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In vitro study of drug-eluting stent coatings based on poly(L-lactide) incorporating cyclosporine A – drug release, polymer degradation and mechanical integrity

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Abstract In this study, absorbable polymer stent coatings for localized drug delivery based on poly(L-lactide) (PLLA) and cyclosporine A (CsA) were developed and tested in vitro. Metallic stents were coated with different compositions of PLLA/CsA (70/30, 60/40, 50/50% w/w) and β-sterilized. The specimens were used to assess the drug release kinetics with HPLC. Sterilization influenced polymer degradation was measured with GPC. Mechanical integrity of the stent coatings was studied with SEM. The interconnection of the coated stents with a balloon-catheter was characterized by the measurement of stent dislodgment force. A migration assay was used to determine the inhibitory effect of the model drug CsA on smooth muscle cell (SMC) migration. The release of CsA was established over time periods up to 24 days in sodium chloride solution and in porcine blood plasma. An inhibition of SMC migration (max. 26–33%) was found for CsA concentrations of 4×10^{-5} to 4×10^{-7} mol/l. Marked molecular weight reduction (70–80%) of the PLLA matrix occurred after β-sterilization. We also observed a substantial decrease of in vitro degradation time. The maintenance of the mechanical integrity of the polymer coating during crimping and dilation of the specimens could be verified, and a sufficient stent dislodgment force of 0.8–0.9 N was measured.

Introduction

Restenosis represents one of the most frequent long-term complications after implantation of coronary stents [1]. The employment of a vascular stent causes a mechanical injury of the blood vessel that may induce neointimal hyperplasia via a chain of immunological and biochemical reactions of the cells in the injured vessel wall [2, 3]. Growth and migration of vascular smooth muscle cells (SMCs) resulting in neointimal proliferation after vascular injury, have been identified as key mechanisms of in-stent restenosis [4].

Avoiding systemic toxicity, stent-based local drug release at the site of vascular injury via a polymer-coated drug-eluting stent is an attractive therapeutic approach to prevent in-stent restenosis. Currently, a number of polymer coating materials, both biodegradable and non-degradable, for metallic stents, as well as different drug classes, such as agents that are anti-inflammatory or immunomodulators, antiproliferative agents, drugs which affect migration and extracellular matrix production, and drugs that promote vascular healing and re-endothelialization are under consideration [5–7]. Parallel approaches pursue fully resorbable drug-eluting stent systems based on polymer materials [8, 9]. However, these concepts are still in a developmental, preclinical phase.

So far, only two different concepts of metallic stents with drug-incorporated polymer coatings have gained

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widespread clinical use [10]. One stent coating is based on the non-degradable polymer blend of poly(ethylene-co-vinylacetate) and polybutylmethacrylate (PEVA/PBMA) and incorporates the immunosuppressant rapamycin [11], while the second stent system employs the non-degradable polymer coating from poly(styrene-β-isobutylene-β-styrene) [SIBS] with the incorporated cytostatic paclitaxel [12].

Although until to date, comprehensive clinical data for these systems has been provided in a number of publications, only few studies describe the general mechanisms of drug-eluting stent systems and the interactions related to the polymer material and the drug with its release kinetics. For biodegradable polymer coatings, however, such in vitro studies must be considered highly important for the understanding of the coating degradation, which should be gradual in order to minimize tissue reaction to degradation by-products. Also, the coating material should allow for an adequate drug delivery to the arterial wall. Furthermore, according to our knowledge, no studies have so far been presented, describing the interference of the coating material and drug release with sterilization protocols, the mechanical integrity of the polymer coating during stent crimping and deployment, and the interconnection of a polymer-coated stent with a balloon catheter.

In the present study, we provide such data for three metallic stent systems coated with a high molecular weight PLLA (M_w = 650,000 g/mol), and incorporated with CsA (PLLA/CsA: 70/30, 60/40, and 50/50% w/w). The immunosuppressant CsA with different inhibitory effects, such as the inhibition of SMC proliferation and migration, was used as a model drug. CsA has been reported to have inhibitory effects on human and rat aortic SMC proliferation [13, 14], and to retard the development of transplant arteriosclerosis after allogeneic heart or aorta transplantation in rats [15, 16].

PLLA was used as coating matrix, because it is known as one of the most common biodegradable polymers, being used in a wide range of medical applications. These include surgical devices [17–19], and sutures [20], urological [21–23] and vascular [24–28] stents, as well as drug delivery implants [29–31]. PLLA has been confirmed as a suitable biodegradable polymer for coronary stents in a number of in vitro and in vivo investigations [32, 33].

Our study describes in detail the following functional characteristics of different PLLA/CsA stent coating systems: (i) interaction between drug loading and release profiles in different media, (ii) in vitro degradation profile of the PLLA coating before and after β-sterilization, (iii) behavior of the coating during crimping and dilation of the specimens, and (iv) interaction of the coated stent with a balloon catheter.

Materials and methods

Materials

Poly(L-lactide) (PLLA, L214 M_w = 650,000 g/mol, M_w/M_n = 1.8) was supplied by Boehringer Ingelheim Pharma (Ingelheim, Germany) and cyclosporine A (CsA, M = 1202 g/mol) was obtained from Synopharm GmbH & Co. KG (Barsbüttel, Germany). Biotronik GmbH & Co. (Erlangen, Germany) supplied the metallic stents. The stents were 3.5×10 mm in size with a strut cross-section of 80×80 μm and a complete stent surface area of 54.3 mm^2.

Polymer drug coating

PLLA was dissolved in chloroform to yield a concentration of 1.7% (w/v). CsA was dissolved in abs. ethanol (17% w/v CsA), which is miscible with chloroform. The drug solutions were mixed into the polymer solution to obtain final CsA concentrations of 30%, 40% and 50% in PLLA (w/w). The polymer drug solution was then applied to the metallic stent surface by a spray process. To remove the solvent, the pure PLLA coated stents were stored in methanol for one day and then in distilled water for another day before drying. All stents were dried in vacuo at 40 °C to achieve a chloroform content of <0.2% as determined by elemental analysis.

The layer thickness of the stent coating was 5–10 μm. The layer masses of the coatings were on an average of 500 μg per stent. The resulting drug contents were 2.8 μg/mm^2 (30% CsA), 3.7 μg/mm^2 (40% CsA) and 4.6 μg/mm^2 (50% CsA), respectively.

The actual amount of CsA in the polymer layer after spraying was verified. The stent coatings were completely dissolved in 2 ml chloroform and brought to 25 ml with acetonitrile. The CsA content was determined by HPLC as described in Section Drug Release.

Light microscopy

The layer quality and structural integrity of the polymer coating were assessed before and after stent crimping and after stent deployment using incident light microscopy (Axioskop, Zeiss, Germany). The stents were crimped on a 3.5×15 mm balloon catheter and then dilated to their nominal diameter with 8 bar balloon pressure.

To determine coating thickness distribution, dilated stents were embedded in an epoxy resin and cured for 24 h. Then the samples were ground and further polished with an aluminum oxide suspension. Representative cross sections of the stent coatings were examined using incident light microscopy with dark field illumination (Axioskop, Zeiss, Germany).

Scanning electron microscopy

The structural integrity of the polymer stent coating was assessed after the stent crimping and deployment procedure described in Section Light Microscopy. Examination was carried out in a Philips XL 30 ESEM (Philips Electron Optics, The Netherlands) operating in the SEM mode.

Stent dislodgment force analysis

The stent dislodgment force was measured to verify the integrity of the catheter/stent system. First, the stents were crimped onto 3.5×15 mm balloon catheters. The catheter/stent system was then mounted in a tensile tester Zwicki ZN 2.5 (Zwick GmbH & Co. KG, Germany) with the catheter shaft clamped at one end while the stent, wrapped in adhesive tape, was attached to the opposite head. The stents were dislodged toward the distal tip of the catheter at a cross-head speed of 20 mm/min and stent dislodgment force was recorded. This test evaluates the potential for a stent loss during the delivery procedure and during system retraction in the case of unsuccessful stent application or emergency.

Drug release

The time course of CsA release from β-sterilized PLLA/CsA coated stents was determined at 37 °C in sodium chloride solution (0.9% NaCl w/w) or porcine blood plasma. The elution media were continuously agitated to maintain a uniform CsA distribution. Individual stents ($n = 3$ in each subgroup) were immersed in 1 ml elution medium for a total duration of 24 days. The elution medium was renewed at periodic intervals, in order to avoid a saturation of the medium with CsA. Thus, the drug release curves result from the accumulation of the measured values per sampling time.

The amount of CsA released at each time period was determined by HPLC. The NaCl solution aliquots containing the released CsA were injected into an Eurospher column 100-5 C18, 250×4 mm ID (Wissenschaftliche Gerätebau Dr.-Ing. Herbert Knauer GmbH, Germany). The chromatographic conditions were: column temperature 70 °C; eluent water-acetonitrile (CH_3CN); 5 min linear gradient 75–90% (v/v) CH_3CN; 3 min 90% (v/v) CH_3CN isocratic; 2 min linear gradient 90–75% (v/v) CH_3CN; flow rate 1.0 ml/min; detection UV, 205 nm; calibrated measurement range 0.1–10.0 mg/l; detection limit approx. 0.01 mg/l. In each case the eluents, water and CH_3CN, contained 0.8 ml ortho-phosphoric acid (85%) per liter.

The CsA blood plasma samples were pre-treated and CsA was determined in blood plasma according to [34]. The aliquots from CsA release in porcine blood plasma were injected into Prontosil column 120–5 Phenyl, 150×4 mm ID (Bischoff Chromatography, Germany). The HPLC system was optimized to detect CsA and to remove the interfering blood plasma peaks. The chromatographic conditions were: column temperature 70 °C; eluent CH_3CN/methanol/water (20/52/28 v/v/v) isocratic; flow rate 1.0 ml/min; detection UV, 205 nm; calibrated measurement range 0.5–20 mg/l; detection limit approx. 0.1 mg/l.

Molecular weight analysis

The molecular weight data of PLLA was obtained at 35 °C using a TSP GPC system with a Shodex RI 71 detector and three PSS SDV 10 μm columns (10^3, 10^5 and 10^6 Å respectively). The system was combined with a WGE Dr. Bures η1000 viscosity detector. Chloroform was used as the eluent at a flow rate of 1 ml/min. The sample concentration was 0.7–1.4 mg/ml, and the injection volume was 0.1 ml. The molecular weights were calculated by the universal calibration method.

Sterilization

Two common β-irradiation processes ($T = 25$ °C, applied surface doses = 25 and 40 kGy) were used to examine the effect of sterilization on the drug-eluting stents. Dosimeters accompanied the samples.

Thermal analysis

Differential scanning calorimetry (DSC) was used to analyze the crystallinity of PLLA films. The scans were made on a Mettler-Toledo Thermosystem FP900 (Mettler-Toledo, Schwerzenbach, Switzerland) operating at ambient atmospheric pressure. Starting at room temperature, the specimens were heated from 30 °C to 60 °C at 20 °C/min, kept isothermal for 2 min, and then heated to 210 °C at 10 °C/min. The sample weight was in the range of 3–6 mg. Two specimen types were

analyzed: unsterile PLLA and β-sterilized PLLA. The degree of crystallinity was calculated using the specific heat of fusion in relation to the reference value for totally crystalline PLLA of 93.7 J/g [35, 36].

In vitro degradation

Each polymer-coated stent was placed in a test tube containing 4 ml Sørensen buffer (0.1 M, pH 7.4) and maintained at 70 °C. Samples were periodically removed, washed with distilled water and dried in vacuo before GPC analysis. The degradation investigations were conducted according to the DIN EN ISO 10993-13 and the ISO 13781 for PLLA.

Cell migration assay

Rat SMCs from embryonic aorta (cell line A7r5, European Collection of Cell Cultures, UK) were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 4.5 g/l glucose, 10% FCS, 25×10^{-3} mol/l HEPES, 100 U/ml penicillin G and 100 μg/ml streptomycin. Primary cultures of human coronary artery SMCs (HCASMC, PromoCell, Germany) were cultured in SMC Growth Medium 2 (PromoCell, Germany). All incubations were performed at 37 °C in a humidified 5% CO_2 atmosphere.

For migration assays, cells were seeded at concentrations of 3×10^4 HCASMCs or 5×10^4 A7r5 cells in 0.5 ml medium with 1% BSA in tissue culture inserts (Nalge Nunc International, Denmark) with collagen coated polycarbonate membrane (pore size 8 μm). HCASMCs were serum-starved for 65 h before migration assays. Then the HCASMCs were incubated for 6 h with CsA solutions, which were prepared from a stock solution of 1×10^{-2} mol/l CsA in ethanol by further dilution with medium down to concentrations of 4×10^{-5} mol/l, 4×10^{-6} mol/l and 4×10^{-7} mol/l. After addition of 25 ng/ml PDGF-BB (Sigma, Germany) to the lower compartment of the chemotaxis chamber, the HCASMCs were allowed to migrate for 18 h. With A7r5 the CsA solutions were added to the cells without further incubation. Immediately PDGF-BB (10 ng/ml) was added to the lower compartment. A7r5 cells were allowed to migrate for 4 h. Controls were performed with and without PDGF-BB and with and without the specific PI3 kinase inhibitor Ly294002 (Sigma, Germany). Controls of the solvent ethanol (max. 0.4% v/v) without cyclosporine A were prepared in parallel. For cell quantification, the non-migrated cells were scraped from the upper surface of the polycarbonate membrane and the migrated cells on the undersurface were stained with Diff-Quik (Dade

Behring Marburg GmbH, Germany). The cells in 15 fields of each membrane were counted under a microscope using a magnification of 400× (Labophot-2, Nikon, Japan).

Results and discussion

Polymer drug coating

Various melt processing techniques have been utilized to fabricate PLLA based implants loaded with bioactive substances [30, 37]. The limiting factor of melt processing of implants for drug delivery is the heat stability of the bioactive agent. Solution casting of polymers is an alternative method for the preparation of drug delivery systems and is one particularly suited for the preparation of drug-eluting stent coatings. In order to incorporate drugs by this method, the polymer is dissolved in a solvent and mixed with the drug solution.

In this study, the high lipid and limited water solubility of CsA proved advantageous for the solution casting type process and the incorporation of the drug into the polymer matrix. Thus, high CsA content in the stent coating could be ensured with good layer quality and integrity. The stent coatings were examined by incident light microscopy (Fig. 1) and by scanning electron microscopy (Fig. 2).

It could be seen that structural integrity of the coating was maintained after crimping and full expansion of the β-sterilized drug-eluting stent prototypes (Fig. 3). Despite the mechanical characteristics and the degree of crystallinity of PLLA (solution cast PLLA films exhibit high brittleness and low flexibility) and the addition of CsA, expansion of the coated stents was
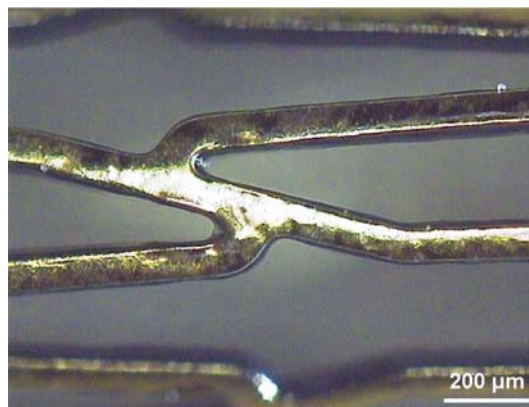

200 μm

Fig. 1 Metallic stent with PLLA/CsA coating (70/30% w/w) before dilation (incident light microscopy)
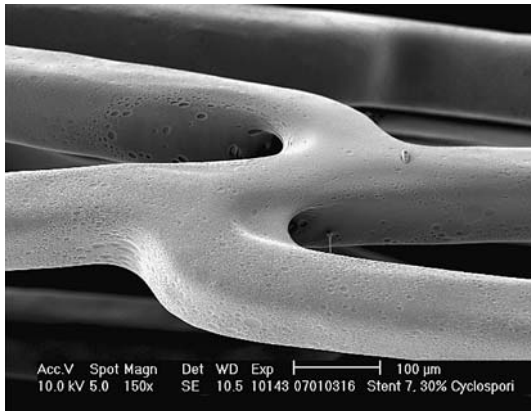
Fig. 2 Metallic stent with PLLA/CsA coating (70/30% w/w) before dilation (SEM)

possible without evident coating cracking or delamination.

Figure 4 shows the typical layer thickness distribution of a coated stent. The coating thickness ranged from 5 μm to 20 μm. The coatings were thicker on the stent exterior surface than on the interior stent surface. It was found that a form fit of the polymer layer around the stent struts is essential for maintaining structural integrity of the coated stents during crimping and dilation.

The mean layer thickness of the stent coating (A_{stent} = 54.3 mm^2, m_{layer} = 0.5 mg, ρ_{PLLA} = 1.2 mg/mm^3) was calculated according to the following relationship:

$$\bar{d}_{layer} = \frac{V_{layer}}{A_{stent}},$$

with

$$V_{layer} = \frac{m_{layer}}{\rho_{PLLA}}$$

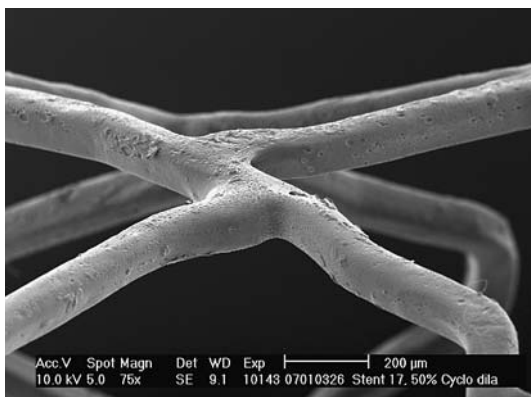**Fig. 3** Metallic stent with PLLA/CsA coating (50/50% w/w) after dilation (SEM)

$$\bar{d}_{layer} = 7.7 \ \mu m$$

Stent dislodgment force

Table 1 shows the measured stent dislodgment forces. The high standard deviations, particularly in the case of the pure PLLA coating, indicate the high variability of the results. This is due to the fact that the stents were manually crimped onto the balloon catheter, as would be the practice in an investigational surgical setting. The measured mean dislodgment values for all PLLA and PLLA/CsA material combinations were found to be in the range of commercially available stent systems [data not shown]. Hence, we can conclude that the investigated polymer coated and drug loaded stents could be delivered safely to or retracted from a stenotic target site without the danger of stent dislodgment or loss.

In vitro drug release

β-sterilized PLLA stent coatings with 30%, 40% and 50% CsA were tested in an in vitro release study (Figs. 5 and 6). Previous studies in this laboratory showed that release in measurable quantities of the relatively large CsA molecule from the hydrophobic PLLA began at a CsA concentration of >20% in the polymer. Thus 30% was chosen as the lowest drug concentration in the present study. The drug release studies were conducted in isotonic NaCl solution (Fig. 5) and porcine blood plasma (Fig. 6) for comparison. It is clearly understood that drug release rates measured in blood plasma would be more relevant than those measured in NaCl solution. CsA is more soluble in blood plasma and more realistic diffusion conditions result from the partial binding of the lipophilic cyclic oligopeptide CsA on the plasma proteins (blood plasma protein bond 33% [38]). However, experiments with blood plasma are more difficult to carry out.

Based on the cumulative mass of released CsA (in μg) and the drug eluent volume (in ml), the concentration of CsA in the eluent volume can then be calculated in mg/l or mol/l, respectively. Table 2 shows the time periods in which relevant CsA concentrations (calculated cumulative concentrations) were achieved based on the amounts of CsA that were released from the different stent coatings.

This method was used to determine whether CsA could be released from the stent coating in amounts necessary to produce concentrations that inhibit SMC proliferation and migration as had been predetermined
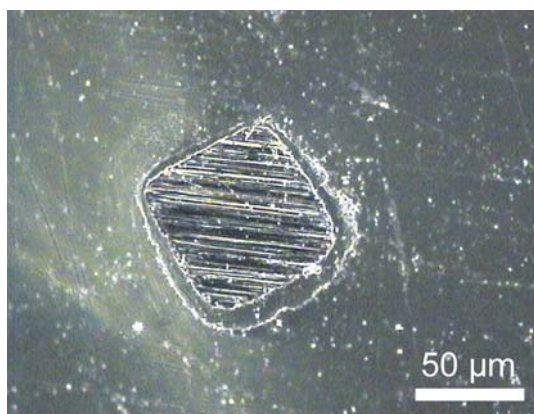
Fig. 4 Representative cross section of a PLLA coating (polished microsection of one stent strut, incident light microscopy, dark field illumination)

Table 1 Stent dislodgment forces ($n = 3$ for each PLLA/CsA combination)

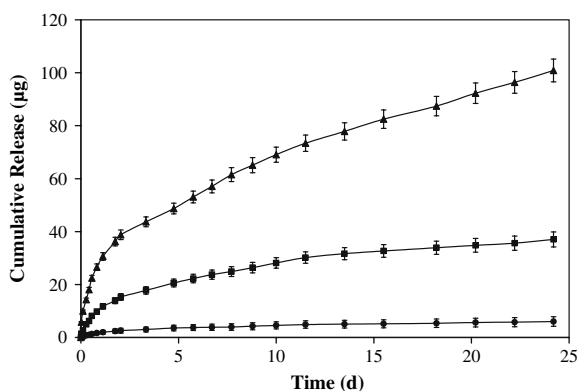|	Dislodgment force (N)	Standard deviation (–)
PLLA	0.77	0.22
PLLA/CsA 70/30	0.84	0.11
PLLA/CsA 50/50	0.94	0.07

Fig. 5 Cumulative CsA release into NaCl solution (0.9% w/w, pH 7.4) at 37 °C as a function of CsA concentration in the coating. (●) PLLA/CsA 70/30% w/w, (■) PLLA/CsA 60/40% w/w, (▲) PLLA/CsA 50/50% w/w. Each curve represents 3 stents. (Mean values and standard deviation)

in the cell culture tests. However, this approach does not account for two potential in vivo phenomena: (1) the permanent release of CsA into the blood stream which would not contribute to the local drug concentration in the vessel wall, and (2) high accumulation of the lipophilic CsA in the vessel wall with potentially cytotoxic effects. Relevant, cumulative dose concentrations of 1×10^{-6} mol/l to 1×10^{-5} mol/l CsA [13] were released in the time periods investigated from all
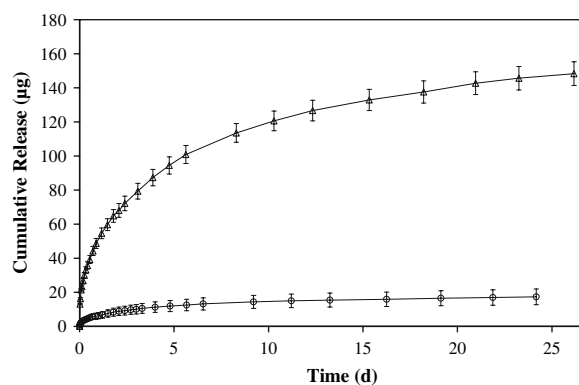

Fig. 6 Cumulative CsA release in porcine blood plasma at 37 °C as a function of CsA content in the coating. (○) PLLA/CsA 70/30% w/w, (△) PLLA/CsA 50/50% w/w. Each curve represents 3 stents. (Mean values and standard deviation)

three coated stent prototypes. From the PLLA/CsA coatings with 30% CsA about 6% of the total drug amount (in NaCl solution) and 17% of the total drug amount (in blood plasma) had been released through the end of the tests (24 days). Higher CsA concentrations (corresponding to 22% of total drug amount) were released from the PLLA/CsA coatings with 40% CsA in NaCl solution after 24 days. For the PLLA/CsA coatings with 50% CsA, the highest CsA concentrations were observed in the elution media. About 33% of the total drug amount was released into the NaCl solution and 71% of the total drug amount was released into the porcine blood plasma at the end of the tests.

A potential risk associated with the large remaining quantities of active agent in the polymer layer, as found for low dose incorporation of 30% and 40% CsA, is a possible high dose late release during degradation (fragmentation) of the polymer layer. This potential risk has to be addressed in further experiments.

With respect to the influence of sterilization we could not identify significant differences in the release kinetics of CsA from β-irradiated and unsterile stents in vitro, as one might have expected resulting from the change in crystallinity [data not shown].

Sterilization

The degradation of PLLA due to γ- or β-irradiation has previously been examined systematically for PLLA powder and sheets [39] and fibers [40]. It is known that the initial molecular weight of the polymer is reduced, the apparent polymer crystallinity is increased and the mechanical properties are affected, all as a result of irradiation sterilization. For these reasons, the impact

Table 2 Dependency between CsA content, elution media (NaCl, plasma), elution time,and CsA concentration in the elution media. Time points are given for relevant CsA concentrations according to [13, 14]

	CsA concentration (mol/l)	Elution time NaCl solution	Blood plasma
PLLA/CsA 70/30	1×10^{-6}	0.5 days	1 h
	1×10^{-5}	–*	5 days
PLLA/CsA 60/40	1×10^{-6}	0.5 h	n. m.**
	1×10^{-5}	2 days	n. m.**
PLLA/CsA 50/50	1×10^{-6}	<0.5 h	0.5 h
	1×10^{-5}	0.25 days	0.5 h

* not reached within 24 days

** not measured

of irradiation on the properties of the PLLA stent coating was studied.

The molecular weight (M_w) decrease of PLLA stent coatings due to β-sterilization was analyzed by GPC (Fig. 7). The M_w reduction averaged 70% with an irradiation dose of 25 kGy and 80% with an irradiation dose of 40 kGy.

The recrystallization of PLLA resulting from the substantial M_w reduction during β-sterilization was shown for PLLA films by thermal analysis (DSC) (Table 3). The influence of the β-sterilization process resulting in an apparent increase of crystallinity was also found by Nuutinen et al. [40] and is described in detail elsewhere [41].

While β-sterilization was shown to have a significant effect on PLLA, CsA appeared to be unaffected by the sterilization process. CsA remained unaltered when examined by HPLC or by NMR spectroscopy. In addition, no impairment of the inhibitory effect of CsA on SMC (A7r5, HCASMC) due to sterilization was found by cell viability assay (MTS) and cell proliferation assay when compared to unsterile controls.
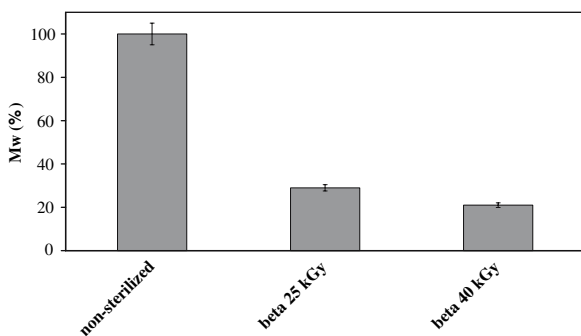

Fig. 7 Decrease of molecular weight (Mw) of PLLA stent coatings as a result of β-sterilization. (Mean values of five measurements and standard deviation)

Table 3 Degree of crystallinity as a function of the β-sterilization process (mean value of three measurements)

	Degree of crystallinity (%)
PLLA unsterile	33
PLLA β-sterilized	49

In vitro degradation

A number of studies relate PLLA degradation to its molecular weight and crystallinity [42–45]. The increase in crystallinity of PLLA due to β-sterilization [41] may retard degradation. Zilberman et al. reported [46] an accelerated degradation of high molecular weight but low crystalline PLLA in comparison to a retarded degradation of low molecular weight but higher crystalline PLLA. Furthermore, these results are consistent with the in vivo studies described by Gogolewski et al. [47], who observed an accelerated degradation of higher molecular weight PLLA after 6 months subcutaneous implantation in the mouse model. Thus, the different tissue reaction to PLLA with different molecular weights, as reported Lincoff et al. [48], cannot solely be attributed to different chain lengths, but may also be influenced by other polymer characteristics such as crystallinity and surface morphology. This underlines the importance of in vitro degradation analysis being performed directly on stent coatings, rather than on arbitrary samples, such as polymer films. However, with respect to the influence on tissue reaction from a low molecular PLLA, as it may result from the molecular weight decrease after β-sterilization, we suggest the use of high molecular PLLA.

In our study we examined the in vitro degradation of unsterile and β-sterilized PLLA stent coatings in an accelerated test at 70 °C in phosphate buffer. It is well-known from the literature [49] that the hydrolysis of PLLA proceeds in two steps. First a reduction of the polymer chain length by randomly distributed cleavage of ester bonds, without the formation of soluble components, takes place. In the second step of the degradation process actual mass loss and release of degradation products occurs. The first step in the degradation process is shown in Fig. 8 for both the sterilized and unsterile samples. Both show that molecular weight decreases with time in the accelerated test. The molecular weight decreases to below the molecular weight detection limit of the GPC after 35 days degradation time for the unsterile samples and already after 21 days for the sterilized samples. It was found that the second step of the degradation process

(actual mass loss) began only after the molecular weight fell below a critical value (approx. 10,000–20,000 g/mol). The results (Fig. 9) clearly show that coating mass begins to decrease earlier in the sterile specimens (after 14 days) as compared to the unsterile specimens (after 28 days). However, the rates of molecular weight reduction and mass loss of PLLA, were found to be the same for unsterile and sterilized samples. As can be seen in Figs. 8 and 9, the slope of the curves for the unsterile and sterilized samples is remarkably similar. The curves are just shifted along the time axis, which is due to the initial difference in molecular weight. This aspect is illustrated in Fig. 8, where the curve of the sterilized PLLA was shifted into the curve for unsterile PLLA.

Comparing the in vitro degradation of β-sterilized samples in the accelerated test at 70 °C with earlier tests of PLLA samples at 37 °C (M_w = 284,000 g/mol, M_w/M_n = 1.5, [50]), a half-life of about 52 weeks at 37 °C can be estimated for the β-sterilized PLLA stent coatings.

Cell migration

CsA has previously been described to inhibit SMC proliferation by Mohacsi et al. [13]. Hence the effects of CsA on SMC (A7r5, HCASMC) viability (MTS assay) and proliferation (BrdU assay) were characterized in this study. Our tests confirmed that CsA in concentrations from 1×10^{-5} mol/l to 1×10^{-6} mol/l CsA inhibit SMC proliferation and further that a concentration of CsA down to 4×10^{-7} mol/l still has an inhibitory effect. This concentration of CsA appears
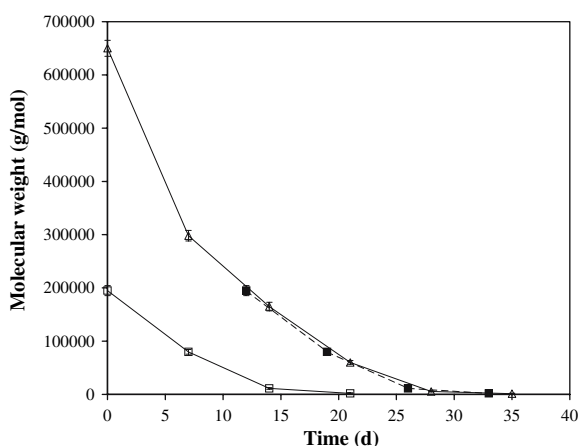

Fig. 8 Molecular weight reduction (Mw g/mol) of non-sterilized and β-sterilized PLLA stent coatings in Sørensen buffer (70 °C, pH 7.4). (□) β-sterilized PLLA coating, (Δ) non-sterilized PLLA coating, (■) shifted curve of β-sterilized PLLA coating to illustrate similar slope. All data points are means of three measurements. (Mean values and standard deviation)
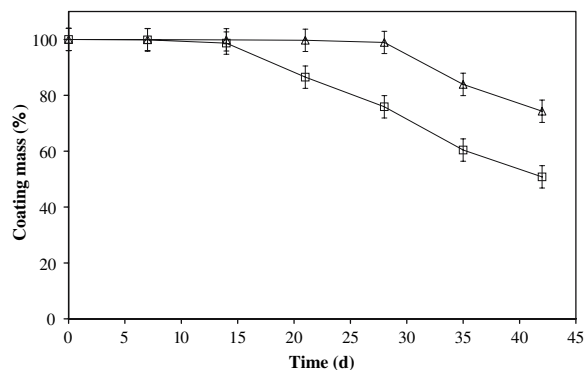

Fig. 9 Changes in coating mass of PLLA stent coatings (in %) after in vitro degradation in phosphate buffer (70 °C, pH 7.4). (□) (-sterilized PLLA coating, (Δ) non-sterilized PLLA coating. All data points are means of three measurements. (Mean values and standard deviation)

to be approaching the lower bound for effectiveness. In studies by Hu et al. [51] in vitro concentrations from 1×10^{-8} mol/l to 1×10^{-12} mol/l CsA yielded no inhibition of the proliferation of SMC.

The effect on SMC migration by CsA (in the concentration range relevant for the inhibition of SMC proliferation) were also analyzed (Fig. 10). It was found that CsA, in concentrations of 4×10^{-5} mol/l to 4×10^{-7} mol/l, resulted in inhibition of the PDGF induced migration of A7r5 cells (14–26%) and the HCASMC (25–33%). A migration inhibition of A7r5 cells (74%) and HCASMC (60%) was reached with the inhibitor Ly 294002 that was used as a positive control in these experiments. The migration-restraining effect of CsA was evident with both cell types, but the effect was not concentration dependent in the range tested.

Investigations of the migration of vascular SMC (rat) under influence of the β-blocker Carvedilol alone or in the presence of CsA were previously described by Kim et al. [52]. Their studies showed that the antimigratory effect of Carvedilol is consistent regardless of the presence of CsA. Careful analysis of their results shows that the single concentration of CsA used in those studies was similar to that used by Hu [51] and was an order of magnitude smaller than the minimum concentration of CsA used in this study. Thus the 10^{-7} mol/l range may also represent a lower bound for the concentration of CsA that has an effect on SMC migration.

Conclusion

In this paper a set of in vitro analyses were conducted, which we deem important for the development and characterization of polymer based drug-eluting stent
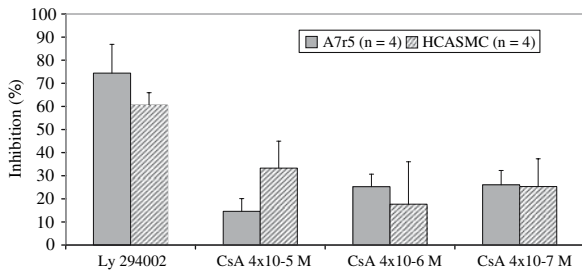
Fig. 10 Effect of CsA on migration of A7r5 cells and HCASMC cells, % inhibition to controls. (Mean values of four measurements and standard deviation)

coatings. We presented data on the in vitro performance of metallic stent prototypes coated with biodegradable PLLA and incorporated with different concentrations of CsA.

The structural integrity of these drug-eluting stent systems after subjection to the mechanical loads occurring during stent crimping and dilation could be demonstrated, and the coated stent systems were found to be safely applicable based on the measured stent dislodgment forces.

Showing that β-irradiation has a strong impact on the physico-chemical properties and degradation of the PLLA coating, it can be concluded that with respect to tissue response and further in vivo degradation behavior of the material, future considerations of a suitable initial molecular weight of the polymer matrix must account for the sterilization method.

The migration assays confirmed the apparently small active concentration interval of CsA. Hence, the release of CsA must be controlled by the type of the polymer, the concentration of the drug in the polymer coating and other parameters, such as the polymer layer thickness. It must be ensured, that the amount of CsA released reliably leads to anti-proliferative rather than cytotoxic concentrations.

Our in vitro data demonstrated that CsA could be released from the PLLA stent coating in doses relevant for the inhibition of SMC proliferation in the short term. However, the issue of late drug release, which may occur during late stage polymer degradation and fragmentation, needs to be addressed in future studies. Furthermore, potential drug accumulation in the vessel wall and the rinsing effect of the blood stream could not be investigated in vitro, and rather have to be studied in vivo.

Altogether, the presented data provide insight into the in vitro function of different PLLA/CsA stent coating systems, and hold general implications for the sterilization and the drug loading and release of polymer based drug-eluting stent coatings.

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