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Elastic multiblock copolymers for vascular regeneration: protein adsorption and hemocompatibility

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Abstract

Hemocompatibility of elastic multiblock copolymers PDC, based on poly(*p*-dioxanone) (PPDO)/poly(ε-caprolactone)-segments, capable of a shape-memory effect, and PDD, based on PPDO/poly((adipinate-*alt*-1,4-butanediol)-*co*-(adipinate-*alt*-ethylene glycol)-*co*-adipinate-*alt*-diethylene glycol)-segments, was studied in order to assess their suitability for an application aiming at blood vessels regeneration. The results were compared with polypropylene (PP) which is a widely used blood-contacting material for devices as blood oxygenators and dialysis tubes. Protein adsorption studies showed diverse blood plasma proteins in a relatively high amount on both elastic polymers compared to the poor amount of plasma proteins adsorbed on PP. Study of the coagulation system revealed high thrombin formation on PDC and no difference in plasma kallikrein activation between elastic multiblock copolymers and the reference PP. Activation of complement system was higher for PDC followed by PDD and lower for PP. However, platelet adhesion and activation were hardly suppressed on the multiblock copolymers compared to the PP surface, where the number of adhered platelets and the activation rate were significant. The present results reveal that the tested multiblock copolymers with improved elastic properties and shape-memory capability (PDC) show low thrombogenicity and are promising candidates for vascular tissue engineering.

Key words: hemocompatibility, degradable elastic polymer, protein adsorption, coagulation, thrombin formation, platelet adhesion/activation, shape-memory polymer

1. Introduction

Cardiovascular diseases remain the leading cause of mortality in western nations. Despite significant improvements and the broad adoption of minimally invasive treatments such as balloon angioplasty and stenting, the therapeutic gold standard for patients with far advanced heart diseases is the coronary artery bypass graft [21]. However, coronary artery bypasses require healthy autologous vessels, which have limited availability and still often do not fit the are not ideal [6]. Efforts at developing bio-durable, synthetic, small diameter vascular grafts for long-term application have been hampered by poor hemocompatibility at the blood–biomaterial interface and mismatch of the mechanical properties, which leads to graft failure and recurrent disease [2,11,23,31]. Regenerative medicine and, in particular, tissue engineering approaches can provide potential solutions to these problems [3,28], in which the new tissue is built around a degradable scaffold material as temporal support. The polymer-based biomaterials will be used to enable growth of endothelial cells, to act as a degradable support in creation of endothelium *de novo*. To match the elastic behaviour of natural tissue a new class of polymers - elastomeric multiblock copolymers [20] are developed that can be tailored to have the desired elasticity and strength. Moreover they are biodegradable and some of them a show shape-memory behaviour. The mechanical properties of these polymers make them extremely attractive for biomedical applications like stents, filling of aneurysms or left atrial appendages, scaffolds for vessel grafts or suture material for blood vessels [18,19]. The multiblock copolymer PDC, which is based on the macrodiols poly(*p*-dioxanone)diol (PPDO) and poly(*ε*-caprolactone)diol and which is capable of a shape-memory effect was shown to be hemocompatible to capillary endothelial cells in the chorioallantois membrane (CAM)

test. Endothelial cells adhered to the polymer surface forming confluent cell layers after 48 h. In the CAM test no cytotoxic effects [22], but an angiogenic effect of the multiblock copolymer PDC was found [9]. In our previous study [27] we investigated the thermoplastic multiblock polymers PDC and PDD, which is based on PPDO/poly((adipinate-*alt*-1,4-butanediol)-*co*-(adipinate-*alt*-ethylene glycol)-*co*-adipinate-*alt*-diethylene glycol)-segments, in order to assess their suitability for an application in blood vessels regeneration. In this respect cell adhesion, growth, and functionality (vWF and PGL_2 production) of human umbilical vein endothelial cells (HUVECs) on these polymers were examined [27]. Our results revealed that both elastic multiblock copolymers are capable for use as scaffolds for vascular regeneration since they support cell adhesion and growth and showed balanced thrombotic and anti-thrombotic activity of seeded endothelial cells. Furthermore, cytotoxicity tests with 3T3 mouse fibroblasts showed cell viability above 90%

Since the polymer scaffolds will directly contact blood flow it is necessary to study their hemocompatibility [16,17]. Thrombus formation is the most common cause of graft failure in contact with blood [24]. The three main events, which are involved in thrombosis are platelet adhesion/activation, coagulation, and complement activation [16]. Adsorption of proteins from blood plasma to the scaffold surface is the first event, which occurs when a foreign material is exposed to blood. The profile of adsorbed proteins and their conformation/orientation state is a function of the physico-chemical properties of the polymer, which determines the cascade of the subsequent main processes upon thrombosis. Studying, understanding, and controlling these processes is a prerequisite for any hemocompatible surface.

In this manuscript we present the results obtained on hemocompatibility of the elastic multiblock copolymers PDD and PDC and compare the results obtained to PP polymer, which is for blood-contacting applications well established. In this regard we studied at first the protein adsorption on the polymer surfaces as well as the composition of the proteins, which was analysed by SDS-PAGE. In addition, the activation of the coagulation system was investigated by determining the thrombin formation and the activation of the complement system. Platelet adhesion and activation was also investigated. In this way we evaluate suitability of the polymers as scaffold material for vascular grafts.

2. Materials and methods

2.1 Preparation and characterization of the polymers

The multiblock copolymer named PDC was synthesized from poly(*p*-dioxanone)diol and poly(*ε*-caprolactone)diol, while PDD was prepared from PPDO diol and poly{(adipinate-*alt*-1,4-butanediol)-*co*-(adipinate-*alt*-ethylene glycol)}diol (PAG) [20,27]. Polymer samples (13 mm in diameter) punched from polymer films were sterilized by ethylene oxide sterilization. Polypropylene (PP, Trespaphan® END 50 without additives; Rehau, Germany) was used as a reference material [17].

2.2 Protein adsorption

2.2.1 Plasma protein adsorption

After pre-wetting with phosphate buffered saline (PBS, 137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4) for 15 min at room temperature (RT), the polymer surfaces (discs with 13 mm in diameter) were incubated in 100% citrated human plasma (Pooled Normal Plasma (PNP), Precision BioLogic/Haemochrom Diagnostica,

Essen, Germany) for 1 h at 37 °C in a humidified atmosphere. The surfaces were washed three times with PBS to remove non-adsorbed proteins and then incubated in 500 μL Laemmli sample buffer (4x) (Novex, Groningen, The Netherlands) at 37 °C for 30 min. Desorbed protein solutions were loaded on a NuPAGE® 4-12% Bis Tris Gel (Novex), and a SDS-PAGE was performed. Gels were silver stained (Silver Xpress, Novex). Densitometry profile plots were constructed from scanned digital images of the gels. Image processing using Quantity One® 1-D analysis software (Version 4.6.3, Bio-Rad Laboratories, Munich, Germany) was limited to background subtraction (one pass).

2.2.2 Fibrinogen adsorption

Pre-wetted polymer surfaces (13 mm in diameter) were incubated in fibrinogen (FNG, Sigma, Taufkirchen, Germany) solution (3 mg/mL FNG in PBS) at 37 °C in a humidified atmosphere for 1 h. Then the surfaces were treated as described above.

2.3 Blood preparation

Blood was collected from healthy volunteers who had not taken any medication for at least 10 days prior the experiments. Sodium citrate was used as anticoagulant (3.9 g/100mL) at a blood:citrate ratio of 9:1. Platelet-rich plasma (PRP) was prepared by centrifugation of blood at 200 *g* for 10 min. The supernatant PRP was collected and the blood was centrifuged at 2000 *g* for 20 min to prepare platelet-poor plasma (PPP). The platelet count was adjusted to 200 000 cells/μL by mixing PRP and PPP. An approval from an ethical board was not appropriate but all experiments were performed in accordance with the ethical guidelines of Clinical Hemorheology and Microcirculation [1].

2.4 Activation of coagulation

2.4.1 Non-activated partial thromboplastin time

Activation of clotting factors in a purified system was determined by measuring the non-activated partial thromboplastin time (nAPTT) as a simple and sensitive test. Plasma (450 µL PNP) was contacted with pre-wetted 13 mm discs of the materials in a 24-well plate for 30 min. 100 µL of this plasma was mixed in the cuvette of a coagulometer (KC 4A, Amelung, Lemgo, Germany) with 100 µL of PTT reagent (Roche Diagnostics, Mannheim, Germany). The PTT reagent was reconstituted with PBS to prevent additional activation by kaolin. After 3 min of incubation 100 µL of 0.025 M $CaCl_2$ was added and the clotting time was determined. All procedures were performed with two samples in quadruplicate at 37 °C.

2.4.2 Formation of thrombin

The pre-wetted the polymer samples (13 mm discs) were in contact with 210 µL PNP in a 24-well plate for 30 min. Afterwards 50 µL of these plasma samples were mixed with 150 µL of Tris buffer (pH 7.9, with 1% human serum albumin) and 50 µL of chromogenic substrate S-2238 (diluted according to the recommendation of the supplier Chromogenix/Haemochrom Diagnostica, Essen, Germany) in a 96-well plate. The method for the determination of activity is based on the difference in absorbance (optical density) between the H-D-Phenylalanyl-*L*-pipecolyl-*L*-arginine-*p*-nitroaniline dihydrochloride (pNA) formed and the original substrate. The rate of pNA formation, i.e. the increase in absorbance per second, is proportional to the enzymatic activity and was determined with a SpectraFluor Plus plate reader (Tecan, Crailsheim, Germany) at 405 nm with a reference wavelength of 492 nm every 5 min for 25 min of incubation. The slope of the increase in optical density is a measure for the activity of thrombin

formation. Plasma without contact to polymer samples served as control. All procedures were performed at 37 °C.

2.4.3 Plasmakallikrein (contact activation)

The contact of blood with foreign surfaces causes the activation of coagulation cascade by the intrinsic pathway. It starts with activation of factor XII to XIIa through the conversion of plasma prekallikrein to kallikrein. The presence of kallikrein in the supernatant was measured spectrophotometrically by the chromogenic substrate S-2302 (Chromogenix/Haemochrom Diagnostica) using a method described previously [7] Briefly, PNP (diluted 1:5 with Tris–HCl, pH 7.8) was brought into contact with pre-wetted polymer samples (13 mm discs) in 24-well plates (1 mL per well) for 30 or 60 min. In 96-well plates, 200 μL of plasma samples were then mixed with 100 μL S-2302 (4 mM) and incubated at 37 °C for up to 25 min. During this time, the reaction kinetics were tracked with a SpectraFluor Plus plate reader every 5 min at 405 nm with a reference wavelength of 620 nm. The kallikrein-like activity was determined from the slope of the increase in optical density over a time of 15 min. Diluted plasma without contact to polymer samples served as control.

2.5 Activation of complement system

The activation of the complement system on foreign surfaces occurs via the alternative pathway that is initiated with the complement component C3. C3 is cleaved to release C3b that binds to the activating surface. Covalently bound C3b binds Factor B. Within the C3bB complex, Factor B is rendered susceptible to cleavage by Factor D into fragments Ba and Bb. Quantification of fragment Bb was performed by an enzyme immuno assay (EIA), the Quidel® Bb fragment EIA Kit (Quidel Corporation, San Diego, CA, USA) containing all necessary reagents. 210 μL PNP was brought into

contact with the pre-wetted polymer samples (13 mm discs) for 15 min. After that time period the generation of complement fragments can decrease and in this way decreases signal intensity. 50 µL of this plasma was diluted for EIA with 450 µL specimen diluent. Briefly, the Bb fragment EIA was performed in the 96-well Quidel® plate, according to the recommendations of the supplier, was washed thoroughly with washing solution (PBS containing 0.05% Tween-20 and 0.01% thimerosal), and 100 µL of specimen diluent, reconstituted standards, controls, or diluted specimens were added to the assigned wells (in triplicate). The plate was incubated for 30 min at RT. After thorough washing Bb conjugate (50 µL) was added and incubated at RT for another 30 min followed by another thorough washing step, and the addition of 100 µL substrate solution. After 30 min the reaction was stopped by the addition of 50 µL Quidel® stop solution (250 mM oxalic acid). The optical density of the solution was measured with the SpectraFluor Plus plate reader at 405 nm.

2.6 Adhesion of platelets

The polymer samples (13 mm discs) were pre-wetted with PBS for 30 min. 300 µL PRP (adjusted to 200 000 cells/µL) was incubated with polymer surfaces for 60 min at RT and washed three times with PBS. Platelet adhesion was assessed by immune fluorescence microscopy of the integrin GPIb expressed as platelets according to reference [26]. Microscopy was carried out with a confocal laser scanning microscope (CLSM, LSM 510, Zeiss, Germany).

2.7 Statistical analysis

All statistical analyses were carried out with GraphPad Prism software (Graph Pad Software Inc., San Diego, USA). The values were considered to be significantly different if the p value was < 0.05.

3. Results

3.1 Protein adsorption

3.1.1 Plasma protein adsorption

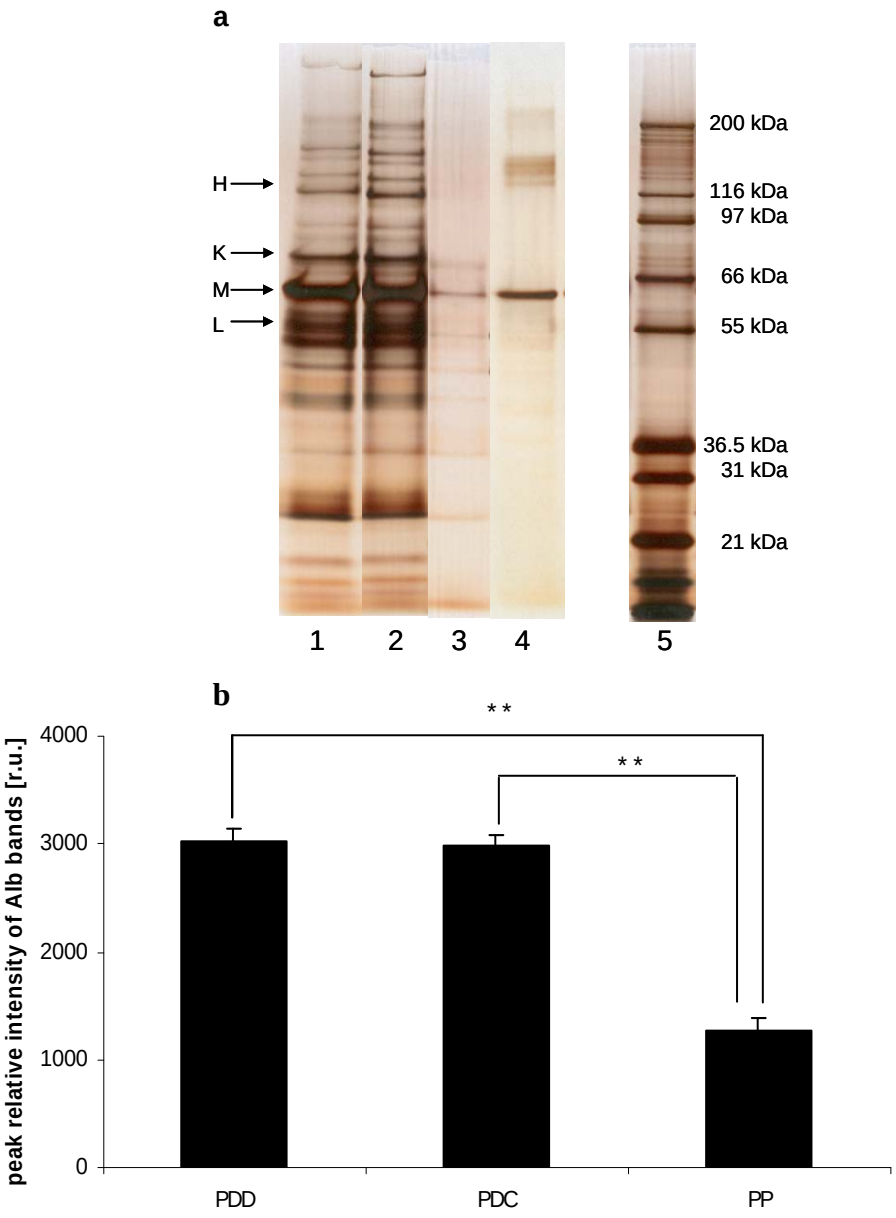


Figure 1: a) SDS-PAGE under reducing conditions of desorbed plasma proteins after incubation for 1 h from PDD50 (Lane 1), PDC50 (Lane 2) and PP (Line 3). Line 4 represents albumin (Alb, 0.5 μg) and Line 5 protein standard Mark 12. This experiment is representative of two others.
b) Alb bands separated by SDS-PAGE under reducing conditions were quantified by densitometry for three independent experiments with Quantity One® 1-D analysis software. ** - $p < 0.001$.

Plasma proteins eluted from the polymer surfaces were analyzed by SDS-PAGE. Figure1 shows a captured digital image of a typical PAGE gel. Many protein bands (120-200, 74, 64, 56, 53, 47, 36, 26 kDa) could be spotted in all of the extracts obtained from the investigated multiblock copolymers in contrast to the reference material PP where only two bands of 64 and 74 kDa with slight intensity (Fig. 1, line 3) were detected. The two major protein bands of 64-66 kDa and 55 kDa can be most probably attributed to Alb (marked with M on the Fig. 1) and heavy chain of immunoglobulin proteins adsorption (marked with L on Fig. 1). Their appearance in the eluates might be caused by their high concentration within plasma[10]. Electrophoresis performed for a single solution of Alb showed the same main band in the range of 64-66 kDa (Fig. 1, line 4). We assume that the α-chain of FNG could have a contribution to the biggest band since our experiment showed that FNG appears on the gel in three main bands of α–, β– and γ-chains of its molecule (Fig. 2): α-chain of FNG can be found to be in the range of 62-64 kDa. A relative quantification of the proteins from the main electrophoretic band (64-66 kDa) obtained from the extracts of the investigated materials by densitometry reveals significantly higher quantities (approximately two times higher) of adsorbed proteins on PDD and PDC compared to PP (Fig. 1 b).

Many other proteins were present in the eluates from both copolymer surfaces as well. The band of 74 kDa is approximately equivalent with the electrophoretic mobility of prothrombin (Fig. 1, marked with K on line 1 and 2), the major protein of clotting system[30]. The 116 kDa band (Fig. 1, marked with H on line 1 and 2) could be the high molecular weight kininogen (HMWK), which has molecular weight of around 120 kDa and is frequently found on biomaterial surfaces [5]. The bands observed at 204 kDa could be related to fibronectin subunits, a protein known to mediate platelet

adhesion [15]. On the other hand, the low molecular weight (MW) bands (approximately 35 kDa and 25 kDa) are possibly related to apolipoproteins [4].

3.1.2 FNG adsorption

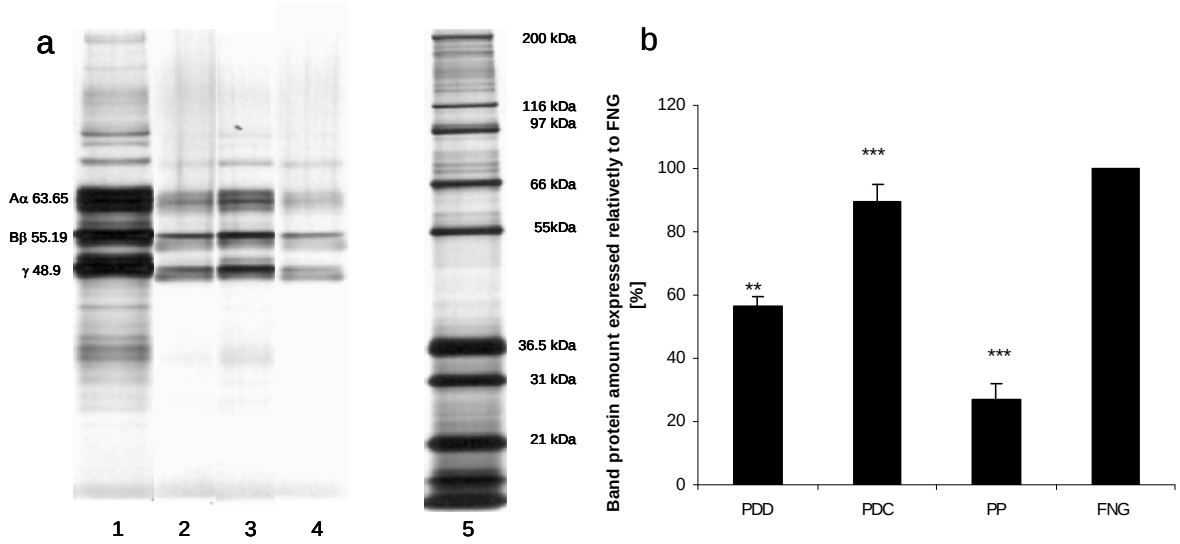


Figure 2: a) SDS-PAGE under reducing conditions of desorbed fibrinogen (FNG, 3 mg/ml) after incubation for 1 h from PDD (Lane 2), PDC (Line 3) and PP (Line 4). Line 1 represents FNG (1.5 mg) directly loaded on the gel and Line 5 protein standard Mark 12. This experiment is representative for three others. b) The protein bands separated by SDS-PAGE under reducing conditions were quantified by densitometry for four independent experiments with Quantity One® 1-D analysis software. The results are expressed relative to the total protein band amount of 1.5 mg FNG. ** - $p < 0.001$.

FNG, an abundant plasma protein (plasma concentration approx. 3 mg/mL), plays a key role in blood coagulation and platelet adhesion, the two most important phenomena in surface-induced thrombosis [8]. Figure 2a shows electrophoretic mobility of human FNG at reducing conditions. Three bands appear with molecular weight of 63 kDa (α-chain), 55 kDa (β-chain), and 48 kDa (γ-chain) on the samples. The most intensive bands were observed in line 3 (FNG adsorbed on PDC50). As can be seen from the densitometry analysis (Fig. 2b) eluates from PDC50 showed the highest content of

FNG, followed by eluates from PDD50, while the lowest quantity of FNG was found in the eluate from PP.

3.2 Activation of coagulation

3.2.1 Non-activated partial thromboplastin time

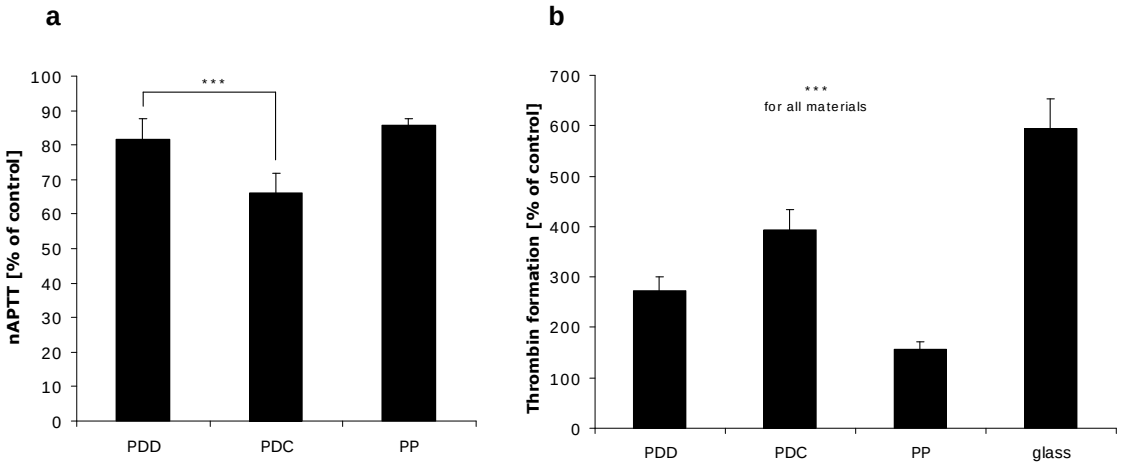


Figure 3: Activation of coagulation a) Non-activated partial thromboplastin time (nAPTT); b) Formation of thrombin in plasma in contact to the surface of the polymer samples. Glass serves as a highly activating reference surface for thrombin formation.

The reduction of non-activated partial thromboplastin time (nAPTT) was highest in plasma with contact to PDC where the coagulation time was reduced to about 66% of the control value (Fig. 3a). The response to PDD and PP was less pronounced, but still a significant shortening of the nAPPT compared to plasma without contact to the materials was found.

3.2.2 Formation of thrombin

The surfaces of PDD and PDC activated the formation of thrombin in plasma to a higher extend than the reference PP, but not as high as the glass surface (Fig. 3 b). Here PDD provoked formation of significantly less thrombin than PDC.

3.2.3 Contact activation

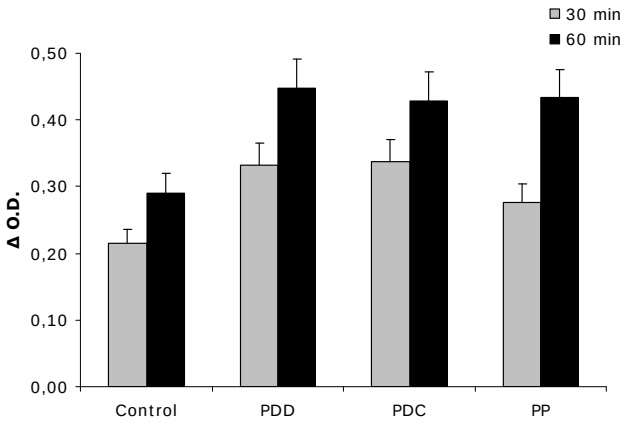


Figure 4: Contact activation as measured by plasma kallikrein formation (increase in optical density O.D. at 405 nm within 15 min) after 30 and 60 min contact of diluted Pooled Normal Plasma with the polymers

Compared to the control all materials showed a contact activation response of the plasmatic coagulation (Fig. 4) without significant differences in the activation of the plasma kallikrein formation between PDD and PDC. A slightly lower response to the reference material PP could be observed for the shorter incubation time interval (30 min).

3.3 Activation of complement system

PDC provoked the highest complement activation of the three polymers assessed, comparable to the response of the reference material PP (Fig. 5). PDD activated the complement to a lower degree than the other polymers.

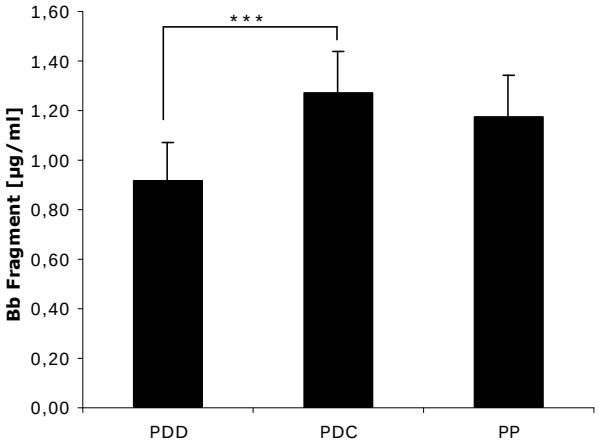
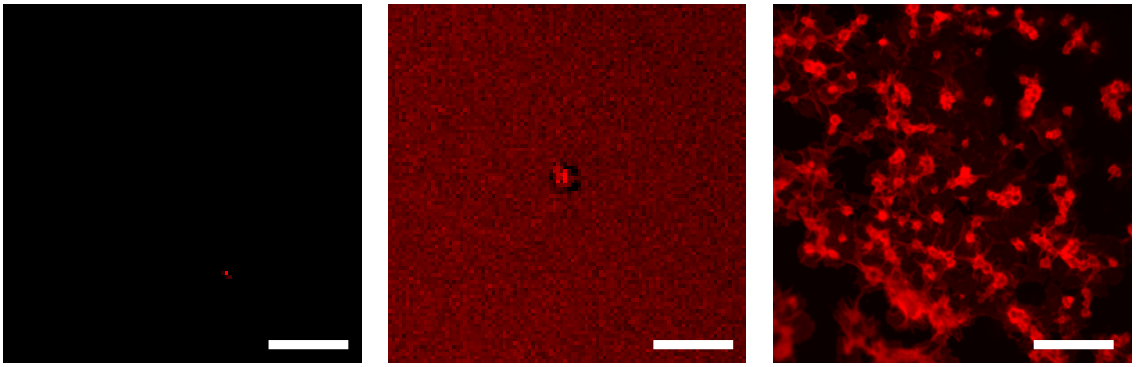


Figure 5: Activation of the complement system indicated by formation of Bb fragment.

3.4 Platelet adhesion and activation

Platelet activation was marked by a morphological change from rest discoid shape to varying degrees of spreading [8]. The platelet morphology and activation on the sample surfaces after incubation with PRP were studied by CLSM (Fig. 6). On both multiblock copolymers we observed only few, single round platelets in the examined sample fields (Fig. 6a, b). In contrast, platelets adhered on PP (Fig. 6c) formed large aggregates and a big fraction of them were well spread suggesting a high activation rate [8].



a b c

Figure 6: Immune fluorescence images of platelet's adhesion, activation and morphology on the sample surfaces PDD (a), PDC (b) and PP (c, from the left to the right; bar = 20 µm).

4. Discussion

In this study we investigated the hemocompatibility of two elastic multiblock copolymers in respect to their possible application for vascular regeneration. One of the main complications by using polymer implants in cardiovascular medicine is surface induced thrombosis [11], which includes the processes of adhesion of platelets, coagulation, and complement activation. Protein adsorption from blood plasma to the polymer surface is the first and the key process occurring on polymer surfaces and determines the subsequent platelet adhesion/activation and activation of coagulation cascade. To be effective in practical applications, protein resistant surfaces must be able to function as such in biological fluids containing many proteins at high concentrations. Blood plasma provides a multiple protein environment to assess protein resistance in a realistic setting for materials that may be used in blood contacting applications as it contains several hundred different proteins [5]. The analysis of plasma protein adsorption showed that both copolymer surfaces are not typical protein resistant surfaces as it can be seen for the non-surface treated PP (Fig.1). The diversity of the adsorbed proteins on both polymers as well as the higher protein quantity found on the

multiblock copolymers could assume activation of coagulation and adhesion of platelets. The protein-repelling property of PP might be considered as function of the crystalline-amorphous microstructure of its surface [12]. However, a higher protein adsorption induced by decreased crystallinity and polydispersity of PP was shown [13] also. There, the lower protein adsorption was found at a crystallinity of about 55 wt% and a polydispersity (M_w/M_n) of 3.7 and only a week shift in that values lead to an increased protein adsorption [13]. The polydispersity of the used multiblock copolymers is in a range of 2.7 (PDC) and 3.5 (PDD) which might be the reason for the increased protein adsorption on PDC. When the proportion between amorphous and crystalline content in multiblock copolymers was changed the protein adsorption was also decreased significantly (data not shown).

Nevertheless, the investigated multiblock copolymers attracted more proteins to the surface but the platelet adhesion was poor on these surfaces. One possible explanation for this result could be the altered protein conformation upon adsorption, which does not favor platelet adhesion. The physico-chemical characteristic of the surface such as surface wettability and the crystalline-amorphous structure were shown to have an impact on the conformational changes upon adsorption of the proteins [14,26]. The more hydrophobic nature of the multiblock-copolymers as well as the mainly amorphous microstructure of the copolymers (at 37 °C) [27] might be the reason for the non-favorable conformational changes of the adsorbed proteins and lack of platelet adhesion. In our previous study on endothelial cell adhesion on multiblock copolymers we also examined comparatively low initial cell adhesion [27]. By studying the activation of coagulation on both elastic multiblock copolymers we observed that the extent to which the different parameters of coagulation system were activated on both

polymers and the control PP surface varied significantly. For instance thrombin formation and complement activation were higher for PDC (Fig.3 and Fig.5). At the same time contact activation (plasma kallikrein) did not differ among the different studied polymers (Fig.4). This ambiguity can also be attributed to the activation of different parts of the coagulation system [25,29]. For instance in many cases the strong thrombin formation did not cause massive platelet adhesion [25]. However, a strong synergistic effect between platelet adhesion and contact activation has to be considered when the hemocompatibilty of the biomaterials is studied. Therefore, successful surface design strategies for hemocompatible materials need to preconceive carefully the interplay of both processes.

5. Conclusion

The plasma protein adsorption onto two biodegradable elastic multiblock copolymers PDD and PDC, from which the latter is also capable of a shape-memory effect, was investigated. In addition, we studied the activation of coagulation cascade as well as platelet adhesion/activation. When compared to PP the thrombin formation and complement activation were found to be higher for PDC than for PDD while the platelet adhesion and activation was lower for both elastic multiblock polymers compared to PP. Therefore the overall low thrombogenicity of these two novel copolymers make them potential candidates as scaffold or support materials for tissue engineering substitutes in blood vessel regeneration.

6. Acknowledgements

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References:

[1] Anonymous, Ethical guidelines for publication in Clinical Hemorheology and Microcirculation, *Clin Hemorheol Microcirc* **44** (2010), 1-2.

[2] O. Basaran, H. Karakayali, R. Emiroglu, S. Belli and M. Haberal, Complications and long-term follow-up of 4416 vascular access procedures, *Transplant Proc* **35** (2003), 2578-9.

[3] A. Battig, B. Hiebl, Y.K. Feng, A. Lendlein and M. Behl, Biological evaluation of degradable, stimuli-sensitive multiblock copolymers having polydepsipeptide- and poly(epsilon-caprolactone) segments in vitro, *Clinical Hemorheology and Microcirculation* **48** (2011), 161-172.

[4] R.M. Cornelius, J. Archambault and J.L. Brash, Identification of apolipoprotein A-I as a major adsorbate on biomaterial surfaces after blood or plasma contact, *Biomaterials* **23** (2002), 3583-7.

[5] M.A. Dobrovolskaia, A.K. Patri, J. Zheng, J.D. Clogston, N. Ayub, P. Aggarwal, B.W. Neun, J.B. Hall and S.E. McNeil, Interaction of colloidal gold nanoparticles with human blood: effects on particle size and analysis of plasma protein binding profiles, *Nanomedicine: Nanotechnology, Biology and Medicine* **5** (2009), 106-117.

[6] M. Grigoni, C. Daniele, G. D'Avenio and V. Barbaro, Biomechanics and hemodynamics of grafting, in vascular grafts: experiment and modeling. . in: A. Tura, (Ed.), Vascular Grafts: Experiment and Modeling., WIT Press, Boston, 2003, pp. 41–82.

[7] T. Groth, J. Synowitz, G. Malsch, K. Richau, W. Albrecht, K.P. Lange and D. Paul, Contact activation of plasmatic coagulation on polymeric membranes measured by the activity of kallikrein in heparinized plasma, *J Biomater Sci Polym Ed* **8** (1997), 797-807.

[8] J.M. Grunkemeier, W.B. Tsai and T.A. Horbett, Co-adsorbed fibrinogen and von Willebrand factor augment platelet procoagulant activity and spreading, *J Biomater Sci Polym Ed* **12** (2001), 1-20.

[9] B. Hiebl, C. Mrowietz, J. Goers, M. Bahramsoltani, J. Plendl, K. Kratz, A. Lendlein and F. Jung, In vivo evaluation of the angiogenic effects of the multiblock copolymer PDC using the hen's egg chorioallantoic membrane test, *Clin Hemorheol Microcirc* **46** (2010), 233-8.

[10] A. Higuchi, K. Sugiyama, B.O. Yoon, M. Sakurai, M. Hara, M. Sumita, S. Sugawara and T. Shirai, Serum protein adsorption and platelet adhesion on pluronic (TM)-adsorbed polysulfone membranes, *Biomaterials* **24** (2003), 3235-3245.

[11] F. Jung, C. Wischke and A. Lendlein, Degradable, Multifunctional Cardiovascular Implants: Challenges and Hurdles, *MRS Bulletin* **35** (2010), 607-613.

[12] N. Kawamoto, H. Mori, M. Terano and N. Yui, Blood compatibility of polypropylene surfaces in relation to the crystalline-amorphous microstructure, *J Biomater Sci Polym Ed* **8** (1997), 859-77.

[13] N. Kawamoto, H. Mori, N. Yui and M. Terano, Mechanistic aspects of blood-contacting properties of polypropylene surfaces--from the viewpoint of macromolecular entanglement and hydrophobic interaction via water molecules, *J Biomater Sci Polym Ed* **9** (1998), 543-59.

[14] J.K. Kim, E.A. Scott and D.L. Elbert, Proteomic analysis of protein adsorption: serum amyloid P adsorbs to materials and promotes leukocyte adhesion, *J Biomed Mater Res A* **75** (2005), 199-209.

[15] M.L. Knetsch, Y.B. Aldenhoff and L.H. Koole, The effect of high-density-lipoprotein on thrombus formation on and endothelial cell attachement to biomaterial surfaces, *Biomaterials* **27** (2006), 2813-9.

[16] B. Leithaeuser, F. Jung and J.W. Park, Rheological and hemostasiological aspects of thrombus formation in the left atrial appendage in atrial fibrillation? A new strategy for prevention of cardioembolic stroke, *Clin Hemorheol Microcirc* **45** (2010), 311-23.

[17] W. Lemm, (Ed.), The Reference Materials of the European Communities: Results of Hemocompatibility Tests Kluwer Academic Publishers, Dordrecht, Boston, London, 1992.

[18] A. Lendlein, M. Behl, B. Hiebl and C. Wischke, Shape-memory polymers as a technology platform for biomedical applications, *Expert Review of Medical Devices* **7** (2010), 357-379.

[19] A. Lendlein and S. Kelch, Shape-memory polymers as stimuli-sensitive implant materials, *Clinical Hemorheology and Microcirculation* **32** (2005), 105-116.

[20] A. Lendlein and R. Langer, Biodegradable, elastic shape-memory polymers for potential biomedical applications, *Science* **296** (2002), 1673-6.

[21] Y. Onuma, J. Daemen, N. Kukreja and P. Serruys, Revascularization in the high-risk patient: multivessel disease, *Minerva Cardioangiol* **55** (2007), 579-92.

[22] D. Rickert, M.A. Moses, A. Lendlein, S. Kelch and R.P. Franke, The importance of angiogenesis in the interaction between polymeric biomaterials and surrounding tissue, *Clin Hemorheol Microcirc* **28** (2003), 175-81.

[23] Y. Song, J. Feijen, D.W. Grijpma and A.A. Poot, Tissue engineering of small-diameter vascular grafts: a literature review, *Clin Hemorheol Microcirc* **49** (2011), 357-74.

[24] Y.Y. Song, Y. Li, C. Yang and X.H. Xia, Surface electric field manipulation of the adsorption kinetics and biocatalytic properties of cytochrome c on a 3D macroporous Au electrode, *Analytical and Bioanalytical Chemistry* **390** (2008), 333-341.

[25] C. Sperling, M. Fischer, M.F. Maitz and C. Werner, Blood coagulation on biomaterials requires the combination of distinct activation processes, *Biomaterials* **30** (2009), 4447-4456.
[26] R. Tzoneva, M. Heuchel, T. Groth, G. Altankov, W. Albrecht and D. Paul, Fibrinogen adsorption and platelet interactions on polymer membranes, *J Biomater Sci Polym Ed* **13** (2002), 1033-50.
[27] R. Tzoneva, C. Weckwerth, B. Seifert, M. Behl, M. Heuchel, I. Tsoneva and A. Lendlein, In Vitro Evaluation of Elastic Multiblock Co-polymers as a Scaffold Material for Reconstruction of Blood Vessels, *J Biomater Sci Polym Ed* **22** (2011), 2205–2226.
[28] D.A. Vorp, T. Maul and A. Nieponice, Molecular aspects of vascular tissue engineering, *Front Biosci* **10** (2005), 768-89.
[29] C. Werner, M.F. Maitz and C. Sperling, Current strategies towards hemocompatible coatings, *Journal of Materials Chemistry* **17** (2007), 3376-3384.
[30] J.P. Wood, J.R. Silveira, N.M. Maille, L.M. Haynes and P.B. Tracy, Prothrombin activation on the activated platelet surface optimizes expression of procoagulant activity, *Blood* **117** (2011), 1710-1718.
[31] L. Xue and H.P. Greisler, Biomaterials in the development and future of vascular grafts, *J Vasc Surg* **37** (2003), 472-80.