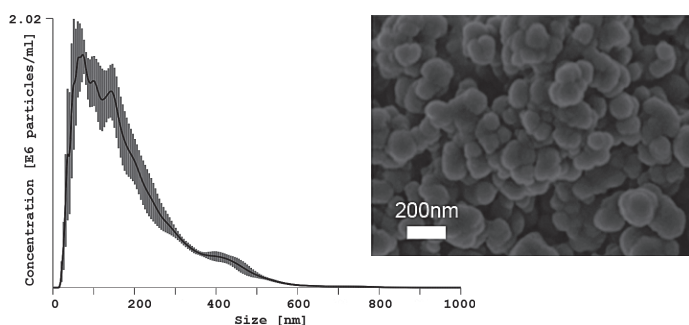


Fig. 2. Size distribution of nanoHAP particles in dispersion solution; embedded: SEM image of nanoparticles

Chemical composition of nanofibrous materials: PLLA and PLLA/nanoHAP nanocomposite, was investigated by FTIR in order to confirm presence of characteristic groups of hydroxyapatite in the structure of PLLA/nanoHAP nanofibrous composite.

Statistical analysis of all the results was performed in *Statistica 10* software (*Statsoft Inc.*, USA). Results are shown as an average fiber diameter (\bar{AV}) \pm sample standard deviation (SD).

Results and discussion

In the present work, the application of nanohydroxyapatite particles (nanoHAP) in nanofibrous polymer/ceramic composite material fabrication by electrospinning was performed. The nanoHAP produced by wet chemical synthesis process, in presence of lecithin, poses good dispersion properties in solvents used for nanofibrous composite preparation by electrospinning. The narrow size distribution of the nanoparticles (Fig. 2) in the dispersion, prior the electrospinning process, allows proper distribution of the nanoHAP in nanofibrous composite. Nanoparticles have a spherical shape and average diameter of the particle 77 ± 13 nm. The size and good suspendability of nanoHAP produced by wet chemical synthesis in presence of lecithin makes them preferable for nanocomposite applications.

Differences in morphology of electrospun PLLA nanofibers and PLLA/nanoHAP nanofibrous composite were investigated based on SEM images in Fig. 3. Presence of nanoHAP is clearly visible as beads in the structure of nanofibers. Also, nanoHAP is embedded into the nanofiber structure, which might be preferred in terms of mechanical properties and chemical availability of hydroxyapatite particles for bone cells attachment. The fibers average diameter (Tab. 1) for PLLA nanofibers is over two times higher than PLLA/nanoHAP nanofibers. The difference between both materials in average size of the fibers is caused by two times lower amount of polymer in nanofibrous composite material. Amounts of dissolved and dispersed materials in solutions for preparation of PLLA nanofibers and PLLA/nanoHAP nanofibers were maintained equal (8%w/w), however, the absolute amount of polymer for both materials was different, causing visible difference in nanofi-

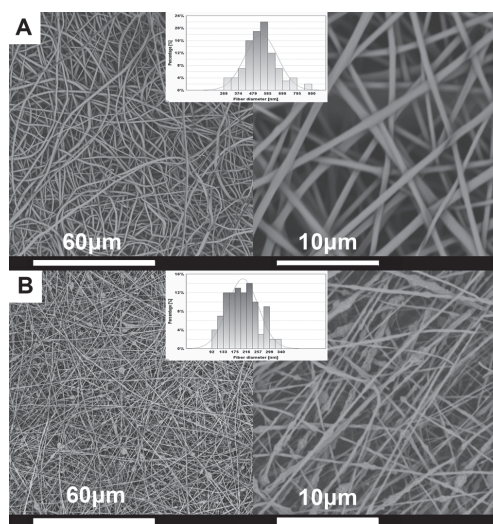


Fig. 3. SEM images of nanofibers: row A – PLLA nanofibers; row B – PLLA/nanoHAP nanofibers; embedded – fiber diameter distribution plots

bers diameter in both investigated materials. The effect of addition of nanoHAP to the electrospinning solution will be investigated in further research. The most important morphological difference between PLLA nanofibers and PLLA/nanoHAP nanofibers is more narrow single fiber distribution for PLLA nanofibers than for PLLA/nanoHAP nanofibers. The addition of nanoHAP results with flattening fiber diameter distribution (Fig. 3).

Chemical composition of nanoHAP was investigated by FTIR spectroscopy and results are shown in Fig. 4. The characteristic peak derived from PO_4^{3-} appears at position of 1030 cm^{-1} and from P-O stretching bonds at 560 cm^{-1} . For PLLA nanofibers, characteristic peaks for ester groups, in transmission spectra, appear at 1755 cm^{-1} for stretching C=O bond, 1182 cm^{-1} and 1086 cm^{-1} both for stretching C-O bond (Fig. 4). Presence of chemical composition of nanoHAP in the PLLA/nanoHAP nanofibers was confirmed by FTIR analysis of the nanofibrous composite sample. The characteristic peaks for nanoHAP and PLLA are present in the spectra of nanofibrous PLLA/nanoHAP material, and the intensity of the nanoHAP peaks is relatively high. Intensity of nanoHAP related peaks suggest chemical presence of ceramic material close to the surface of nanofibrous scaffold. This property may lead to creation of adhesion sites for cells in tissue engineering and bone filling applications. However, the availability of ceramic material as a cell signaling site needs to be further investigated.

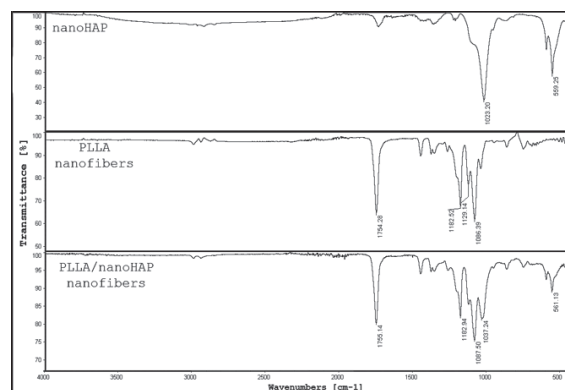


Fig. 4. Transmittance spectra of nanoHAP, PLLA nanofibers, PLLA/nanoHAP nanofibers

Conclusion

In this paper, the method for synthesis of nanohydroxyapatite particles in presence of lecithin and their application to nanofibrous composite material production by electrospinning were reported.

The resulting poly-L-lactic acid/nanohydroxyapatite nanofibrous composite material poses good chemical availability of nanoHAP embedded into PLLA nanofibrous structure and high porosity, properties preferred for bone substitute applications.

Thus, use of the nanofibrous composite material should be considered for further test as a material for the applications like dental filling, bone replacement material, bone tissue engineering, etc.

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