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Hydrolysis of Bone-Replacing Materials Based on Polylactic Acid and Containing Hydroxyapatite in an *In Vitro* Experiment

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We studied the features of hydrolytic degradation of polylactic acid (PLLA) implants depending on their structural filling with hydroxyapatite (HA). The resistance to *in vitro* hydrolysis was tested for the following samples: PLLA without HA (control; group 1), PLLA/HA 25 wt% (group 2), and PLLA/HA 50 wt% (group 3). Samples were incubated at 37°C. In the hydrolysate, lactate, calcium ions, and inorganic phosphate were determined. Additionally, the time of appearance of visual deformation and sample disintegration was recorded. PLLA degradation was higher in samples saturated with HA. The highest resistance to deformation was noted for samples without HA. Samples with a PLLA/HA 50 wt% demonstrated the maximum degradation of PLLA in combination with lower resistance to deformation and the highest bioavailability of calcium and phosphate. Group 2 samples are most promising for clinical use.

Key Words: *implant; polylactic acid (PLLA); hydroxyapatite (HA); hydrolytic degradation*

The development of synthetic bone substitute materials based on degradable polymers, including polylactic acid (PLLA), is a modern trend in tissue engineering [1]. The ligation of such materials with various fillers makes it possible to create composites with desired properties [2-4]. Of particular interest are composites filled with hydroxyapatite (HA) exhibiting osteoconductive properties and capable of inducing bone formation processes. The percentage of HA in relation to the main substance PLLA in these composites can vary [5-7]. It is known that HA reduces the degree of acidification of surrounding tissues with lactic acid released during PLLA degradation [8]. Composites with slower rate of hydrolytic degradation are more promising for clinical use, because rapid degradation leads to early impairment of the mechanical properties of implanted products [1,9,10]. Thus, evaluation of the PLLA degradation rate and dynamics of the release of osteotropic elements depending on their concentration in the material is of great clinical importance.

Our aim was to study hydrolytic degradation of PLLA implants depending on the content of HA in their structure.

MATERIALS AND METHODS

The experiments were performed *in vitro*. We compared 3 types of implant samples made by extrusion of pure PLLA and a composite based on PLLA with

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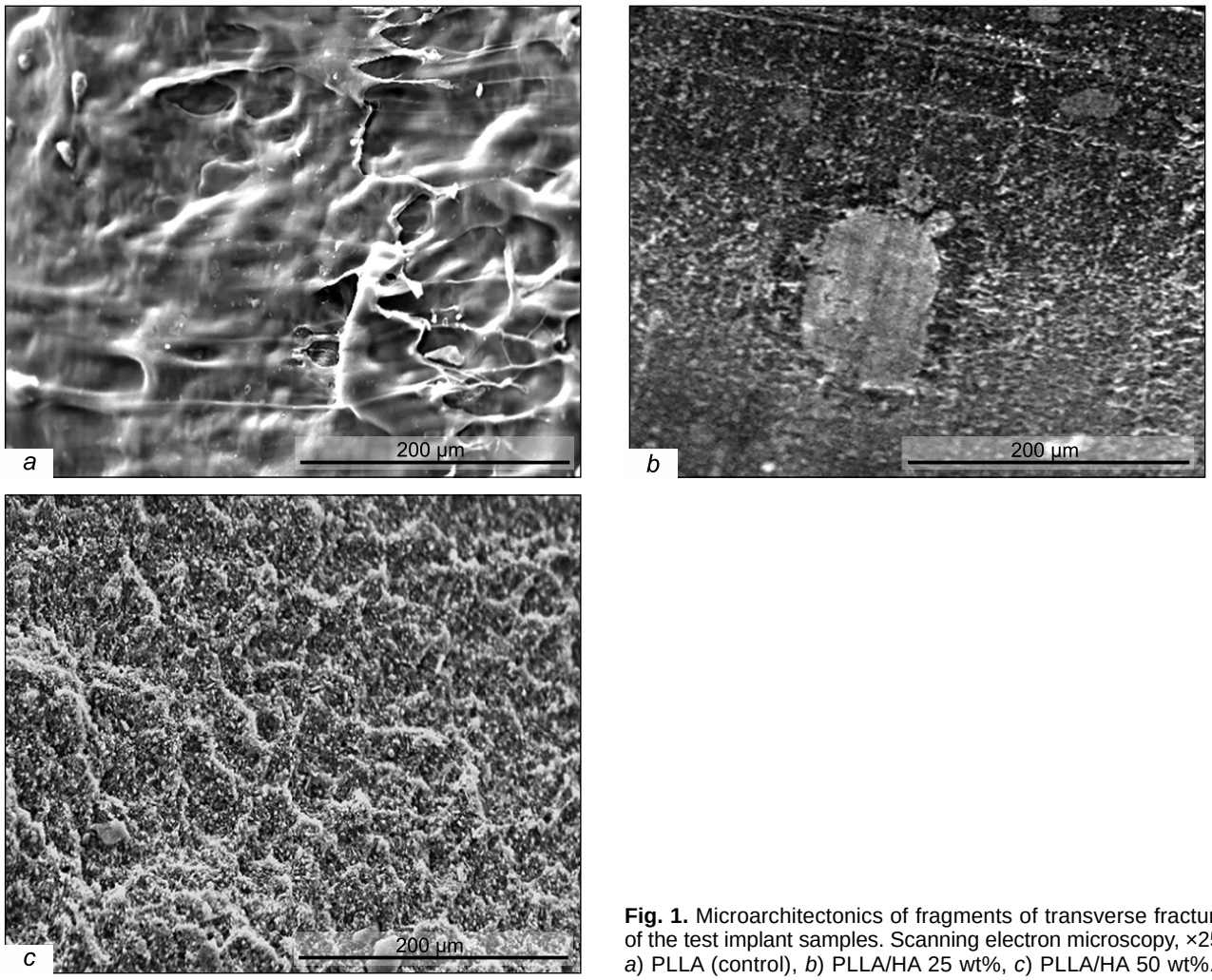

Fig. 1. Microarchitectonics of fragments of transverse fractures of the test implant samples. Scanning electron microscopy, ×250. *a*) PLLA (control), *b*) PLLA/HA 25 wt%, *c*) PLLA/HA 50 wt%.

25 and 50 wt% HA filling. All samples had cylindrical shape 1 cm long and 2 mm in diameter. Samples of all groups were subjected to annealing to increase the PLLA crystallinity. The first group (control) consisted of PLLA samples without HA (Fig. 1, *a*) and groups 2 and 3 were PLLA/HA samples containing 25 and 50 wt% HA, respectively (Fig. 1, *b, c*). In each group, 6 samples were examined.

To study hydrolytic degradation, each sample was placed in a separate measuring cell filled with distilled water (4 ml per 1 cm^2 sample surface). Then, the samples were incubated in a thermostat at 37°C. After a week of incubation, the medium was changed. The concentration of lactate, calcium ions, and inorganic phosphate was determined in the hydrolysate. A new portion of distilled water was added to the cells. The duration of incubation was 81 weeks (for samples of groups 2 and 3) or 12 weeks (for group 1 samples; the hydrolysate at this time did not contain detectable substances).

Lactate, calcium, and phosphate ions were assayed using Vital Diagnostic reagent kits on a Hitachi 902 biochemical analyzer. pH of the hydrolysate was estimated using a solution pH calculator (https://

TABLE 1. pH Values in the Hydrolysate

Sample	Time of pH decrease, weeks	Minimum pH, weeks	Range of hydrolysate pH (min-max)	Mean pH
PLLA (control)	1-8	4	3.629-3.964	3.761
PLLA/HA 25 wt%	1-8	1	3.512-4.210	3.803
PLLA/HA 50 wt%	1-7	1	3.375-4.219	3.831

planetcalc.ru/8840/). The integrity of products at all stages was assessed visually. The time of appearance of visible deformation or disintegration of the products was recorded. Electronic images from the sections of transverse fractures of the samples were obtained using a JSM-840 electron microscope (Jeol).

Statistical analysis of the obtained results was carried out using the AtteStat 13.1 add-on for Microsoft Excel spreadsheets. For quantitative parameters, the arithmetic mean (*M*) and standard deviation (*SD*) were determined. The significance of intergroup differences was assessed using the Kruskal–Wallis *H* test. The differences were considered significant at $p<0.05$.

RESULTS

Analysis of the dynamics of PLLA hydrolysis by the accumulation of lactate in the hydrolysate showed that polymer hydrolysis was maximum at the 4th week of incubation in control samples and at the 1st week of incubation in the experimental samples of both groups (Fig. 2, *a*). Over the 1st week, 69% (PLLA/HA 25 wt%) and 90% (PLLA/HA 50 wt%) of the total volume of the polymer was hydrolyzed (Fig. 2, *b*). In the PLLA/HA 50 wt% samples, polymer hydrolysis stopped most early.

Hydrolysis of the products in the aqueous medium shifted pH to the acidic zone for all samples to practically the same values (Table 1), but the some differences were noted for duration of pH decline (the longest period was observed for the control and PLLA/HA 25 wt% samples and the minimum for the PLLA/HA 25 wt% samples) and the time of reaching the minimum pH (the 4th week in the control and the 1st week for the test samples).

In the control samples, only trace amounts of calcium and inorganic phosphate ions were found in the hydrolysate during the entire period of incubation (Fig. 3), while the release of these ions from the test samples was significant. The volume and rate for calcium ion release decreased in the series: PLLA/HA 50 wt%>PLLA/HA 25 wt%. The release of

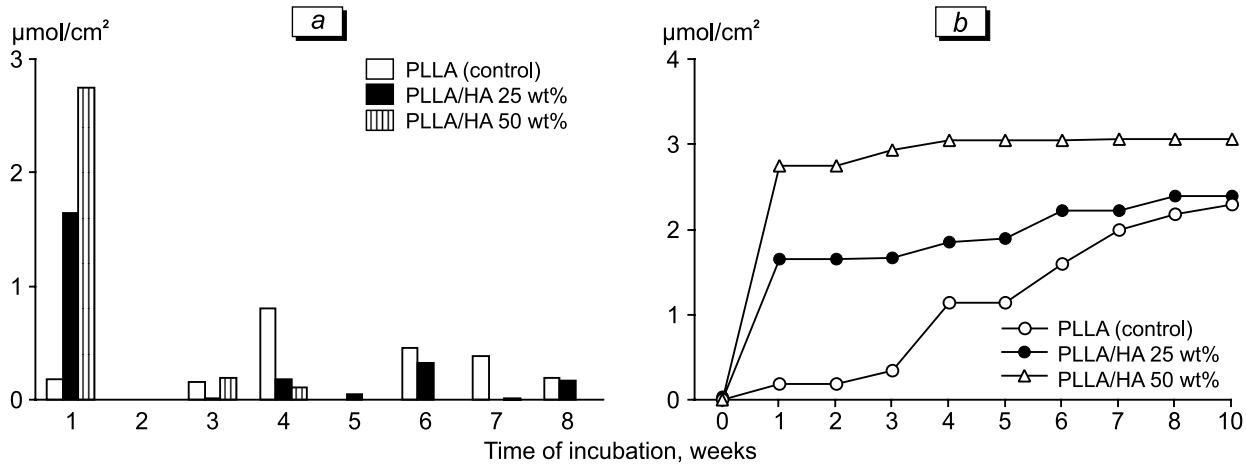

Fig. 2. Lactate release from the studied samples over 1 week (*a*) and throughout the incubation (*b*).

TABLE 2. Characteristics of Sample Resistance to Hydrolysis

Agent	Sample	Relapse period, weeks	Maximum release, weeks	Amount of released substance, μmol/cm² (*M*±*SD*)	Time of detection of the first deformation and/or fracture of the product, weeks
Lactate	PLLA (control)	1-8	4	2.297±0.950	28
	PLLA/HA 25 wt%	1-8	1	2.392±0.845	18
	PLLA/HA 50 wt%	1-7	1	3.062±0.679	18
Calcium	PLLA (control)	trace 1-11	—	trace	—
	PLLA/HA 25 wt%	6-42	26	2.827±0.340	—
	PLLA/HA 50 wt%	5-42	10	4.378±0.820*	—
Phosphate	PLLA (control)	trace 1-10	—	trace	—
	PLLA/HA 25 wt%	1-46	1	6.181±0.686	—
	PLLA/HA 50 wt%	1-46	1	14.314±0.494*	—

Note. *$p<0.05$ in comparison with PLLA/HA 25 wt%.

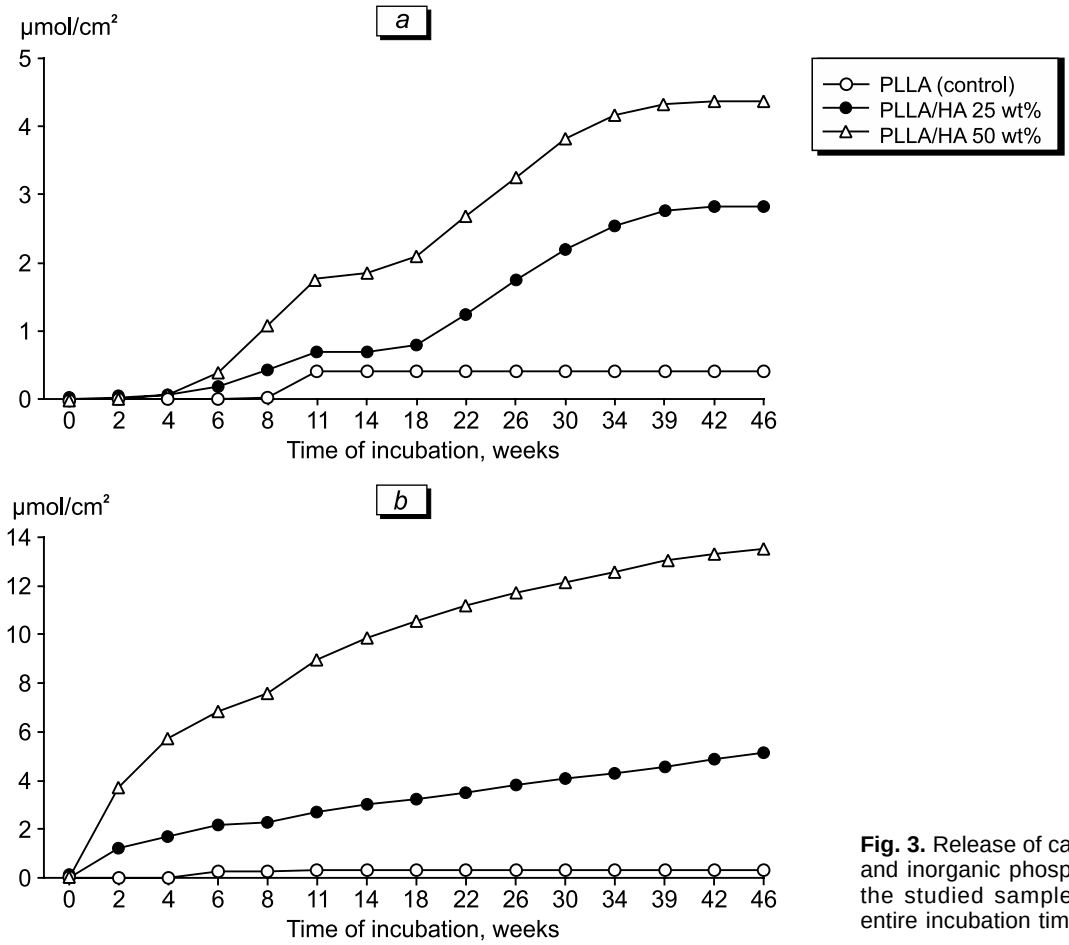

Fig. 3. Release of calcium ions (*a*) and inorganic phosphate (*b*) from the studied samples during the entire incubation time.

calcium from the test samples was uniform throughout the incubation, while a significant amount of phosphate was released as soon as after 1 week of incubation.

The hydrolysis resistance characteristics for all tested samples are presented by the key operational data: the time of maximum release of the analytes from the product, the duration of the release, the total amount of ion released (bioavailability), and resistance to deformation (Table 2).

Thus, it was found that samples saturated with HA demonstrated more active degradation of the polymer. The polymer saturated in equal ratios with HA (PLLA/HA 50 wt%) was characterized by the greatest degradation of PLLA along with lower resistance to deformation; the highest bioavailability of calcium and phosphate was also observed in these samples. The PLLA/HA 25 wt% samples are more promising for clinical use due to lower activity of polymer degradation and lower bioavailability of calcium and phosphate, which reduces the risk of early undesirable impairment of the mechanical properties of implanted devices and the risk of excessive tissue ossification.

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