ChemPubSoc Europe

DOI: 10.1002/cmdc.201400078

VIP

The Integrin Ligand *c*(RGDf(*N*Me)Nal) Reduces Neointimal Hyperplasia in a Polymer-Free Drug-Eluting Stent System

Florian Rechenmacher,[a] Kristin Steigerwald,[b] Burkhardt Laufer,[a] Stefanie Neubauer,[a] Tobias G. Kapp,[a] Liang Li,[b] Carlos Mas-Moruno,[a, c] Michael Joner,[b, d] and Horst Kessler*[a, e]

The use of highly active and selective integrin ligands in combination with stent implantation is emerging as a promising alternative to the release of classical immunosuppressive drugs by current drug-eluting stents (DES), which has been associated with delayed vascular healing and late stent thrombosis. Herein we present the development and biological evaluation of the integrin ligand *c*(RGDf(*N*Me)Nal) as a potent anti-proliferative molecule that targets coronary artery smooth muscle cells (CASMCs). This peptide showed an antagonistic activity for $\alpha v\beta 3$ and $\alpha v\beta 5$ in the low-nanomolar range, and selectivity against the platelet receptor $\alpha IIb\beta 3$. In vitro, it efficiently inhibited the proliferation of CASMCs, displaying higher potency than the anti-tumor drug candidate cilengitide. This peptide was then loaded into a polymer-free bare metal stent (BMS), and its release studied at different time points. Up to seven days of elution, the peptide-coated stents retained high anti-proliferative activity toward CASMCs. Finally, the peptide was examined in vivo in a polymer-free DES system in a rabbit iliac artery model. After 28 days of implantation, histopathological analysis revealed that the peptide clearly decreased neointimal growth and improved vessel healing and re-endothelialization compared with the FDA-approved Cypher DES. Our study shows that this type of lipophilic integrin ligand, when eluted from a polymer-free stent system, has the potential to successfully decrease in-stent restenosis in the absence of delayed vascular healing.

Introduction

The development of drug-eluting stents (DES) was a major breakthrough in the interventional treatment of myocardial infarctions and coronary artery disease, as it significantly decreased the risk of restenosis, the most significant limitation of percutaneous coronary intervention using bare metal stents (BMS).[1] The principle of DES is the deferred release of anti-proliferative drugs such as Rapamycin (sirolimus) or Taxol (paclitaxel) to avoid re-narrowing of the vessel (Figure 1). In this regard, the released drug limits proliferation and migration of smooth muscle cells (SMCs) and thus tissue growth of the newly built intimal layer, the so-called neointima, an important contributor

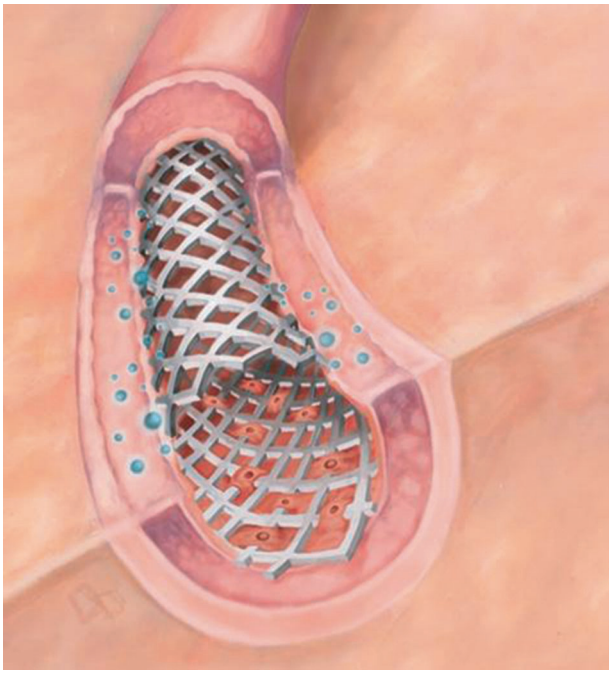


Figure 1. Schematic presentation of a drug-eluting stent in a blood vessel. The released drug (cyan) targets VSMCs to decrease their proliferation in order to prevent restenosis.

[a] *Dr. F. Rechenmacher,[+] Dr. B. Laufer,[+] Dr. S. Neubauer, T. G. Kapp, Dr. C. Mas-Moruno, Prof. Dr. H. Kessler*
Institute for Advanced Study at the Department of Chemistry
Technische Universität München
Lichtenbergstraße 4, 85748 Garching (Germany)
E-mail: Kessler@tum.de

[b] *Dr. K. Steigerwald,[+] L. Li, Dr. M. Joner*
Department of Cardiology, Deutsches Herzzentrum München
Lazarettstraße 36, 80636 München (Germany)

[c] *Dr. C. Mas-Moruno*
Biomaterials, Biomechanics and Tissue Engineering Group
Department of Materials Science and Metallurgical Engineering
Technical University of Catalonia (UPC)
Av. Diagonal 647, 08028 Barcelona (Spain)

[d] *Dr. M. Joner*
CVPath Institute Inc.
19 Firstfield Road, Gaithersburg, MD 20878 (USA)

[e] *Prof. Dr. H. Kessler*
Faculty of Science, King Abdulaziz University
P.O. Box 80203, Jeddah 21589 (Saudi Arabia)

[+] *These authors contributed equally to this work.*

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201400078.

of in-stent restenosis.[2] However, the major drawback of this technology is the unspecific inhibition of various cell types. In particular, most drugs in current use also adversely affect endothelial cells that are necessary to maintain anti-thrombotic properties and vasomotor function of the artery,[2,3] thus impairing stent re-endothelialization and vascular healing. The endothelial layer represents the physiological layer of the inner vessel wall, and has important functions such as regulating thrombosis, inflammation, and vessel tonus.

Furthermore, most of the currently approved DES use permanent polymers to facilitate drug coating and modulate drug release kinetics. This represents another potential issue, because it has been reported that the permanent presence of these polymers is associated with inflammatory and hypersensitivity reactions, important drivers of delayed vascular healing.[4] Delayed arterial healing has been identified as the key risk factor for late stent thrombosis, an often fatal event in patients,[5] and new evidence has emerged that this may also be the underlying cause for late restenotic events owing to premature neoatherosclerotic changes in patients treated with DES.[6] For these reasons, current research has focused on the improvement of polymer-free or biodegradable DES systems to enhance and accelerate vessel wall healing.[7]

Integrins are of crucial relevance for the interaction between vascular cells and the extracellular matrix (ECM) during the process of neointima formation after coronary interventions. Especially integrins $\alpha v\beta 3$, $\alpha v\beta 5$, and $\alpha 5\beta 1$ are key players in migration, proliferation, and survival of cells.[8] The fact that integrins are promising targets for blocking proliferation, and as a consequence angiogenesis, has stimulated the development of integrin ligands in medicinal chemistry.[9] One of the most prominent binding motifs in ECM proteins is the RGD sequence,[10] which is recognized by nearly half of the 24 heterodimers known to date, including all αv integrins binding to vitronectin, the fibronectin binding integrin $\alpha 5\beta 1$, and the platelet integrin $\alpha IIb\beta 3$. Thus, mimicking the RGD binding motif with peptides or peptidomimetics possessing defined integrin subtype-selectivity profiles is a powerful approach to target these integrins.[11] However, the interplay of integrins during vascular development is very complex, and it has not yet been fully elucidated which integrin subtype plays the most important role in this context.[12] The levels of different integrin subtypes, such as $\alpha v\beta 3$, $\alpha v\beta 5$, or $\alpha 5\beta 1$, expressed on vascular smooth muscle cells (VSMCs) as well as on endothelial cells may vary during the process of neointima formation, leading to a dynamically changing integrin pattern.

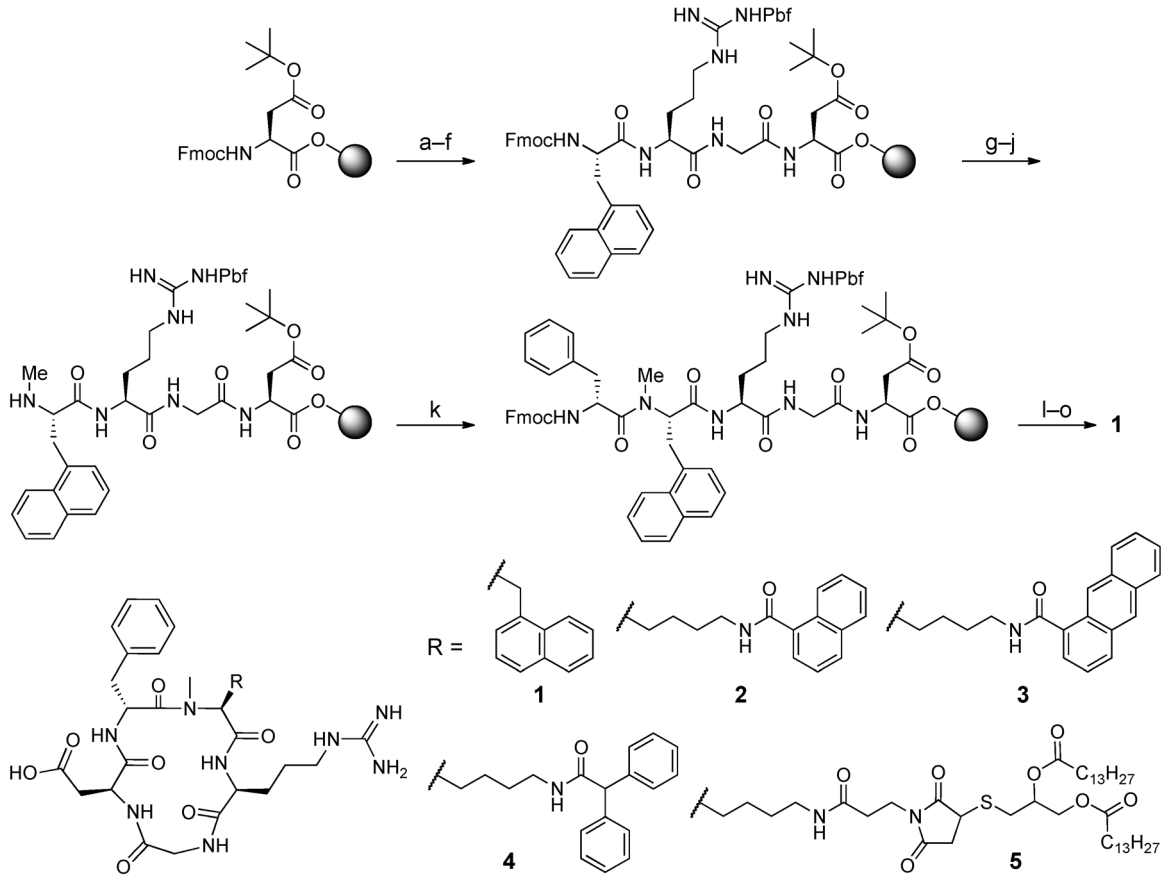
Owing to the shortcomings of first-generation DES, alternative coating strategies of coronary stents were focused on a more specific disruption of neointimal growth without causing excess collateral damage including delayed vascular healing. In this regard, combining stent therapy with systemic integrin inhibitors or direct stent coating with integrin ligands was reported to result in a more favorable reduction of neointimal growth relative to contemporary DES releasing immunosuppressive drugs.[13] Because RGD peptides can interact with integrins in a potent manner and block cell proliferation, and as SMCs strongly express the $\alpha v\beta 3$ subtype,[14] we aimed at targeting this subtype with a highly $\alpha v\beta 3$-active peptide released from a DES. Furthermore, a compound that can be used in a polymer-free stent system would be of high interest, because of the above-mentioned side effects of polymers applied in this context.

In this work, we designed and synthesized the $\alpha v\beta 3$ integrin ligand *c*(RGDf(*N*Me)Nal) as an anti-proliferative molecule (Scheme 1) and evaluated its biological potency when released from a polymer-free DES in a rabbit iliac artery model. The peptide, which is a derivative of the anti-angiogenic drug candidate cilengitide,[15] was first assessed in vitro regarding its anti-proliferative potential to inhibit coronary artery smooth muscle cell (CASMC) proliferation. In a further experiment, the integrin ligand was loaded onto a microporous BMS using a previously established customizable spray-coating technology.[7a] Following implantation of these novel DES in healthy iliac arteries of New Zealand white rabbits, histopathological assessment was performed at follow-up of 28 days relative to BMS as well as to the FDA-approved Cypher DES.

Results and Discussion

In our study, we aimed at targeting the $\alpha v\beta 3$ integrin known to play an important role in VSMC proliferation, but in a polymer-free approach, in contrast to previously reported studies. Starting with the drug candidate cilengitide *c*(RGDf(*N*Me)V), which is active for $\alpha v\beta 3$ in the sub-nanomolar range and for $\alpha v\beta 5$ and $\alpha 5\beta 1$ in the low-nanomolar range,[15b] we tried to introduce lipophilic amino acids without affecting its biological properties. Lipophilicity is an important criterion for this preclinical application, as the compound must be slowly eluted from the polymer-free surface of a microporous BMS. In this context, enhanced hydrophilicity of the drug would lead to rapid elution from the stent during blood passage. On the other hand, very high lipophilicity often leads to excessive serum binding, thus lowering the biological activity.

It has been shown that the valine residue of cilengitide can be easily substituted with other amino acids without a notable loss in activity, offering a suitable position for functionalization purposes.[16] Thus, a series of lipophilic residues were introduced in such position leading to a small library of cilengitide derivatives with increased lipophilicity (peptides **1**–**5**, Scheme 1). In preliminary cell proliferation assays (data not shown), compounds **1**–**3** showed excellent and similar anti-proliferative properties; however, whereas in peptide **1** the L-2-naphthylalanine building block could be incorporated directly on solid phase (Scheme 1), the functionalization of **2** and **3** required an extra synthetic step in solution. Peptide **4** failed to reproduce the same biological activity due to insufficient loading onto the stent surface, and **5** was insoluble in water as a consequence of the high lipophilicity of its acyl chains. For these reasons, peptide **1** c(RGDf(*N*Me)Nal) was chosen as promising candidate and evaluated in a solid-phase integrin binding assay regarding its biological activity (Scheme 1). The binding affinity for integrins $\alpha v\beta 3$ and $\alpha v\beta 5$ was retained almost completely relative to cilengitide. Furthermore, **1** showed no activity for the blood platelet integrin $\alpha IIb\beta 3$



compound	IC_{50} ($\alpha v\beta 3$) [nM]	IC_{50} ($\alpha v\beta 5$) [nM]
1	1.72 ± 0.19	9.53 ± 0.83
cilengitide	0.54 ± 0.01	1.68 ± 0.12

Scheme 1. Fmoc-based, solid-phase peptide synthesis of **1**, structures of peptides **1**–**5**, and activities of **1** for the integrin subtypes $\alpha v\beta 3$ and $\alpha v\beta 5$ relative to cilengitide. a) piperidine/DMF (1:4); b) Fmoc-Gly-OH, HATU, HOAt, DIEA, DMF; c) piperidine/DMF (1:4); d) Fmoc-Arg(Pbf)-OH, HATU, HOAt, DIEA, DMF; e) piperidine/DMF (1:4); f) Fmoc-Nal-OH, HATU, HOAt, DIEA, DMF; g) piperidine/DMF (1:4); h) NBS-Cl, collidine, NMP; i) Ph_3P, MeOH, DIAD, THF; j) $HS(CH_2)_2OH$, DBU, NMP; k) Fmoc-D-Phe-OH, HATU, HOAt, DIEA, DMF; l) piperidine/DMF (1:4); m) HFIP/CH_2Cl_2 (1:4); n) DPPA, $NaHCO_3$, DMF; o) TFA/CH_2Cl_2/H_2O/TIS (60:35:2.5:2.5). Fmoc: 9-fluorenylmethoxycarbonyl, DMF: *N*,*N*-dimethylformamide, HATU: *O*-(7-azabenzotriazol-1-yl)-tetramethyluronium hexafluorophosphate, HOAt: 1-hydroxy-7-azabenzotriazole, DIEA: ethyldiisopropylamine, NBS-Cl: nitrobenzylsulfonyl chloride, NMP: *N*-methyl-2-pyrrolidone, DIAD: diisopropylazodicarboxylate, DBU: 1,5-diazabicyclo[5.4.0]undec-5-ene, HFIP: hexafluoroisopropanol, DPPA: diphenylphosphorylazide, TFA: trifluoroacetic acid, TIS: triisopropylsilane.

($IC_{50} > 1000$ nM), which is necessary for both preclinical and clinical applications.

To further evaluate the inhibitory activity of **1**, we performed a cell proliferation assay with CASMCs (Figure 2). After seeding, CASMCs were incubated with **1** or cilengitide for 72 h in concentrations ranging from 10 nM to 100 μM. Proliferation was clearly inhibited at a concentration of $\geq$10 μM and $\geq$100 μM for **1** and cilengitide, respectively. Surprisingly, **1** clearly inhibited CASMCs at a concentration of 10 μM, while cilengitide remained less effective, thus giving striking evidence for the superior anti-proliferative properties of the lipophilic derivative (Figure 2).

In a follow-up experiment, RGD-peptide-coated stents were tested for their release kinetic profile of **1** and were examined in vitro regarding their potential to inhibit CASMC proliferation. For that purpose, stents were spray-coated with a polymer-free solution of **1** as previously reported,[7a] resulting in a total surface load of 100 μg per stent. To examine the inhibitory effect of **1** in vitro, RGD-peptide-coated stents (+RGD) were placed in sterile cell culture media in the absence of additives for 1, 7, and 14 days. The supernatant was subsequently incubated with CASMCs, and cell proliferation assessed after 0, 24, and 72 h (Figure 3). The control groups consisted of supernatant media derived from coated stents with the same coating solution but without RGD peptide (−RGD) and of untreated cell culture medium (untreated). Treatment of CASMCs with supernatant media from RGD-peptide-coated stents (days 1 and 7) revealed a sustained anti-proliferative effect up to 72 h (Figure 3). This effect was decreased when supernatant from the day-14 group was used. Notably, all supernatant media resulted in decreased cell numbers at 72 h relative to control (−RGD). To summarize, supernatant media from RGD-peptide-

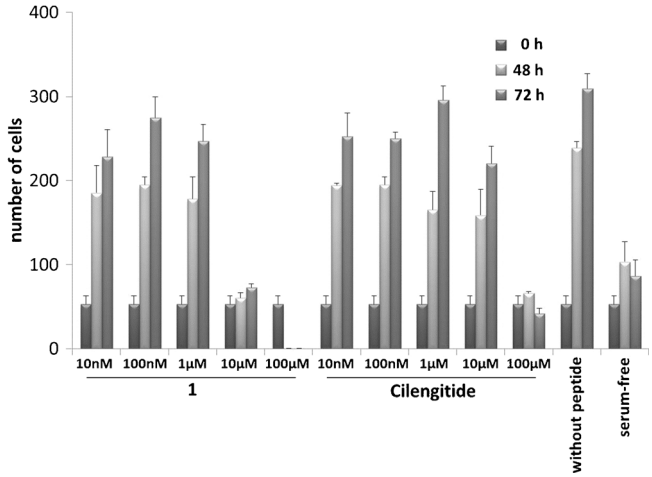


Figure 2. In vitro proliferation assay of CASMCs incubated with **1** or cilengitide. Cell culture medium (without peptide) and serum-free medium served as positive and negative controls, respectively.

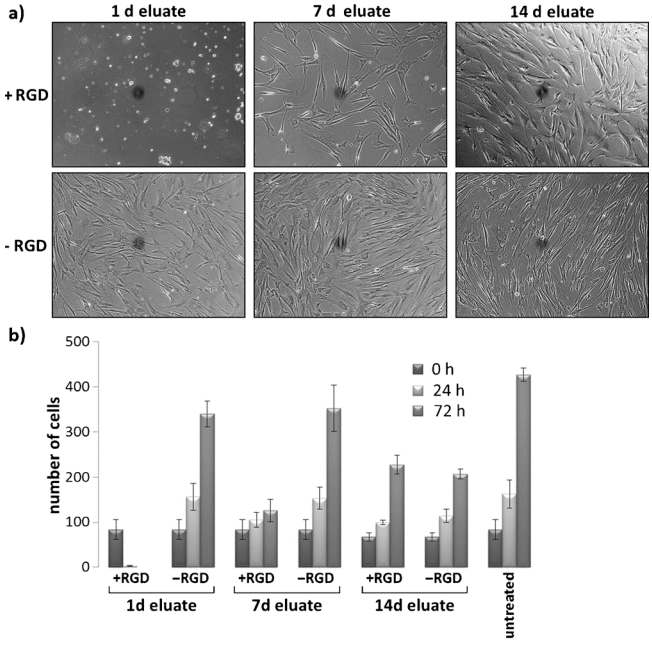
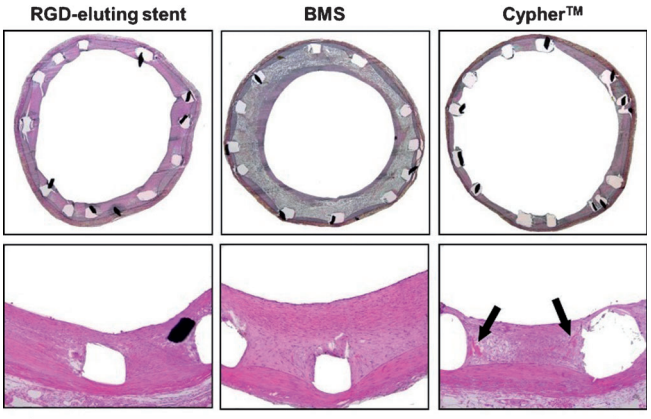


Figure 3. Inhibitory effects of supernatant media derived from RGD-peptide-coated stents. a) CASMC density and morphology 72 h after exposure to supernatant media derived from coated stents incubated in media for 1 (left), 7 (middle), and 14 days (right). b) Quantification of cell proliferation at various time points.

coated stents **1** resulted in sustained abolishment of SMC proliferation up to 7 days in vitro.

Following successful in vitro testing, the RGD-peptide-coated stents were evaluated in a proof-of-concept study in vivo (Figure 4). Six healthy New Zealand white rabbits received bilateral stent implantation in the external iliac arteries. Stents (Yukon DES, Translumina, 3 mm × 12 mm) were either coated with peptide **1** (RGD-coated stent) or remained uncoated (BMS) to serve as control. Animals were randomized to three different treatment groups consisting of the RGD-coated stent, the BMS and the Cypher DES: an FDA-approved sirolimus-eluting stent known to inhibit neointimal growth and display delayed arterial healing in the applied animal model.[17] The treated arteries were histopathologically evaluated 28 days after stent deployment regarding neointimal growth and vessel healing parameters. The assessment of percent luminal endothelialization and degree of fibrin deposition in the neointimal tissue were of special interest. Low percent endothelialization and persisting fibrin deposition are indicators of delayed vessel healing and important predictors of stent safety with regards to thrombotic risk.[18]



Parameter	RGD-eluting stent	BMS	Cypher™ DES
Neointimal thickness [mm]*	0.11 ± 0.05	0.14 ± 0.06	0.09 ± 0.02
Stenosis [%]*	23.42 ± 7.00	28.76 ± 10.03	19.59 ± 2.3
Endothelialization [%]*	88.75 ± 8.16	84.17 ± 12.66	77.22 ± 5.09
Struts with fibrin [%]**	3.70 ± 7.79	13.19 ± 12.0	74.32 ± 10.22

Figure 4. Histopathological evaluation of the stent eluting **1**. Upper panel: Overview images of the RGD-, bare metal (BMS), and Cypher drug-eluting stents at 40× magnification, Movats Pentachrome staining. Lower panel: Images at 200× magnification, hematoxylin and eosin stain. Black arrows indicate fibrin persistence; *p value not significant; **$p < 0.0001$ for Cypher versus others.

Histopathological evaluation revealed the percentage of stenosis was greatest in the BMS and lowest in Cypher, whereas the RGD-peptide-coated stent showed an intermediate reduction in percent stenosis (Figure 4). Vascular healing was completed in the RGD-coated stent and BMS at 28 days, as markers of delayed healing were almost absent. In contrast, Cypher DES showed evidence of delayed arterial healing displayed by incomplete endothelialization and persistence of fibrin (Figure 4, black arrows). The peptide-eluting stent showed an almost complete re-endothelialization (88.8%) and low percentage of exposed struts. In contrast, after four weeks almost 50% of the struts in the Cypher stents were uncovered, and incomplete re-endothelialization (77.2%) was observed (Figure 4). To date, incomplete re-endothelialization and exposed stent struts were often reported as important factors that increase the risk of late or very late stent thrombosis. Additionally, Cypher showed greater delayed healing characterized by persistent fibrin accumulation, whereas the fibrin deposition at strut level of the peptide-eluting stent was very low, at 3.7%. Taken together, these results demonstrate that coating stents with *c*(RGDf(*N*Me)Nal) is feasible, safe, and at the same time effective in terms of neointimal growth reduction.

Conclusions

In summary, we have demonstrated that coating of DES with the lipophilic integrin targeting peptide *c*(RGDf(*N*Me)Nal) **1** is a promising alternative to the FDA-approved medical devices coated with immunosuppressive drugs such as Rapamycin. This peptide clearly inhibited CASMC proliferation and migration in vitro and was successfully loaded onto microporous stents without the need for a polymer. In a rabbit model, stents coated with **1** clearly decreased neointimal growth and improved vessel healing and re-endothelialization relative to Cypher DES. The cyclic peptide was able to induce a substantial decrease in neointimal growth in the novel DES group relative to control, at the same time showing a favorable healing profile compared with commercially available Cypher DES, thereby giving striking evidence for its potential in the use of polymer-free DES.

Animal study

The study protocol was approved by the responsible authority (Regierung von Oberbayern, AZ 55.2-1-54-2531-30-09) implementing the German Animal Welfare Act. Animal housing and care were in accordance with the Directive 2010/63/EU of the European Parliament, compliant with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Acknowledgements

We thank the International Graduate School of Science and Engineering (IGSSE), the Bund der Freunde der TU München e.V., and Materials Science of Complex Interfaces (CompInt) of the Elite Network of Bavaria for funding, and the Institute for Advanced Study (IAS) of Technische Universität München and the Center for Integrated Protein Science Munich (CIPSM) for financial support. We thank Philipp Ganthaler for illustrating Figure 1.

Keywords: drug-eluting stents · integrin ligands · myocardial infarction · peptides · RGD · smooth muscle cells

[1] a) P. W. Serruys, M. J. B. Kutryk, A. T. L. Ong, *N. Engl. J. Med.* **2006**, *354*, 483 – 495; b) R. Tung, S. Kaul, G. A. Diamond, P. K. Shah, *Ann. Intern. Med.* **2006**, *144*, 913 – 919.

[2] T. J. Parry, R. Brosius, R. Thyagarajan, D. Carter, D. Argentieri, R. Falotico, J. Siekierka, *Eur. J. Pharmacol.* **2005**, *524*, 19 – 29.

[3] S. Fuke, K. Maekawa, K. Kawamoto, H. Saito, T. Sato, T. Hioka, *Circ. J.* **2007**, *71*, 220 – 225.

[4] a) R. Virmani, A. Farb, G. Guagliumi, F. D. Kolodgie, *Coron. Artery Dis.* **2004**, *15*, 313 – 318; b) R. A. Byrne, M. Joner, A. Kastrati, *Minerva Cardioangiol.* **2009**, *57*, 567 – 584.

[5] a) J. R. Nebeker, R. Virmani, C. L. Bennett, J. M. Hoffman, M. H. Samore, J. Alvarez, C. J. Davidson, J. M. McKoy, D. W. Raisch, B. K. Whisenant, P. R. Yarnold, S. M. Belknap, D. P. West, J. E. Gage, R. E. Morse, G. Gligoric, L. Davidson, M. D. Feldman, R. Virmani, *J. Am. Coll. Cardiol.* **2006**, *47*, 175 – 181; b) M. Joner, A. V. Finn, A. Farb, E. K. Mont, F. D. Kolodgie, E. Ladich, R. Kutys, K. Skorija, H. K. Gold, R. Virmani, *J. Am. Coll. Cardiol.* **2006**, *48*, 193 – 202; c) T. F. Lüscher, J. Steffel, F. R. Eberli, M. Joner, G. Nakazawa, F. C. Tanner, R. Virmani, *Circulation* **2007**, *115*, 1051 – 1058.

[6] a) R. A. Byrne, R. Iijima, J. Mehilli, S. Pinieck, O. Bruskina, A. Schömig, A. Kastrati, *JACC Cardiovasc. Interv.* **2009**, *2*, 291 – 299; b) G. Nakazawa, F. Otsuka, M. Nakano, M. Vorpahl, S. K. Yazdani, E. Ladich, F. D. Kolodgie, A. V. Finn, R. Virmani, *J. Am. Coll. Cardiol.* **2011**, *57*, 1314 – 1322.

[7] a) J. Hausleiter, A. Kastrati, R. Wessely, A. Dibra, J. Mehilli, T. Schratzenstaller, I. Graf, M. Renke-Gluszko, B. Behnisch, J. Dirschinger, E. Wintermantel, A. Schömig, *Eur. Heart J.* **2005**, *26*, 1475 – 1481; b) N. Tada, R. Virmani, G. Grant, L. Bartlett, A. Black, C. Clavijo, U. Christians, R. Betts, D. Savage, S. H. Su, J. Shulze, S. Kar, *Circ. Cardiovasc. Interv.* **2010**, *3*, 174 – 183; c) L. Pendyala, X. Yin, J. Li, T. Shinke, Y. Xu, J. P. Chen, S. B. King, K. Colley, T. Goodchild, N. Chronos, D. Hou, *EuroIntervention* **2010**, *6*, 126 – 133; d) T. Koppara, M. Joner, G. Bayer, K. Steigerwald, T. Diener, E. Wittchow, *Thromb. Haemostasis* **2012**, *107*, 1161 – 1171.

[8] C. J. Avraamides, B. Garmy-Susini, J. A. Varner, *Nat. Rev. Cancer* **2008**, *8*, 604 – 617.

[9] a) D. Zimmermann, E. W. Guthöhrlein, M. Malešević, K. Sewald, L. Wobbe, C. Heggemann, N. Sewald, *ChemBioChem* **2005**, *6*, 272 – 276; b) D. Heckmann, A. Meyer, L. Marinelli, G. Zahn, R. Stragies, H. Kessler, *Angew. Chem. Int. Ed.* **2007**, *46*, 3571 – 3574; *Angew. Chem.* **2007**, *119*, 3641 – 3644; c) S. Urman, K. Gaus, Y. Yang, Y. U. Strijowski, N. Sewald, S. De Pol, O. Reiser, *Angew. Chem. Int. Ed.* **2007**, *46*, 3976 – 3978; *Angew. Chem.* **2007**, *119*, 4050 – 4053; d) L. Manzoni, L. Belvisi, D. Arosio, M. Civera, M. Pilkington-Miksa, D. Potenza, A. Caprini, E. M. V. Araldi, E. Monferini, M. Mancino, F. Podestà, C. Scolastico, *ChemMedChem* **2009**, *4*, 615 – 632; e) A. Tolomelli, L. Gentilucci, E. Mosconi, A. Viola, S. D. Dattoli, M. Baiula, S. Spampinato, L. Belvisi, M. Civera, *ChemMedChem* **2011**, *6*, 2264 – 2272; f) L. Manzoni, L. Belvisi, D. Arosio, M. P. Bartolomeo, A. Bianchi, C. Brioschi, F. Buonsanti, C. Cabella, C. Casagrande, M. Civera, M. De Matteo, L. Fugazza, L. Lattuada, F. Maisano, L. Miragoli, C. Neira, M. Pilkington-Miksa, C. Scolastico, *ChemMedChem* **2012**, *7*, 1084 – 1093; g) F. Curnis, A. Cattaneo, R. Longhi, A. Sacchi, A. M. Gasparri, F. Pastorino, P. Di Matteo, C. Traversari, A. Bachi, M. Ponzoni, G. P. Rizzardi, A. Corti, *J. Biol. Chem.* **2010**, *285*, 9114 – 9123; h) S. L. Goodman, M. Picard, *Trends Pharmacol. Sci.* **2012**, *33*, 405 – 412.

[10] M. D. Pierschbacher, E. Ruoslahti, *Nature* **1984**, *309*, 30 – 33.

[11] a) J. M. Smallheer, C. A. Weigelt, F. J. Woerner, J. S. Wells, W. F. Daneker, S. A. Mousa, R. R. Wexler, P. K. Jadhav, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 383 – 387; b) R. Stragies, F. Osterkamp, G. Zischinsky, D. Vossmeyer, H. Kalkhof, U. Reimer, G. Zahn, *J. Med. Chem.* **2007**, *50*, 3786 – 3794; c) L. Gentilucci, G. Cardillo, S. Spampinato, A. Tolomelli, F. Squassabia, R. De Marco, A. Bedini, M. Baiula, L. Belvisi, M. Civera, *J. Med. Chem.* **2010**, *53*, 106 – 118; d) C. Mas-Moruno, J. G. Beck, L. Doedens, A. O. Frank, L. Marinelli, S. Cosconati, E. Novellino, H. Kessler, *Angew. Chem. Int. Ed.* **2011**, *50*, 9496 – 9500; *Angew. Chem.* **2011**, *123*, 9668 – 9672; e) F. Rechenmacher, S. Neubauer, J. Polleux, C. Mas-Moruno, M. De Simone, E. A. Cavalcanti-Adam, J. P. Spatz, R. Fässler, H. Kessler, *Angew. Chem. Int. Ed.* **2013**, *52*, 1572 – 1575; *Angew. Chem.* **2013**, *125*, 1612 – 1616; f) S. Neubauer, F. Rechenmacher, A. J. Beer, F. Curnis, K. Pohle, C. D'Alessandria, H.-J. Wester, U. Reuning, A. Corti, M. Schwaiger, H. Kessler, *Angew. Chem. Int. Ed.* **2013**, *52*, 11656 – 11659; *Angew. Chem.* **2013**, *125*, 11870 – 11873; g) S. Neubauer, F. Rechenmacher, R. Brimioulle, F. S. Di Leva, A. Bochen, T. R. Sobahi, M. Schottelius, E. Novellino, C. Mas-Moruno, L. Marinelli, H. Kessler, *J. Med. Chem.* **2014**, DOI: 10.1021/jm500092w.

[12] A. van der Flier, K. Badu-Nkansah, C. A. Whittaker, D. Crowley, R. T. Bronson, A. Lacy-Hulbert, R. O. Hynes, *Development* **2010**, *137*, 2439 – 2449.

[13] a) S. S. Srivatsa, L. A. Fitzpatrick, P. W. Tsao, T. M. Reilly, D. R. Holmes, R. S. Schwartz, S. A. Mousa, *Cardiovasc. Res.* **1997**, *36*, 408 – 428; b) R. Blindt, F. Vogt, I. Astafieva, C. Fach, M. Hristov, N. Krott, B. Seitz, A. Kapurniotu, C. Kwok, M. Dewor, A. K. Bosserhoff, J. Bernhagen, P. Hanrath, R. Hofmann, C. Weber, *J. Am. Coll. Cardiol.* **2006**, *47*, 1786 – 1795; c) S. R. Meyers, D. J. Kenan, M. W. Grinstaff, *ChemMedChem* **2008**, *3*, 1645 – 1648; d) A. Braun, L. Dofiles, S. Rousselle, L. Guerrero, J. Gunther, T. Yednock, A. Stricker-Krongrad, E. Messersmith, *PLoS One* **2010**, *5*, e14314; e) S. R. Meyers, D. J. Kenan, X. Khoo, M. W. Grinstaff, *Biomacromolecules* **2011**, *12*, 533 – 539; f) M. Joner, Q. Cheng, S. Schönhofer-Merl, M. Lopez, S. Neubauer, C. Mas-Moruno, B. Laufer, F. D. Kolodgie, H. Kessler, R. Virmani, *J. Biomed. Mater. Res. Part B* **2012**, *100B*, 637 – 645; g) W. Zheng, Z. Wang, L. Song, Q. Zhao, J. Zhang, D. Li, S. Wang, J. Han, X.-L. Zheng, Z. Yang, D. Kong, *Biomaterials* **2012**, *33*, 2880 – 2891; h) C. McNichols, J. Wilkins, A. Kubota, Y. T. Shiu, S. M. Aouadi, P. Kohli, *J. Biomed. Mater. Res. Part A* **2014**, *102*, 532 – 539.

[14] M. P. Bendeck, C. Irvin, M. Reidy, L. Smith, D. Mulholland, M. Horton, C. M. Giachelli, *Arterioscler. Thromb. Vasc. Biol.* **2000**, *20*, 1467 – 1472.

[15] a) M. A. Dechantsreiter, E. Planker, B. Mathä, E. Lohof, G. Hölzemann, A. Jonczyk, S. L. Goodman, H. Kessler, *J. Med. Chem.* **1999**, *42*, 3033 – 3040; b) C. Mas-Moruno, F. Rechenmacher, H. Kessler, *Anti-Cancer Agents Med. Chem.* **2010**, *10*, 753 – 768.
[16] R. Haubner, R. Gratias, B. Diefenbach, S. L. Goodman, A. Jonczyk, H. Kessler, *J. Am. Chem. Soc.* **1996**, *118*, 7461 – 7472.
[17] M. C. John, R. Wessely, A. Kastrati, A. Schömig, M. Joner, M. Uchihashi, J. Crimins, S. Lajoie, F. D. Kolodgie, H. K. Gold, R. Virmani, A. V. Finn, *JACC Cardiovasc. Interv.* **2008**, *1*, 535 – 544.
[18] A. V. Finn, G. Nakazawa, M. Joner, F. D. Kolodgie, E. K. Mont, H. K. Gold, R. Virmani, *Arterioscler. Thromb. Vasc. Biol.* **2007**, *27*, 1500 – 1510.

Received: January 31, 2014
Published online on April 6, 2014