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Biological Evaluation of Degradable, Stimuli-sensitive Multiblock Copolymers having Polydepsipeptide- and Poly(*ε*-caprolactone) segments *in vitro*

Alexander Battig[1,2], Bernhard Hiebl[1], Yakai Feng[2,3], Andreas Lendlein[1,2], Marc Behl[1,2]*

[1]Center for Biomaterial Development and Berlin Brandenburg Center for Regenerative Therapies, Helmholtz-Zentrum Geesthacht, Kantstr. 55, 14613 Teltow, Germany

[2]Tianjin University-Helmholtz-Zentrum Geesthacht, Joint Laboratory for Biomaterials and Regenerative Medicine, Weijin Road 92, 300072 Tianjin, China. Kantstr. 55, 14513 Teltow, Germany

[3]School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, P. R. China

* To whom correspondence should be addressed. Email: andreas.lendlein@hzg.de, Tel: (0049) 03328-352450, Fax: (0049) 03328-352452

Abstract:

Polydepsipeptides, alternating copolymers consisting of *α*-amino acids and *α*-hydroxy acids, are degradable polymers. Depsipeptide-based polymers of varied architectures can be synthesized via ring-opening polymerization of various morpholine-2,5-dione derivatives. Thermoplastic phase-segregated multiblock copolymers with poly(*ε*-caprolactone) (PCL) and poly(*iso*-butyl-morpholindion) segments have been synthesized from macrodiols and an aliphatic diisocyanate as a coupling agent. The respective multiblock copolymers showed shape-memory capabilities and good elastic properties, making them attractive candidates for potential application as biomaterials for controlled drug release systems, scaffolds to

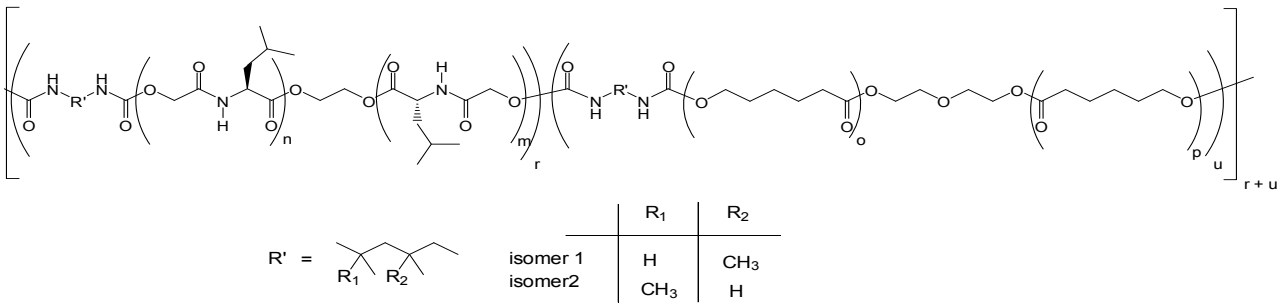
be applied in tissue engineering or biofunctional implants. Thus, these abilities cumulate to form multifunctional materials, combining degradability with shape-memory capability. The advantage of depsipeptide-based multiblock copolymers compared to previously reported poly(ether)ester-derived biomaterials having shape-memory property may result from their different degradation products, as the resulting α-amino acids may act as a buffer for the hydroxy acids, thereby stabilizing pH values. In this context, we report on the biological evaluation of material samples in accordance with international standards (ISO10993). Here, extracts of the substrates were exposed to a continuous fibroblast like cell line (L929) to study cytocompatibility of the extractable substrates. Cell viability, morphology, LDH-release (as a parameter for the functional integrity of the cell membrane), activity of the mitochondrial dehydrogenases (as a parameter of the cell activity) and assembly of the actin- and vinculin cytoskeleton indicated no incompatibilities between extracts and L929 cells. These results suggest that depsipeptide-based multiblock copolymers are promising candidates for soft substrates in use as multifunctional cell culture devices or *in vivo* implants.

1. Introduction:

Poly(*α*-hydroxy alkanoates) like poly(*L*-lactide) or copolymers from *L,L*-dilactide and diglycolide have widespread potential applications in biomedicine as resorbable implants, biodegradable sutures, and matrices for controlled drug release.[33; 42] Polydepsipeptides are alternating copolymers of α-amino acids and α-hydroxy acids. They are known to be non-toxic and degradable *in vitro* and *in vivo.* Synthetic biodegradable polydepsipeptides have been investigated for biomedical and pharmaceutical applications.[14; 23; 36] Different combinations of an α-amino acid (e.g., *L*-leucine, *L*-valine, glycine, *L*-lysine or *L*-glutamic acid) and an α-hydroxy acid (e.g., glycolic acid, *L*-lactic acid, or *rac*-lactic acid) result in materials, in which hydrophilicity, crystallinity, mechanical properties and degradation properties can be varied in a wide range. A common synthetic route towards polydepsipeptides is the ring-opening polymerization (ROP) of morpholine-2,5-dione derivatives in the presence of tin dioctanoate ($Sn(oct)_2$) as catalyst.[27; 26; 31; 37]

A strategy for improving the elastic properties of copoly(ether)esters is the synthesis of multiblock copolymers containing two different (co)poly(ether)ester segments.[20] The starting materials for the preparation of these phase segregated polymers are dihydroxy telechelic oligomers.[19] Thermoplastic multiblock copolymers of semicrystalline poly(*ε*-caprolactone) (PCL) and poly(*p*-dioxanone) blocks were reported as biodegradable shape-memory polymers[18] and their adsorption of adhesive proteins as well as the interaction with human umbilical endothelial cells was explored.[32] Biodegradable poly[(*rac*-lactide)-*ran*-glycolide]-urethane networks with and without shape-memory properties).[2; 39] as well as poly(ε-caprolactone)/poly(ω-pentadecalactone)-based polymer networks having triple-shape properties were synthesized via coupling of star-shaped oligomers using a racemic mixture of 2,2,4- and 2,4,4-trimethylhexamethylene diisocyanate

(TMDI).[41; 40] Using well-defined star-shaped telechelic oligomers automatically defines the functionality of netpoints introduced, which is determined by the number of arms having functional endgroups.[21] While the shape-memory capability is of great interest for biomedical applications such as self-tightening knots in suture materials or smart medical devices[4; 18] other application fields are automotive, textiles and sensors.[3; 5; 11; 13; 15] Recently, multiblock copolymers named PCL-PIBMD50 having polydepsipeptide segments based on 3-(*S*)-*iso*-butyl-morpholine-2,5-dione and PCL segments capable of a shape-memory effect were reported (Scheme 1).[8] The incorporation of a polydepsipeptide segment in such multiblock copolymers is thought to combine the advantageous degradation behavior of the depsipeptide segment with the shape-memory capability of multiblock copolymers with PCL switching segment.[9] Furthermore, it is expected that the α-amino acids from the degradation of the depsipeptides may buffer the hydroxy acids resulting from the degradation of the PCL segment and may therefore minimize or avoid inflammation reactions during degradation of an implant.[1]



Scheme 1. Multiblock copolymer PCL-PIBMD, which were prepared using TMDI (2,2,4 and 2,4,4 isomers) to build up junction units

In the multiblock copolymers, the polydepsipeptide segments forming crystalline domains with a high melting temperature (T_m = 170 °C) act as hard segments. The crystallizable PCL segments work as switching segment. $T_{m,PCL}$ can be adjusted

between room temperature and body temperature by adjusting the molecular weight of the PCL-diols used in the synthesis. PCL-diol with a molecular weight of 2900 $g \cdot mol^{-1}$ was selected as it exhibits a T_m around body temperature. The polydepsipeptide segments also form amorphous domains with a glass transition temperature around $T_{m,PCL}$ to support the stabilization of the temporary shape. The macroscopic shape-memory effect of the multiblock copolymer is shown in figure 1.

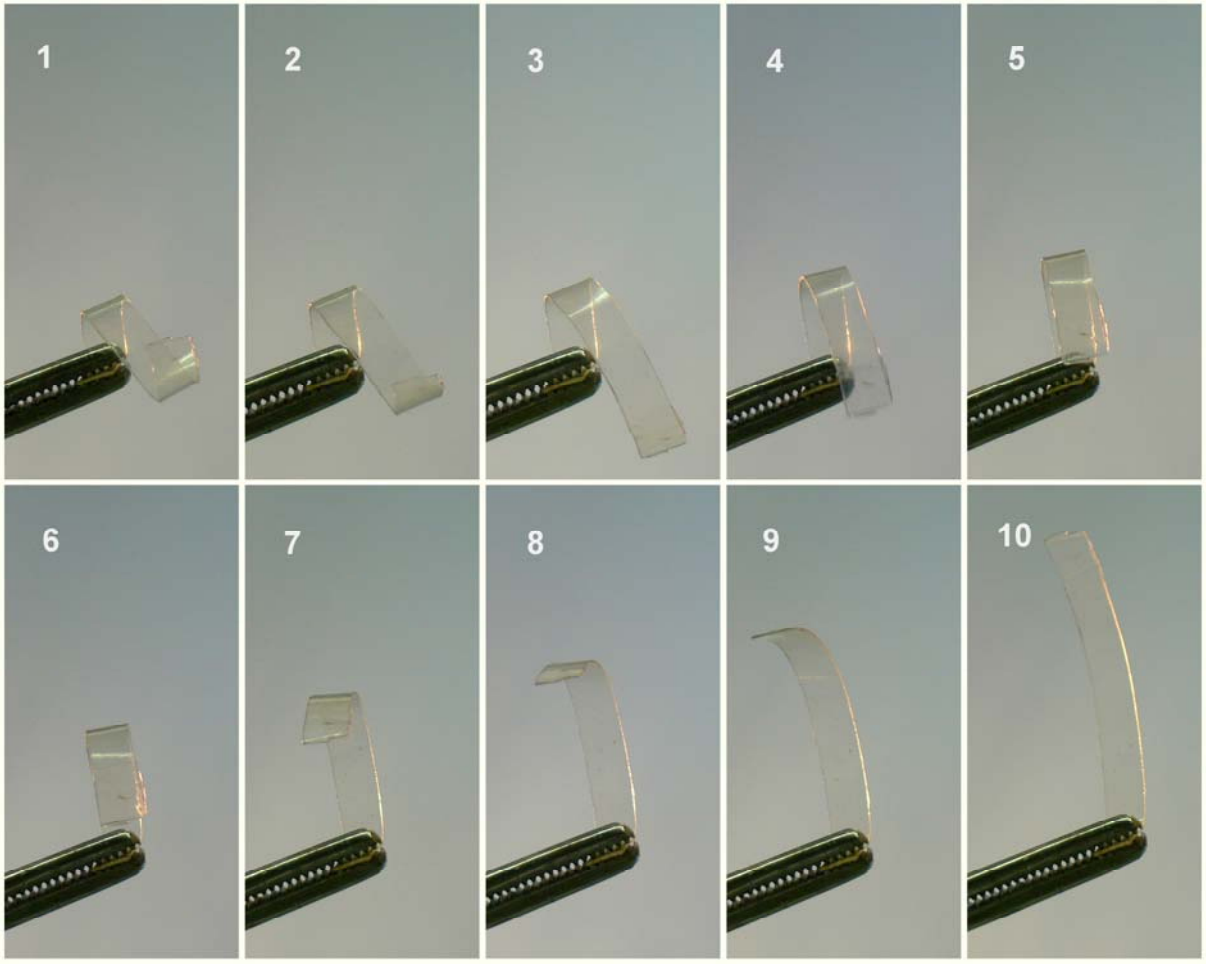


Figure 1. The series of photographs (1-10) demonstrates the macroscopic shape-memory effect for PCL-PIBMD50. The transition from the temporary shape (“corkscrew’’, 1) to the permanent shape (“stripe” 10) took approx. 30 s at 60 ºC.

In this paper we report about the biological evaluation of PCL-PIBMD50 multiblock copolymers. Chemical, thermal and mechanical properties of the multiblock copolymers were determined to provide a basic material characterization.[8] Viability,

morphology, lactate dehydrogenase (LDH)-release and mitochondrial activity (MTS-assay) of L929 cells after exposure to PCL-PIBMD50 extracts were studied in strong accordance to EN DIN ISO 10993-5 and 10993-12. The integrity of the cell-contacts to the substrate and to neighbored cells was also addressed by analysis of the cytoskeletal proteins actin and vinculin those arrangement is an indicator for focal adhesions. The biological tests were performed in order to establish depsipeptide-based multiblock copolymers as non-toxic, medical grade materials. The results of these studies and their meaning are discussed in this paper.

2. Experimental Part

2.1. Materials

PCL-diol with number average molecular weight (M_n) 2900 g:mol^{-1} and a polydispersity of 1.72 was obtained from Solvay Caprolactones (Warrington, UK). The other reagents, such as, TMDI, *iso*-leucin (both *Aldrich*, Munich, Germany), and solvents (*Merck*, Darmstadt, Germany), were of commercial grade and used without further purification.

2.2. Synthesis

The monomer 3-(*S*)-Isobutyl-morpholine-2,5-dione (IBMD), (yield: 29%, m.p. 129-130 °C) and the dihydroxy telechelic depsipeptide oligo(3-(*S*)-*iso*-butyl-morpholine-2,5-dione) diol (PIBMD-diol) (yield: 80%, m.p. 170 °C) were synthesized as previously described.[12]

The synthesis of PCL-PIBMD50 multiblock copolymer from PCL-diol and PIBMD-diol was conducted with a slight variation to the procedure as previously described.[8] Here, *N*-Methyl-2-pyrrolidone (NMP) was used as a solvent instead of dimethylformamide (DMF). Yield: 90%, m.p. 37 °C (PCL-blocks), 170 °C (PIBMD blocks) for PCL-PIBMD50.

2.3. Methods

GPC

Molecular weights of depsipeptide oligomers and multiblock copolymers were determined on a multidetector GPC, which consisted of a GRAM VS1 precolumn (40 mm x 4.6 mm), a GRAM 30Å 5091312 and a GRAM 1000Å 71111 column (both 250 mm x 4.6 mm) (all *PSS*, Mainz, Germany), a CO-200 column oven (*W.O. electronics*, Langenzersdorf, Austria), an isocratic pump 980, an automatic injector 851-AS, a LG 980-02 ternary gradient unit, a multiwave length detector MD-910, a RI detector RI-930 (all *Jasco*, Gross-Umstadt, Germany), a differential viscometer η-1001 (*WGE Dr. Bures*, Dallgow-Doeberitz, Germany), a Wyatt miniDawn Tristar light scattering detector (*Wyatt Technology Corporation*, Santa Barbara, USA), a degasser ERC-3315α (*Ercatech*, Berne, Switzerland), and dimethylformamide (0.4 wt% toluene as internal standard, 35 °C, 1.0 $mL \cdot min^{-1}$) as eluent by universal calibration with polystyrene standards using WINGPC 6.2 (*PSS*) software.

DSC

DSC was performed on a Netzsch (Selb, Germany) DSC 204 apparatus, equipped with a low temperature cell. 5 to 15 mg of the sample was heated from -100 °C to 200 °C at a heating rate of 10 $K \cdot min^{-1}$, and kept at 200 °C for 2 min. Subsequently, it was cooled at the same rate. After 2 min at the low temperature, a second heating run was performed. The thermal transitions were evaluated from the second heating run.

Films

Films (400 µm thick) were prepared from PCL-PIBMD50 by compression moulding at 180 °C, 90 bar using a Dr. Collin (Ebersberg, Germany) P200E laboratory press. The films were tempered at 100 °C for 30 min then at 80 °C for 24 h, and finally slowly cooled to room temperature.

Mechanical and thermomechanical testing

Mechanical properties at different temperatures were assessed by tensile tests using a ZWICK1425 tensile tester (*Zwick Roell GmbH*, Ulm, Germany) equipped with a load cell capable of forces up to 50 N combined with thermo chamber (Climatic Systems LTD, model 091250) and controlled by a Eurotherm 902-904 unit (*Eurotherm LTD*, Worthing, UK). The deformation rate was 10 $mm \cdot min^{-1}$. The samples were annealed for 20 min at the operating temperature before each experiment. Bone-shaped samples with dimensions of 30 mm × 3 mm (parallel area), a thickness of 0.1 mm - 0.3 mm and a free length of the clamped samples of 20 mm were used. Tensile tests for each sample were repeated four times.

The stress-controlled cyclic thermomechanical test was performed as follows: the sample of PCL-PIBMD50 was stretched at T_{high} (50 °C) to a maximum elongation ε_m of 50% at an elongation rate of 10 $mm \cdot min^{-1}$. After 5 min the stretched sample was cooled down to T_{low} (-5 °C) under stress-control and kept for 15 min at this temperature. The fixed sample was unloaded to σ = 0 MPa and the temperature was raised to T_{high} at a heating rate of 5 $K \cdot min^{-1}$ and held there for 10 min. This program was repeated five times with the same sample (N = 5).

The strain fixity rate R_f and the strain recovery rate R_r were calculated from Eq. (1) and (2), respectively.

$$R_f(N) = \frac{\varepsilon_u(N)}{\varepsilon_l(N)} \qquad (1)$$

$$R_r(N) = \frac{\varepsilon_l(N) - \varepsilon_p(N)}{\varepsilon_l(N) - \varepsilon_p(N-1)} \qquad (2)$$

where $\varepsilon_l(N)$ is the tensile strain of a loaded sample after cooling in a cyclic, thermomechanical experiment in the N^{th} cycle, $\varepsilon_u(N)$ is the strain in the stress-free

state after the retraction of the tensile stress in the N^{th} cycle, $\varepsilon_p(N-1)$ and $\varepsilon_p(N)$ are the strain of the sample in two successively passed cycles in the stress-free state.

Headspace GC

The samples were injected into the headspace (Headspace Sampler HP7694, *Hewlett Packard*, Santa Clara, USA) and were equilibrated for 30 min at 90 °C and were finally transferred into the GC (Gaschromatograph 5890 Series II, *Hewlett Packard*, Santa Clara,USA) where they were heated from 100 °C to 200 °C. For detection purposes, a column (DB 624, *J&W Scientific*, Folsom USA) equipped with a flame ionization detector was used.

Sample sterilization and preparation

All samples were sterilized by dry heat sterilization (160 °C, exposure time: 120 min) prior to the biological tests. Cytotoxicity tests were performed using disc shaped samples (diameter: 13.0 mm, thickness: 1.0 mm). The samples (n = 16) were exposed to serum-free cell culture medium under permanent stirring at 37 °C for three days. The resulting extract was used as cell culture medium for the L929 cells. The serum-free cell culture medium served as control.

Culture of L929 cells

The substrate was tested for cytotoxic effects using *in vitro* cell tests under static conditions. According to the international standard EN ISO 10993-5 cytotoxicity tests were performed with cells derived from normal subcutaneous areolar and adipose tissue of a 100-day-old male C3H/An mouse (L929 cells provided by the American Type Culture Collection, *ATCC*, Wesel, Germany). For cell expansion continuous subcultures of these cells were maintained under standard environmental conditions (humidified atmosphere, 5 vol% CO_2 in air, 37 °C) on polystyrene using DMEM cell culture medium (A*TCC*) supplemented with 10% horse serum (*ATCC*) and 1% penicillin/streptomycin (*Invitrogen*, Darmstadt, Germany). Every second day, the

culture medium was changed. The cells were subdivided when they reached a subconfluence of about 80% of the growth area by rinsing them with phosphate buffered saline, and by subsequent trypsinization using trypsin-EDTA solution (trypsin 0.05% and ethylene-diaminetetraacetic acid 0.02%). Cell tests were performed with L929 cells which were seeded on a polystyrene based cell culture 24-multiwell plate (*Corning*, Wiesbaden, Germany) and which established a subconfluence cell layer of about 80% of the growth area. For all biological tests serum-free cell culture medium was used.

Endotoxin load

The endotoxin load of the samples was measured with the QCL-1000® Limulus Amebocyte Lysate Test (U.S. License No. 1775, *Lonza*, Cologne, Germany) in accordance to the “Guideline on Validation of the Limulus Amebocyte Lysatetest as an End-product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices” (U.S. Department of Health and Human Services, Public Health Service, Food and Drug Administration, 1987).

Cell viability

Cell viability was assessed using fluorescein diacetate (FDA) and propidium iodide (PI) staining. Adherent cells were rinsed with PBS and immersed in 500 µl/well FDA/PI solution made by diluting 5 µL × 5 mg FDA/mL acetone and 2 µL × 1 mg PI/mL PBS in Eagle's minimal essential medium (EMEM, *Biochrom*, Berlin, Germany). After 15 min at 37 °C in the dark, cells were rinsed twice in PBS. While still immersed in PBS, cells were then subjected to fluorescence microscopy with excitation at 488 nm and detection at 530 nm (FDA) and 620 nm (PI).

Cell morphology assessment

Using phase contrast microscopy in transmission (*Zeiss*, Jena, Germany) the morphology of the cells was evaluated based on the cell shape, the formation of

intracellular vacuoles and the organization of the cell layer. This parameter portfolio was chosen according to the USP 23-NF18 (US Pharmacopeial Convention) and the EN DIN ISO 10993-5.

Lactat dehydrogenase (LDH)-release measurement

After incubating the L929 cells with the sample extracts for 48 h at 37 °C in a humidified atmosphere with 5% CO_2, the integrity of the cell plasma membrane was tested by measuring the activity of the LDH in the cell culture medium by an enzymatic method (LDH-assay, *Roche*, Mannheim, Germany) at 492 nm using a TECAN SpectraFluor Plus spectrophotometer (*TECAN*, Männedorf, Swiss). LDH acts as a catalyst for the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD+. LDH is located solely within the confines of the cell; by testing for any LDH in the extracellular matrix (ECM), an assessment of LDH release can be studied. If elevated levels of LDH are found in the ECM, the integrity of the cells has been compromised.

Mitochondrial function studies

Study of the mitochondrial function by analyzing the activity of the mitochondrial dehydrogenases is a measurement of cell activity. As many cell processes require the activity of mitochondria, a measurement of mitochondrial dehydrogenase helps to establish the level of cell activity. After 48 h of cell exposure to the sample extract the mitochondrial activity was measured by the tetrazolium compound 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS), which is reduced by cells into a coloured formazan product that is soluble in tissue culture medium (CellTiter 96® AQueous One Solution Cell Proliferation Assay, *Promega*, Mannheim, Germany). This conversion is presumably accomplished by NADPH or NADH produced by dehydrogenase enzymes in metabolically active

cells.[6] The absorbance of the colored formazan product was measured at 490 nm using the TECAN SpectraFluor Plus spectrophotometer.

Fluorescent staining

Fluorescent staining was used to visualize vinculin and actin filaments (F-actin). The cells were fixed with paraformaldehyde (3 vol%, 0.9 vol% NaCl, 30 min, 0 °C) and pre-treated with Triton X-100 (0.5 vol%). Vinculin was immunochemically visualized (polyclonal rabbit anti-vinculin IgG, 1:50; polyclonal goat anti-rabbit IgG, conjugated with Cy2, 1:20; both Abcam, USA). For F-actin staining BODIPYTM 558/568 phalloidin (1:20, Invitrogen, Germany) was used. From every sample five different fields of view were analyzed with a confocal laser scanning microscope (LSM 510 META, Zeiss, Germany).

Statistics

Data are given as mean value ± standard deviation for continuous variables and analyzed by a two-sided Student's t-test for paired samples. A p value of less than 0.05 was considered significant.

3. Results and Discussion

Properties of PCL-PIBMD50 (Structural, mechanical, thermal, shape-memory)

The molecular weight M_n of PCL-PIBMD50 was equal to 62000 $g \cdot mol^{-1}$, as was determined by GPC using DMF as eluent, with a molecular weight distribution of D = 1.7. In DSC experiments PCL-PIBMD50 displayed a glass transition temperature T_g = -60 °C. In addition, a melting temperature T_{m1} = 38 °C, with a heat of fusion ΔH_{m1} = 53.2 $J \cdot g^{-1}$, which corresponds to the PCL segments of the multiblock copolymer and a second melting peak at T_{m2} = 170 °C with a heat of fusion ΔH_{m2} = 4.8 $J \cdot g^{-1}$, which corresponds to the PIBMD segments, was found. Mechanical testing showed a Young's modulus of E = 200 ± 50 MPa at 25 °C with a tensile stress of σ_b = 14.5 ±

2.1 MPa and a resulting elongation ε_b = 680% ± 80% at the breaking point. At 75 °C, a Young's modulus of E = 30 ± 9.0 MPa with a tensile stress of σ_b = 2.8 ± 0.1 MPa and a resulting elongation ε_b = 70% ± 11% at the breaking point was determined. The strain fixity rate and the strain recovery rate of the first cycle was R_f = 97.8% and R_r = 97.1%, respectively. From the chemical, physical and mechanical properties it can be concluded that the material is quite similar to the previously reported material.[8]

Residual solvent and endotoxin load

In head-space GC measurements the residual amounts of solvents used during synthesis such as DMF and diethylether was below the detection limit while for NMP a slight residual content of 79 ppm was determined. The endotoxin content of the sample extract was <0.06 EU/mL (n=2) and thus below the threshold value (0.5 EU/ml) given by the FDA guidelines for medical products, which qualified the material for further biocompatibility investigations. The multiblock copolymer PCL-PIBMD50 was tested for cytocompatibility with L929 cells via the study of the cell viability, the cell morphology including the assembly of the actin- and vinculin assembly, the LDH-release and the mitochondrial activity. These tests aimed to study the effects of the polymer's performance *in vitro* prior to actual implantation. All cytotoxicity examinations were performed according to the EN DIN ISO 10993-5 and 10993-12.

Cell viability and morphology

Viability staining of the cells revealed that 97.2% were viable after 48 h of cell exposure to an extract of PCL-PIBMD50. The viability was not significantly different to that of the control (96.7%, p>0.05). This shows that the extract of PCL-PIBMD50 does not exhibit cytotoxic effects on the L929 cells.

The morphological phenotype of the L929 cells gave also no hint to a cytotoxic effect. Cell exposure to the PCL-PIBMD50 extract for 48 h caused no cell lysis, only

rare round and loosely attached cells and only small and discrete intracellular vacuoles (Fig. 2). Similar to the control the cells showed a typical spreaded and spindly (sparse cells) or ovoid (confluent L929 layer) morphology. Cells exposed to the sample extract established even a more tightly packed cell layer than after exposure to the cell culture medium only. Cell density after exposure to the PCL-PIBMD50 extract for 48 h was 1356±22 cells·mm^{-2} and significantly higher ($p<0.05$) than after cell exposure to pure cell culture medium only (904±17 cells·mm^{-2}) for the same period of time.

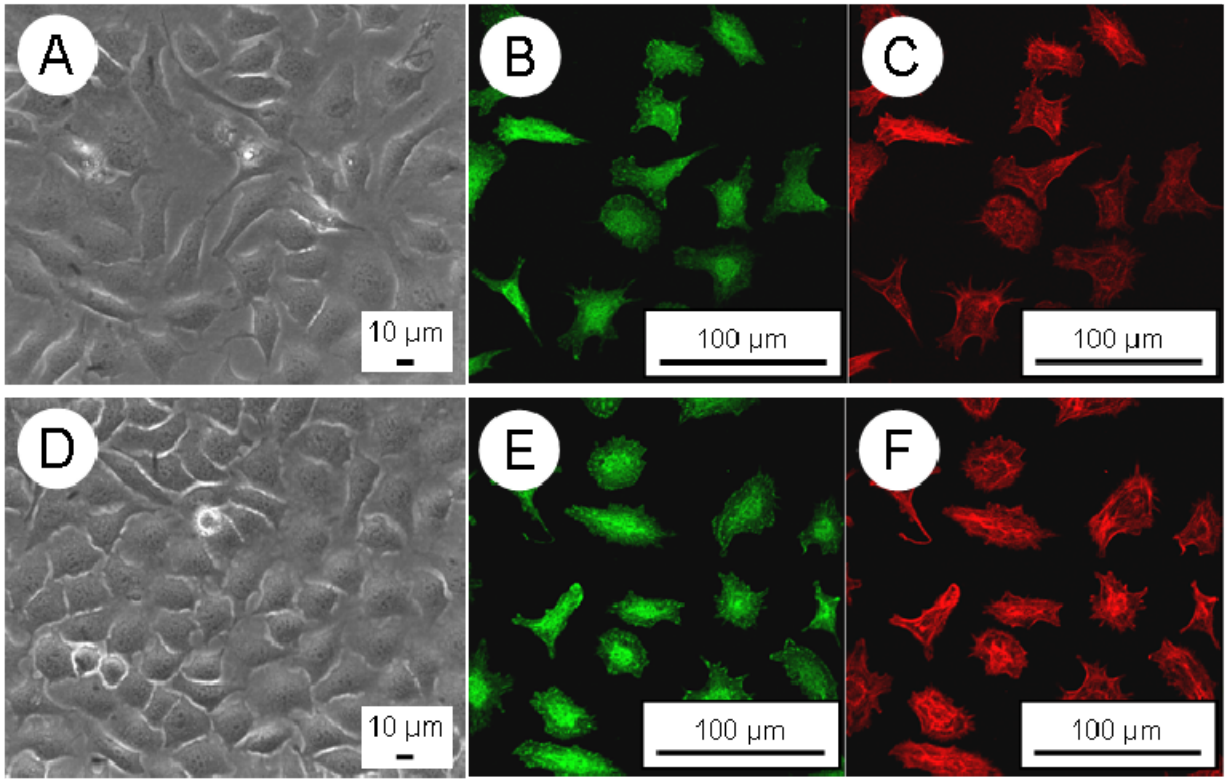


Figure 2. L929 cells 48h after culturing with pure culture medium (A, B, C, control) and a 72 h extract of PCL-PIBMD50 (D, E, F), primary magnification 40× ; (A, D) phase contrast microscopy in transmission; confocal laser-scanning microscopy after vinculin staining (B, E) and actin staining (C, F).

LDH-release and mitochondrial activity

After cell exposure to the PCL-PIBMD50 extract for 48 h the LDH-release was not significantly ($p>0.05$) different to the LDH-release seen 48 hours after culturing L929 with pure cell culture medium (control) (Fig. 3). This indicates that the sample had no negative influence on the functional integrity of the outer cell membrane. In contrast cell exposure to the PCL-PIBMD50 extracts caused an significant increase of the mitochondrial activity ($p<0.05$) and cellactivity respectively.

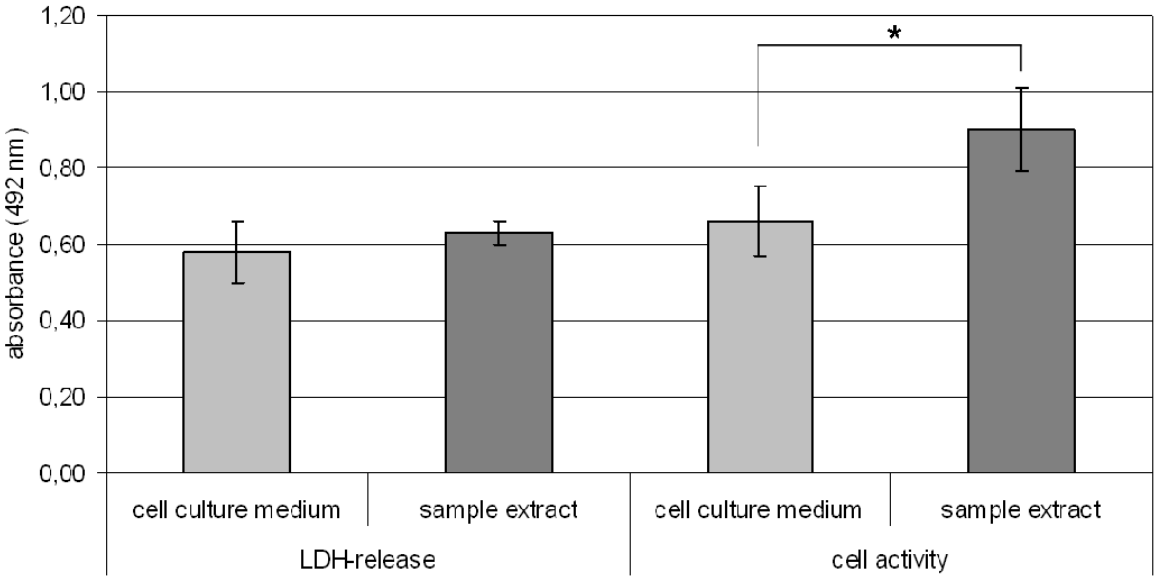


Figure 3. Lactate dehydrogenase release (LDH, light grey) and mitochondrial activity (dark grey) 48 h after culturing L929 cells with pure cell culture medium (control) and with a 72 h extract of the sample; LDH-release assay (Roche, Germany), MTS-assay (Promega, Germany) means ± standard deviation (SD), n=8.

Cell contact assessment

F-actin and vinculin staining revealed no obvious differences in the arrangement of both cytoskeletal proteins between the cells which were cultured with pure cell culture medium (control) and the PCL-PIBMD50 extract. Vinculin as well as F-actin were almost homogenously distributed in the cell body (fig. 2 B, E). However, after cell exposure to the PCL-PIBMD50 extract vinculin and stress fibres were more

prominent (Fig. 2 F) than in the cells which were grown in cell culture medium only (Fig. 2 C).

4. Conclusion

Multiblock copolymers containing polydepsipeptide PIBMD and PCL segments, previously synthesized and characterized, underwent biological evaluation and their effects on cell viability, morphology, LDH-release, mitochondrial activity, and assembly of the F-actin and vinculin-cytoskeleton were examined.

Biological studies showed that cell exposure to sample extracts did not cause decreased cell viability. 97% of the cells, which were grown in PCL-PIBMD50 extract or in pure cell culture medium were viable. This shows that the sample extracts did not increase cell death by apoptosis. According to the cytotoxicity levels given by the USP 23-NF18 (US Pharmacopeial Convention) and the EN DIN ISO 10993-5 the morphological phenotype of the cells and of the (subconfluent) cell layer exhibited no hints to cytotoxicity after exposure to the PCL-PIBMD50 extracts. Additionally PCL-PIBMD50 extracts did not cause increased LDH-release and disturbance of the integrity of the outer cell membrane respectively.

However, cell exposure to sample extracts resulted at least in an increase of the cell activity (MTS test), which was probably due to a higher cell proliferation and followed by a more tightly packed cell layer than seen in the control. The higher cell activity resulted also in changes of the vinculin and actin assembly. Vinculin is a ubiquitously expressed protein in focal adhesion plaques that is involved in linkage of integrin adhesion molecules to the actin cytoskeleton.[16; 25] For this reason vinculin is frequently used as a marker for both cell–cell and cell–extracellular matrix (focal adhesion) adherens-type junctions.[38] Actin is the main element stabilizing cell-contacts[29] and composed of netlike oriented actin filaments in the cell cortex with

increased actin density at the cell-cell- and cell-ECM-contact points.[10; 22; 34] The cell-ECM contacts are further stabilized by contractile and linearly oriented actin filament bundles called stress fibers.[7; 17] These actin stress fibers are known to make connections with focal adhesion complexes (FA),[28] which are known to link between the ECM and the cytoskeleton regulating cell functions including proliferation and migration.[30; 35] PCL-PIBMD50 extracts did not cause changes in the vinculin and actin assembly in the cell body. Both cytoskeletal proteins were almost homogenously distributed after sample extract exposure. However, vinculin and actin were more prominent in the cells which were grown with the PCL-PIBMD50 extracts. This might hint to stronger focal adhesions and stronger cell-substrate interaction respectively.

These results show that multiblock copolymers based on the polydepsipeptide PIBMD with PCL show a very promising level of cytocompatibility. Previous examinations found these materials to possess good thermomechanical properties, good biodegradability and shape-memory properties with switching temperatures within range of body temperature. These newest findings help to ascertain the biocompatibility of these versatile multiblock copolymers and lead to the establishment of multifunctional polymers with a wide range of biomedical applications including self anchoring implants for controlled drug release.[24]

Acknowledgement

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