INVESTIGATION OF THE 3-D POLYMER SCAFFOLDS WITH MINERALIZED SURFACE FOR TISSUE ENGINEERING VIA ELECTROSPINNING TECHNOLOGY

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ИССЛЕДОВАНИЕ ВОЛОКНИСТЫХ 3-Д ПОЛИМЕРНЫХ СКЭФФОЛДОВ ПОЛУЧЕННЫХ МЕТОДОМ ЭЛЕКТРОФОРМИРОВАНИЯ С МИНЕРАЛИЗОВАНОЙ ПОВЕРХНОСТЬЮ ВОЛОКОН ДЛЯ КОСТНОЙ ИНЖЕНЕРИИ

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Аннотация. *В настоящей работе приведены результаты получения нетканых волокнистых трехмерных скэффолдов на основе полигидроксибутирата или полигидроксибутират-гидроксивалерата с минерализованной поверхностью волокон по всему объему скэффолда, и исследования их морфологии, химического состава и структуры. Скэффолды были получены методом электроформирования при скорости вращения коллектора 1200 или 600 об/мин. Для получения полностью покрытых волокон по всему объему скэффолда частицами карбоната кальция использовалась ультразвуковая обработка в растворах хлористого кальция и карбоната натрия. Осуществлялся контроль массы образцов до и после каждой обработки. Для исследования морфологии скэффолдов использовалась сканирующая электронная микроскопия. Для исследования структуры и химического состава образцов использовались рентгеновский фазовый анализ и энергодисперсионная рентгеновская спектроскопия, соответственно.*

Introduction. Manufacturing of implants with predetermined architectonics and individually optimized functionality is one of the most important challenges today. 3D scaffolds of polymers can be used for bone tissue engineering. Electrospinning is the most broadly used technique and allows obtaining the most promising results for different tissue engineering applications [1]. Polyhydroxyalkanoates (PHAs) are the most prospective class of materials that possess good biocompatibility as well as physical and mechanical properties that are similar to that of the hard tissue in the human body. Compared with other PHAs, poly(hydroxybutyrate) (PHB) and poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) of a natural origin are simultaneously degradable and piezoelectric, which might promote bone growth *in vivo* [2]. Normally, cell affinity toward polymers is poor as a result of their low hydrophilicity and lack of surface cell recognition sites [3]. A tendency in technological development of implantable materials is to functionalize of bio-constructions. Fibers with $CaCO_3$ can be successfully used in bone tissue repair and substitution [4]. To our knowledge, a study of the 3-D scaffolds from PHB and PHBV with different structure and with $CaCO_3$ coatings has not been reported yet. The objective of

this project is to fabricate and investigate the morphology, chemical composition and structure of electrospun 3-D fiber PHBV and PHB scaffolds with mineralized surface via biomimetic coating for biomedical application.

Materials and methods. The fabrication of scaffolds involved the dissolution of poly[(R)3-hydroxybutyrate] and poly[3-hydroxybutyrate-co-3-hydroxyvalerate] powders in chloroform ($CHCl_3$). A collector was rotated at 600 and 1200 rotation per minute (rpm). The mineralization of PHB and PHBV scaffolds was carried out using a typical process of $CaCO_3$ particles crystallization from a mixture of Na_2CO_3 and $CaCl_2$ solutions (1 M). PBH and PHBV scaffolds were soaked in 1 ml of $CaCl_2$ and 1 ml of Na_2CO_3 solutions. This system was subjected to ultrasound for 30 sec. After treatment the treated scaffolds were washed and dried at 55 °C for 60 min. The SEM investigations were carried out with an ESEM Quanta 400 FEG instrument equipped with an energy-dispersive X-ray analysis (EDX; EDS analysis system Genesis 4000, SUTW-Si(Li) detector) operated in a high vacuum. X-ray diffraction analysis (XRD) of prepared samples was performed using a D8 Advance Bruker equipped with a Cu anode.

Results and discussion. Fig. 1 shows the SEM images of PHBV and PHB scaffolds prepared at different rotation speed of the collector. Successful fabrication of electrospun scaffolds without any beads was observed. The average diameter of PHBV and PHB scaffolds fibrous prepared at the 1200 rpm was 6.1±1.9 μm and 4.3±1.0 μm, respectively, at the 600 rpm was 7.6±2.7 μm and 4.6±1.2 μm, respectively. From the Fig.1, the morphology of the scaffolds has become more oriented along the longitudinal axis, which resulted in a more aligned structure of the scaffolds, which was in accordance with the results reported elsewhere [5].

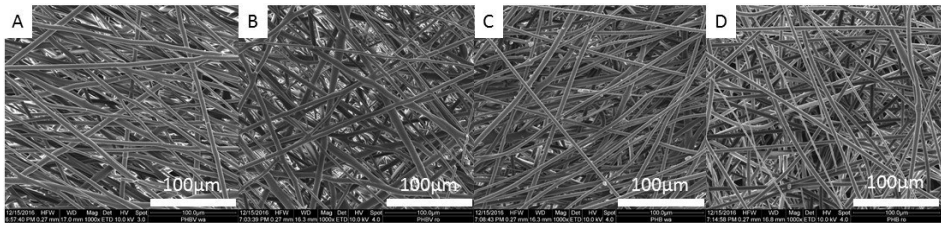

Fig. 1. SEM images of 3-D PHBV (a,c) and PHB (b,d) scaffolds prepared at the rotation speed of the collector of 1200 (a,c) and 600 rpm (b,d)

Fig. 2 represents SEM images of the scaffolds treated 3 times to attain uniform coatings of calcium carbonate throughout the scaffold. The ultrasonic treatment provides the penetration of solutions into pores and interior of the fibrous material, which facilitates the homogenous mineralization of the scaffold [4]. The EDX analysis resulted in the presence of Ca, C, O, Na and Cl.

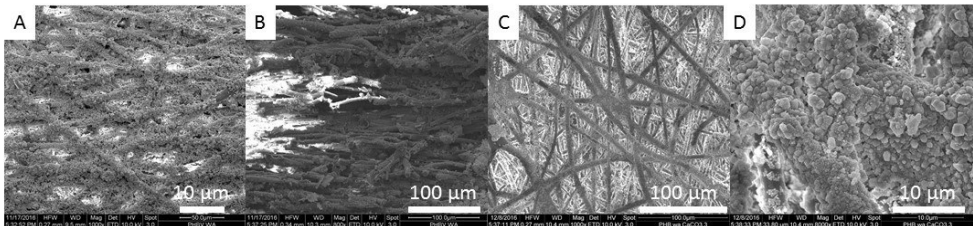

Fig. 2. SEM images of 3-D PHBV (a,b) and PHB (c,d) scaffolds prepared at the rotation speed of the collector of 1200 rpm: A,C – top views, B,D – side views

The increase of the mass after each treatment, independence of the polymer and morphology, were observed, as shown in the Fig.3a. Fig. 3b represents XRD patterns of the treated and untreated scaffolds. The peaks

correspond to calcite rhombohedra (№ 01-072-1652 PDF 4+ ICDD) and NaCl (№ 00-05-0628 PDF 4+ ICDD) were observed after three treatment procedures of the scaffolds.

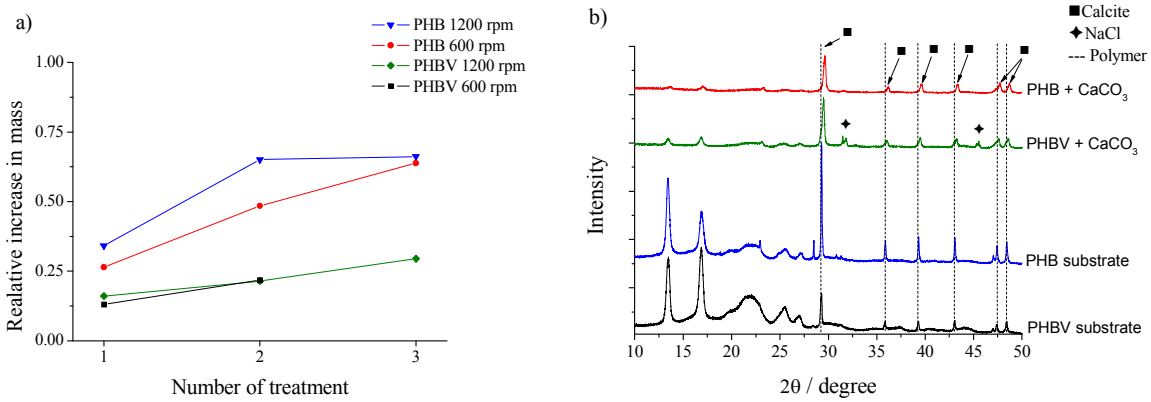

Fig. 3. Dependence of the relative increase in mass from the number of treatments (a), and XRD patterns (b) of scaffolds before and after three treatment procedures.

Conclusion. The PHBV and PHB scaffolds were successfully prepared via electrospinning with two different rotation speeds of the collector, which allows changing fiber size distribution and scaffolds structure. The ultrasound treatment in Na_2CO_3 and $CaCl_2$ solutions allows preparing uniform coated scaffolds with calcium carbonate via 3 times repetition of the treatment procedure, independently of the used polymer. However, at the same treatment conditions, the relative increase in mass for PHB scaffolds after each treatment procedure was significantly larger than that of PHBV scaffolds. The mechanical behavior, wettability, piezoelectric activity and biological response of scaffolds with mineralized fibers will be investigated in the future.

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