In vitro hemocompatibility studies
of (poly(L-lactide) and poly(L-lactide-co-glycolide)
as materials for bioresorbable stents manufacture

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Vascular stents are one of the most popular modern methods of dealing with advanced coronary artery disease. At the same time stents are foreign objects to the body, they can stimulate proliferation of vascular endothelium and finally lead to restenosis. Biomaterials designed for temporary and permanent contact with an organism should be biologically inert especially in the case of contact with liquid tissue such as blood.

The study aims to assess the impact of resorbable polymers: poly(L-lactide) (PLLA) and poly(L-lactide-co-glycolide) (PLGA), intended for the construction of bioresorbable stents, on activation of coagulation, haemolysis, and morphology of blood cells.

The test results showed that in terms of haemolytic activity the samples selected from PLLA and PLGA, shaped by compression pressure followed by laser cutting, do not cause changes in the structural elements of blood and meet the biocompatibility requirements for materials intended for use in the circulatory system.

Key words: bioresorbable stents, bioresorbable polymers, hemocompatibility, blood

1. Introduction

Vascular stents are one of the most popular modern methods of dealing with advanced coronary artery disease. The most commonly used stents are made of 316L stainless steel, Co-Cr and Pt-Cr alloys, nitinol, and titanium. Over the last decade, there has been a significant increase in the popularity of metal stents with polymer coatings releasing anti-inflammatory or anticoagulant drugs (DES – Drug eluting stents) [1], [10]. Stents perform multiple tasks. First of all, stents restore vessel patency and free flow of blood. Their second task is to pulverise and push atherosclerotic plaque into the vessel wall [5]. Additionally, stents prevent dissection of the vessel wall and its elastic deformation immediately after angioplasty. The "scaffolding" function of a stent is most important during the first few days after surgery. Later on it provides no beneficial effect; on the contrary, its presence as a foreign body can generate adverse effects. Since stents are foreign objects to the body, they can stimulate proliferation of vascular endothelium. This phenomenon leads to restenosis, i.e., repeat narrowing of the blood vessel lumen and thrombosis, typically resulting in acute coronary syndromes [6]. Since the first endovascular stent implantation in the mid-1980s stent thrombosis has been the main factor limiting

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long-term success of coronary stents [12], [17]. During the first 2 weeks after implantation, subacute stent thrombosis is caused mainly by thrombus formation developing as a result of an adverse interaction between blood and the stent surface [8], [15]. Although vascular stent implantation has been widely used for many years, scientific studies show that full hemocompatibility of steel stents has not yet been achieved [3], [9], [11].

Therefore, it can be concluded that an ideal stent should support vessel wall after the implantation procedure and should biomechanically support functioning of the vessel for the time needed for its regeneration; after the stent has fulfilled the above task, it would be advisable to remove it. In practice, such a stent can be made by using biodegradable materials for its construction [13]. One example of such materials are aliphatic polymers degrading in the environment of body fluids: PLLA (poly(L-lactide)), PCL (poly(ε-caprolactone)), or PLGA (poly(L-lactide-co-glycolide)) copolymer, which are used in the production of vascular stents [19]. The use of bioresorbable polymers in stent design is important in terms of mechanobiology of the stent-vessel system. Polymer degradation is associated with a gradual decrease of mechanical properties over the length of stay in tissue environment [4]. This enables gradual reduction of radial stent stiffness and activation of the load bearing function of the vessel wall.

Biomaterials designed for temporary and permanent contact with an organism should be biologically inert. This is particularly important in the case of contact with liquid tissue such as blood, whose cells are particularly sensitive to any negative factors and the resulting disturbances may cause systemic effects [7], [12]. Products of material biodegradation, either by themselves or in protein complexes, can also cause early local or distant reactions at various times after implantation.

The study aims to assess the impact of resorbable polymers: poly(L-lactide) (PLLA-Resomer L 210S, Evonik Biomaterials) and poly(L-lactide-co-glycolide) 85/15 (PLGA-Resomer LG 857S, Evonik Biomaterials) on activation of coagulation, haemolysis, and morphology of blood cells.

2. Material and method

The research was conducted on samples made of poly(L-lactide) (PLLA) and poly(L-lactide-co-glycolide) (PLGA). Disc samples with diameters of 10 and 16 mm

were cut out from film approx. 0.25÷0.30 mm in thickness. The films were produced in a pressure casting die in the form of two flat and parallel sheets. During the first stage, the polymers were dried for 3 hours at a temperature of 100 °C in order to reduce the moisture content to less than 250 ppm. The inner walls of the die were lined with Teflon spacers and the die was heated to a temperature of 210 °C. After an appropriate amount of granulated mass was placed between the die plates and plasticized (approx. 3 min), the plates were subjected to a pressure of approx. 50 bar. The cast film was cooled inside the die at a rate of 20 °C/min. The final circular form was obtained with laser treatment technique using a beam generated by a CO_2 laser. The discs were sterilised using plasma sterilisation technique.

Testing of PLLA and PLGA samples obtained in the above process included an assessment of the haemolytic activity and analyses of activation of the plasma coagulation system. The research was conducted at the Department of Experimental Surgery and Biomaterials Research at the Wrocław Medical University.

2.1. Tests in contact with blood

Testing was carried out on the human blood group 0 Rh+ collected on CPD (citrate-phosphate-dextrose) preservative fluid obtained from Regional Blood Donation and Haemotherapy Centre in Wrocław, USP). The tests were approved by the Bioethics Committee in Wrocław (No. KB 426/2012).

Haemolytic activity was analysed on the basis of isotonic extracts obtained from the evaluated polymers, which were subjected to temporary contact and incubated with diluted blood and compacted erythrocytes. Haemoglobin concentration was determined in the liquid above the blood sediment (supernatant), whose values were used to calculate the haemolytic index (HI) and determine the haemolytic class. The HI parameter was calculated for the tested polymers and for the control group – the PBS solution. The haemolytic class was determined as the difference between the average haemolytic index for the tested sample and the control sample (PBS solution) [14].

Haemolytic activity was also examined with the use of red blood cells.

Testing involved erythrocytes obtained from whole human blood after centrifugation and suction of plasma. Compacted red blood cells were added in the amount of 0.02 ml to 5 ml of PBS, where the test ma-

terials had been incubated for 24 hours. The samples were closed and left at a temperature of 37 ± 1 °C for 24 h. Subsequently, the liquid with red blood cells was sucked from above the tested polymers. The obtained material was used to make preparations that enabled assessment of red and white blood cells and platelets using a light microscope. The ready wet preparations were centrifuged at $366 \times$ g for 10 min. Absorption of centrifuged fluids (supernatant) was measured against PBS at a wavelength of 540 nm. The percentage of haemolysis (%*H*) was calculated according to the formula

$$\%H = \frac{(AB - AK) \cdot 100}{A100 - AK}$$

where:

AB – absorbance of the tested sample,

AK – absorbance of the control sample,

*A*100 – absorbance of the sample with 100% haemolysis.

2.2. Diagnostic blood tests

Polymer samples were incubated in citrated blood (at a ratio of 3 cm^2/1 ml) for 4 and 24 h at a temperature of 37 ± 1 °C, similarly to blood alone, which was the control sample. Subsequently, the material samples were taken out and blood was collected for haematological, coagulation, and plasma haemoglobin tests.

Haematological tests

The red blood cell system was tested by determining the value of the haematocrit (HCT), haemoglobin (HGB) concentration, red blood cell (RBC) count, and red cell indices: mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red blood cell distribution width (RDW).

The white blood cell system was tested by determining the white blood cell (WBC) count including leukogram: the percentage of lymphocytes (LYM), the percentage of mixed cells (MXD – monocytes, basophils, and eosinophils), and the percentage of neutrophils (NEUT). Blood platelets were tested on the basis of determination of the blood platelet (PLT) count. The tests were performed on the K-4500 haematology analyser. The assay procedures are consistent with the methods described in the device manual.

The morphological evaluation of blood cells – red blood cells, nucleated cells – granulocytes, monocytes, lymphocytes, and platelets was made using the May Grünwald/Giemsa (MGG) stained smears [14]. The preparations were examined under oil immersion magnification (1000×) of a light microscope.

Tests of plasma coagulation system

Whole human blood 0 Rh+ after temporary (4 and 24) h contact with polymers and control blood were centrifuged at 3000 rpm for 10 min. Next, plasma was separated from the mass of formed blood elements, providing platelet medium rich plasma. In plasma, activated partial thromboplastin time (APTT) and prothrombin time (PT) were determined, including prothrombin index PT and prothrombin ratio INR defined as

$$INR = \left(\frac{PT_{\text{tested}}}{PT_{\text{control}}} \right)^{ISI}$$

where: *ISI* – international sensitivity index of the thromboplastin tested.

Thrombin time (TT) and fibrinogen (Fb) concentration were also determined. Measurements of parameters of the plasma coagulation system were performed on Coag Chrom 3003 coagulometer at a temperature of 37 ± 1 °C at a wavelength of 405 nm.

Results of the experiments are expressed as standard deviation of the mean. Data from *in vitro* studies were analyzed using two-way analysis of variance (ANOVA) to assess differences in haemolytic activation between the two sample groups PLLA, PLGA and control. Results with $p < 0:05$ were considered to be statistically significant.

3. Results

3.1. Tests in contact with blood

Haemoglobin concentrations in supernatant Hb(S), the HI index, and the haemolytic class in haemolytic activity testing using diluted blood are shown in Table 1.

The obtained polymer extracts were clear and colourless. Sedimentation of whole blood in the extract proceeded similarly as in the PBS solution.

The calculated values of the haemolytic index (H) for the evaluated polymers were within the normal range (Table 1).

Table 1. Haemoglobin concentration
in supernatant Hb(S), HI index, and haemolytic class

Polymer	Time [h]	Hb (S) [mg/ml]	IH	Hemolytic class
PLGA	3	0.008 ± 0.002	0.060	absence of hemolysis
	24	0.012 ± 0.002	0.090	absence of hemolysis
PLLA	3	0.008 ± 0.002	0.060	absence of hemolysis
	24	0.014 ± 0.002	0.105	absence of hemolysis
Control	3	0.007 ± 0.003	0.052	absence of hemolysis
	24	0.012 ± 0.002	0.090	absence of hemolysis
Polyethylene HD (6)	3	0.0064 ± 0.002	0.019	absence of hemolysis

In the study of haemolytic activity using compacted red blood cells, percentages of haemolysis did not exceed standard values, i.e., 3%. The percentages of haemolysis for the tested polymers are shown in Table 2.

Table 2. The percentages of haemolysis

Polymer	%H ± SD
PLGA	0.48 ± 0.08
PLLA	0.42 ± 0.06

3.2. Diagnostic blood tests

Observation of the shape of blood cells revealed that their picture was similar in both the test using diluted blood and the test using concentrated cells. Red blood cells after incubation in PBS (the control) had the correct shape with short cytoplasmic projections – echinocytes. Erythrocytes after incubation with the tested polymers also had echinocyte form (Fig. 1).

Haematological tests

Parameter values of the red blood cell system for the tested polymers and the control are shown in Tables 3–5. The red blood cell (RBC) count, haematocrit (HCT) value, haemoglobin (HGB) concentration,

Fig. 1. Whole blood after incubation (37 ºC) in extract from PLGA (a), PLLA (b) and the control – PBS (c).
Erythrocytes with short cytoplasmic projections (echinocytes).
Unstained preparation. Magnification 1000×

Table 3. Red blood cell (RBC) count, haemoglobin (HGB) concentration, and haematocrit (Ht) value
in control blood and after contact with polymers – (4, 24) h at a temperature of 37 ± 1 °C

Polimer	Time [h]	RBC [10^6/μL] $X \pm$ SD	p	HGB [g/L] $X \pm$ SD	p	HCT [%] $X \pm$ SD	p
PLGA	4	4.49 ± 0.04	0.213	13.30 ± 0.05	0.457	38.57 ± 1.02	0.405
	24	4.16 ± 0.12	0.912	12.37 ± 0.41	0.912	35.97 ± 1.24	0.757
PLLA	4	4.34 ± 0.19	0.676	13.47 ± 0.15	0.289	35.86 ± 1.32	0.089
	24	4.15 ± 0.15	0.885	12.90 ± 0.10	0.549	34.40 ± 1.54	0.505
Control	4	4.31 ± 0.20	–	13.20 ± 0.35	–	37.87 ± 0.81	–
	24	4.18 ± 0.27	–	12.66 ± 0.61	–	35.50 ± 2.10	–

Range: RBC 4 h: 4.15–4.54 10^6/μL,   HGB 4 h: 13.00–13.60 g/L, HCT 4 h: 37.00–38.60%;
24 h: 4.00–4.49 10^6/μL,   24 h: 12.00–13.20 g/L,   24 h: 33.10–37.00%;
X – average , ± SD – standard deviation, p – level of significance $p < 0.05$

mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red blood cell distribution width (RDW) after 4 h and 24 h were at a comparable level. The mean values of the determined parameters were not significantly different from the values recorded in the control group.

Values of parameters of the white blood cell system and blood platelet count for the control and test groups are shown in Tables 6 and 7. White blood cell (WBC) count, percentage of lymphocytes (LYM), neutrophils (NEUT), and other cells as well as blood platelet count were similar to the values of those parameters in the control groups.

Table 4. Red blood cell index values: mean corpuscular volume (MCV),
mean corpuscular haemoglobin (MCH) in control blood and after contact
with polymers – (4, 24) h at a temperature of 37 ± 1 °C

Polymer	Time [h]	MCV [μm] $X \pm$ SD	p	MCH [pg] $X \pm$ SD	p
PLGA	4	85.90 ± 2.51	0.358	29.03 ± 0.15	0.510
	24	85.90 ± 0.10	1.000	28.50 ± 1.33	0.515
PLLA	4	89.57 ± 1.32	0.856	29.93 ± 0.20	0.193
	24	88.00 ± 3.61	0.608	29.67 ± 1.15	0.145
Control	4	89.70 ± 4.59	–	28.27 ± 1.83	–
	24	89.90 ± 0.10	–	27.66 ± 1.53	–

Range: MCV: 4 h: 85.40–94.20 μm   MCH 2 h: 26.20–29.70 p;
              24 h: 81.00–94.00 μm   24 h: 26.00–29.00 pg;
X – average , ± SD – standard deviation, p – level of significance $p < 0.05$

Table 5. Values of mean corpuscular haemoglobin concentration (MCHC)
and red blood cell distribution width (RDW) in control blood and after contact
with polymers (4, 24) h at a temperature of 37 ± 1 °C

Polymer	Time [h]	MCHC [g/dl] $X \pm$ SD	p	RDW [%] $X \pm$ SD	p
PLGA	4	33.83 ± 0.87	0.439	12.90±0.17	0.135
	24	33.07 ± 1.27	0.521	12.33±0.42	0.069
PLLA	4	33.37 ± 0.31	0.711	14.33±0.74	0.782
	24	32.33 ± 2.08	0.963	13.33±0.70	0.291
Control	4	33.07 ± 1.27	–	14.63±1.59	–
	24	32.80 ± 0.46	–	14.90±2.19	–

Range: MCHC 4 h: 31.60–33.90 g/dl   RDW 4 h: 13.30–16.40%;
              24 h: 31.20–33.00 g/dl   24 h: 13.30–16.00%;
X – average, ± SD – standard deviation, p – level of significance $p < 0.05$

Table 6. White blood cell (WBC) count, percentage of lymphocytes (LYM),
in control blood, and after contact with polymers – (4, 24) h at a temperature of 37 ± 1 °C

Polymer	Time [h]	LYM [%] $X \pm$ SD	p	MXD [%] $X \pm$ SD	p	NEUT [%] $X \pm$ SD	p
PLGA	4	36.33 ± 5.50	0.576	6.83 ± 3.15	0.524	56.83 ± 6.06	0.874
	24	36.40 ± 7.52	0.717	7.20 ± 2.10	0.213	58.16 ± 2.96	0.796
PLLA	4	40.10 ± 1.77	0.601	2.50 ± 1.10	0.150	57.40 ± 7.21	0.911
	24	39.30 ± 2.96	0.756	2.63 ± 0.75	0.181	58.30 ± 2.25	0.751
Control	4	38.70 ± 3.89	–	5.30 ± 2.13	–	56.00 ± 6.00	–
	24	38.33 ± 4.16	–	4.66 ± 2.08	–		–

Range: LYM 4 h: 35.70–43.10%,   MXD 4 h: 3.40–9.00%,   NEUT 4 h: 47.30–60.90%
              24 h: 35.00–37.00%,   24 h: 3.00–7.00%, 24 h: 50.00–62.00%;
X – average, ± SD – standard deviation, p – level of significance $p < 0.05$

Table 7. Blood platelet (PLT) count in control blood and after contact
with polymers – (4, 24) h at a temperature of 37 ± 1 °C

Polymer	Time [h]	WBC [10^5/µL] $X \pm$ SD	p	PLT [10^3/µL] $X \pm$ SD	p
PLGA	4	5.77 ± 0.06	0.751	159.66 ± 4.51	0.932
	24	5.40 ± 0.52	0.296	158.33 ± 5.51	0.242
PLLA	4	5.60 ± 0.20	1.000	160.00 ± 1.73	0.822
	24	5.10 ± 0.07	0.742	148.00 ± 2.65	0.256
Control	4	5.60 ± 0.60	–	159.33 ± 4.50	–
	24	4.80 ± 0.69	–	150.33 ± 1.58	–

Range: WBC 4 h: 5.00 – 6.20 ×10^5/µL, PLT 4 h: 155.00 – 164.00 ×10^3/µL;
24 h: 4.00 – 5.20 ×10^5/µL, 24 h: 149.00 – 152.00 ×10^3/µL;
X – average, ± SD – standard deviation, p – level of significance $p < 0.05$

Haemoglobin concentration in blood plasma was determined after contact of whole citrated blood with polymers. The values were compared to haemoglobin concentration in the control blood plasma (Table 8). The observed plasma haemoglobin concentrations were within the range observed in the control group and the obtained values did not exceed 40 mg/dl, i.e., the value recognized as normal.

Table 8. Values of haemoglobin concentration
in citrated blood plasma

Polymer	Time [h]	Hb [g/dL] $X \pm$ SD p	
PLGA	4	30.93 ± 2.49	0.358
	24	34.99 ± 2.06	0.083
PLLA	4	31.62 ± 2.51	0.222
	24	33.70 ± 2.73	0.284
Control	4	29.35 ± 0.14	–
	24	31.36 ± 1.80	–

A microscopic examination of stained smears (MGG) of citrated blood after 4 and 24 hours of incubation of whole blood (CPD) found that the shape of red blood cells, white cell lymphocytes, and blood platelets was comparable to the shape of cells in the control blood. Red blood cells were correctly shaped and had the form of an acanthocyte.

A microscopic examination of stained smears after 24 h of incubation of whole blood (CPD) found white blood cells (granulocytes, monocytes, and lymphocytes) that were correctly shaped and had intact nuclei. Red blood cells were correctly shaped and had the form of an acanthocyte.

Tests of plasma coagulation system

The reference values of coagulation indices for human plasma and citrated plasma used for the test (time 0) and determined on a Coag Crom 3003 coagulometer are shown in Table 9.

Table 9. Reference values of coagulation system parameters
for the Coag Crom 3003 coagulometer

Denoted parameters	Range of reference values for human plasma	Parameter values for plasma
APTT [s]	26.0–32.6–38.9	34.27 ± 0.61
APTT [–]	0.81–1.01–1.21	1.01 ± 0.02
PT [s]	9.9–12.4–14.9	15.06 ± 0.06
PT [%]	86–108–130	88.66 ± 0.59
PT, INR	0.74–0.93–1.11	1.17 ± 0.03
TT [s]	11.0–13.0–15.0	15.76 ± 0.25
TT [–]	0.85–1.00–1.16	1.22 ± 0.03
Fb [g/l]	2.55–3.19–3.82	2.06 ± 0.04

Table 10. Values of partial thromboplastin time
after APTT activation for control citrated blood plasma
and after contact with polymers at a temperature of 37 ± 1 °C

Polymer	Time [h]	APTT [s] $X \pm$ SD	p	[–] $X \pm$ SD	p
PLGA	4	34.23±0.50	0.810	1.01±0.02	0.829
	24	41.77±0.61*	0.037	1.14±0.05	0.610
PLLA	4	34.53±0.38	0.588	1.02±0.01	0.482
	24	41.80±0.52*	0.025	1.17±0.04	0.696
control	4	34.33±0.45	–		
	24	40.27±0.55	–	–	–

Range: APTT: 4 h: 33.90–34.80 s; Ratio: 4 h: 1.00–1.03;
24 h: 38.60–40.70 s; Ratio: 24 h: 1.13 – 1.16;
X – average, ± SD – standard deviation, p – level of significance,
* $p < 0.05$ – significant difference compared to control

Values of parameters of the coagulation system for the control and test groups are shown in Tables 10–12. Activated partial thromboplastin times (APTT) in the test groups after 4 h were comparable to the values obtained in the control group. After 24 h APTT

was found to be prolonged. A statistically significant ($p < 0.05$) APTT prolongation was recorded in the range of values of the control group (Table 10).

The values of the prothrombin time (PT, INR, and WSK) after 4 h were comparable to the values in the control group. However, after 24 h a statistically significant ($p < 0.001$) increase was found in the PT

time, slightly in excess of the range of values in the control group.

Thrombin time (TT) values and fibrinogen concentration after 4 h were similar to the values in the control group. After 24 hours TT was found to be significantly prolonged ($p < 0.01$, $p < 0.0001$), slightly in excess of the range of values in the control group. The Fb concen-

Table 11. The values of prothrombin time (PT), INR, and WSK
for control citrated blood plasma and after contact with polymers at a temperature of 37 ± 1 °C

Polymer	Time [h]	PT					
		[s]		INR		WSK %	
		$X \pm SD$	p	$X \pm SD$	p	$X \pm SD$	p
PLGA	4	15.37 ± 0.40	0.272	1.15 ± 0.03	0.419	88.66 ± 1.53	1.000
	24	28.67 ± 0.06***	0.0000	2.07 ± 0.01***	0.0000	45.67 ± 0.58**	0.003
PLLA	4	15.06 ± 0.06	1.000	1.13 ± 0.06	0.643	87.33 ± 1.15	0.148
	24	28.53 ± 0.35***	0.0002	2.11 ± 0.03***	0.0001	46.00 ± 1.00**	0.006
Control	4	15.06 ± 0.06	–	1.13 ± 0.01	–	88.66 ± 0.58	–
	24	25.23 ± 0.23	–	1.82 ± 0.02	–	51.63 ± 1.53	–

Range: PT 4 h: 15.00–15.20 s; INR 1.12–1.14; WSK 88–89%;
          24 h: 25.10–25.20 s; INR 1.83–1.84; WSK 51–53%;
X – average, ± SD – standard deviation, p – level of significance, * $p < 0.05$, ** $p < 0.01$,
*** $p < 0.001$ – significant difference compared to control

Table 12. Thrombin time (TT) values and fibrinogen (Fb) concentration for control citrated blood plasma
after contract with polymers at a temperature of 37 ± 1 ºC

Polymer	Time [h]	TT				Fb [g/l]	
		[s]		[–]			
		$X \pm SD$	p	$X \pm SD$	p	$X \pm SD$	p
PLGA	4	15.46 ± 0.35	0.783	1.17 ± 0.03	0.119	2.05 ± 0.06	0.342
	24	19.76 ± 0.06***	0.0000	1.54 ± 0.01***	0.0003	1.30 ± 0.03**	0.007
PLLA	4	15.67 ± 0.06	0.065	1.23 ± 0.01	0.055	2.02 ± 0.03	0.384
	24	20.10 ± 0.40***	0.002	1.54 ± 0.01***	0.0005	1.28 ± 0.02**	0.003
Control	4	15.40 ± 0.17	–	1.21 ± 0.01	–	1.71 ± 0.05	–
	24	18.40 ± 0.10	–	1.43 ± 0.01	–	1.44 ± 0.04	–

Range: TT 4 h: 15.30–15.60 s; Ratio 1.20–1.22;
          24 h: 18.30–18.50 s; Ratio 1.43–1.47;
X – average, ± SD – standard deviation, p – level of significance, * $p < 0.05$, ** $p < 0.01$,
*** $p < 0.001$ – significant difference compared to control

Table 13. The recalcification times (RT) of citrated plasma
with polymer and control plasma after 60 min of intubation
at a temperature of 37 ºC

Polymer	Clotting time [s]		Clotting time relative to PS [%]
	$X \pm SD$	p	
PLGA	321.33 ± 9.02 *	0.103	−11
PLLA	318.33 ± 8.51**	0.084	−10
Control (PS)	358.00 ± 28.79	–	–

Range: PS: 325.00–378,00 s;
* $p < 0.05$, ** $p < 0.001$ – significant difference compared to PS (control).

trations decreased ($p < 0.01$) in terms of the values of the control group (Table 13).

The values of citrated blood coagulation time after calcification, determined for the evaluated polymers, were compared to the clotting time in the presence of polystyrene (PS).

The tested polymers showed slightly significant shortening of the clotting time compared to the clotting time in the presence of polysterene ($p < 0.05$ in the case of PLGA and $p < 0.01$ in the case of PLLA).

4. Discussion

This paper presents hemocompatibility tests of two aliphatic polymers: poly(L-lactide) (PLLA-Resomer L 210S, Evonik Biomaterials) and poly(L-lactide-co-glycolide) 85/15 (PLGA-Resomer LG 857S, Evonik Biomaterials) intended for the construction of bioresorbable stents. Hemocompatibility of synthetic materials in contact with blood is an important property allowing for an assessment of the suitability of the tested material for use in implants in the circulatory system. Assessment of blood compatibility is particularly important in the case of polymers, where the final physico-chemical properties depend to a large extend on the technological processes shaping the implants [13], [18], [19]. In the case of small implants such as stents, consisting of tiny elements, laser treatment is often used. Focused working beam delivers to the workpiece the energy fluence of 10–100 J/cm^2 that creates intense thermal effect zones at the treated site. It causes local changes in the structure of the treated polymer, resulting in a change of its physical properties [13]. Researchers often overlook this aspect and assume that physico-chemical properties of the material from which the stent is made are identical to the properties of the starting material – granulated mass. As is clear from the conclusions contained in the [2] study, in the case of PLLA or PLGA it is not necessarily so. In our view, changes in the structure of the material caused by thermal effects may also affect hemocompatibility of bioresorbable polymers. Therefore, we conducted *in vitro* studies of PLLA and PLGA compatibility with blood.

The primary *in vitro* study of material compatibility with blood is the study of haemolytic activity, which together with the microscopic evaluation of red blood cell morphology allows to demonstrate the toxic effect of the evaluated biomaterials on red blood cells and pre-select them before further studies.

Some interesting results were obtained in studies of haemolytic activity with the use of diluted and whole blood. Regardless of the assay method, the observed haemolysis values did not exceed the standard range of values. The haemolysis percentages of 0.48% for PLGA and 0.42% for PLLA are lower than the values determined by Yang et al. [18] for samples collected from the 316L stainless steel – 0.6%. Shen et al. [16] examined the haemolytic impact of samples prepared from PLLA, observing a higher percentage of haemolysis than in our tests, amounting to 0.677%. Despite the fact that the same test method was used, there is a significant difference in the value of this parameter. In our view, this confirms the importance of the impact of technological processes related to the processing of the polymer on its physicochemical properties, as highlighted in the work by [2].

Morphological pictures of red blood cells after temporary contact with the examined polymers were comparable. The morphological picture of red blood cells revealed changes in the shape of cells. Tests in contact with blood involved haematological, coagulation, recalcification time, and extracellular haemoglobin tests. The tests were performed after 4 h and 24 h of incubation of blood with polymer and without material – the control at a temperature of 37 °C in a static system.

The impact of polymers on citrated blood was assessed on the basis of haematological and coagulation testing. The tests were performed after 4 h and 24 h of incubation of blood with material and without material – the control at a temperature of 37 °C in a static system.

Haemoglobin concentration, values of haematocrit and red blood cell indices (MCV, MCH, and MCHC) as well as RBC, WBC, and PLT blood count after 4 h and 24 h of contact with the tested polymers were comparable and similar to the control value and did not exceed the limit values in the standard. A microscopic examination of formed blood elements after 4 h and 24 h of contact with the tested polymers revealed the correct shape of red blood cells, white blood cells, and blood platelets. Plasma concentration of extracellular haemoglobin after 4 h and 24 h of blood contact with polymers was within the range of values for the control group.

Assessment of the plasma coagulation system for the whole blood after 4 hours of contact of blood with the material did not demonstrate any changes in the APTT, PT, and TT values and in fibrinogen concentration. However, after 24 h a prolongation of APTT, PT, and TT was observed as well as reduced fibrinogen concentration. Changes in the values of parameters fall within the reference range or exceed them slightly.

Tests of recalcification time also point to activation of coagulation. A comparison of polymers found that PLGA prolonged coagulation time by 9% compared to the PLLA polymer.

5. Summary

1. Studies of haemolytic activity with the use of diluted blood and erythrocytes have demonstrated

that PLGA and PLLA polymers do not change the morphological picture of erythrocytes and the haemolysis values are in the normal range.

2. Morphological blood tests have shown that the tested polymers PLGA and PLLA do not affect the qualitative changes in the selected blood parameters or the morphological picture of cells.

3. Tests of activation of the plasma coagulation system have demonstrated that PLGA and PLLA polymers activate the endogenous (APTT), exogenous (PT), and thrombin coagulation systems after prolonged contact with blood. The process of activation is characterized by reduced recalcification times (CT) and prolonged APTT, PT, and TT; however, they fall within the range of reference values.

The test results showed that in terms of haemolytic activity the samples selected from PLLA and PLGA, shaped by compression pressure followed by laser cutting, do not cause changes in the structural elements of blood and meet the biocompatibility requirements for materials intended for use in the circulatory system.

Acknowledgements

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