Effect of annealing on mechanical and morphological properties of Poly(L-lactic acid)/Hydroxyapatite composite as material for 3D printing of bone tissue growth stimulating implants

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Abstract. In this work, effect of additional annealing on mechanical and morphological properties of 3D-printed PLLA/HAp composite scaffolds of three compositions (12.5, 25, and 50 wt.% of HAp) was investigated. Morphology and Young's modulus of 3D-printed scaffolds were investigated by scanning electron microscopy and nanoindentation. It has been shown that additional annealing does not have an effect on the homogeneous distribution of HAp powder in the PLLA-matrix. Results of nanoindentation showed growth of Young's modulus after annealing. The maximum value of 9393 ± 709 MPa Young's modulus was reached for the annealed composite with 50 wt.% of HAp.

1. Introduction

Reducing polytrauma and congenital diseases treatment duration is one of the most difficult and important objectives of orthopedics and traumatology. Unstable osteosynthesis and such related complications as delayed consolidation and nonunion of bone fragments often lead to a significant increase in the duration of treatment. One of the most effective approaches in the treatment of congenital and acquired diseases of the musculoskeletal system is the osteosynthesis with the use of metal implants. However, despite the effectiveness, metal implants have a number of drawbacks such as negative impact on the functioning of the musculoskeletal system and high probability of permanent implants rejection due to metallosis. Moreover, removing temporary metal implant from the body requires an additional surgical intervention.

One of the most promising alternative approaches in the treatment of musculoskeletal system diseases is the use of biodegradable implants made of thermoplastic polymers and polymer composites [1]. Advantages of such materials are their complete enzymatic bioresorbability in body fluids and non-toxicity [2]. However, poor mechanical properties and uncontrolled degradation of the polymer matrix restrict the use of such materials in orthopedics [3]. Used in orthopedics in the form of pins and screws for fixing bone fragments [4], poly(L-lactic acid) (PLLA) has mechanical characteristics that are significantly inferior to those of natural bone. The low elastic modulus of this polymer does not ensure the preservation of the implant morphology, thereby limiting its use as a bulk osteoconductive implant. It was shown that filling of the polylactic acid with bioactive hydroxyapatite (HAp) up to 50% by weight increases the stiffness of composite scaffold by 2 times [5]. Moreover, maintaining the melt flow rate at the level acceptable for extrusion allows creating of PLLA/HAp composite scaffolds by Fused Deposit Modeling (FDM) [6,7]. 3D printing of polymer composites allows implementing the personalized approach in traumatology and orthopedics [8], which, presumably, will have a positive effect on the reduction of treatment time.

In this research, the effect of annealing on the morphology and mechanical properties of the PLLA/HAp composite material with a HAp filling up to 50% by weight is investigated. Additional annealing of 3D-printed scaffolds allows obtaining PLLA/HAp composite with enhanced Young's modulus.

2. Materials and methods

2.1. Materials
In this work, poly(L-lactic acid) (USA, melting point ~ 175...180°C) was used as the polymer matrix. Biological hydroxyapatite (HAp) (Russia, fraction size 12.56 μm ± 5.28) was used as a reinforcing bioactive filler. Purchased from Ecros-Analytics (Russia) chloroform was used to dissolve PLLA pellets. Dimethyl sulfoxide (DMSO) (Russia,) was used as non-ionic surfactant to prevent aggregating of HAp particles. Composites were prepared in the weight ratio at 87.5% PLLA/12.5% HAp; 75% PLLA/25% HAp and 50% PLLA/50% HAp.

2.2 Preparation of filament and 3D printing
PLLA/HAp composites were prepared according to the following method. HAp was preliminarily dried at 150°C and mixed with DMSO for 3 hours in a ball mill. HAp weight fractions were 14.3 g, 33.3 g and 100 g for PLLA12.5, PLLA25 and PLLA50, respectively. Then, 15% solution of PLLA in chloroform was added into the mixture of HAp with DMSO. The composition was mixed for 8 hours on ball mill with zirconia balls. After mixing composite was granulated and dried in a vacuum oven at 60°C and $5 \cdot 10^{-3}$ mbar. Dried pellets were extruded on the horizontal single-screw extruder Filabot EX2 (Filabot, USA) at 175°C. Pristine PLLA filament for printing of reference scaffolds was also extruded. The diameter of the extruded filament was 1.75 mm.

Obtained PLLA and composite filaments were used to print scaffolds on FDM 3D printer PrintBox3D One (RGT, Russia). Printing was performed by extrusion of PLLA and composite melts on glass substrate through 0.2 mm nozzle. Temperature of the nozzle was 220°C, thickness of layers – 50 μm, infill – 30%.

After printing five samples of each composition were annealed in the air sterilizer GP-40-SPU (Smolensk SKTB SPU, Russia) for 12 hours at 110°C.

All scaffolds were divided into two groups: group 1 – 3D-printed scaffolds, group 2 – annealed 3D-printed scaffolds (table 1).

2.3 Scaffolds morphology
Scaffolds surface morphology was investigated by scanning electron microscopy (SEM). Preliminarily, all scaffolds were sputter coated with gold for 2 minutes on JEOL Smart Coater (JEOL USA, USA) magnetron sputter coater. SEM at 22x magnification was performed on JEOL JCM-6000 (JEOL USA, USA), at 4000× magnification – on Quanta 200 3D (FEI Company, USA). Sizes of HAp particles and scaffolds pores were estimated using ImageJ software (National Institute of Mental Health, USA).

Scaffolds deformation after annealing was evaluated as deviation from plane-parallelism using calipers.

2.4 Mechanical tests
Young's modulus of obtained materials was investigated by the method of nanoindentation on the Nanoindenter G200 (Agilent's Electronic Measurement, USA) according to the ISO 14577. Indentation was performed with triangular Berkovich pyramid at 10 mN.

Table 1. Groups of materials and compositions.

Group	Composition	PLLA, wt.%	HAp, wt.%
1	PLA100	100	0
	PLA87.5	87.5	12.5
	PLA75	75	25
	PLA50	50	50
2	PLA100an	100	0
	PLA87.5an	87.5	12.5
	PLA75an	75	25
	PLA50an	50	50

3. Results and discussion

3.1. Scaffolds morphology

Porous disk-shaped scaffolds of 20 ± 0.15 mm diameter and 2 ± 0.08 mm thickness were successfully prepared from pristine PLLA and composite filaments using FDM 3D printing. The average size of pores in scaffolds is 1.096 ± 0.181 mm. No influence of HAp weight fraction or additional annealing on the pores size was observed.

After annealing, bending and deviation of scaffolds from the original were observed. Presumably, the deformation was caused by volumetric changes in the crystal structure of the semicrystalline polymer matrix. The scaffold surface was rapidly cooled by airflow in the process of FDM 3D printing, which led to decrease of the size and increase of the density of crystallites in the surface layers of the extruded polymer. In the bulk of the polymer, layer cooling occurred with a lower degree of undercooling due to additional heating from the substrate and previous layers. As a result of the undercooling gradient, the difference in the densities and sizes of crystallites on the surface and in the bulk of polymer appeared. Heating above the glass transition temperature during annealing initiated diffusion processes leading to the recrystallization and redistribution of crystallite densities in the surface layers. Volumetric changes in the crystalline structure initiated tensile structural stresses on the surface of scaffolds and their deformation.

The deformation of scaffolds was evaluated as the deviation from flatness using the formula 1:

$$\Delta X = \frac{X_1 - X}{X} \times 100\% \qquad (1)$$

where X – scaffold thickness after 3D printing, X_1 – the distance between most distant point in the cross section of a scaffold after annealing (figure 1).

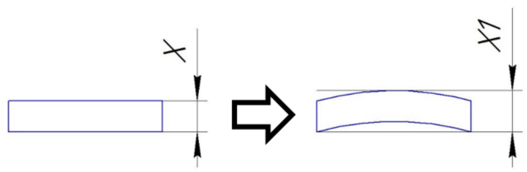

Figure 1. Method for determining deformation of scaffolds.

Deformation of reference scaffolds made of PLLA is 6.41 ± 0.11% (figure 2). Deformation increased up to 8.33 ± 0.08% for annealed composite filled with 12.5% by weight of HAp, but with further increase in the weight fraction of HAp, deformation decreased to 4.19 ± 0.06% and 1.86 ± 0.06% for 25 and 50 wt.% of HAp, respectively.

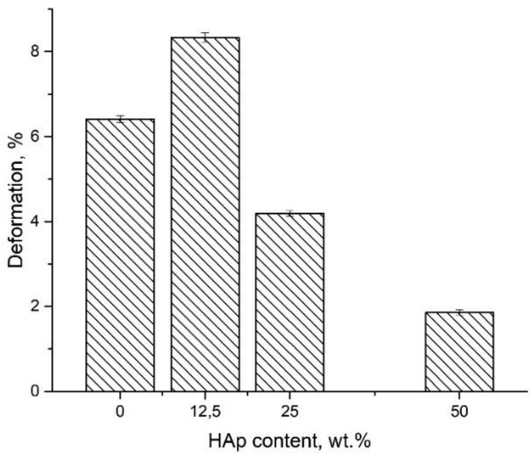

Figure 2. Scaffolds deformation after annealing.

SEM images (figure 3) demonstrated the presence of a large amount of homogeneously distributed hydrophilic HAp particles in the surface layer of scaffolds. The average particle size was 309 ± 71 nm, which indicates the successful grinding of HAp in the process of composites mixing.

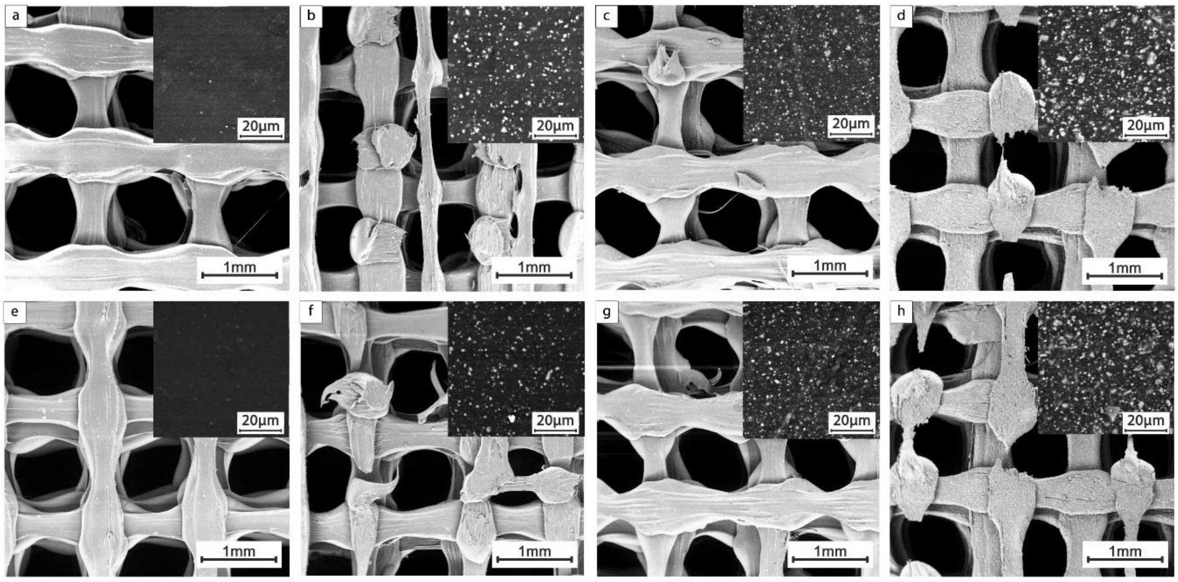

Figure 3. SEM images of 3D-printed scaffolds at 22× and 4000× magnification: (a) PLA100, (b) PLA87.5, (c) PLA75, (d) PLA50, (e) PLA100an, (f) PLA87.5an, (g) PLA75an, (h) PLA50an.

3.2. Mechanical tests

Young's modulus variation before and after annealing of obtained materials is shown in figure 4. As can be seen, Young's modulus of all compositions increases with increasing of HAp weight fraction. It is also seen that the module of all compositions, including reference pure PLLA, increased after annealing. The value of Young's modulus for pristine PLLA is 2468 ± 237 MPa. After annealing, the module of pure PLLA increased to 3725 ± 287 MPa. The addition of 12.5 and 25 wt.% of HAp into the polymer matrix led to a further increase in the modulus to 4774 ± 848 and 6138 ± 309 MPa, respectively. However, subsequent annealing did not show a significant increase in Young's modulus for the composite with 25 wt.% of HAp. The highest value of Young’s modulus 9393 ± 709 MPa was observed for the annealed composite with 50 wt.% of HAp. Presumably, the growth of Young's modulus with an increase in the HAp weight fraction means reinforcing effect in the polymer matrix as a result of the introduction of highly dispersed HAp powder. Additional annealing initiated heterogeneous recrystallization on the surface of HAp. Presumably, in the process of heterogeneous recrystallization, an increase in the degree of crystallinity of the polymer matrix occurred proportionally to the increase in the weight fraction of HAp, which led to the increase in the Young's modulus of the composites.

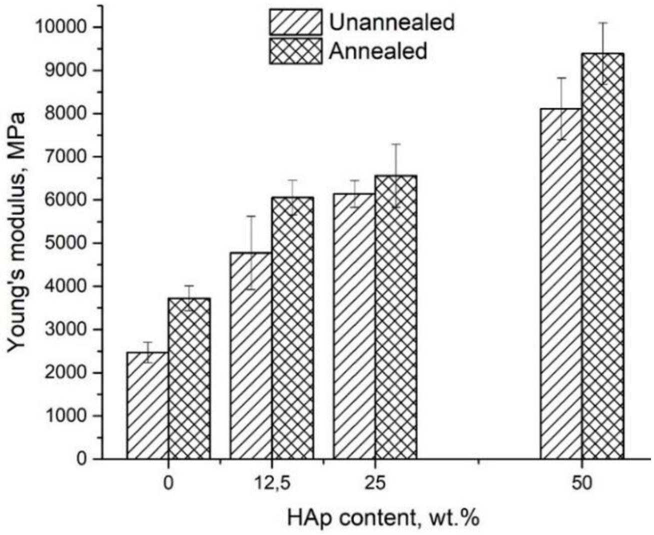

Figure 4. Young's modulus of obtained materials.

4. Conclusion

In this paper effect of annealing on morphology and elasticity of PLA/HAp composite investigated. It was shown that additional annealing does not influence the homogeneous distribution of HAp powder in the PLLA matrix. Deformation of 3D-printed scaffolds decreased with the increase of HAp weight fraction. Moreover, annealing led to the growth of Young's modulus up to 9393 ± 709 MPa for the annealed composite with 50 wt.% of HAp.

Acknowledgements

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