Biodegradable Drug Depots on Coronary Stents – Local Drug Delivery in Interventional Cardiology

T. DIENER, B. HENSEL
Department of Biomedical Engineering, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany

B. HEUBLEIN, S. BARLACH, R. ROHDE
Hannover Medical School, Hannover, Germany

K. STERNBERG, K.-P. SCHMITZ
Institute for Biomedical Engineering, University of Rostock, Rostock, Germany

C. HARDER, S. HARTWIG, M. TITTELBACH
Biotronik Center for Research and Technology, Erlangen, Germany

Summary

Cardiovascular diseases rank among the leading causes of death in Western industrialized countries. In the field of minimally invasive therapies, both percutaneous transluminal coronary angioplasty and the stabilization of the redilated vessel with the help of vessel scaffolding (i.e., coronary stents) have become well-established procedures. Drug-coated stents are intended to further reduce the rate of late complications, especially in high-risk patients. However, most of the approaches pursued so far to achieve a local active agent release have paid little attention to the biocompatibility of the carrier system (e.g., when using acrylates as carriers). This article presents a stent carrier system based on polymers (polyesters) that not only possesses outstanding biocompatible properties, but also allows for the realization of a completely biodegradable drug depot.

Key Words

Coronary stents, drug-coated stents, local drug delivery, degradation, polymer

Introduction

The interaction between the implant and the surrounding tissue or body fluids frequently leads to undesirable side effects, such as restenosis in stented vessels or the formation of fibrotic tissue around pacemaker electrodes. Such side effects can be treated medicinally. In the systemic application of drugs, high doses must be applied over a long period of time, but this approach is often accompanied by undesirable adverse reactions. Another approach is the local supply of pharmacological active agents. In addition, an implant with a coating of biodegradable polymers, which acts as a carrier matrix for drugs, is available.

It is characteristic of degradable and resorbable materials that many of their properties change during a given period of time under the environmental conditions of the human body, up to a complete resorption of the material itself. The degradation products produced during the degradation of bioresorbable materials must themselves be biocompatible, i.e., they must be carried away in vivo by natural metabolic processes without any harmful effects. However, it has to be principally assumed that all foreign non-resorbable and resorbable polymers induce at least an initial tissue reaction. In the case of resorbable polymers, further reactions are to be expected in the final degradation stage (release of soluble components, fragmentations).

In the research literature, poly-L-lactide (PLLA) is regarded as the most suitable resorbable polymer for

coronary stents, based on various in vitro and in vivo studies on biocompatibility [1]. Incorporated active agents are released from the polymer matrix by diffusion according to the existing concentration gradient, as well as during the biodegradation of the coating in the case of degradable polymers. The controlled diffusion of the pharmaceutical agent and the degradation and resorption of the polymer are thus the basis for a targeted release of drugs into the immediate surroundings of the material surface (local drug delivery = LDD). Possible applications are stent coatings with antiproliferative substances for prophylaxis of restenosis or coating of the surfaces of electrodes with anti-inflammatory agents.

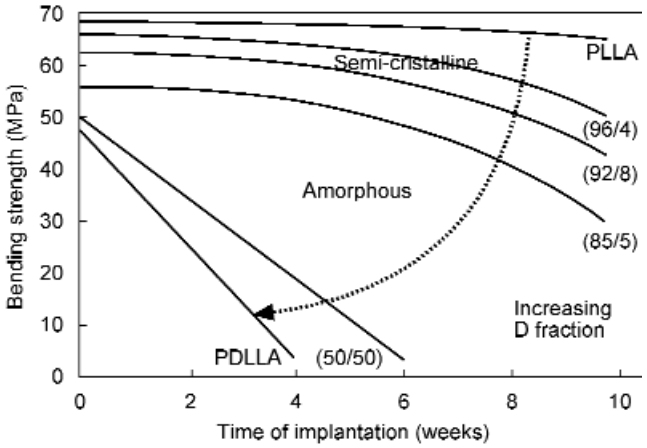

Figure 1. Dependence of cristallinity, bending strength and degradation rate of an copolymer out of poly-L lactide (PLLA) and of poly-D,L lactide (PDLLA) with different (L/D, L) -ratios [5,13].

The Polymer Matrix

Many polymers have already been studied by a multitude of research groups worldwide in regard to their suitability as a polymer matrix for stent coating. The following polymers are examples of suitable carrier matrices for systems capable of releasing drugs [2-4]:

- Non-degradable polymers:
  Polyurethane, polymethacrylate, silicone, polyorgano-phosphacene, polymethacrylate, polyethylene terephthalate, and phosphorylcholine;
- Degradable polymers:
  Polylactide, polyhydroxybutyrate, hyaluronic acid, polycaprolactone, polyorthoester, and fibrin.

Stents have also been coated with the anticoagulative polysaccharide heparin to prevent thrombosis after stent implantation.

The bioresorbable polylactide used as a polymer carrier for the LDD coating in this study belongs to the group of polyesters, in particular to polyhydroxy alkanoate, with lactic acid as its monomer building block. Due to the chiral character of lactic acid (D and L enantiomers), a complex stereochemistry of the lactides (DD, LD, and LL lactides) and polylactides, respectively, results. While polymerization of pure DD or LL lactide leads to completely syndiotactic polymers, mixing these monomers disturbs the strict stereoregularity of the forming material. Co-polymerization of the different stereochemical forms of the lactide dimers allows for the development of a tailored property profile of the polylactides in regard to their mechanical characteristics and degradation behavior [5,6]. In general, the polymer properties, especially of polylactides, depend on the following parameters:

- Molecular weight
- Crystallinity
- Residual monomer content
- (L/D) ratio

Whereas the mechanical properties increase with raising molecular weight, a more narrow distribution and increasing crystallinity, the content of monomer or low-molecular components may act as plasticisers and thus accelerate the degradation process [5].

The main difference between pure PLLA and racemic poly-D,L-lactide (PDLLA) lies in its morphology, as shown in Figure 1. While poly-L-lactide can become highly crystalline with a crystallization grade of up to 80%, poly-D,L-lactide – with 50% each of the L and the D component – is intrinsically amorphous. Therefore, the co-polymer of both lactides shifts between a semi-crystalline and an amorphous appearance according to the (L/D) ratio, with amorphous polylactides being present from a D percentage of about 15 mol% [5].

Generally, increasing L content leads to an increase in the following parameters:

- Crystallization tendency
- Mechanical stability

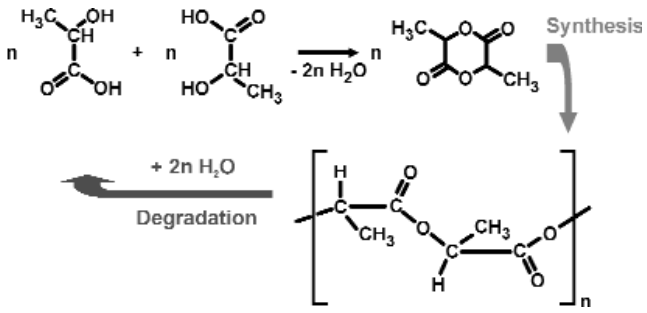

Figure 2. Synthesis and degradation path of polylactide [13].

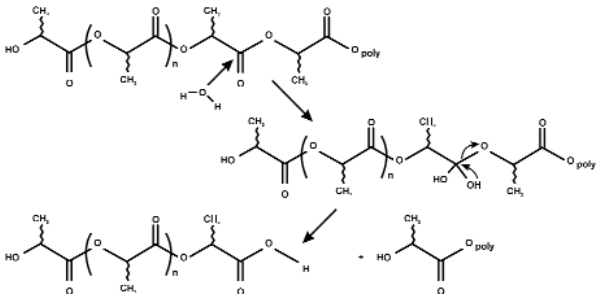

Figure 3. Schematic depiction of hydrolytic degradation [8].

- Degradation time
- Glass transition temperature [7,8]

Figure 2 shows the life cycle of a polylactide, starting at the synthesis path, disregarding the different enantiomer and dimer structures, to the hydrolytic degradation. Polymerization takes place in the melted mass at temperatures between 140°C and 180°C, leading first to the formation of the lactide dimers from the respective lactic acid monomers by means of a condensation reaction, followed by the catalytic opening of the lactide ring using tin catalysts, such as tin-2-ethylhexanoate, which finally results in the polylactide [6,8].

A special characteristic of polylactides – which enables them to be used as temporarily implant materials – is its degradation and resorption ability in the living organism under the influence of water.

Besides the hydrolytic degradation, the degradation of polyesters is also initiated by temperature and radiation, which occur during the process of sterilization.

Although the hydrolytic degradation of polylactide results in non-toxic degradation products, the biocompatibility of the degraded implant itself and its degradation products in the vital organism evokes questions. In order to examine this phenomenon, it is necessary to reveal precisely the degradation products, respectively, within the degradation process. Figure 3 shows the mechanism of the hydrolytic ester cleavage of a polylactide [8].

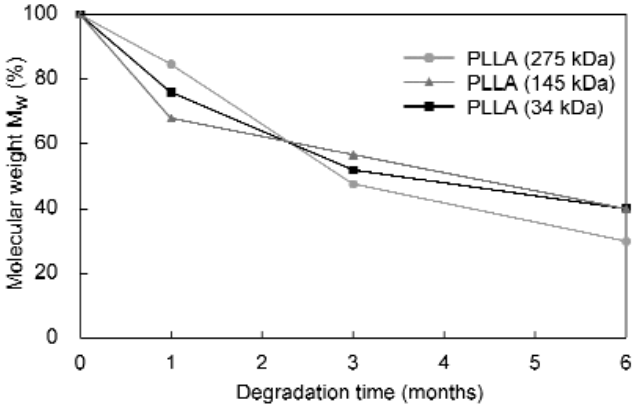

Figure 4. Degradation course of different molecular poly-L lactides (PLLA) [10].

This basic effect of hydrolytic polymer degradation, which is called "random chain scission," is based on water adsorption on the polymer chain, leading to chain cleavage and thus the generation of hydroxyl as well as carboxyl end groups [9]. The oligomeric and monomeric degradation products can be expected to have good biocompatibility, because these non-toxic degradation products can be either metabolized to carbon dioxide and water in the living organism through the citric acid cycle [6] or excreted by the kidneys. Since any ester cleavage is accompanied by an increase in acidic groups and a decrease of the pH value, the degradation process can cause inflammatory tissue reactions due to exceeding the local tissue tolerance. However, the buffer capacity of the local tissue will not be exceeded when using high-molecular, i.e., slowly degrading polylactides. Figure 4 shows the degradation course of different polylactic acids and the decrease in molecular weight in relation to the initial molecular mass [10,11]. The main factors influencing the polylactide degradation [5] are presented in the following subsections.

Geometry of the Implant, Thickness of the Layer

By reducing the component thickness, the autocatalysis is inhibited and, therefore, a decelerated degradation is achieved. On the other hand, a faster degrada-

tion by enlarging the surface/bulk relationship is also achieved. Investigations with PDLLA show the result of the autocatalytic effect, which leads to a greater loss of molecular weight in the interior of the implant than in its exterior regions [4]. This generally valid process can be classified with a compact polylactide component in two degradation mechanisms:

- the surface degradation, the so-called bulk erosion, and
- the internal degradation, the bulk degradation [17].

The most important difference between both mechanisms is the competition between the diffusion velocity of water vw into the polymer and the velocity of hydrolysis of polymer degradation vp. Accordingly, the surface degradation appears in the case of $v_w < v_p$, and the bulk degradation, respectively, the other way round. While the bulk erosion mechanism will start continuously from the moment of implantation, the bulk degradation can be divided into different phases: Within the first stage, water uptake leads to the cleavage of weak van der Waals` and hydrogen bonds. In the second stage, covalent bonds of the main chain are also split, which finally leads to a loss in stability, and the first signs of a bimodal molecular weight distribution are recognizable. At this time, carboxyl end groups at the implant's surface can be neutralized or buffered by the surrounding medium. Therefore a type of diaphragm accrues that prevents the diffusion of autocatalytic degraded components from the interior to the outside. With proceeding chain cleavage, the material loses its consistency and its mechanical integrity due to the release of acid components from the interior of the implant [5,9,15,17].

Structure of the Polymer Chain, Crystallinity
Semi-crystalline polyesters degrade slower than amorphous ones, since less free volume is available for water diffusion. The degradation speed can be regulated by the degree of co-polymerization.

Molecular Weight, Monomers, or Low-molecular Components
The slower the degradation proceeds, the higher the initial molecular weight. If the polymer contains low-molecular components, the degradation speed is accelerated by the increased number of carboxyl end groups.

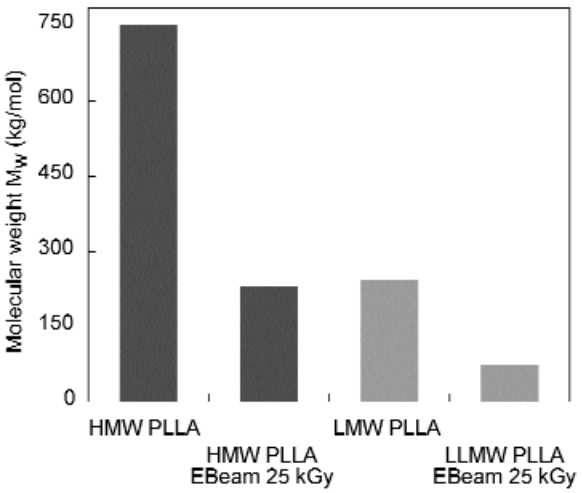

Figure 5. Influence of electron beam sterilization (dose 25 kGy) on a high-molecular (HMW) and a low-molecular (LMW) poly-L-lactide (PLLA).

Density
Similar to decreasing crystallinity, the free volume available for water diffusion and thus also the degradation speed increase with falling material density.

Sterilization
During sterilization with γ- or electron radiation, molecular chains are split, reducing the molecular weight and thus, as described above, increasing the degradation speed. γ sterilization can also lead to increased polymer degradation due to the longer duration of the process. Figure 5 demonstrates the radiation-induced decrease in molecular weight on a high-molecular weight (HMW) poly-L-lactide and a low-molecular weight (LMW) poly-L-lactide. Gas sterilization with ethylene oxide may have a softening effect, aside from leaving behind toxic components in the matrix [10,12]. Therefore, further investigations focus on a high-molecular, semi-crystalline polylactide. On the one hand, degradation within a time window relevant for the LDD application can be expected due to its crystallinity [10]; on the other hand, the high initial molecular weight promises a better biocompatibility, which has already been shown in animal studies as described in the research literature [11]. The poly-L-lactide (approx. 80.000 g/mol) was analyzed in a study [11] and showed an intense neointimal response in comparison to a higher molecular weight PLLA (approx. 321.000 g/mol). It has been suggested that prolonga-

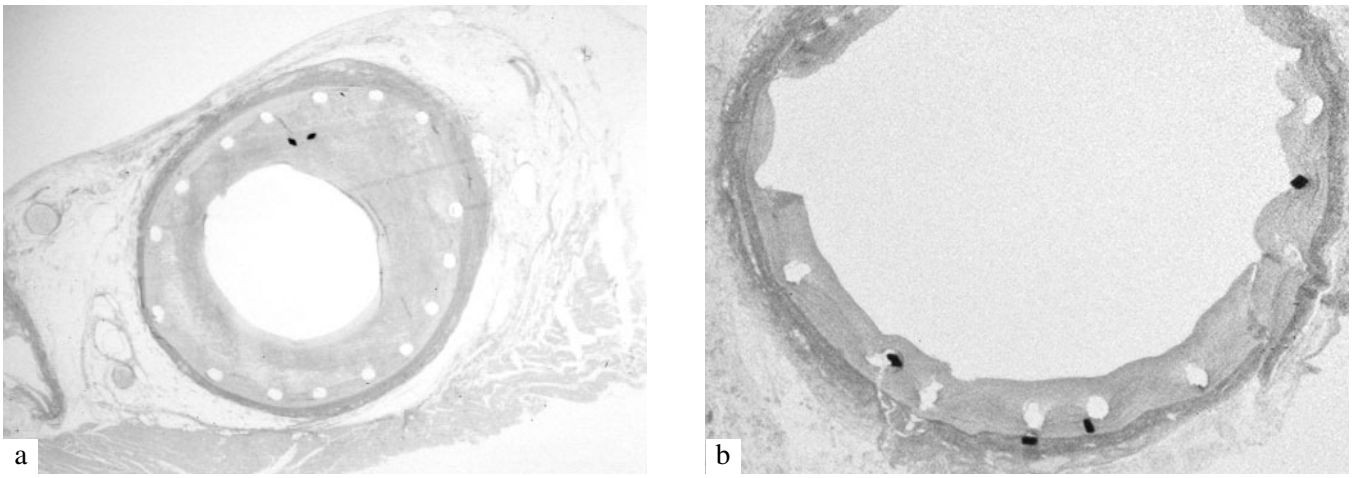

Figure 6. Histological Images. Panel a) Histological cross section of a porcine coronary artery stented with a poly-D,L lactide coated stent. Panel b) Histological cross section of a porcine coronary artery stented with a high-molecular of poly-L lactide coated stent.

tion of the biodegradation time with increasing molecular weight most probably minimizes the concentrations of degradation products, which may play a causal role in tissue inflammation. Nevertheless, the LMW PLLA falls below the described critical molecular weight following the sterilization process (Figure 5).
Figures 6a and 6b show the results of our own trials with a poly-D,L-lactide and an HMW poly-L-lactide. Figure 6a shows a hematoxiline eosine (HE) stained histological cross section of a stented porcine left anterior descending artery (LAD) after a four-week follow-up. The circularly arranged white spots within the vessel wall mark the former locations of the stent struts, which have mostly dropped out from the section during the cutting process. Two residual strut discs (black), which are displaced by the cutting process, are seen in the upper half of the cross section. The stent has been coated with poly-D,L-lactic acid. At the interior side of the struts, a thick neointimal tissue area has formed, which narrows the vessel lumen to a large degree. Figure 6b is a cutout from an Elastica van Gieson (EvG) stained cross section of a stented porcine right coronary artery (RCA) after a 4-week follow-up period. The display detail shows the main part of the cross section. As in Figure 6a, the circularly arranged white spots mark the former stent strut locations. Most of the strut disks have completely dropped out of the section during the cutting process. Four residual strut discs (black) are left in the cross section, of which three in the lower part of the picture are displaced by the cutting process. The struts are completely endothelialized and a reasonably thin neointimal layer has formed after four weeks which does not distinctly narrow the vessel lumen.

Active Agents

Aside from the physiologic tasks of the pharmaceutical agent incorporated in the polymer matrix, which are inhibition of inflammatory reactions, cell division, and cell migration [2], the chemical properties of the active substance are of decisive importance for an LDD system. As a precondition, the substance must retain its pharmacologic activity after sterilization. It is also necessary to evaluate the therapeutic window within the drug proves its efficacy and does not show any toxic effects. Finally, the following are among the decisive factors for the release kinetics, aside from the consistency of the matrix material:

- Molecular size and molecular charge
- Hydrophilic or hydrophobic character of the substance
- Layer thickness
- Active agent content in the polymer layer

The effects of the drug on the growth factor-induced proliferation of human vascular smooth muscle cells

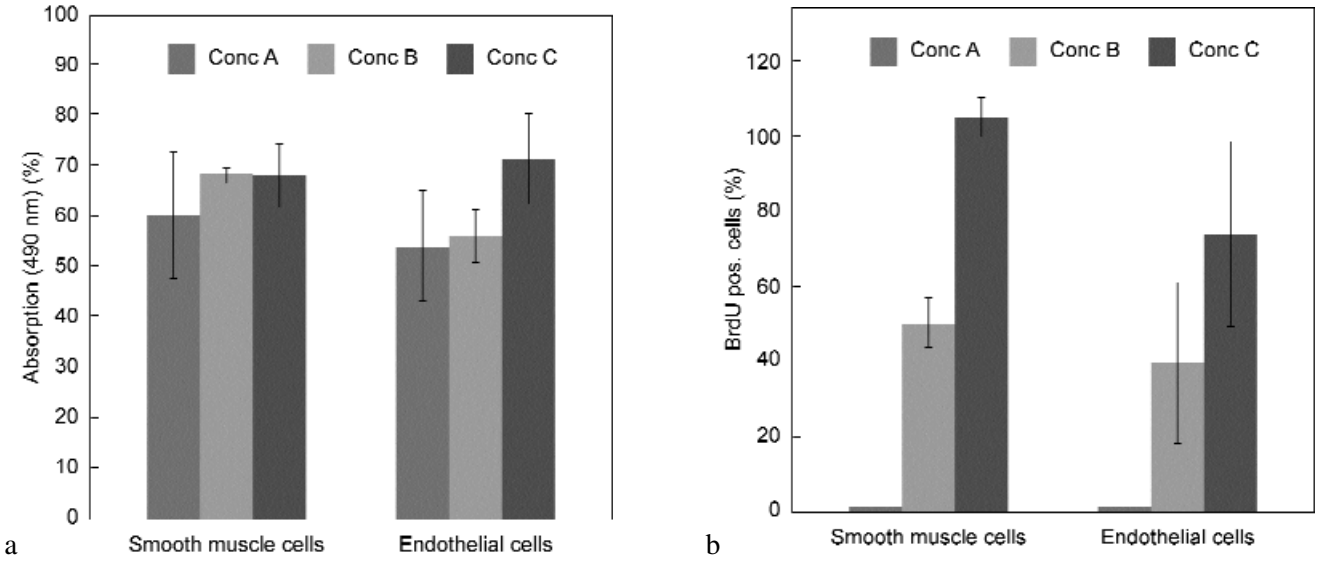

Figure 7. Cell Culture. Panel a) Drug effect on the vitality of human vascular smooth muscle cells and endothelial cells. Panel b) Drug effect on the growth factor induced proliferation of human vascular smooth muscle cells and endothelial cells. See text for detailed information.

(SMC) and endothelial cells (EC) were tested in vitro. The cells were made quiescent in defined serum-free media, and were stimulated by the addition of growth factors (PDGF/EGF). The drug was tested in a defined concentration range. With an immunofluorescence assay based on the incorporation of 5-bromo-2'-deoxy-uridine (BrdU) into cellular DNA, the DNA synthesis was measured as an indicator for cell proliferation. The DNA building block BrDU is incorporated into the DNA molecule during DNA replication. When processing the samples, a fluorescing antibody recognizes and binds to BrDU. For analysis, the number of fluorescent cells is counted under the microscope.

The effects on cell vitality were determined with an assay based on a tetrazolium compound (MTS). This reagent is reduced by vital cells and shows an absorption maximum at the wavelength λ = 490 nm. The absorption is directly proportional to the number of proliferating cells.

The control groups for SMC and EC are acquired without adding the drug and set to 100%. SMC incubated with the drug showed vitality between 60% and 70% in comparison to control cells (Figure 7a). With the highest tested concentration of the drug, proliferation was completely inhibited (Figure 7b). Lower concentrations resulted in an increasing proliferation. Endothelial cells revealed nearly the same results: 50 – 70% vitality and increasing proliferation from 0% to 70%. In regard to in-stent restenosis, the inhibition of smooth muscle cells by this drug could contribute to a reduced neointimal hyperplasia.

Coating of Coronary Stents

The coating of coronary stents with high-molecular weight polymers has been realized using the coating technology shown in Figure 8. The main principle is to expose the stent to a finely dispersed spray of the polymer solution. Related to the strut, a shape-adhering coating of the stent can thus be achieved. Especially when coating filigreed stent structures with narrow radii and small distances between the individual struts, an extremely narrow droplet spectrum is necessary to avoid adhesions between the stent-struts (Figure 9). The self-contained construction of the unit guarantees the coating process without any contamination. Moreover, the refeeding of the fluid is ensured by a closed-circuit construction, whereby a constant layer-composition of successive coated stents is obtained.

By varying the process parameters, any desired thickness of the polymer layer, which constitutes the reservoir for the drug, can be achieved. The section of a stent strut depicted in Figure 10 shows a maximum layer thickness of 15 µm towards the vessel wall, and

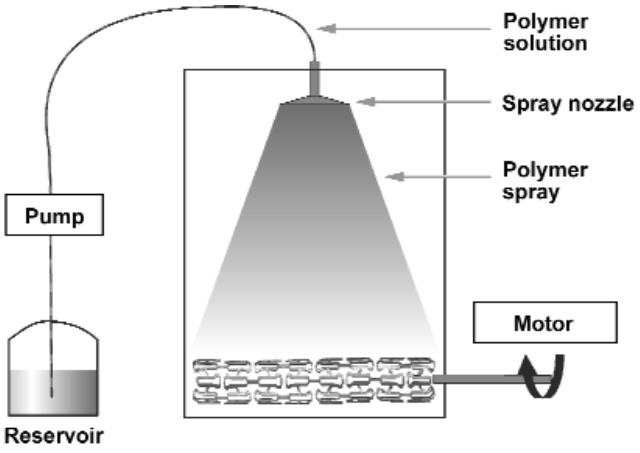

Figure 8. Schematic depiction of the coating technology.

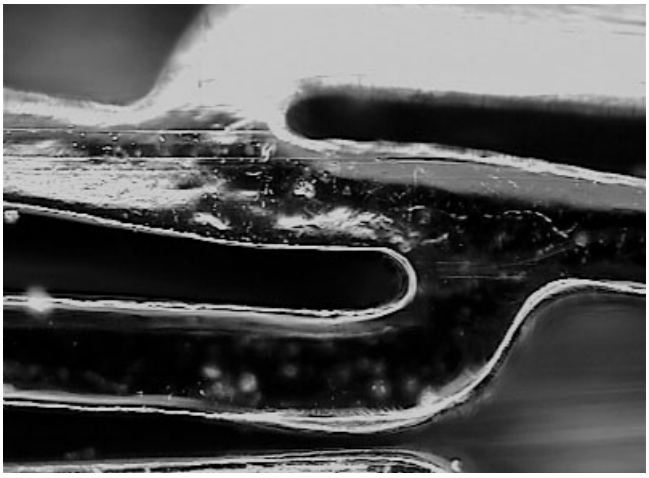

Figure 9. Light-microscopic image of a stent segment. 220 times magnified.

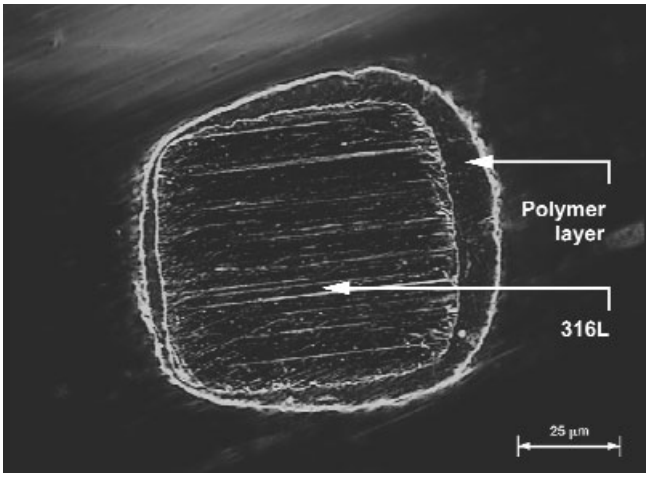

Figure 10. Light-microscopic image of a stent strut section [2]. 500 times magnified.

of about 5 µm towards the lumen. Since the antiproliferative active drug must be introduced into the vessel wall anyway, this distribution of the layer thickness is suited for an LDD system.

Potential risks during stent implantation and balloon dilatation are, among others, the above-mentioned agglutinations, which might lead to a peeling of the stent coating (Figure 11). However, the mechanical integrity of the coating discussed here has already been proven by employing application-relevant stability tests. The polymer layer shows no peeling off from the substrate surface and no chipping. Even in regions of high tension, like the two-dimensional finite element analysis (FEA) of a dilated stent shown in Figure 12, no damage to the layer can be found. Thus, the optimum mechanical and biological behavior of the vessel scaffolding continues to be guaranteed.

Active Agent Elution

The coated, drug-containing stents are stored in vitro (physiologic saline solution and porcine plasma, both 37 °C in agitated medium) to determine the release of the active agent. The rinsing medium is examined for its active agent content by means of high-performance liquid chromatography (HPLC).

Figure 13 clarifies how decisive the choice of the medium is with regard to the elution of the drug in vitro, respectively, on the interpretation of the received release curve. Stents which have been coated with a high-molecular polylactide with the same drug content were rinsed in a physiological saline solution and in plasma. At any measuring point in the chart, a complete change of the rinsing medium was carried out and the taken medium was quantitatively analyzed by means of HPLC on its active agent content. The determined content of the released drug was accumulated and then plotted. Just 9% of the active agent could be determined in the physiological saline solution in comparison with the elution in plasma. While the drug reservoir of the stent, which is rinsed in plasma, is exhausted within 25 days – recognizably at the transition of the release curve into a plateau – the stent that is rinsed in physiological saline solution steadily releases active agent. Proceeding a complete exhaustion of the active agent reservoir, both graphs will result in the same mass of eluted drug after a longer trial duration, at the latest in the complete degradation of the polymer. The dis-

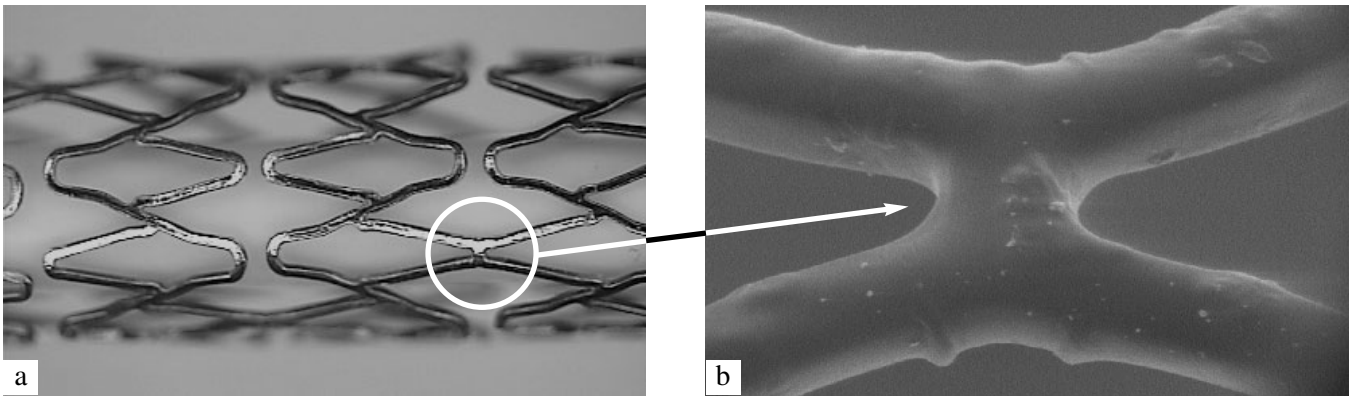

Figure 11. Light-microscopic and scanning electron microscopic image of a dilated coated stent. Panel a) 7 times magnified, panel b) 153 times magnified.

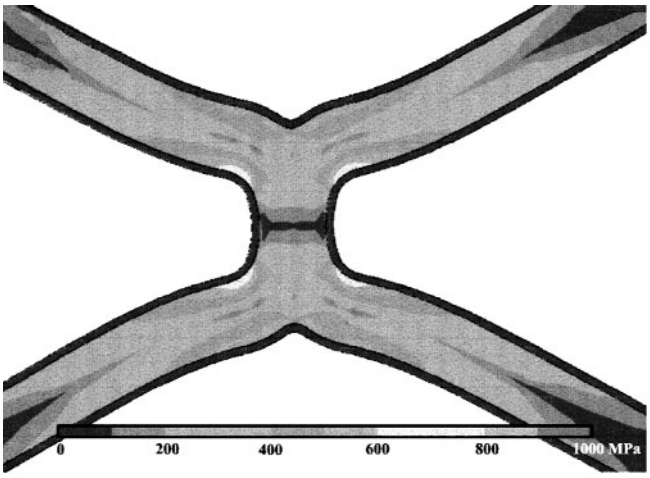

Figure 12. Finite element analysis: Tensions (v. Mises) of a dilated (3.5 mm) stent.

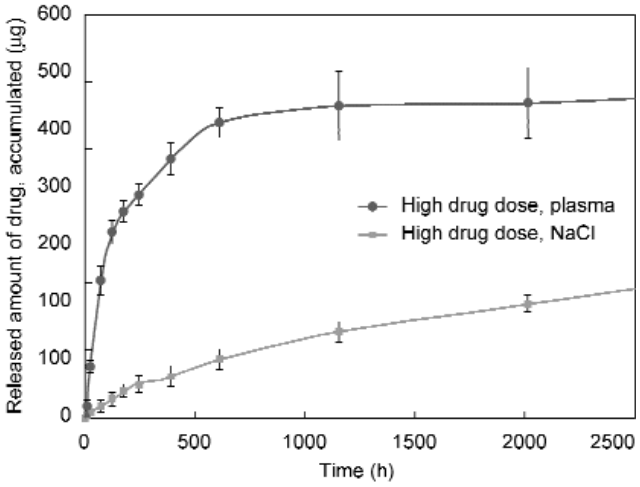

Figure 13. Elution characteristics of high drug-loaded stents in physiologic saline and plasma.

crepancy of the two elution processes is a strong evidence for the influence and the interaction of the drug with the rinsing medium. Factors such as chemical potential of the medium, buffer ability of the medium, adhesion to proteins, solubility, etc. play a prominent roll in these diffusion processes. To design the in vivo situation as realistically as possible, the studies conducted later were carried out exclusively in plasma.

Serving as examples, Figure 14 shows the release characteristics of two stents that were coated with a high-molecular, poly-L-lactide with different drug dosages. The percentage of released drug that was already eluted from the stent is presented in this diagram. The standard plotting of the active agent elution of the different high drug-loaded stents shows approximately the same characteristics with about 50% active agent release within the first 25 days.

The dependency of varying high drug loads results in significant differences in the course of the two curves when plotting the accumulated eluted concentration (Figure 15). According to the drug's action mode in connection with the therapeutic window, it is possible to design a "tailored" active agent release based on these analysis results. Interpretation of existing studies shows that the dependency on the medium (physiologic saline solution, buffer solution, human or animal plasma and serum) must, of course, also be considered,

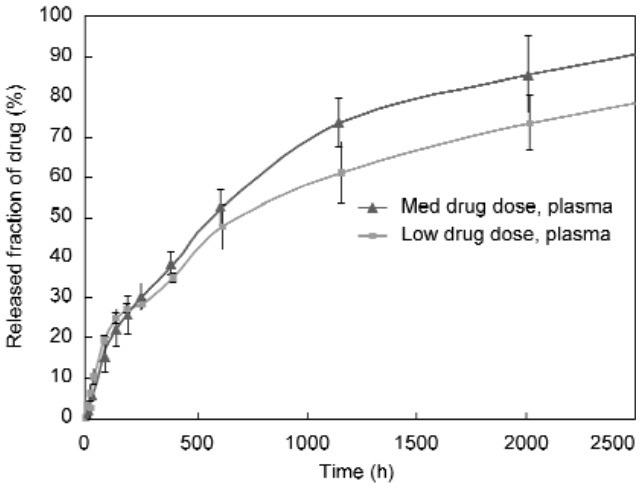

Figure 14. Elution characteristics of two differently high drug-loaded stents.

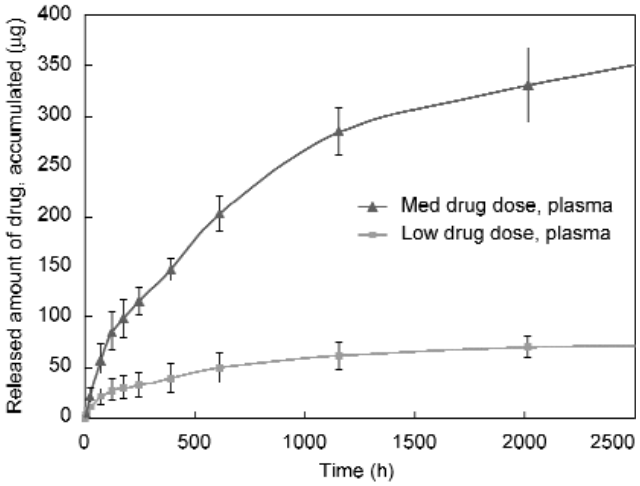

Figure 15. Eluted quantity of differently high drug-loaded stents.

in addition to the influence of the active agent on the elution characteristics described above. Other issues to be considered are, e.g., the bioavailability of the active agent or the consistency of the endothelium (endothelial barrier or injured endothelium). Another point to consider is the behavior of the solubility of the drug, whether it is hydrophobic or hydrophilic. While hydrophobic active agents potentially show prolonged tissue residence (partitioning phenomenon), hydrophilic compounds can distribute freely, but are cleared rapidly [18, 19].

Conclusion

This research study shows that bioresorbable polymers offer good preconditions as a carrier matrix of highly effective drug depots. The already guaranteed biocompatibility of the high molecular poly-L-lactide within animal trials (rabbit subcutaneous, pig coronary artery) together with the variable release kinetics and the results from cell culture tests of the active agent indicates a promising system for local drug delivery application.

As the exact distribution of drug in the vessel wall and its metabolism in the vital organism are yet unknown, and the long-term effects of the polymer that was used have not been demonstrated to date, it will be the task of further experiments and clinical studies to examine the developed system in vivo in greater detail. However, the highly successful developmental steps so far already point towards the future potential of LDD technology with polylactides, especially within the field of interventional cardiology. The final goal for application of biodegradable and drug eluting coatings will be their usage on a completely degradable stent.

References

[1] Labinaz M, Zidar JP, Stack RS, et al. Biodegradable stents: The future of interventional cardiology? J Intervent Cardiol. 1995; 8: 395-405.

[2] Heublein B, Rohde R, Barlach S, et al. Stents as carriers for applying a local active agent: The concept of the "drug-eluting stent." Prog Biomed Res. 2002; 7: 216-225.

[3] Wintermantel E, Suk-Woo H. Biokompatible Werkstoffe und Bauweisen: Implantate für Medizin und Umwelt. Berlin: Springer-Verlag. 1996.

[4] Freier T, Sternberg K, Behrend D, et al. Health Issues of Biopolymers: Polyhydroxybutyrate, Biopolymers, Volume 10. Weinheim: Wiley-VCH. 2003: 247.

[5] Ruffieux K. Degradables Osteosynthesesystem aus Polylactid für die maxillofaciale Chirurgie. (Dissertation). Zürich: Eidgenössische Technische Hochschule Zürich. 1994.

[6] Dorgan JR, Williams JS, Lewis DN, et al. Melt rheology of poly(lactic acid): Entanglement and chain architecture effects. J Rheol. 1999; 43: 1141-1155.

[7] Joziasse CAP, Grijpma DW, Pennings AJ. On the chain stiffness of poly(lactide)s. Macromol Chem Phy. 1996; 197: 2219-2229.

[8] Lunt J. Large-scale production, properties and commercial application of polylactic acid polymers. Polym Degrad Stab. 1998; 59: 145-152.

[9] Bellenger V, Ganem M, Mortaigne B, et al. Lifetime prediction in the hydrolytic aging of polyesters. Polym Degrad Stab.1995; 49: 91-97.

[10] Gogolewski S, Jovanovic M, Perren SM. Tissue response and in vitro degradation of selected polyhydroxyacids: Polylactides (PLA), poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate)(PHB/VA). J Biomed Mat Res. 1993; 27: 1135-1148.

[11] Lincoff AM, Furst JG, Ellis SG. Sustained local delivery of dexamethason by a novel intravascular eluting stent to prevent restenosis in the porcine coronary injury model. J Am Coll Cardiol. 1997; 29: 808-816.

[12] Henn GG, Birkinshaw C, Buggy M, et al. A comparison of the effects of g-irradiation and ethylene oxide sterilization on the properties of compression moulded poly-d,l-lactide. J Mat Sci: Mater Med. 1996; 7: 591-595.

[13] Diener T. Entwicklung eines Polylactid/Calciumphosphat Kompositwerkstoffes als resorbierbares Implantatmaterial. (Diploma thesis). Erlangen: Friedrich-Alexander University Erlangen-Nuremberg (Lehrstuhl für Polymerwerkstoffe). 2001.

[14] Henn GG, Birkinshaw C, Buggy M, et al. A comparison of the effects of g-irradiation and ethylene oxide sterilization on the properties of compression moulded poly-d,l-lactide. J Mat Sci: Mater Med. 1996; 7: 591-595.

[15] Pistner H. Osteosynthese mit Blinddübeln und Platten aus biodegradierbarem Block-Poly(L-Lactid). München: Akademischer Verlag. 1992.

[16] Kopinke FD, Remmler M, Mackenzie K, et al. Thermal decomposition of biodegradable polyesters – II. Poly(lactic acid). Polym Degrad Stab. 1996; 53: 329-342.

[17] Li S, McCarthy S. Further investigations on the hydrolytic degradation of poly (DL-lactide). Biomat. 1999; 20: 35-44.

[18] Creel CJ, Lovich MA, Edelman ER. Arterial paclitaxel distribution and deposition. Circ Res. 2000; 86: 879-884.

[19] Hwang CW, David W, Edelman ER. Physiological transport forces govern drug distribution for stent-based delivery. Circulation. 2001; 104: 600-605.

Contact
Dipl.-Ing. Tobias Diener
Department of Biomedical Engineering
Friedrich-Alexander-University Erlangen-Nuremberg
Turnstrasse 5
D-91054 Erlangen
Germany
Phone: +49 9131 85 2 2417
Fax: +49 9131 27196
E-mail: tobias.diener@biomed.uni-erlangen.de