AAPS PharmSciTech, Vol. 14, No. 3, September 2013 (© 2013)
DOI: 10.1208/s12249-013-0011-9

Research Article

Development of Hydrophobized Alginate Hydrogels for the Vessel-Simulating Flow-Through Cell and Their Usage for Biorelevant Drug-Eluting Stent Testing

Beatrice Semmling,[1] Stefan Nagel,[1] Katrin Sternberg,[2] Werner Weitschies,[1] and Anne Seidlitz[1,3]

Received 11 March 2013; accepted 15 July 2013; published online 6 August 2013

Abstract. The vessel-simulating flow-through cell (vFTC) has been used to examine release and distribution from drug-eluting stents in an *in vitro* model adapted to the stent placement *in vivo*. The aim of this study was to examine the effect of the admixture of different hydrophobic additives to the vessel wall simulating hydrogel compartment on release and distribution from model substance-coated stents. Four alginate-based gel formulations containing reversed-phase column microparticles LiChroprep® RP-18 or medium-chain triglycerides in form of preprocessed oil-in-water emulsions Lipofundin® MCT in different concentrations were successfully developed. Alginate and modified gels were characterized regarding the distribution coefficient for the fluorescent model substances, fluorescein and triamterene, and release as well as distribution of model substances from coated stents were investigated in the vFTC. Distribution coefficients for the hydrophobic model substance triamterene and the hydrophobized gel formulations were up to four times higher than for the reference gel. However, comparison of the obtained release profiles yielded no major differences in dissolution and distribution behavior for both fluorescent model substances (fluorescein, triamterene). Comparison of the test results with mathematically modeled data acquired using finite element methods demonstrated a good agreement between modeled data and experimental results indicating that gel hydrophobicity will only influence release in cases of fast releasing stent coatings.

KEY WORDS: biorelevant dissolution testing; drug-eluting stent; hydrophobized hydrogel; release; vessel-simulating flow-through cell.

INTRODUCTION

Local drug delivery systems such as drug-eluting stents have widely been used for the treatment of arteriosclerotically narrowed blood vessels. Cardiovascular stents are balloon-expandable tiny mesh wire tubes classified as medical devices class III. After placement within coronary arteries, stents help to prevent renarrowing of the previously stented vessel section by mechanically supporting the vessel wall to avoid collapse (1,2). In the early days of the stenting therapy, drug-free stent scaffolds (bare metal stents) were used. In consequence of the resulting high rates of in-stent restenosis, drug-coated stent scaffolds (drug-eluting stents, DES) were developed. These implants are coated with antiproliferative or immunosuppressive therapeutic agents such as rapamycin (also known as sirolimus) or paclitaxel (3–5) in order to suppress proliferation as well as migration of smooth muscle cells. The therapeutic agent can be coated directly upon stent surfaces (6) or mixed with biodegradable (7–9) or non-biodegradable (3,10) coating polymers.

To characterize *in vitro* drug release from drug-eluting stents, *in vivo* studies in animals (9,10) as well as various kinds of *in vitro* dissolution test methods (11–14) have been employed. However, *in vivo* investigation of drug release in humans is only possible as long as plasma concentrations are quantifiable. For sirolimus-eluting stents, plasma profiles over at maximum 7 days have been reported in literature, a time period after which the drug concentration in the blood samples dropped below the limit of quantification (15,16). However, drug-eluting stents are intended to release their drug load over periods of weeks and months.

The United States Pharmacopoeia (USP) and the European Pharmacopoeia (Ph. Eur.) provide a range of *in vitro* test setups intended for dissolution testing of pharmaceutical dosage forms. From these test setups, USP apparatus 4 (flow-through cell) (11) and 7 (reciprocating holder with a specialized stent holder) (12) have been used to investigate drug release from DES. Additionally, non-compendial dissolution test methods are described in literature such as incubation setups (agitated reagent tubes with a small volume of release media) (13,14) or a modified flow-through cell setup referred to as the vessel-simulating flow-through cell developed by our group (17). The vessel-simulating flow-through cell (vFTC) is based on the compendial standard flow-through cell for tablets and allows

[1] Institute of Pharmacy, Center of Drug Absorption and Transport, University of Greifswald, Felix-Hausdorff-Straße 3, 17487 Greifswald, Germany.
[2] Institute for Biomedical Engineering, University of Rostock, Friedrich-Barnewitz-Straße 4, 18119 Rostock, Germany.
[3] To whom correspondence should be addressed. (e-mail: anne.seidlitz@uni-greifswald.de)

for the embedding of DES into a gelled calcium alginate compartment, which serves as an acceptor compartment for the delivered drug mimicking some of the properties of the tissue of the vessel wall. Due to the structural redesign, the vFTC enables the investigation of drug dissolution as well as distribution between the compartments drug-eluting stent coating, release media, and hydrogel under *in vitro* test conditions which are adapted to some of the aspects of the situation *in vivo*. The utility of a vessel-simulating hydrogel for a biorelevant dissolution test setup has been demonstrated in *in vitro* studies (17,18). In order to improve the predictive value of such a test system, a refinement of the composition of the gel compartment to further adapt its properties to the situation *in vivo* is highly desirable.

Hydrogels are swellable, three-dimensional networks prepared from natural (*e.g.*, agarose, alginate, chitosan, collagen) or synthetic polymers (*e.g.*, poly(vinyl alcohol), polyacrylamide, poly(hydroxyethyl methacrylate), polyethylenglycol, and derivates) (19–21). Lateral polymer chains or polymeric backbones are either physically (22–24) or chemically cross-linked (25,26) allowing the network structure to swell but not to dissolve upon contact with aqueous solutions or body fluids. Due to the three-dimensional architecture and various physicomechanical properties such as tensile and compressive gel strength, elasticity, plasticity, and various physicochemical properties such as diffusability, hydrophilicity, and non-toxicity, polymeric gel formulations are widely used in food and biomedical applications (27). Hydrogels allow for the inclusion of additives to attain desirable functionality such as the incorporation of high molecular weight dextran in alginate hydrogels to increase the viscosity (23) or the encapsulation of large molecules such as insulin (28) or cells (29). Recently, polymeric materials have been used as scaffolds for cells in tissue engineering applications (30). Most of all alginate hydrogels have extensively been used to mimic cell and organ functions serving as synthetic extracellular matrix material (29,31). Hydrogels create an aqueous environment for cell growth and the pores allow for the transport of nutrients as well as waste products of the embedded cells. Moreover, a great number of hydrogel-based, drug-loaded networks have been developed for medical and pharmaceutical applications, especially for the use as controlled drug delivery system (26,28,32). Comparable to the use in the vFTC, hydrogels have been used in the field of *in vitro* dissolution testing to study drug release processes from parenteral dosage forms into a tissue-simulating environment (33–35).

Additives may modify the physical and chemical hydrogel properties for example by altering the network structure, the rheological gel properties (23), or the hydrophobicity. To provide a more biorelevant DES environment, this study focused on the approach of modifying the conventional hydrogel compartment of the vFTC apparatus. For this purpose, the calcium alginate hydrogel was modified by the addition of different hydrophobic additives. The modifications were performed by the addition of an emulsifying agent (L-α-phosphatidylcholine), of reversed-phase microparticles (LiChroprep® RP-18) or by replacing the water as the liquid gel phase with oil-in-water emulsions (Lipofundin® MCT 10% and 20%). The distribution coefficient of the selected model substances, fluorescein sodium (log *P*, −1.52 (36)) and triamterene (log *P*, 1.25 (37)), between the modified hydrogels and a buffer compartment and especially the distribution between the three compartments dissolution media, hydrogel, and stent coating upon implantation of model substance-coated stents in the vFTC were investigated.

MATERIALS AND METHODS

Materials

Sodium alginate was purchased from Fagron GmbH & Co. KG (Barsbüttel, Germany), L-α-phosphatidylcholine (type IV-S, ≥30% TLC) was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and LiChroprep® RP-18 (diameter of 5–20 μm) was purchased from Merck KGaA (Darmstadt, Germany). Commercially available emulsions of Lipofundin® MCT 10% as well 20% (B. Braun Melsungen AG, Melsungen, Germany) were used as received. Fluorescent model substance fluorescein sodium was purchased from Fluka (Buchs, Switzerland) and triamterene was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). All other chemicals used were of analytical grade.

Phosphate-buffered saline according to Ph. Eur. with a pH of 7.4 (PBS pH 7.4) was prepared by dissolving 2.38 g $Na_2HPO_4 \cdot 12\ H_2O$, 0.19 g KH_2PO_4, and 8.00 g NaCl in purified water and further water was added until reaching 1,000 mL. pH was adjusted using the respective buffer salt (Na_2HPO_4 or KH_2PO_4).

Methods

Drug-Eluting Stents

A comprehensive description of the coating procedure has already been reported previously (17). Briefly, study devices were prepared by dip-coating stainless steel bare metal stents (length, 18 mm; nominal expanded diameter, 3.5 mm, BIOTRONIK SE & Co. KG, Berlin, Germany) with a model substance embedded in a non-biodegradable model polymer matrix, a blend of ammonio methacrylate copolymer types A and B (Eudragit® RL/RS ratio 3/7 *w*/*w*, Evonik Industries AG, Essen, Germany) dissolved in a mixture of purified water and isopropanol (75/25% *v*/*v*). Fluorescein sodium, a hydrophilic fluorescent substance, and triamterene, a hydrophobic fluorescent drug, were selected as model substances. Model substance content of each coated stent was set to 20–25% *w*/*w* of total coating layer mass.

Preparation of Hydrogels

Reference Hydrogel. In the presence of divalent cations such as Ca^{2+} alginate solutions form a gel based on a cross-linked network. An internal gelation method (22) was used to delay gelling in order to enable pouring of the hydrogel into the chamber of the vFTC prior to gelation. Calcium alginate hydrogels (3% *w*/*w*, alginate gel) were prepared by dissolving sodium alginate powder in purified water.

Phosphatidylcholine Gels. Calcium alginate hydrogels were loaded with L-α-phosphatidylcholine. The fraction of the emulsifier in the hydrogel formulation was set above the critical micellar concentration of 0.1% *w*/*w* (38) to 1% *w*/*w* of the final gel formulation. Phosphatidylcholine gels were prepared by mixing the emulsifying agent with purified water and subsequent addition of 3% *w*/*w* alginate powder to the solution. L-α-Phosphatidylcholine was necessary to

achieve sufficient wetting and to avoid flotation of the LiChroprep® RP-18 particles during gel solidification.

LiChroprep Gels. LiChroprep® RP-18 was selected to introduce a hydrophobic component within the alginate gel. Gels were prepared with different fractions of the microparticles (1, 5, 10, 20, 30, or 50%) to identify suitable gel compositions. To prepare LiChroprep® RP-18 hydrogels, the respective mass of LiChroprep® RP-18 microparticles (diameter of 5–20 μm) was poured onto the phosphatidylcholine preparation (see above). Microparticles were carefully dispersed within the gel to prevent the formation of air bubbles.

Lipofundin 10 and Lipofundin 20 Gels. To introduce an oily component within the hydrogel compartment, the water was replaced in the gel compositions as the solvent for the gelling agent alginate by the oil-in-water (o/w) emulsion Lipofundin® MCT, either in concentrations of 10 or 20% of medium-chain triglycerides (Lipofundin® MCT 10%, Lipofundin® MCT 20%), respectively. Lipofundin 10 and Lipofundin 20 gels were formed by dissolving sodium alginate powder in the respective oil-in-water emulsion under stirring and cooling with ice in order to prevent coalescence of oil droplets.

All preparations were gelled by weighing 16.5 g into a 50-mL glass dish and adding an aqueous suspension of 0.15 g $CaSO_4$ dispersed in 1.5 mL purified water and 470 μL of a 10% *w/w* solution of $Na_3PO_4{\cdot}12H_2O$ in purified water. The components were carefully mixed avoiding the formation of air bubbles in the gel.

The prepared hydrogels were examined regarding their gelation time, transparency, flexibility, and stability under perfusion. Gelation time was defined as period of time after pouring of the gels after which the gels retained their shape when a placeholder rod originally inserted centrally in the cylindrical gel container was removed thus creating an opening in the gels which served as the simulated vessel lumen in the studies with the vFTC (see below). Transparency and flexibility of the prepared gel bodies were evaluated by visual examination as well as by applying manual pressure. Stability of hydrogels under perfusion conditions was examined visually after perfusion in the vFTC for 24 h. In detail, good stability was assumed when no signs of optically visible erosion of the gel bodies especially at the gel/media boundary and no washed-out microparticles within the media container were observed.

Distribution Coefficient

For evaluation of the distribution coefficient (DC) of fluorescein and triamterene between phosphate-buffered media and samples of the different gel formulations, gels were prepared as described above and cast in the chamber of the vFTC. After gelling, each sample was removed from the vFTC, placed in a 250-mL glass flask, and incubated with solutions of the model fluorescent substances in PBS pH 7.4 (concentration 300 μg/L for fluorescein and 81 μg/L for triamterene). Distribution studies were conducted at 37±0.5°C in a shaking incubator (Heidolph Instruments Titramax 1000, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) with 300 rpm for 24 h in case of fluorescein and for 72 h in case of triamterene. The tightly screwed flasks were protected from light. Model substance content of the outer media was quantified fluorimetrically using a microplate reader (Varioskan Flash, Thermo Scientific, Waltham, USA). Fluorescence of fluorescein was recorded at an excitation wavelength of 485 nm and an emission wavelength of 538 nm. Fluorescence of triamterene was measured at an excitation wavelength of 370 nm and an emission wavelength of 434 nm. DCs were calculated by Eq. 1

$$\mathrm{DC} = \frac{V_2}{V_1} \times \frac{c_0 - c_t}{c_t} \tag{1}$$

in which V_1 and V_2 represent the volume (in liter) of the gel (V_1) and the model substance-loaded buffer solutions (V_2), respectively. c_0 (in microgram per liter) is defined as model substance concentration in the buffer phase at the beginning of experiment and c_t (in microgram per liter) is defined as model substance concentration in the buffered media at time point t=24 h (fluorescein) or t=72 h (triamterene). DC studies were conducted in duplicate for the hydrophilic substance (mean of n=2 reported) and in triplicate for the hydrophobic substance (mean± SD of n=3 reported).

Vessel-Simulating Flow-Through Cell Apparatus

To examine *in vitro* release and distribution processes from model substance-coated stents, the vFTC was used. A detailed description of the vFTC and the dissolution setup is given elsewhere (17). A schematic representation of the vFTC and an overview of the experimental setup are depicted in Fig. 1.

The chamber of the vFTC was filled either with the reference or with one of the developed modified alginate hydrogels. After solidification, the placeholder rod was removed and a fluorescent model substance-coated stent was expanded into the resulting lumen *via* a balloon catheter (Biotronik, Berlin, Germany). Balloon inflation and dwell time was set to a pressure of 6 atm and 15 s, respectively. Dissolution media phosphate-buffered saline with a pH of 7.4 was pumped through the lumen of the vFTC from the bottom to the top at 35 mL/min, a flow rate that is adapted to the blood flow rate in coronary arteries (39). The volumes of release media were set to 250 mL for fluorescein and 350 mL for triamterene thus maintaining sink conditions while also allowing for exact quantification. Experiments were conducted in a closed loop system. Dissolution studies were performed in a water bath at 37±0.5°C. Experiments were terminated after perfusion times of 2, 5, 10, 15, 20, 30, 45, and 60 min (fluorescein) or 5, 15, 30, 60, 120, 240, 480, 720, 960, and 1,560 min (triamterene) and the amounts of model substance in each compartment (dissolution media, hydrogel, and residual amount in coating) were quantified independently of each other as follows. To determine respective fluorescent drug amount released into PBS pH 7.4, two 200 μL media samples were collected from the stirred media container. Furthermore, at each time interval, the respective gel compartment was liquefied in tenfold concentrated phosphate buffer pH 7.4 (USP, 68.05 g KH_2PO_4 and 15.64 g NaOH dissolved in 1,000 mL purified water) and the amount of fluorescein or

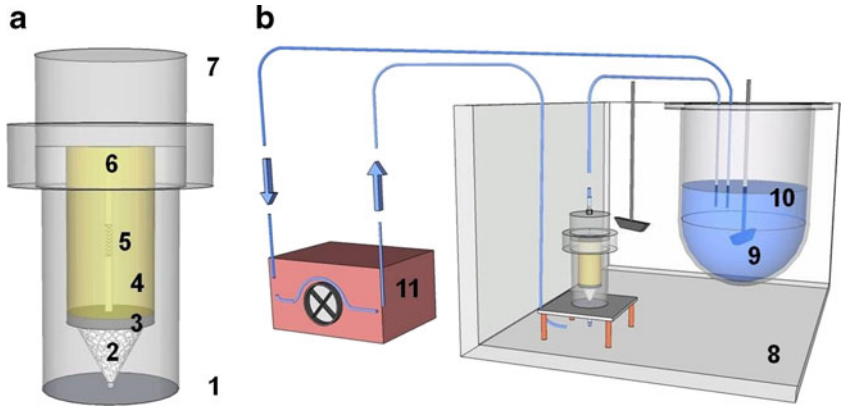

Fig. 1. Schematic of the vessel-simulating flow-through cell (**a**) and the *in vitro* testing methodology (**b**): *1* bottom of the vFTC, *2* glass beads, *3* stainless steel disk (diameter of 22.5 mm), *4* hydrogel compartment, *5* drug-eluting stent, *6* vessel-simulating lumen (diameter of 3 mm), *7* top of the vFTC, *8* water bath (37.0±0.5°C), *9* paddle, *10* media container (PBS pH 7.4), and *11* peristaltic pump (flow rate of 35 mL/min)

triamterene released into the hydrogel was measured. The residual model substance content of the DES coating was examined by incubation of the explanted stents for at least 48 h in 2 mL PBS pH 7.4 at 37±0.5°C and, where appropriate, transferred into freshly prepared phosphate buffer. Transfer into fresh buffer was performed until fluorescence of the buffer after an incubation period of 24 h was below the quantification limit defined as a signal to noise ratio of 3:1. Model substance content of samples of dissolution media, liquefied gels, and residual DES coating were analyzed fluorimetrically with a microplate reader (Varioskan Flash, Thermo Scientific, Waltham, USA) at the respective excitation and emission wavelengths (fluorescein sodium λ_{Ex}, 485 nm; λ_{Em}, 538 nm and triamterene λ_{Ex}, 370 nm; λ_{Em}, 434 nm). Calibration was performed for each fluorescence measurement studies using a dilution series of either model substance-loaded PBS pH 7.4 solutions (samples of media and stent) or model substance solutions in the respective liquefied hydrogels (gel samples). The model substance fractions (in percent) in the respective compartments were calculated based on the sum of the detected amounts of substance in the dissolution media, hydrogel, and stent coating taking previous sampling and evaporation into account. All dissolution studies were performed in triplicate (three stents per time point) and results are reported as mean±SD (n=3 stents for each data point).

Theoretical Modeling of Release and Distribution

In a simplified theoretical model, release and distribution processes in the vFTC were described as diffusion processes by finite element (FE) calculations using the commercial software ABAQUS (Dassault Systèmes Simulia Corp., Providence, USA, version 6.11). Details of the numerical calculation are given elsewhere (18). Briefly, the two-dimensional model consists of a homogeneously loaded coating layer of thickness l around a quadratic strut (edge length of 100 μm) which is placed in contact with a segment of a hollow cylinder ($d_{outside}$=22.6 mm, d_{inside}=3 mm, segment angle calculated according to volume ratio of stent coating and hydrogel). The release into stirred media is roughly described *via* the boundary condition c=0 for the inner surface of the hollow cylinder and the luminal side of the coating. The mass flux vanishes at the inner surface of the coating (no flux into the metal) and the outer surface of the hollow cylinder as well as at the side edges of the model due to symmetry reasons. The entire model contains a mesh of 7300 eight-node biquadratic elements.

The coating layer thickness l was determined by differential weighing prior to and after coating taking the stent surface according to manufacturer specification and the density of the coating into account. For the FE calculations, the concentrations were normalized by the solubility s_i of the model substance in the compartment i. The solubility in the polymer layer was assumed to equal the amount of model substance incorporated. The solubility of the model substance in the reference alginate gel (S_{h1}) was set approximately to the solubility in water (27.7 μg/mL (40)). For the solubility in the modified LiChroprep gel (S_{h2}), a four times higher value was assumed taking the experimental findings for the gel–water DC into account. The diffusion coefficient of the model substance in the coating layer and in the reference (alginate gel) was determined in previous experiments as described elsewhere (18). In a rough approximation, diffusion velocity is assumed to be unaffected by the hydrogel modification and both gel types are described by the same diffusion coefficient. The model parameters were used as follows: coating thickness l=3 μm, diffusion coefficient in coating $D_c=2\times10^{-10}$ mm^2/s, diffusion coefficient in hydrogel $D_h=5\times10^{-4}$ mm^2/s, solubility in coating S_c=0.3 g/mL, solubility in reference hydrogel $S_{h1}=3\times10^{-5}$ g/mL, and solubility in LiChroprep hydrogel $S_{h2}=1.2\times10^{-4}$ g/mL.

RESULTS

For the purpose of gel composition refinement, hydrogels were prepared with various amounts of LiChroprep® RP-18 or Lipofundin® MCT in order to introduce hydrophobic domains within the hydrogels. Gel compositions suitable for the vFTC were identified and the effect of these modifications on drug release and distribution from fluorescein or triamterene-coated stents was examined.

Modified Hydrogels

To identify a suitable LiChroprep® RP-18 particle-containing gel composition, six concentrations (1, 5, 10, 20, 30, and 50% *w*/*w*) of LiChroprep® RP-18 were investigated. The desired gel formulation should be elastic under external force (stenting procedure), dimensionally stable after solidification, and under perfusion (shear stress) during dissolution testing within the vFTC. In order to facilitate the stenting procedure, it is also considered favorable if the gels are transparent. Furthermore, transition time from pourable (liquid state) to form-stable gel bodies had to be maintained allowing an easy filling of the vFTC chamber and a timely start of the experiment. An overview of the characteristics of the developed LiChroprep gel formulations is listed in Table I.

With an increasing microparticle concentration of 1, 5, 10, 20, and 30% *w*/*w*, gelation time increased to 15, 20, 60, 300, and 480 min, respectively. Following the same order, flexibility and transparency were reduced leading to more brittle and opaque gel bodies. Disintegration and erosion of the cylindrical gel body were detected during hydrogel perfusion for formulations with a solid fraction of 20% *w*/*w* or more. In these gels, washed-out microparticles were detectable within media container after 24 h of perfusion. At solid-phase concentrations of 10 and more percent *w*/*w* LiChroprep® RP-18, an increasing number of air bubbles dispersed within the gel bodies were observed. The development of a gel containing 50% *w*/*w* LiChroprep® RP-18 failed since gels did not solidify in acceptable time spans. All further experiments were conducted using the formulation containing 5% *w*/*w* LiChroprep® RP-18.

Gels containing the surfactant phosphatidylcholine gelled within 15 min. Both Lipofundin formulations gelled within 20 min. Flexibility and stability of the gel bodies under flow conditions was comparable to the reference. Similarly, no air pockets were entrapped within the hydrogels after solidification. However, the lipophilized gels are not transparent. A photograph of the gel bodies of the final hydrogel formulations is depicted in Fig. 2.

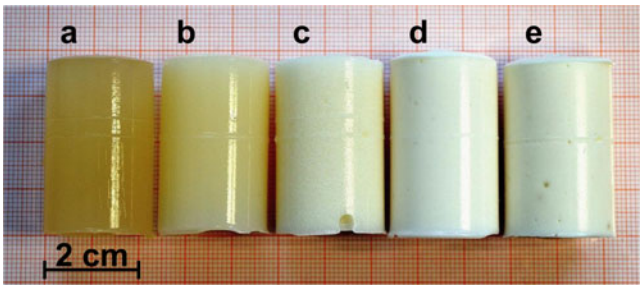

Fig. 2. Photograph of the solidified hydrogels: alginate (*a*), phosphatidylcholine (*b*), LiChroprep containing 5% *w*/*w* (*c*), Lipofundin 10 (*d*), and Lipofundin 20 (*e*)

Distribution Studies

To evaluate the potential of the developed gel formulations as more hydrophobic gel compartments for release studies in the vFTC, modified hydrogels were characterized by determination of the DCs of the model substances between the gel and media. The DCs obtained for the hydrophilic model substance fluorescein (mean of $n=2$) did not differ considerably for the different gel formulations (DC reference gel = 0.9, phosphatidylcholine gel = 0.8, LiChroprep gel = 1.0, Lipofundin 10 gel = 1.0, and Lipofundin 20 gel = 1.0). The DCs of triamterene (mean of $n=3\pm$SD) between the respective modified gel formulations and buffered media were higher compared to the reference. DC increased approximately by a factor of 5 (DC, 4.7±0.5) for the microparticle containing gel and by a factor of 3 (DC, 3.1±0.8 (Lipofundin 10 gel); DC, 2.6±0.1 (Lipofundin 20 gel)) for both oil droplet containing gel samples.

Vessel-Simulating Flow-Through Cell Apparatus

The incorporation of hydrophobic agents within the hydrogel compartment of the vFTC may influence drug release from cardiovascular implants and the resulting distributions. A comparison of the release profiles obtained for fluorescein-coated stents with the reference hydrogel (alginate) and the modified gel formulations with 1% *w*/*w* L-α-phosphatidylcholine is given in Fig. 3a and a comparison of the reference with 5% *w*/*w* LiChroprep® RP-18 is given in Fig. 3b.

As shown in Fig. 3a, b, the fraction (in percent) of hydrophilic model substance detected within the release media increased continuously whereas the fraction of residual model substance in the stent coating decreased independent of the gel formulation. The model substance fraction detected in the hydrogel ranged between 20 and 40% throughout the time span of the release study (1 h). The highest fractional fluorescein

Table I. Results of Gelation Time Measurement and Organoleptic Evaluation of Critical Properties of the Prepared LiChroprep Gel Formulations with Different Percentage Mass Fractions of Microparticles (x). Score: +++ = Good, ++ = Acceptable, and + = Unsatisfactory

x	Gelation time (min)	Flexibility	Transparency	Stability under perfusion[a]	Remarks
1% *w*/*w*	15	+++	++	+++	–
5% *w*/*w*	20	+++	+	+++	A few, small air bubbles dispersed within the gel body
10% *w*/*w*	60	+	+	++	Small air bubbles dispersed within gel body
20% *w*/*w*	300	+	+	+	Many small air bubbles dispersed within gel body
30% *w*/*w*	480	+	+	+	Large number of air bubbles and pores dispersed within gel body
Reference (0% *w*/*w*)	15	+++	+++	+++	–

[a] Perfusion duration of 1 day

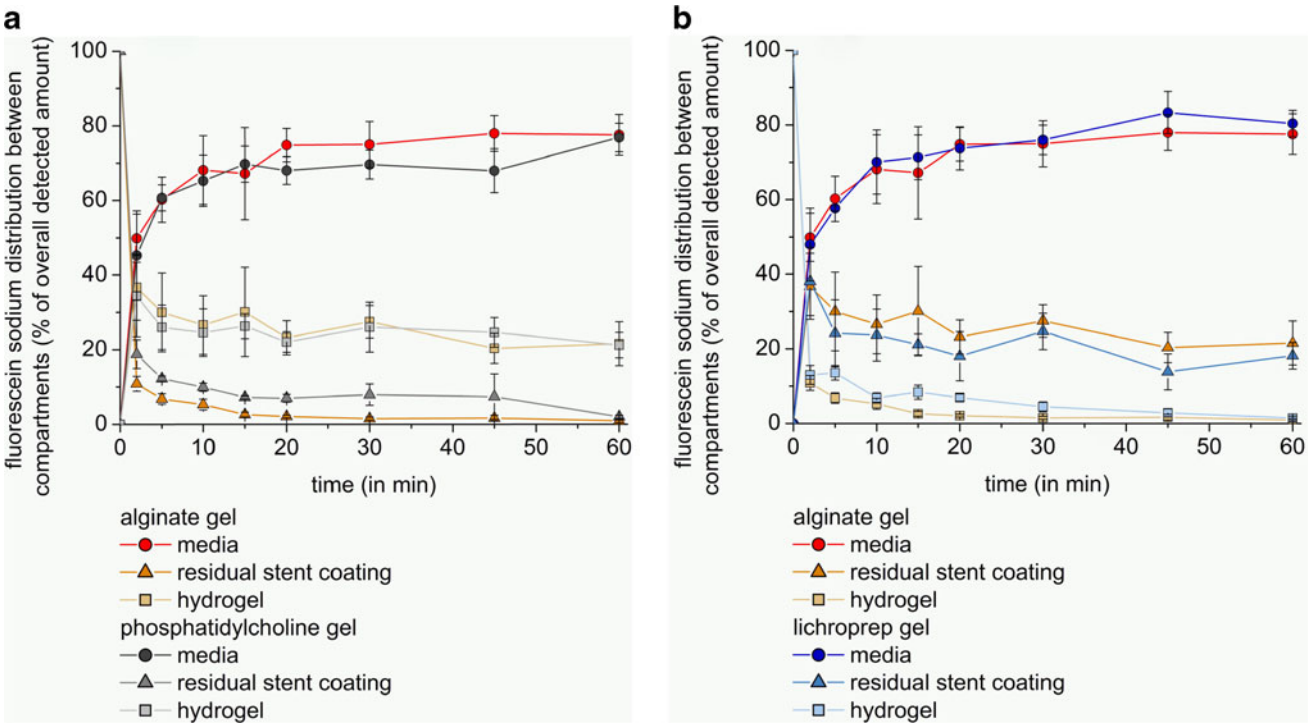

Fig. 3. **a**, **b** Comparison of reference and modified hydrogels. **a** Alginate gel *versus* phosphatidylcholine gel and **b** alginate gel *versus* LiChroprep gel containing 5% *w/w* LiChroprep® RP-18. Cumulative amounts (in percent) of fluorescent model substance fluorescein detected in PBS pH 7.4, respective gel formulations, and residual model substance fractions within the stent coating. Experimental data are given as mean±SD (n=3 stents for each data point)

loads of the gels were observed after only 2 min. After 1 h, 78±5% (alginate gel, n=3), 77±4% (phosphatidylcholine gel, n=3), and 80±4% (LiChroprep gel, n=3) of the total detected amount of fluorescein were dissolved in the perfusion media PBS pH 7.4. At that time point, fluorescein release was also almost completed with a fraction remaining in the polymeric coating layer of 1±1% (alginate gel, n=3), 2±1% (phosphatidylcholine gel, n=3), and 2±1% (LiChroprep gel, n=3), respectively. The fraction of fluorescein detected in the gel compartment was determined to be 22±6% (alginate gel, n=3), 21±3% (phosphatidylcholine gel, n=3), and 18±4% (LiChroprep gel, n=3) at the end of experiment.

The comparison of experimental data of triamterene release and distribution from DES using the reference (alginate) and the modified gel formulations is given in Fig. 4, in which alginate gel *versus* 5% *w/w* LiChroprep® RP-18 hydrogel is shown in Fig. 4a and alginate gel *versus* Lipofundin 10 or Lipofundin 20 hydrogel is shown Fig. 4b.

As indicated in Fig. 4, the release of the hydrophobic fluorescent model substance occurred much slower compared to the hydrophilic model compound. After 1 h, the percentage of triamterene in the PBS pH 7.4 compartment ranges between 18±2% (Lipofundin 20, n=3) and 24±3% (Lipofundin 10, n=3), while the percentage of triamterene remaining in the stent coating ranges between 79±2% (Lipofundin 20, n=3) and 72±4% (Lipofundin 10, n=3). Of total triamterene, 3±1% (Lipofundin 20, n=3) and 4±1% (Lipofundin 20, n=3) were detected in the hydrogel compartment at the same time. After 26 h, 89±2% (alginate gel, n=3), 89±1% (LiChroprep gel, n=3), 85±3% (Lipofundin 10 gel, n=3), and 80±3% (Lipofundin 20 gel, n=3) of total triamterene were released into the media compartment. At the same time, 6±2% (alginate gel, n=3), 6±1% (LiChroprep gel, n=3), 9±3% (Lipofundin 10 gel, n=3), and 15±3% (Lipofundin 20 gel, n=3) remained within the stent coating.

Since the determination of the DC yielded the highest DC for the triamterene LiChroprep gel—media distribution, this case was also exemplarily modeled mathematically. A comparison of experimental and FE modeled data for triamterene distribution upon release in the vFTC with the reference and LiChroprep hydrogel is given in Fig. 5. Modeling was performed using solubility values of triamterene in the reference and the LiChroprep gels that were calculated based on the solubility in water *via* the DCs. Data are plotted as fraction (in percent) of triamterene detected in buffered media, hydrogel, and stent coating over square root of time.

The results of theoretical FE modeling showed only minor deviations from the experimental data with a slightly higher fraction contained in the media and correspondingly a slightly lower fraction remaining in the stent coating in the modeled data. In addition, wide linear ranges in both experimental and theoretical distribution curves in a plot over square root of time can be observed which are typical for diffusion-controlled release and distribution processes. An increase in solubility of a factor of 4 within the LiChroprep gel compared to the alginate yields no clear changes in the calculated distributions between the media, gel, and DES compartments. These findings are in accordance with our experimental results, where no influence of the gel modification on the release and distribution behavior was detected.

DISCUSSION

The aim of this study was to examine the effect of the admixture of different hydrophobic additives to the vessel wall

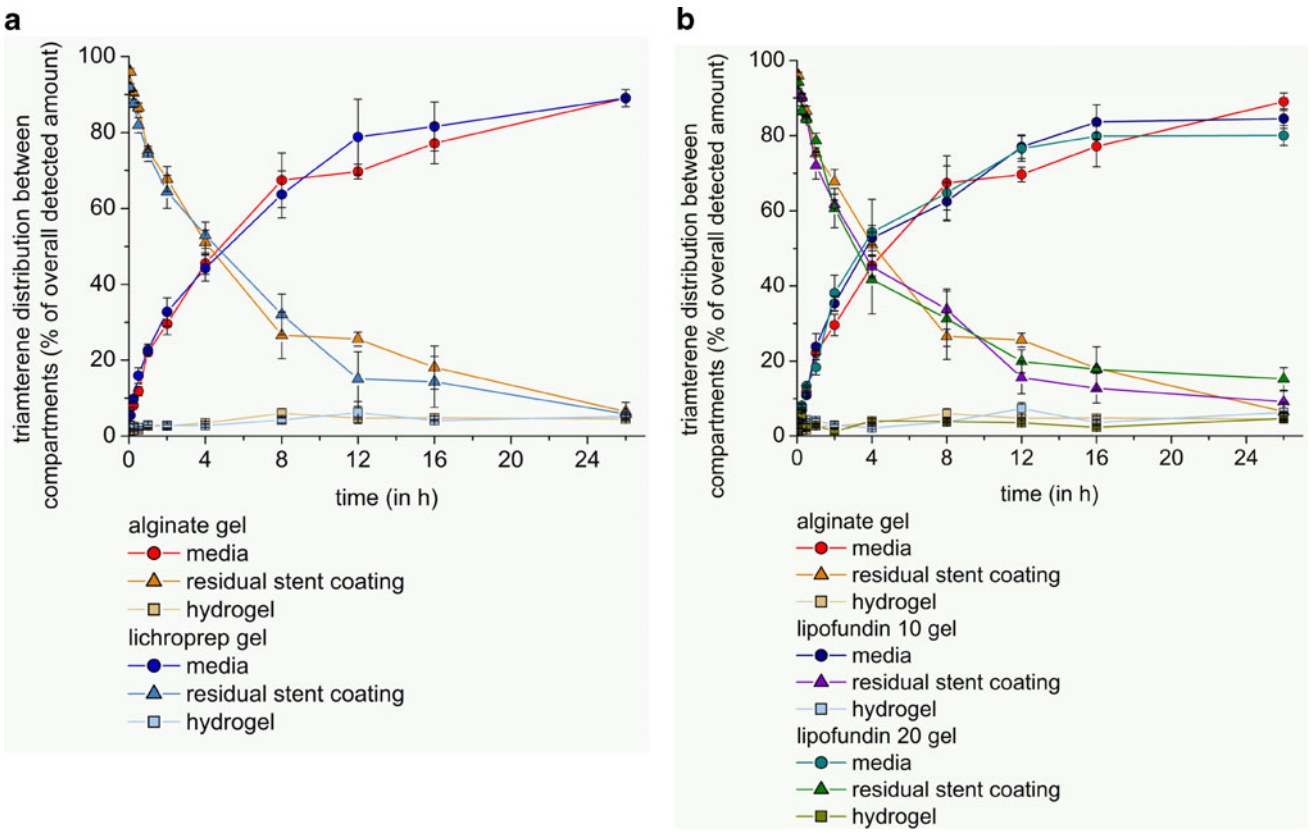

Fig. 4. **a**, **b** Comparison of reference and modified hydrogels. **a** Alginate gel *versus* LiChroprep gel containing 5% *w/w* LiChroprep® RP-18 and **b** alginate gel *versus* Lipofundin 10 and Lipofundin 20 gel. Cumulative amounts (in percent) of triamterene detected in PBS pH 7.4, respective gel formulations, and residual model substance fractions within the stent coating. Experimental data are given as mean±SD (n=3 stents for each data point)

simulating hydrogel compartment on release and distribution from model substance-coated stents. Four alginate-based gel formulations containing reversed-phase column microparticles LiChroprep® RP-18 or medium-chain triglycerides in form of preprocessed oil-in-water emulsions Lipofundin® MCT in different concentrations were successfully developed.

Modified Hydrogels

Hydrogels are a simplified approach to simulating tissue in an *in vitro* setup. They lack many features of tissue such as diffusion barriers, active transport mechanisms, or specific binding sites. Unless the released substance is subject to active transport, the dominant transport mechanism in tissue is diffusion. Diffusion is also the predominant transport mechanism in hydrogels opposed to convection, which is most likely dominant in stirred *in vitro* dissolution setups. Therefore, the use of a hydrogel compartment is a valuable approach when differences in release result from different transport phenomena. The idea of including a gel compartment in a dissolution test setup for DES has also been adopted by O'Brien *et al.* (35) who examined release from a model single stent strut in a geometrically different setup. The goal of the study presented here was to include hydrophobic domains inside the hydrophilic gel compartment to further adapt the test conditions to the situation *in vivo*.

Gel formulations containing LiChroprep® RP-18 particles up to a concentration of 30% *w/w* were successfully developed. The biggest problem associated with the rise of microparticle content was the observed loss of stability under perfusion for gels with 20% *w/w* microparticles or more. Furthermore, a rise in gelation time was observed for these gels. Delay of solidification and loss of stability of the hydrophobized gel formulations from 15 min (reference) up to more than 60 min (fraction of microparticles of 10% *w/w* and above) may be due to the rise of the solid fraction. In the swollen state, alginates usually form a coherent network architecture in which lipid droplets or particles are finely dispersed. The presence of hydrophobic particles such as LiChroprep® RP-18 seems to influence the three-dimensional network structure. In a pasty system with a particle fraction of ≥20% *w/w*, the formation of the structure-giving chelate complexes between two polymer chains of homopolymeric regions of L-α-guluronate acid (G-block) and calcium ions (egg-box model) (22,41) or, in a more general sense, the number of junction zones may be reduced which leads to a decreased gel strength. As the number of these junction zones decreased by an increased in percentage of microparticles (0–30% *w/w* RP-18 column material), the gel bodies also became more brittle. Similar results were observed by Zhang *et al.* (23) who report on a decrease of shear moduli, which represents a decreasing cross-linking density, as a result of the addition of high molecular dextran to 1.25% *w/w* alginate hydrogels in relation to increased dextran concentrations.

In addition, gel bodies became more opaque as the LiChroprep® RP-18 fraction increased. The gel formulation containing 5% *w/w* LiChroprep® RP-18 includes the positive characteristics of the alginate gel combined with the maximal amount of RP-18 material and, therefore, the largest degree of hydrophobicity, which can be dispersed within the hydrogel while retaining gelation time of approximately 15 min, flexibility,

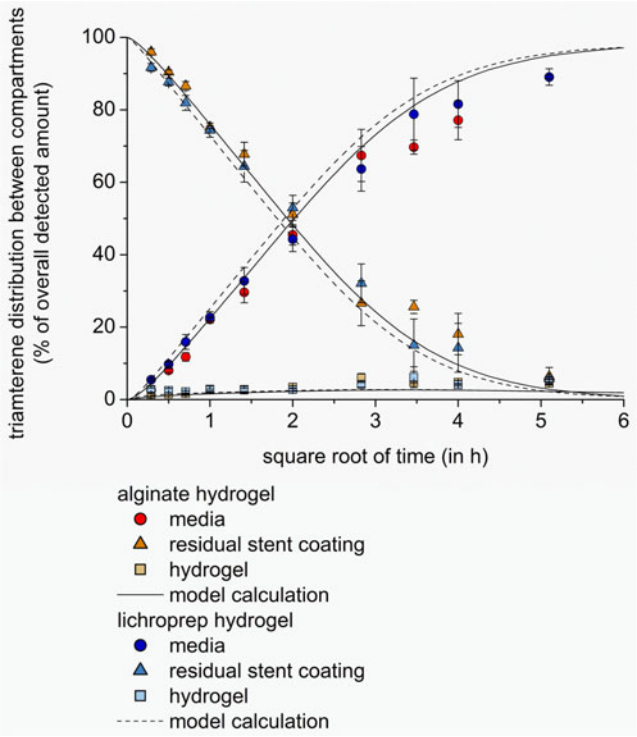

Fig. 5. Triamterene distribution between the compartments release media, stent coating, and hydrogel formulation (reference or LiChroprep gel containing 5% *w/w* microparticles) in the vessel-simulating flow-through cell apparatus. Comparison of experimental (*dots*) and mathematically modeled (*lines*) data where alginate gel data set is represented by *solid lines* and LiChroprep gel data set by *dashed lines*. Experimental data are given as mean±SD (n=3 stents for each data point)

and stability under flow conditions. Therefore, this formulation was identified as the most suitable gel for the vFTC.

Compared to the reference, both lipophilized hydrogels containing 10 or 20% medium-chain triglycerides showed a slight increase in sol-gel transition times from 15 to 20 min. Also, the gels are fully opaque due to the inclusion of the emulsion. Using the developed method of preparation under ice cooling, coalescence of the oil droplets could successfully be avoided.

Distribution Studies

In previous experiments, it was demonstrated (data not shown) that the equilibrium distribution of the fluorescent compounds between the two compartments hydrogel and aqueous media was reached within t=24 h for fluorescein or t=72 h for triamterene. Due to the selection of fluorescein with a log P value of −1.52 and triamterene with a log P value of 1.25, two contradictory model substances were selected in terms of their physicochemical properties such as their water solubility, with fluorescein as a highly water-soluble substance (0.1–1 g/mL (42)) and triamterene as a poorly water-soluble model substance (27.7 μg/mL (40)). The amount of water included in a gel body ranges from 97% *w/w* of the total gel mass for the reference to approximately 77% *w/w* for the Lipofundin 20 gel. Due to the high water solubility of fluorescein and the fact that the gelled compartment consists primarily of water, similar fluorescein distributions independent of the gel formulation may be predicted. On the opposite, a greater accumulation of the hydrophobic model substance triamterene in the hydrophobized hydrogels might be expected.

DC determined for the hydrophilic drug substance between media and respective modified gel formulations did not differ distinctively from the DC of the alginate gel. This phenomenon can possibly be explained by the ratio of additive to gel fraction. The fraction of added reversed-phase microparticles or micro droplets of MCT is too low to induce a hydrophobic gel environment and subsequently to measure a noticeable change to DC values of fluorescein. The comparison of DC of the hydrophobic model drug triamterene between media and reference or modified hydrogels shows that the additives influence the distribution behavior. DC increased by a factor of almost 5 for the LiChroprep gel as well as approximately 3 for the Lipofundin 10 and 20 gels and the phosphatidylcholine gel. The influence of additives in hydrogels on DCs has also been reported by Guilherme *et al.* (43). With an increasing fraction of poly(*N*-isopropylacrylamide), the gel environment became more hydrophobic leading to a decreased DC of the more hydrophilic dye orange II and to an increased DC of the less hydrophilic dye of methylene blue. This finding shows that the environment of the respective hydrogel may have a distinct influence on the distribution behavior of a dissolved substance.

Release Study

Vessel-Simulating Flow-Through Cell

Fluorescein sodium was released very fast in the vFTC irrespective of the gel formulation which lead to initial maxima in the fractions detected in the gels after 10 min. Based on redistribution processes between media and gel compartment, the initially high model substance fraction in the gel was reduced during further perfusion eventually leading to an equilibrium distribution, which has already been reported previously (17). The comparison of distribution profiles obtained with the reference formulation and the phosphatidylcholine as well as the LiChroprep gels yielded no marked differences which had to be expected from the results of the DC studies. It is likely that the hydrophilic properties of the gel remain dominant in the employed concentration range of the hydrophobic additive. Accordingly, it seems difficult to modify hydrogels to an extent that these gel properties change the release and distribution behavior of extremely hydrophilic model substances such as fluorescein in the vFTC.

Compared to the hydrophilic model substance, triamterene release occurs more slowly and initial peak gel concentrations were not observed. For this hydrophobic model substance, a rise of the gel–water DC was achieved with the employed additives. It is conceivable that such a rise in DC may influence release and distribution in the vFTC in different ways. On the one hand, the triamterene fraction detected in the gel compartment will increase while the media fraction decreases at the end of the release from the coating when approaching equilibrium distributions. Moreover, the release of the substance from the stent coating into the gel will directly be influenced, since this DC between gel and coating is also expected to change with the gel modification. An increase in gel hydrophobicity may lead to a faster transport of the substance from the coating into the gel.

The experimental data, however, shows neither an increase in triamterene fraction in the gel compartment nor a faster depletion of the stent coating in spite of the essentially increased DC. It must be assumed that in the case of triamterene the release and distribution from the stent is substantially governed by the properties of the coating. An increase in gel hydrophobicity does not result in a distinct increase in the release rate from the stent. The observed maximum gel concentration of approximately 6% does not change in the dependency of the gel formulation, either, since it is also governed by the release rate from the coating. In the respective time period (26 h), the release from the stent coating was not completed and an equilibrium distribution was not yet achieved. This may, at least in part, be the case because of the fairly small contact surfaces which are available for mass transfer. The calculated equilibrium concentration of triamterene in the hydrogel is also fairly small (5%) due to the chosen volume ratio between gel and perfusion media in the experiments with the vFTC. However, sink conditions caused by the large volume of the systemic circulation in combination with the clearance must be expected *in vivo*. An equilibrium distribution will not be achieved at all.

Results of the FE simulation reinforce the interpretation of the experimental results. The release from the stent coating is parameterized by the diffusion coefficient. The calculated diffusion coefficient for the stent coating ($D_c=2\times10^{-10}$ mm^2/s) is about 6 orders of magnitude smaller compared to the diffusion coefficient of the gel ($D_h=5\times10^{-4}$ mm^2/s). Thereby, the diffusion from the coating governs all subsequent processes in the simulation. The calculation shows that a distinct influence of the hydrophobization of the gel compartment on the distribution processes is to be expected for triamterene in cases of faster release, *i.e.*, greater diffusion coefficients for the coatings (data not shown).

The calculated model substance fractions for the gel compartment are slightly lower than the experimentally determined values. In the simulation, the perfusion media is modeled *via* the boundary condition $c=0$ resulting in a complete model substance depletion of the whole system in infinite time. Such a "maximum" sink condition is not representative of the experiment in which finite media volumes are used and redistribution from the media to the gel may occur. Therefore, the model substance fraction in the gel is underestimated in the simulation. The transport processes are not terminated in the simulation after 26 h. Only after much longer periods of time the last model substance fractions are eluted from coating and gel.

It has to be noted that it was assumed for the FE simulation that the modification of the hydrogel changed the equilibrium DC but not the diffusion coefficient of the model substance triamterene; the mean pore size of the gel is supposed to be unaffected by the addition of hydrophobic areas. Due to the high water content of a hydrogel, the diffusion coefficient of dissolved small molecules is often only slightly lower than the diffusion coefficient in water (44). Differences regarding the transport characteristics have to be expected if the water content of the gel is markedly decreased or if interactions between charged polymer chains and charged dissolved substances occur. The experimental data presented does not indicate that this is the case.

CONCLUSION

Hydrogel formulations containing hydrophobic microparticles or oil droplets were developed and used to examine release and distribution from model substance-coated stents in the vFTC. Furthermore, DCs were determined experimentally and mathematical modeling was performed for the gel/model substance combination with the greatest variation in DC compared to the reference. The results of mathematical modeling indicate that the hydrophobization of the vessel-simulating compartment may influence the release from the coating and the distribution into the gel especially when the dosage form is a fast releasing system or if phases of fast initial (burst) release occur. This finding makes the model also very promising for release testing of drug-coated balloons. Hydrophobic modifications of the gel compartment do not seem to influence release and distribution from stents with very slow release profiles in spite of differences in the DC. In these cases, the inclusion of specific binding partners for the released substances seems a very promising approach, since the presence and distribution of binding proteins has also been shown to impact on drug distribution *in vivo* (45).

ACKNOWLEDGMENTS

The authors thank Biotronik SE & Co. KG (Berlin, Germany) for supplying bare metal stent platforms. We are also grateful for the supply of Eudragit® by Evonik Industries AG (Essen, Germany). Additionally, the authors would like to acknowledge the laboratory work of Katja Semper, Marcus Schewe, and Thomas Brand. Furthermore, thanks are given to Grzegorz Garbacz for proposals regarding this work. This research was funded by the Federal Ministry of Education and Research (BMBF) within REMEDIS "Höhere Lebensqualität durch neuartige Mikroimplantate".

REFERENCES

1. Fischman DL, Leon MB, Baim DS, Schatz RA, Savage MP, Penn I, *et al.* A randomized comparison of coronary-stent placement and balloon angioplasty in the treatment of coronary artery disease. N Engl J Med. 1994;331(8):496–501. doi:10.1056/NEJM199408253310802.
2. Serruys PW, de Jaegere P, Kiemeneij F, Macaya C, Rutsch W, Heyndrickx G, *et al.* A comparison of balloon-expandable-stent implantation with balloon angioplasty in patients with coronary artery disease. N Engl J Med. 1994;331(8):489–95. doi:10.1056/NEJM199408253310801.
3. Morice M-C, Serruys PW, Sousa JE, Fajadet J, Ban Hayashi E, Perin M, *et al.* A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. N Engl J Med. 2002;346(23):1773–80. doi:10.1056/NEJMoa012843.
4. Moses JW, Leon MB, Popma JJ, Fitzgerald PJ, Holmes DR, O'Shaughnessy C, *et al.* Sirolimus-eluting stents versus standard stents in patients with stenosis in a native coronary artery. N Engl J Med. 2003;349(14):1315–23. doi:10.1056/NEJMoa035071.
5. Khan W, Farah S, Domb AJ. Drug eluting stents: developments and current status. J Contr Release. 2012;161(2):703–12. doi:10.1016/j.jconrel.2012.02.010.
6. Steigerwald K, Merl S, Kastrati A, Wieczorek A, Vorpahl M, Mannhold R, *et al.* The pre-clinical assessment of rapamycin-eluting, durable polymer-free stent coating concepts. Biomaterials. 2009;30:632–7.
7. Westedt U, Wittmar M, Hellwig M, Hanefeld P, Greiner A, Schaper AK, *et al.* Paclitaxel releasing films consisting of poly(-vinyl alcohol)-graft-poly(lactide-co-glycolide) and their potential as biodegradable stent coatings. J Contr Release. 2006;111(1–2):235–46. doi:10.1016/j.jconrel.2005.12.012.

8. Guo Q, Knight PT, Mather PT. Tailored drug release from biodegradable stent coatings based on hybrid polyurethanes. J Contr Release. 2009;137(3):224–33. doi:10.1016/j.jconrel.2009.04.016.
9. Tzafriri AR, Groothuis A, Price GS, Edelman ER. Stent elution rate determines drug deposition and receptor-mediated effects. J Contr Release. 2012;161(3):918–26. doi:10.1016/j.jconrel.2012.05.039.
10. Kleinedler JJ, Foley JD, Orchard EA, Dugas TR. Novel nanocomposite stent coating releasing resveratrol and quercetin reduces neointimal hyperplasia and promotes re-endothelialization. J Contr Release. 2012;159(1):27–33. doi:10.1016/j.jconrel.2012.01.008.
11. Merciadez M, Alquier L, Metha R, Patel A, Wang A. A novel method for the elution of sirolimus (rapamycin) in drug-elution stents. Dissolution Technol. 2011; November: 37–42.
12. Kamberi M, Nayak S, Myo-Min K, Carter TP, Hancock L, Feder D. A novel accelerated in vitro release method for biodegradable coating of drug eluting stents: Insight to the drug release mechanisms. Eur J Pharm Sci. 2009;37(3–4):217—22. doi:10.1016/j.ejps.2009.02.009.
13. Sirianni RW, Jang E-H, Miller KM, Saltzman WM. Parameter estimation methodology in a model of hydrophobic drug release from a polymer coating. J Contr Release. 2010;142(3):474–82. doi:10.1016/j.jconrel.2009.11.021.
14. Minghetti P, Cilurzo F, Selmin F, Casiraghi A, Grignani A, Montanari L. Sculptured drug-eluting stent for the on-site delivery of tacrolimus. Eur J Pharm Biopharm. 2009;73(3):331–6. doi:10.1016/j.ejpb.2009.08.004.
15. Thakkar AS, Abhyankar AD, Dani SI, Banker DN, Singh PI, Parmar SA, *et al*. Systemic exposure of sirolimus after coronary stent implantation in patients with de novo coronary lesions: Supralimus-Core® pharmacokinetic study. Indian Heart J. 2012;64(3):273–9.
16. Vetrovec GW, Rizik D, Williard C, Snead D, Piotrovski V, Kopia G. Sirolimus PK trial: a pharmacokinetic study of the sirolimus-eluting Bx velocity stent in patients with de novo coronary lesions. Catheter Cardiovasc Interv. 2006;67(1):32–7. doi:10.1002/ccd.20565.
17. Neubert A, Sternberg K, Nagel S, Harder C, Schmitz K-P, Kroemer HK, *et al*. Development of a vessel-simulating flow-through cell method for the in vitro evaluation of release and distribution from drug-eluting stents. J Contr Release. 2008;130(1):2–8. doi:10.1016/j.jconrel.2008.05.012.
18. Seidlitz A, Nagel S, Semmling B, Grabow N, Martin H, Senz V, *et al*. Examination of drug release and distribution from drug-eluting stents with a vessel-simulating flow-through cell. Eur J Pharm Biopharm. 2011;78(1):36–48. doi:10.1016/j.ejpb.2010.12.021.
19. Peppas NA, Bures P, Leobandung W, Ichikawa H. Hydrogels in pharmaceutical formulations. Eur J Pharm Biopharm. 2000;50(1):27–46. doi:10.1016/S0939-6411(00)00090-4.
20. Hoare TR, Kohane DS, Hoare TR, Kohane DS. Hydrogels in drug delivery: progress and challenges. Polymer. 2008;49(8):1993–2007. doi:10.1016/j.polymer.2008.01.027.
21. Hoffman AS. Hydrogels for biomedical applications. Adv Drug Deliv Rev. 2002;43:3–12.
22. Draget KI, Smidsrød O, Skjåk-Bræk G. Alginates from algae. Biopolymers Online. 2005. DOI: 10.1002/3527600035.bpol6008
23. Zhang J, Daubert CR, Mulligan JH, Foegeding EA. Additive effects on the rheological behavior of alginate gels. Journal of Texture Studies. 2008;39(5):582–603. doi:10.1111/j.1745-4603.2008.00159.x.
24. Otsuka E, Suzuki A. A simple method to obtain a swollen PVA gel crosslinked by hydrogen bonds. J Appl Polym Sci. 2009;114(1):10–6. doi:10.1002/app.30546.
25. Kanakasabai P, Vijay P, Deshpande AP, Varughese S. Crosslinked poly(vinyl alcohol)/sulfonated poly(ether ether ketone) blend membranes for fuel cell applications—surface energy characteristics and proton conductivity. J Power Sourc. 2011;196(3):946–55. doi:10.1016/j.jpowsour.2010.08.094.
26. Ganguly S, Dash AK. A novel in situ gel for sustained drug delivery and targeting. Int J Pharm. 2004;276(1–2):83–92. doi:10.1016/j.ijpharm.2004.02.014.
27. Hoffman AS. Hydrogels for biomedical applications. Ann N Y Acad Sci. 2001;944(1):62–73. doi:10.1111/j.1749-6632.2001.tb03823.x.
28. Zentner GM, Rathi R, Shih C, McRea JC, Seo M-H, Oh H, *et al*. Biodegradable block copolymers for delivery of proteins and water-insoluble drugs. J Contr Release. 2001;72(1–3):203–15. doi:10.1016/S0168-3659(01)00276-0.
29. Rowley JA, Madlambayan G, Mooney DJ. Alginate hydrogels as synthetic extracellular matrix materials. Biomaterials. 1999;20(1):45–53. doi:10.1016/S0142-9612(98)00107-0.
30. Lee H-R, Park KM, Joung YK, Park KD, Do SH. Platelet-rich plasma loaded hydrogel scaffold enhances chondrogenic differentiation and maturation with up-regulation of CB1 and CB2. J Contr Release. 2012;159(3):332–7. doi:10.1016/j.jconrel.2012.02.008.
31. Augst AD, Kong HJ, Mooney DJ. Alginate hydrogels as biomaterials. Macromol Biosci. 2006;6(8):623–33. doi:10.1002/mabi.200600069.
32. Terry CM, Li L, Li H, Zhuplatov I, Blumenthal DK, Kim S-E, *et al*. In vivo evaluation of the delivery and efficacy of a sirolimus-laden polymer gel for inhibition of hyperplasia in a porcine model of arteriovenous hemodialysis graft stenosis. J Contr Release. 2012;160(3):459–67. doi:10.1016/j.jconrel.2012.03.011.
33. Allababidi S, Shah JC. Kinetics and mechanism of release from glyceryl monostearate-based implants: evaluation of release in a gel simulating in vivo implantation. J Pharm Sci. 1998;87(6):738–44. doi:10.1021/js9703986.
34. Hoang Thi TH, Chai F, Leprêtre S, Blanchemain N, Martel B, Siepmann F, *et al*. Bone implants modified with cyclodextrin: study of drug release in bulk fluid and into agarose gel. Int J Pharm. 2010;400(1–2):74–85.
35. O'Brien C, Finch C, Barber T, Martens P, Simmons A. Analysis of drug distribution from a simulated drug-eluting stent strut using an in vitro framework. Ann Biomed Eng. 2012;40(12):2687–96. doi:10.1007/s10439-012-0604-6.
36. Sakai M, Imai T, Ohtake H, Azuma H, Otagiri M. Effects of absorption enhancers on the transport of model compounds in Caco-2 cell monolayers: assessment by confocal laser scanning microscopy. J Pharm Sci. 1997;86(7):779–85. doi:10.1021/js960529n.
37. Zhao Y, Jona J, Chow DT, Rong H, Semin D, Xia X, *et al*. High-throughput logP measurement using parallel liquid chromatography/ultraviolet/mass spectrometry and sample-pooling. Rapid Comm Mass Spectrom. 2002;16(16):1548–55. doi:10.1002/rcm.749.
38. McKee MG, Layman JM, Cashion MP, Long TE. Phospholipid nonwoven electrospun membranes. Science. 2006;311(5759):353–5. doi:10.1126/science.1119790.
39. Di Mario C, Meneveau N, Gil R, de Jaegere P, de Feyter PJ, Slager CJ, *et al*. Maximal blood flow velocity in severe coronary stenoses measured with a Doppler guidewire: limitations for the application of the continuity equation in the assessment of stenosis severity. Am J Cardiol. 1993;71(14):D54–61. doi:10.1016/0002-9149(93)90134-X.
40. Watanabe E, Takahashi M, Hayashi M. A possibility to predict the absorbability of poorly water-soluble drugs in humans based on rat intestinal permeability assessed by an in vitro chamber method. Eur J Pharm Biopharm. 2004;58(3):659–65. doi:10.1016/j.ejpb.2004.03.029.
41. Grant GT, Morris ER, Rees DA, Smith PJC, Thom D. Biological interactions between polysaccharides and divalent cations: the egg-box model. FEBS Lett. 1973;32(1):195–8. doi:10.1016/0014-5793(73)80770-7.
42. European Directorate for the Quality of Medicines & Healthcare. European Pharmacopoeia and Supplements 7.8 ed. Strassbourg.
43. Guilherme MR, Silva R, Girotto EM, Rubira AF, Muniz EC. Hydrogels based on PAAm network with PNIPAAm included: hydrophilic–hydrophobic transition measured by the partition of Orange II and Methylene Blue in water. Polymer. 2003;44(15):4213–9. doi:10.1016/S0032-3861(03)00370-7.
44. Muhr AH, Blanshard JMV. Diffusion in gels. Polymer. 1982;23(7):1012–26. doi:10.1016/0032-3861(82)90402-5.
45. Levin AD, Vukmirovic N, Hwang C-W, Edelman ER. Specific binding to intracellular proteins determines arterial transport properties for rapamycin and paclitaxel. Proc Natl Acad Sci U S A. 2004;101(25):9463–7. doi:10.1073/pnas.0400918101.