Coating of Poly(*p*-xylylene) by PLA-PEO-PLA Triblock Copolymers with Excellent Polymer−Polymer Adhesion for Stent Applications

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Poly(*p*-xylylene) (PPX) was deposited by chemical vapor deposition (CVD) on stainless steel substrates. These PPX films were coated by solution casting of poly(lactide)-poly(ethylene oxide)-poly(lactide) triblock copolymers (PLA-PEO-PLA) loaded with ^{14}C-labeled paclitaxel. Adhesion of PLA-PEO-PLA on PPX substrate coatings was measured using the blister test method. Excellent adhesion of the block copolymers on PPX substrates was found. Stress behavior and film integrity of PLA-PEO-PLA was compared to pure PLA on unexpanded and expanded stent bodies and was found to be superior for the block copolymers. The release of paclitaxel from the biodegradable coatings was studied under physiological conditions using the scintillation counter method. Burst release of paclitaxel was observed from PLA-PEO-PLA layers regardless of composition, but an increase in paclitaxal loading was observed with increasing content of PEO.

Introduction

Recent developments in the treatment of atherosclerosis have focused on the use of metallic implants (stents) to reduce restenosis after percutaneous transluminal coronal angioplasty (PTCA). First attempts by Sigwart[1] showed that soon after PCTA restenosis occurred in many cases (30−60%) by proliferation of smooth muscle cells. This proliferation and adhesion of the smooth muscle cells on stents could be significantly reduced by passivation of the metal surface using polymer coatings.[2−4] Poly(*p*-xylylene) (PPX) has been used successfully for stent coating. PPX protects the metal from oxidation and forms a smooth surface reducing inflammatory reactions with the surrounding tissue, however, restenosis cannot be prevented in all cases.[5,6] Antiproliferative agents, e.g., paclitaxel or sirolimus, and antiinflammatory drugs, e.g., dexamethasone, have been used to generate drug eluting stents.[7,8] Locoregional drug release from the stent surface is usually preferred over systemic delivery. Presently, drug release from stent coating represents an area of great activity. Commercial products (e.g., Cypher stent) are already on the market. Mostly biocompatible and/or biodegradable polymers have been investigated and have been used for drug release.[9] Poly(lactide) (PLA) is a biodegradable and biocompatible polyester and therefore a promising candidate for drug release applications.[10−13] Properties of PLA, e.g., rate of degradation, can be modified by copolymerization, e.g., by block copolymerization with poly(ethylene oxide) (PEO).[14,15]

Here we describe the use of partially biodegradable triblock copolymers PLA-PEO-PLA as a drug containing layer on PPX coated stents. The newly designed stent coating consists of the well-established PPX coating as a permanent coating on the surface of a metal stent covered by an additional biodegradable polymer layer, which controls release of an anti-proliferative drug over an initial period of several weeks by biodegradation. Thereafter, a permanent PPX layer remains firmly attached to the stent surface. Particular focus of this work is on the adhesion and stress resistance of the coatings on PPX substrates compared to standard PLA. Drug release is shown to be exemplarily but is not within the scope of the paper.

Experimental Section

Materials. PPX coatings were prepared by vapor phase pyrolysis of [2.2]paracyclophane (Specialty Coating Systems (SCS), DPX-N) and subsequent chemical vapor deposition (CVD) of quinodimethane according to the Gorham process.[16] A commercially available adhesion promoter (A-174, SCS) was used without further modification. Commercial α-,ω-dihydroxyterminated poly(ethylene oxide) (PEO), M_n = 4000 g/mol (Fluka) was repricipitated from chloroform/hexane, dried in vacuum, and stored under argon. D,L-Lactide (Aldrich) was recrystallized from ethyl acetate and stored under argon. D,L-PLA for comparison of the release properties on the PPX-coated steel substrate was prepared according to literature.[17] Unlabeled and ^{14}C-labeled paclitaxel was obtained from Angiotech Pharmaceuticals Inc. and used as received.

Measurements. IR spectra were recorded on a Perkin-Elmer FT-IR 1600. Gel permeation chromatography (GPC) was measured using a setup of SDV 5A Phenomenex columns (8 × 600 mm), Knauer UV detectors, Gynkotech IR detectors, and THF as solvent (c = 1 g/L). The standards for calibration were poly(styrene)s of molecular weights between 1000 and 12000 D obtained from PSS. ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ using a 300 MHz AMX300 (Bruker). T_g and T_m were recorded by differential scanning calorimetry (DSC), using a Mettler Toledo 812c between −100 and +100 °C with a cooling ratio of 10 °C/min. Release studies were performed using a liquid scintillation counter (TriCarb/QuantaSmart, Canberra Packard Bioscience) and an Ultima Gold scintillation cocktail (Canberra Packard

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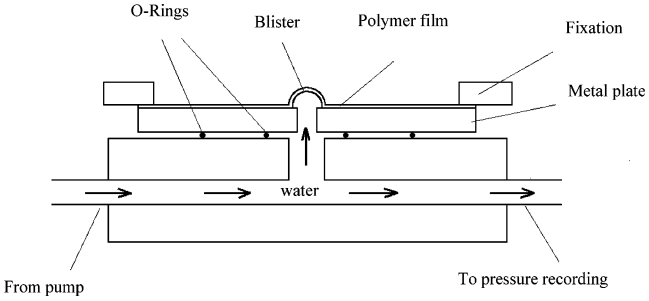


Figure 1. Schematic drawing of the Blister test apparatus.

Table 1. Molecular Weight of the PLA-PEO-PLA Triblock Copolymers and Ratios of PEO and PLA in the Copolymer

		PLA-PEO-PLA					
PEO [g/mol OH]	D,L-LA [g/mol]	sample no.	yield[a] [%]	PLA/PEO [mol/mol]	M_n [d]	M_w [d]	M_w/M_n
5.0	0.44	A	20	22:1[c]	4400	4900	1.13
5.0	0.90	B	95	10.9:1[b]	4900	5400	1.11
5.0	1.80	C	90	5.2:1[b]	5400	5900	1.09
5.0	3.80	D	94	2.2:1[b]	6200	7300	1.17

[a] Yield is based on the total amount of educts used. [b] The molar ratio of PLA and PEO moieties was analyzed by quantitative NMR. [c] The molar ratio of PLA and PEO moieties was analyzed by GPC due to the small amount of PLA. [d] Based on experimental data obtained by GPC analysis.

Bioscience). For SEM pictures, a CamScan 4 scanning electron microscope with 15 mV anodic potential was used. The thickness of the polymer films was measured using a Sloan Dektak profilometer 3 ST.

Coating of Steel Substrates by PPX. Coating of the clean stainless steel substrates (stents and stainless steel plate as model substrate for monitoring of drug release) was performed according to the Gorham process. A total of 1.25 g of paracyclophane was vaporized at 175 °C and pyrolyzed at 650 °C to form the reactive monomer, *p*-quinodimethane. The deposition took place at low pressure ($p = 20$ mbar) for 2−4 h. The samples were kept at 25 °C during deposition.

Blister Test. Polished steel plates (7 cm diameter, 0.5 cm thickness) with a hole in the exact center were completely coated with the polymer to be measured. The hole was sealed with silicone fat during the coating to provide a surface (film) free of holes. After the coating, the silicone fat was removed carefully. The polymer film was laminated with tape of known tensile strength for reinforcement of the film during the experiment (here: Tesa from Henkel Co.) and the plate was placed in the apparatus (Figure 1).

The water pressure in the system was increased at a steady rate and was recorded online. At a certain pressure, a blister formed and delamination between the polymer and the metal occured. The pressure droped until a steady state was reached. The slope of the curve obtained was plotted against the time (plot p/T) after the delamination and it can be used to calculate the absolute force of adhesion. For the calculation of the values, the following equation was used:

$$G_a = 0.398\sqrt[5]{\frac{F^2}{n^2 E h_p}}$$

where G is the force of adhesion, h_p is the thickness of the polymer film, F is the flux of water, n is the slope of the graph (p/T), and E is Young's modulus (see the auxiliary part).

Synthesis of PLA-PEO-PLA Triblock Copolymers. The PLA-PEO-PLA triblock copolymers were synthesized according to a previously published procedure.[18] α,ω-Hydroxyl-terminated PEOs were used as initiators for the preparation of PLA-PEO triblock copolymers of different composition by ring opening polymerization of D,L-lactide (Table 1).

A defined amount of dry PEO ($M_w = 4000$ g/mol) and D,L-lactide were placed in a round-bottom flask and stored under argon. Highly viscous Sn(II)octuate was added via a syringe, and the mixture was heated to 180 °C under low pressure ($p = 4 \times 10^{-4}$ bar). Melting of the polymers occurred at ∼90 °C when a clear liquid was obtained. After 1 h, a white solid started to form, and after 2 h, the suspension was cooled to 130 °C and stirred for another 2 h.

The crude polymer was dissolved in 30 mL chloroform and precipitated twice in 500 mL of diethyl ether.

Characterization of PLA-PEO-PLA Triblock Copolymers. Molecular weight data for all samples are given in Table 1.

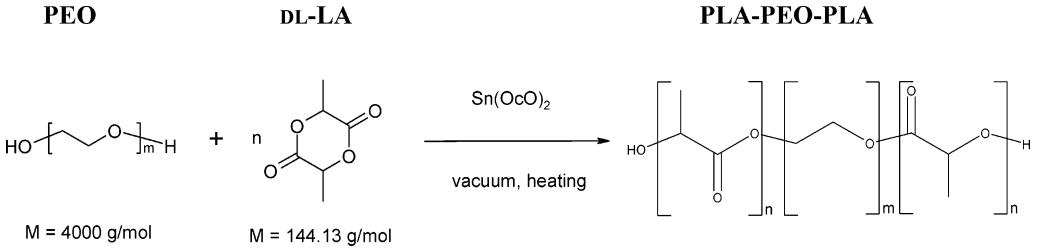
Sample A, Exemplarily for all Samples. ^{1}H NMR (300 MHz, $CDCl_3$): δ/ppm = 1.51 (s, CH_3 PLA), 3.86 (s, CH_2 PEO), 5.37 (m, CH PLA). ^{13}C NMR (300 MHz, $CDCl_3$): δ/ppm = 70.9 (CH_2 PEO). IR(ν/cm^{-1}), KBr: 2883 (b, s) = CH-valence bond, 1757 (s) = C═O-valence bond, 1559/1457 (s) = C−H-deformation bond, 1343 (w) = symmetric C−H-deformation bond, 1115 (s) = C−O-valence bond. GPC $M_n = 4300$, $M_w = 4900$, PD = 1.13. DSC $T_m = 47$ °C. TGA $T_{5\%} = 208$ °C.

Characterization of D,L-PLA Used for Release Studies. ^{1}H NMR (300 MHz, $CDCl_3$): δ/ppm = 1.52 (s, 3H, CH_3), 5.10 (q, 1H, CH, $^3J = 5.7$ Hz). ^{13}C NMR (300 MHz, $CDCl_3$): δ/ppm = 17.0 (1C, CH3), 69.4 (1C, CH), 170.0 (1C, C═O). IR(ν/cm^{-1}), Film on NaCl: 2995, 2945 (s) = CH-valence bond, 1757 (s) = C═O-v.b., 1454 (s) = C−H-d.b., 1364 (s) = symmetric C−H-d.b., 1184, 1089 (s) = C−O-v.b. GPC $M_n = 17200$, $M_w = 30200$, PD = 1.76.

Coating of Stents. Stents (Jomed Stent on catheter, diameter 20 mm, length 100 mm) were coated with a PPX layer of 1.4 μm thickness. Afterward, the stents were immersed in chloroform for 30 s and coated with an additional layer of PLA or PLA-PEO-PLA triblock copolymer by an airbrush technique (2 wt % polymer in chloroform, 2 mL per stent). The stents were expanded after coating using a balloon catheter in order to investigate the response of coatings to the expanding procedure.

Coating of PPX Films by Paclitaxel-Loaded PLA-PEO-PLA Triblock Copolymers and PLA. Polished stainless steel plates (1 mm thick, 15 mm diameter, polished by grinding paper P2000) were coated by a 0.6 μm thick layer of PPX as described before. These plates were spin-coated at 3000 rpm for 10 s. A total of 100 μL of a 3 wt % solution of the copolymer in dichloromethane, which contained 2.5 wt % paclitaxel related to the copolymer. The ratio of ^{14}C-labeled and unlabeled paclitaxel was set to 1:100. The resulting polymer film on the substrate was smooth and approximately 1.2 μm thick.

Scheme 1



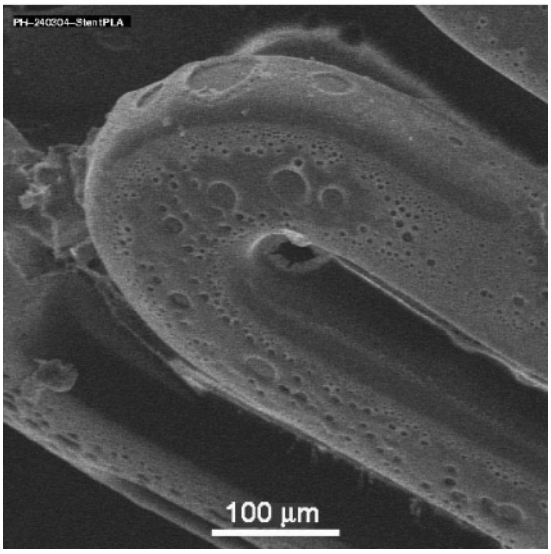
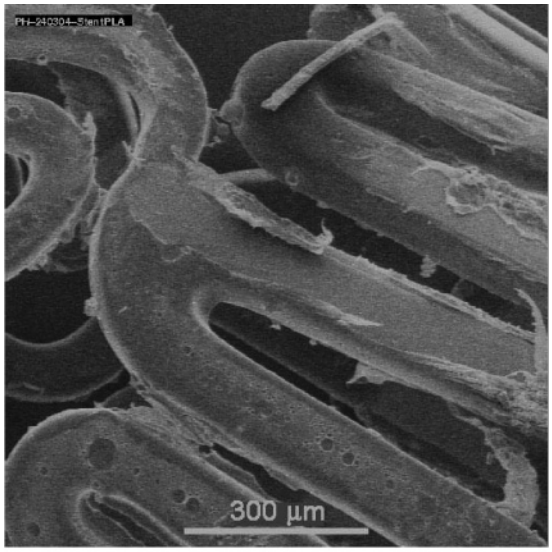


Figure 2. SEM microphotographs of unexpanded stents coated by PPX/PLA at different magnifications.



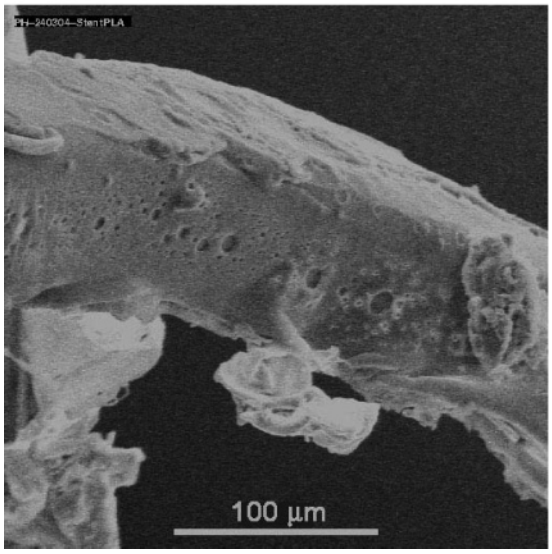


Figure 3. SEM microphotographs of expanded stents coated by PPX/PLA at different magnifications.

Release of Paclitaxel. The substrates were placed in a closed glass vessel and covered by 1 mL of buffer solution. The buffer solution contained of 150 mM of standard-phosphate-buffer (pH 7.4) and 40 vol % of ethanol. After defined periods of time, the solution was exchanged completely and the amount of paclitaxel in the solution was measured by liquid scintillation counter. The polymer films were weighted before and after the release (after drying at 80 °C for 6 h), and no loss of weight could be observed within the accuracy of the balance.

Results and Discussion

Materials Synthesis. ABA-triblock copolymers of PLA-PEO-PLA were prepared by melt-polycondensation of α,ω-dihydroxyterminated PEO M_n = 4000 g/mol and D,L-dilactide-catalyzed by Sn(II)-octuate at 180 °C according to Scheme 1 following a previously published procedure.[18] The resulting block copolymers were purified by reprecipitation from chloroform/diethyl ether. The presence of PEO and PLA moieties in the resulting polymers was verified by means of IR and NMR spectroscopy and GPC analysis. The molar quantities of PEO and PLA in the product were analyzed by quantitative ^{1}H NMR spectroscopy. Theoretical and experimental ratios of the components were identical within the range of error (3%). It should also be noted that monomodal molecular weight distributions were observed by GPC for all block copolymer samples. The samples showed no signs of crystallinity and were found to be a tacky solid. The reduction in crystallinity when compared to PEO can be explained by the good mixing behavior of PLA and PEO. Therefore, PLA suppresses the crystallization of PEO.

Table 2. Blister Test of Coatings of PPX on Steel, PLA on PPX, PLA-PEO-PLA on PPX

test	thickness PPX	second layer	Ga_a (N/m^2)	note
1	650 nm	none	5.0	reference, only PPX
2	550 nm	PLA	0.0	not soaked in chloroform
3	500 nm	PLA	4.9	soaked in chloroform
4	400 nm	PLA-PEO-PLA 5:1	0.7	swells in water, value too low
5	400 nm	PLA-PEO-PLA 10:1	0.6	swells in water, value too low

Adhesion Tests. Adhesion to substrate under wet conditions is crucial for all stent coatings. Therefore, adhesion testing was performed in order to analyze adhesion of the coating materials used here on given substrates. All adhesion tests were performed using the blister-test method, which provides quantitative data compared to the tape test.[19−21] Very good adhesion of pure PPX on metal plates as well as on stents was observed with adhesion

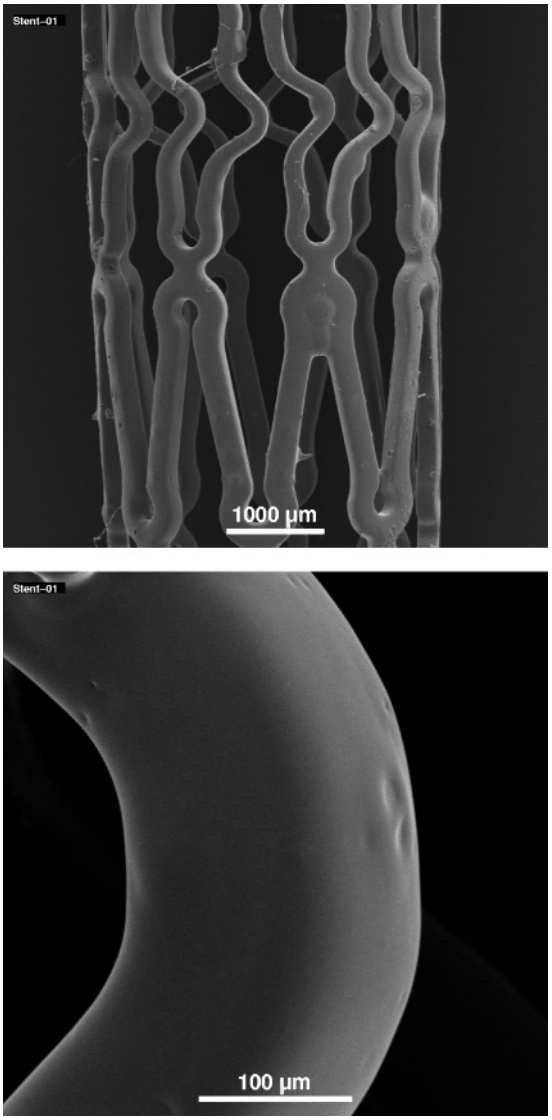


Figure 4. SEM pictures of unexpanded stents coated with PPX/PLA-PEO-PLA triblock copolymer (sample B) at different magnifications.

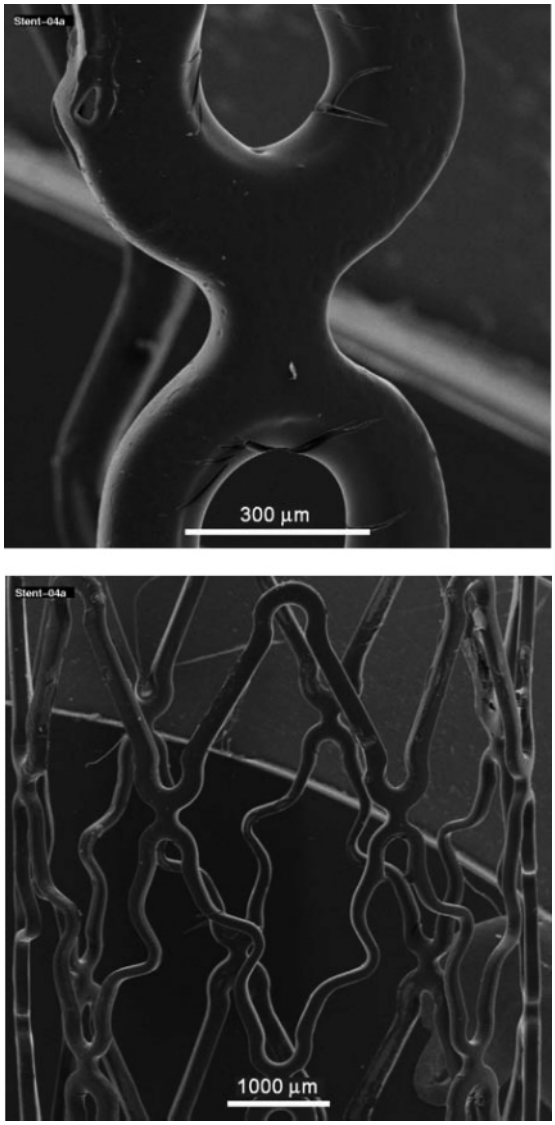


Figure 5. SEM pictures of expanded stents coated with PPX/PLA-PEO-PLA triblock copolymer (sample B) at different magnifications.

promoter (A-174). Consequently, no detachment of PPX was observed within the limits of the Blister test setup (Table 2). The adhesion between PLA and PPX under physiological conditions was found to be weak (see test 2, Table 2). The adhesion between PLA and PPX was improved by immersing PPX with chloroform and applying the biodegradable polymer layer (PLA or PLA-PEO-PLA) while PPX was still wet. Speculatively, it can be anticipated that a stronger physisorption through partial entanglement between the polymer layers occurred, which led to a stronger overall adhesion (see test 3, Table 2). It should be noted here that the penetration of PPX films by small molecules such as, e.g., styrene assisted by supercritical CO_2, has been used previously for the preparation of PPX blends which are otherwise insoluble.

In another set of experiments, the PLA-PEO-PLA triblock copolymer was cast on prewetted PPX films. In this case, the values obtained for the total adhesions were small because the copolymer significantly swelled in water used by the Blister test method (see test 4 and 5, Table 2). This led to penetration of water through the polymer film before the maximum value of the test run could be recorded. The films stayed intact on the surface of the PPX indicating that adhesion itself was good but the values gained in the experiment were too low.

Stents are usually expanded upon implantation, which results in mechanical stress for stent coatings. To visualize the response of stent coatings upon stent expansion, stents were coated by PPX and by PLA-PEO-PLA triblock copolymer or by PLA. The stents were expanded by 40% total in diameter. PLA coatings showed an uneven coating prior to expansion and severe damage after expansion according to SEM analysis (Figures 2 and 3). In contrast, the block copolymers (sample B was used) showed smooth coatings prior to expansion with significantly less damage even after 70% expansion procedure (Figures 4 and 5). Only very few small cracks were visible of the triblock copolymer coatings. It should be noted here that the coating thickness of block copolymers and PLA were of the same order ($\sim 2-3\ \mu$m) and that no cracks were observed here in the PPX substrate coatings.

Release of Paclitaxel from PLA and PLA-PEO-PLA Triblock Copolymer Coatings. Release studies of paclitaxel from the PLA and block copolymer coatings were performed as described above under physiological conditions in order to explore the effect of different PEO contents in the block copolymers on the paclitaxel release profiles. Figure 6 shows that 90% of the paclitaxel was released within the first 4 h, which clearly does not meet the demands for a successful drug eluting stent coating. However, increasing the PEO content

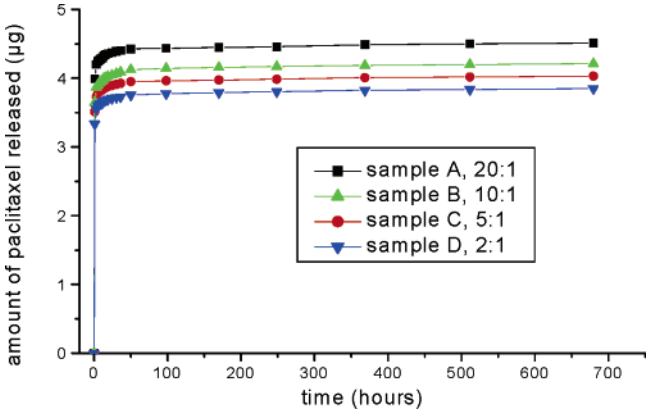


Figure 6. Release of paclitaxel from copolymers of different composition.

resulted in increase of the total amount of paclitaxel release. Most likely, the total amount of paclitaxel loaded in the polymer layer is governed by different sorption and swelling behavior of the polymer layers.

Conclusions

Biodegradable polymers such as PLA and ABA triblock copolymers of PLA-PEO-PLA were used as drug carriers on PPX layers coated on metal stent surfaces. Among others crucial parameters for these coatings are wet adhesion to PPX layers, mechanical response to stent expansion, and rate of drug release under physiological conditions. Experiments showed that wet adhesion of block copolymer layers can be improved significantly by coating of prewetted PPX surfaces. Mechanical expansion of stents revealed smooth coatings formed by the block copolymers but rough coatings with cracks and lesions when the well-established PLA was used. Rate of drug release from layers of biodegradable polymers was independent of polymer composition. The maximum period of drug release observed here was 3 days which is clearly too short to be of practical use. Extension of drug release period based on the system presented here, e.g., by additional PPX coatings, will be the topic of forthcoming papers.

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