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The influence of polylactide/hydroxyapatite composite implant crystallinity on the polymer structure degradation

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Abstract

Introduction Assessment of biological characteristics of polylactide/hydroxyapatite (PLLA/HA) biodegradable materials is requiered to specify indications for the use of PLLA/HA composite implants in clinical practice. **The present study** was aimed to measure the kinetics of calcium and phosphate release from PLLA and its dependence on polymer structure crystallinity. **Material and methods** Four types of biodegradable materials were studied *in vitro*. Samples of type 1 and type 3 made of crystalline PLLA after annealing contained 25 % and 50 % of HA mass fraction, respectively. Samples of type 2 and type 4 made of amorphous PLLA (without annealing) contained 25 % and 50 % of HA mass fraction, respectively. In every group, 6 samples were tested. The samples were incubated in an aqueous medium at 37 °C for 52 weeks. The rate of PLLA degradation was assessed by the accumulation of lactate monomer in the hydrolysate. The concentrations of calcium ions and phosphate ions were determined for assessment the HA hydrolysis rate. The degree of crystallinity of the polymer matrix was evaluated by scanning calorimetry. **Results** The hydrolysis of PLLA and HA in the samples was not simultaneous. The PLLA was hydrolyzed first followed by HA hydrolysis. By the moment of complete hydrolysis of PLLA, there was only 15 % of hydrolyzed HA. The release of calcium ions occurred from the sixth week of incubation for all tested samples, that of phosphate ions from the third week. The total amount of the released calcium ions and phosphate ions decreased in the line: material 3 > material 4 > material 1 > material 2. Calcium ions in the hydrolysates were detected up to 42 weeks of incubation, phosphate ions up to the 52nd week. **Conclusion** Higher crystallinity of PLLA achieved by annealing results in increased rate of hydrolysis of HA from PLLA matrix. Biological activity of PLLA/HA implants can be determined by degree of polymer crystallinity and saturation with HA.

Keywords: biodegradable implant, polylactide (PLLA), hydroxyapatite (HA), crystallinity, hydrolytic degradation

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INTRODUCTION

Recently developed biomaterials are designed to stimulate regeneration of host tissues providing the surgeon with new options for restoring shape and function. Medical devices made of polymeric biodegradable materials based on polylactide (PLLA) with inclusions of hydroxyapatite (HA) are promising for clinical practice [1-4]. The hydroxyapatite in the polymer content provides osteoinductive and osteoconductive characteristics of the products based on PLLA [5]. It was demonstrated that osteoinductive properties of the material amplify along by increasing the saturation of HA in PLLA due to greater elimination of calcium and phosphate from its composition [6]. However, an increase in the content of HA in the polymer decreases its biomechanical properties. Therefore, to date, along with an increase in the osteogenic properties of PLLA-based materials, their porosity and mechanical resistance are important and required characteristics [7, 8]. Numerous studies have demonstrated the role of crystallinity of PLLA for mechanical stability and resistance of PLLA to hydrolysis [9-11]. It can provide even greater potential for the use of structured PLLA for bioengineering purposes, such as artificial bones and tissue scaffolds [12]. Thereby, the assessment of the relationship between the rate of hydrolysis of PLLA/HA composite materials and their crystallinity presents a problem of relevance.

Our study evaluates the kinetics of calcium and phosphate release from PLLA and its dependence on crystallinity of the polymer structure.

MATERIALS AND METHODS

Product samples were extruded from PLLA-based composite filled with 25 % HA and 50 % mass fractions (wt. %). To increase the crystallinity of PLLA, one part of samples was annealed at the temperature of 110 °C. The samples had cylindrical shape and were 1 cm long and 2 mm in diameter.

Four types of materials were studied. Samples of materials 1 and 3 made of crystallized PLLAc (by annealing) contained 25 % and 50 % of mass fractions of HA, respectively. Samples of materials 2 and 4 based on amorphous PLLAa (without annealing) contained 25 % and 50 % of mass fractions of HA,

respectively. In each material group, 6 samples were examined.

The differential scanning calorimetry (NETZSCH DSC 204 F1 Phoenix apparatus, Germany) was applied to measure the crystallinity of PLLA polymer matrix in the groups of samples (Table 1).

Table 1

Crystallinity of the tested samples

Material	Degree of crystallinity, %
Material 1 – PLLAc/HA25 %	46.1 ± 3.0
Material 2 – PLLAa/HA25 %	34.3 ± 2.7
Material 3 – PLLAc/HA50 %	17.6 ± 3.2
Material 4 – PLLAa/HA50 %	12.4 ± 1.8

Each sample was placed in a separate measuring cell filled with distilled water, the volume of which was determined at the rate of 4 ml per 1 cm^2 of the sample surface. Next, the samples were incubated in a thermostat at the temperature of 37 °C. After a week of incubation, the medium was changed. A new solution was poured in. The hydrolysate was subjected to chemical analysis for lactate, calcium ions and inorganic phosphate. The rate of PLLA degradation was assessed by the accumulation of its monomer in the hydrolysate. The concentration of calcium ions and phosphate ions reflected the progress of HA hydrolysis. The duration of incubation of the samples of all the materials was 52 weeks. The reagents of BioSystems (Spain) on Hitachi 902 biochemical analyzer (Hitachi Ltd., Japan) were used for analysis of lactate, calcium, and phosphate.

The arithmetic mean (M) and standard deviation (SD) were determined for quantitative parameters. The reliability of intergroup differences was assessed using the Kruskal-Wallis H-test. Differences were considered statistically significant at $p < 0.05$. Statistical analysis was performed using AtteStat 13.1 for Excel.

RESULTS

The hydrolysis of PLLA in material 1 (crystalline phase, HA content of 25 wt. %) increased during the first and the second week of incubation. In contrast to that, the samples of group 3 (PLLAc/HA 50 % sample) had an increased hydrolysis only the first week (Table 2). The hydrolysis of amorphous PLLA samples (HA content of 25 wt. %, material 2 and PLLAa/HA 50 %, material 4) was significant throughout the first three weeks.

The dynamics of the release of calcium ions and phosphate ions is shown in Figure 1.

Table 2

PLLA hydrolysis progression; % of total lactate formed as a result of hydrolysis

Week(s) of incubation	Material 1 PLLAc/HA 25 %	Material 2 PLLAa/HA 25 %	Material 3 PLLAc/HA 50 %	Material 4 PLLAa/HA 50 %
1	47.9 ± 5,5	47.4 ± 6.0	90.2 ± 8.2	67.0 ± 4.7
2	20.9 ± 3.2	15.0 ± 3.3	3.3 ± 1.1	10.8 ± 3.6
3	7.5 ± 1.1	19.7 ± 4.9	3.0 ± 0.7	16.2 ± 2.0
4	9.5 ± 2.0	10.9 ± 3.0	3.5 ± 1.8	3.4 ± 0.9
5	7.2 ± 1.4	7.9 ± 2.0	0	1.3 ± 0.5
6	4.2 ± 0.9	4.7 ± 1.8	0	1.2 ± 0.3
7	2.7 ± 0.8	3.9 ± 0.6	0	0
8	0	2.2 ± 0.7	0	0
10	0	5.9 ± 1.0	0	0
11	0	0	0	0
Total duration of hydrolysis (weeks)	7	10	4	6

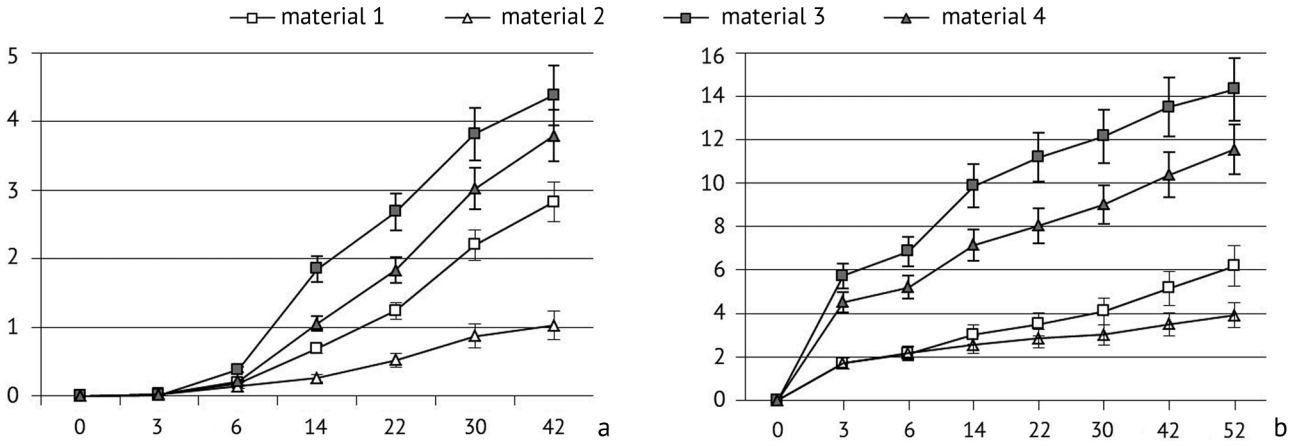

Fig. 1 Diagram of accumulation: a – calcium ions released from the samples during incubation; b – released inorganic phosphate ions from the samples during incubation. OX axis – weeks of incubation; OY axis – the number of ions (μmol/cm^2)

We stated that significant release of calcium ions began from the sixth week of incubation for all the samples while that of phosphate ions occurred from the third week. There was at the same time, the total number of released calcium ions and phosphate ions decreased in the line: material 3 > material 4 > material 1 > material 2.

Calcium ions in the hydrolysates of the samples of all materials were detected up to 42 weeks of incubation, phosphate ions – up to the 52nd week. The total amount of the released calcium and phosphate is presented in the Table 3. We noticed higher release of ions from the samples with high content of HA (samples 3 and 4) ($p < 0.05$). The accessibility of these ions from the crystalline polymers was higher relative to the samples of amorphous PLLA phase.

The comparison of kinetics in hydrolysis of PLLA and HA are presented in Table 4. During the period of PLLA of degradation (the duration of hydrolysis is shown in Table 1), no more than 15 % out of the total released calcium and from 40.9 % to 61.1 % of phosphate were released

Table 3

Total amount of released calcium and phosphate from the studied materials

Ion	Material 1	Material 2	Material 3	Material 4
Ca, μmol/cm^2	$2.83 \pm 0.34^{2,3,4}$	$1.03 \pm 0.21^{1,3,4}$	$4.38 \pm 0.82^{1,2}$	$3.79 \pm 0.46^{1,2}$
P, μmol/cm^2	$6.18 \pm 0.68^{2,3,4}$	$3.92 \pm 0.23^{1,3,4}$	$14.31 \pm 0.49^{1,2,4}$	$11.55 \pm 1.06^{1,2,3}$

Notes: superscript – the material relative to which there were significant differences at $p < 0.05$.

Table 4

Percentage of released calcium and phosphate for the period of complete decay of PLLA

Ion	Material 1	Material 2	Material 3	Material 4
Ca, %	$11.03 \pm 1.52^{3,4}$	$15.12 \pm 1.81^{3,4}$	$5.46 \pm 0.62^{1,2}$	$5.75 \pm 0.69^{1,2}$
P, %	40.9 ± 2.8^{2}	$61.1 \pm 4.1^{1,3,4}$	41.6 ± 3.0^{2}	48.2 ± 2.5^{2}

Notes: superscript – the material relative to which there were significant differences at $p < 0.05$.

DISCUSSION

Our studies revealed that the degree of PLLA crystallinity had an impact on the kinetics of HA release from the samples. In particular, the progression of HA hydrolysis correlates with the crystallinity of PLLA. This finding can be explained by the fact that the polymer in the crystalline phase underwent hydrolysis faster than in the amorphous one.

A typical feature of the hydrolysis kinetics found in the study is that the hydrolysis of PLLA and HA occurred stepwise. The polymer hydrolyzed earlier than HA. This is confirmed by the fact is that at the time of complete PLLA hydrolysis, the hydrolyzed HA was no more than 15 %. In general, such a sequence of the breakdown process of PLLA and filler (HA) degradation represents an important feature of the material for practical application. This fact implies a delayed release of calcium ions into host tissue environment after implantation.

We found that the saturation of crystalline PLLA with hydroxyapatite might be a promising option for production of materials designed for orthopedics. Although the issue of optimal amount of HA in PLLA remains open. On the one hand, the biological efficiency of materials containing both a small amount of HA (up to 10 wt. %) and its significant portion (up to 80 wt. % HA) was demonstrated [13, 14]. On the other hand, the osteogenic activity of the materials based on PLLA improves with an increase HA amount in the polymer composition [6].

Findings of our study are consistent with the study of study of Zhang et al. [15]: the crystallinity of the polymer saturated with HA determines its degradability and consequently the biological characteristics of the PLLA/HA material. That is why the PLLA crystallinity and its saturation with HA are the main characteristics of the PLLA/HA material, which determine its effectiveness and indications for use.

Based on the results of the analysis performed, it is not correct to speak about the exceptional superiority of any of the materials we studied. We believe that all the studied materials and products based on them could be used in clinical practice. It all depends on the indications for their use. The clinical experience in the use of products made from the PLLA/HA material shows that the choice of a biodegradable product should be made after considering the nature of degradation of its polymer [16].

In this regard, we believe that the indications for the use of PLLA products saturated with HA by more than 50 wt. %, might be cases of large defect management as the HA in the polymer actually becomes a calcium reserve for local bone tissue formation [17]. An additional advantage of PLLA/HA implants saturated with HA is their ability to reduce development of biofilms formed by S. aureus and P. aeruginosa on the surface of PLLA materials [18]. Less HA-saturated materials (< 25 wt. %) might address small bone defects [19].

Obviously, varying the content of HA and the Obviously, varying the content of HA and the degree of PLLA crystallinity may ensure options for using PLLA/HA materials for creating customized implants [14, 20, 21]. The material might be also promising for creation of scaffolds providing controllable degradation rate to compliment cell/tissue in-growth and maturation [22, 23].

Nevertheless, we should mention potential risks of the materials with high HA saturation for clinical use. The accelerated PLLA/Ha degradation results in the risks of early mechanical instability of implanted devices. The risk of heterotopic ossification should not be negligible either.

CONCLUSION

Thus, the performed study showed that increased crystallinity of PLLA treated by annealing increases the rate of hydrolysis of HA included in the PLLA matrix. Changes in the HA content and in PLLA crystallinity allow control over the biological characteristics (mechanical stability, calcium release, osteogenic properties) of the PLLA/HA composite materials. This also expands the indications for their possible clinical use.

Conflict of interest *Authors declare no conflict of interest.*

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REFERENCES

1. Alizadeh-Osgouei M, Li Y, Wen C. A comprehensive review of biodegradable synthetic polymer-ceramic composites and their manufacture for biomedical applications. *Bioact Mater*. 2018;4(1):22-36. doi: 10.1016/j.bioactmat.2018.11.003
2. Bharadwaz A, Jayasuriya AC. Recent trends in the application of widely used natural and synthetic polymer nanocomposites in bone tissue regeneration. *Mater Sci Eng C Mater Biol Appl*. 2020;110:110698. doi: 10.1016/j.msec.2020.110698
3. Fairag R, Li L, Ramirez-GarciaLuna JL, et al. A Composite Lactide-Mineral 3D-Printed Scaffold for Bone Repair and Regeneration. *Front Cell Dev Biol*. 2021;9:654518. doi: 10.3389/fcell.2021.654518
4. Popkov AV, Popkov DA, Kononovich NA, et al. Biological activity of the implant for internal fixation. *J Tissue Eng Regen Med*. 2018;12(12):2248-2255. doi: 10.1002/term.2756
5. Tayton E, Purcell M, Aarvold A, et al. A comparison of polymer and polymer-hydroxyapatite composite tissue engineered scaffolds for use in bone regeneration. An in vitro and in vivo study. *J Biomed Mater Res A*. 2014;102(8):2613-24. doi: 10.1002/jbm.a.34926
6. Woźna AE, Junka A, Hoppe VW. Influence of the different composites (PLA/PLLA/HA/β-TCP) contents manufactured with the use of additive laser technology on the biocompatibility. *Acta Bioeng Biomech*. 2021;23(2):169-180.
7. Murugan S, Parcha SR. Fabrication techniques involved in developing the composite scaffolds PCL/HA nanoparticles for bone tissue engineering applications. *J Mater Sci Mater Med*. 2021;32(8):93. doi: 10.1007/s10856-021-06564-0
8. Ngo HX, Bai Y, Sha J, et al. A Narrative Review of u-HA/PLLA, a Bioactive Resorbable Reconstruction Material: Applications in Oral and Maxillofacial Surgery. *Materials* (Basel). 2021;15(1):150. doi: 10.3390/ma15010150
9. Purnama P, Samsuri M, Iswaldi I. Properties Enhancement of High Molecular Weight Polylactide Using Stereocomplex Polylactide as a Nucleating Agent. *Polymers* (Basel). 2021;13(11):1725. doi: 10.3390/polym13111725
10. Samsuri M, Iswaldi I, Purnama P. The Effect of Stereocomplex Polylactide Particles on the Stereocomplexation of High Molecular Weight Polylactide Blends. *Polymers* (Basel). 2021;13(12):2018. doi: 10.3390/polym13122018
11. Zhao X, Liu J, Li J, et al. Strategies and techniques for improving heat resistance and mechanical performances of poly(lactic acid) (PLA) biodegradable materials. *Int J Biol Macromol*. 2022;218:115-134. doi: 10.1016/j.ijbiomac.2022.07.091
12. He Y, Xu WH, Zhang H, Qu JP. Constructing Bone-Mimicking High-Performance Structured Poly(lactic acid) by an Elongational Flow Field and Facile Annealing Process. *ACS Appl Mater Interfaces*. 2020;12(11):13411-13420. doi: 10.1021/acsami.0c01528
13. Bernardo MP, da Silva BCR, Hamouda AEI, et al. PLA/Hydroxyapatite scaffolds exhibit in vitro immunological inertness and promote robust osteogenic differentiation of human mesenchymal stem cells without osteogenic stimuli. *Sci Rep*. 2022;12(1):2333. doi: 10.1038/s41598-022-05207-w
14. Pandele AM, Constantinescu A, Radu IC, et al. Synthesis and Characterization of PLA-Micro-structured Hydroxyapatite Composite Films. *Materials* (Basel). 2020;13(2):274. doi: 10.3390/ma13020274
15. Zhang Y, Wang J, Ma Y, et al. Preparation of poly(lactic acid)/sintered hydroxyapatite composite biomaterial by supercritical CO_2. *Biomed Mater Eng*. 2018;29(1):67-79. doi: 10.3233/BME-171713
16. Kim YM, Lee JH. Clinical courses and degradation patterns of absorbable plates in facial bone fracture patients. *Arch Craniofac Surg*. 2019;20(5):297-303. doi: 10.7181/acfs.2019.00409
17. Retegi-Carrión S, Ferrandez-Montero A, Eguiluz A, et al. The Effect of Ca2+ and Mg2+ Ions Loaded at Degradable PLA Membranes on the Proliferation and Osteoinduction of MSCs. *Polymers* (Basel). 2022;14(12):2422. doi: 10.3390/polym14122422
18. Woźna AE, Junka AF, Szymczyk PE. The influence of different composite mixtures (PLA/HA) manufactured with additive laser technology on the ability of S. aureus and P. aeruginosa to form biofilms. *Acta Bioeng Biomech*. 2018;20(4):101-106.
19. Zimina A, Senatov F, Choudhary R, et al. Biocompatibility and Physico-Chemical Properties of Highly Porous PLA/HA Scaffolds for Bone Reconstruction. *Polymers* (Basel). 2020;12(12):2938. doi: 10.3390/polym12122938
20. Sakamoto A, Okamoto T, Matsuda S. Unsintered Hydroxyapatite and Poly-L-Lactide Composite Screws/Plates for Stabilizing β-Tricalcium Phosphate Bone Implants. *Clin Orthop Surg*. 2018;10(2):253-259. doi: 10.4055/cios.2018.10.2.253
21. Wu D, Spanou A, Diez-Escudero A, Persson C. 3D-printed PLA/HA composite structures as synthetic trabecular bone: A feasibility study using fused deposition modeling. *J Mech Behav Biomed Mater*. 2020;103:103608. doi: 10.1016/j.jmbbm.2019.103608
22. Liu Z, Chu W, Zhang L, et al. The effect of enhanced bone marrow in conjunction with 3D-printed PLA-HA in the repair of critical-sized bone defects in a rabbit model. *Ann Transl Med*. 2021;9(14):1134. doi: 10.21037/atm-20-8198

23.Oryan A, Hassanajili S, Sahvieh S, Azarpira N. Effectiveness of mesenchymal stem cell-seeded onto the 3D polylactic acid/polycaprolactone/hydroxyapatite scaffold on the radius bone defect in rat. *Life Sci*. 2020;257:118038. doi: 10.1016/j.lfs.2020.118038

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Stogov M.V. – formulation or evolution of the overarching goals and objectives of the study; development and design of methodology; creation of models; preparation and writing of the initial draft (draft) of the work.
Kireeva E.A. – application of statistical, mathematical, computational or other formal methods for analysis or synthesis of research data; carrying out the research process.
Dubinenko G.E. – development or design of methodology; application of statistical, mathematical, computational or other formal methods to analyze or synthesize research data; conducting the research process; making adjustments to the original version, preparing the work for publication.
Tverdokhlebov S.I. – development or design of methodology; making adjustments to the original version, preparing the work for publication.