Sabine Kischkel*, Niels Grabow, Carsten M. Bünger and Anja Püschel

Impact of statins on vascular scaffolds response in porcine carotid arteries

Impact of statins on vascular scaffolds response

Abstract: Surgical treatments of arterial occlusive disease with fully absorbable polymeric scaffolds, as a potential alternative to permanent metallic stents, are increasingly penetrating the clinical field. An addition part of the management of patients suffering from vascular diseases is the administration of statins.

In this study, absorbable x-ray marked PLLA-based polymer scaffolds and permanent bare-metal stents (BMS) were implanted interventionaly into both common carotid arteries (CCA) of 6 healthy female pigs via the left common iliac artery (8F-sheath). The pigs were administered dual antiplatelet drugs oral starting 3 days before the procedure until the end of the study. In Addition, the pigs received atorvastatin orally, beginning 5 days prior to surgery and lasting until the study ended. Stented CCA segments were explanted after 4 weeks, and processed for quantitative histomorphometry, and estimation of vascular inflammation and injury scores.

Polymer scaffolds showed a decreased residual lumen area and higher stenosis after 4 weeks ($6.41 \pm 0.83 \text{ mm}^2$ and $40.52 \pm 5.01\%$) as compared to the bare-metal reference stent ($15.17 \pm 0.896 \text{ mm}^2$ and $7.80 \pm 0.88\%$). After 4 weeks, inflammation score were higher in the polymer group (1.30 ± 0.37) compared to the BMS group (0.42 ± 0.18). In contrast, the BMS showed a slightly elevated vascular injury score (0.85 ± 0.12), as compared to the polymer (0.60 ± 0.23) group.

In this preclinical model, the new absorbable polymeric scaffolds showed similar technical feasibility and safety for vascular application as the permanent metal stents. Although no positive trends were observed with oral treatment with

atorvastatin, further optimization with a dual-loaded coating is still reasonable. In addition, reduced strut thickness of the polymer scaffolds would have potential to positively impact tissue ingrowth between struts and should be considered in future work on stent design.

Keywords: absorbable polymeric scaffold, x-ray marked, statin, carotid artery stenting, experimental porcine model.

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1 Introduction

Surgical treatment of arterial occlusive disease is now routinely performed by scaffold insertion. They are designed to provide temporary scaffolding, in order to prevent acute recoil. Fully absorbable polymeric scaffolds, as a potential alternative to permanent metallic stents, are increasingly penetrating the clinical field.

The first FDA-approved absorbable vascular scaffold (Absorb-BVS), could not confirm the initially good results in the long-term course, so that the device was withdrawn from the market in 2017 [1]. Hence, new devices with improved features, novel design and materials are always under development.

Drug treatment with statins is another important component in the management of patients with vascular diseases. In addition to lipid-lowering, statins show numerous other positive effects, the so-called "statin pleotropic effects" [2]. Routine use of statins in vascular patients is associated with several beneficial effects, whether they are treated conservatively or undergo open surgical or endovascular procedures. Statins have been shown to improve outcomes in patients with peripheral arterial disease just like in patients with carotid artery disease [2].

The aim of this study was to evaluate feasibility and biocompatibility of a novel x-ray marked PLLA-based polymer scaffold for peripheral vascular application in the non-diseased porcine carotid artery model after 4 weeks. In addition, the influence of an orally administered statin on histomorphometry will be investigated.

*Corresponding author: **Sabine Kischkel:** Institute for Biomedical Engineering, Rostock University Medical Center, Rostock, Germany, e-mail: sabine.kischkel@uni-rostock.de

Niels Grabow: Institute for Biomedical Engineering, Rostock University Medical Center, Rostock, Germany

Carsten M. Bünger: Department of Vascular Medicine, Vivantes Humboldt-Klinikum, Berlin, Germany

Anja Püschel: Department of General, Thoracic, Vascular and Transplantation Surgery, Rostock University Medical Center, Rostock, Germany

2 Materials and Methods

2.1 Scaffolds

The absorbable PLLA-based polymer scaffolds used in this study were developed, produced and tested *in vitro* at the Institute for Biomedical Engineering at Rostock University Medical Center. In brief, the polymer scaffolds were produced in an automated, PC-assisted immersion process and cut from tiny tubes (I.D. = 2.2 mm, A.D. = 2.7 mm) using a CO₂ laser with a nominal dimension of 5.0 x 20 mm and a wall thickness of 250 µm [3]. All polymer scaffolds were hand-mounted on standard 5.0 x 40 mm balloon catheters (Passeo-35, Biotronik SE Co. KG, Berlin, Germany). Intravascular polymer scaffolds expansion was achieved within one minute by increasing balloon pressure of up to 8 bar. In addition, all polymer scaffolds were x-ray marked at the distal and proximal ends (**Figure 1**).

Figure 1: X-ray marked absorbable PLLA-based polymer scaffold hand-mounted on standard balloon catheter.

Commercial permanent BMS systems from stainless steel (316L) with an amorphous silicon carbide coating (Dynamic 5.0 x 20 mm, wall thickness 160 µm; Biotronik SE & Co. KG) were used as a control. Metal stents were deployed instantaneously, using inflation pressure of 9 bar.

2.2 Animals and experimental procedure

Animal experiments were approved by the governmental ethical board for animal research (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei, Mecklenburg-Vorpommern, Germany; No: 7221.3-1.1-027/12) and were carried out in accordance with the EU-directive 2010/63/EU. Six young adult German Landrace pigs with a mean body weight (BW) of 26.2 ± 2.0 kg were the subjects of this study.

Animal preparation and stent implantation was performed as described previously [4]. A total of 12 stents were implanted interventionally into both CCA.

During the entire intervention the animals received continuous intravenous heparin (50 IU/kg per hour). Oral dual antiplatelet drugs comprising 250 mg acetylsalicylic acid (ASA; Aspirin®, Bayer, Leverkusen, Germany) and 75 mg clopidogrel bisulfate (Plavix®; Sanofi-Synthelabo, Berlin, Germany) were administrated daily from postoperative day 3 until the end of the experiment. In Addition, the pigs received 60 mg/day atorvastatin (Sortis, Pfizer, Germany) orally, beginning 5 days prior to surgery and lasting until the study ended.

After 4 weeks, the pigs were euthanized in deep sedation, and the stented CCA were surgically explanted, and processed for quantitative histological and histomorphometric analysis using hematoxylin & eosin (H&E), as described in [5].

Vascular injury was semi-quantitatively evaluated as described by Schwartz et al. [6]. Inflammatory response was categorized as described by Kornowski et al. [7].

2.3 Statistical analysis

Calculations were performed using SigmaPlot 13.0 (Systat Software Inc., Richmond, CA, USA). Differences between two groups were compared using t-test for normal distributed values or the Mann-Whitney Rank Sum test for nonparametric data. Results are presented as mean $\pm$ standard deviation (SD). A p-value <0.05 was considered statistically significant.

3 Results

All animals survived until the end of the study without developing neurological complications. No wound complications were observed. After a weight gain of 12.8 kg, the animals reached a body weight of 39.1 ± 3.2 kg at the end of the study. A total of 6 permanent BMS as well as 6 absorbable PLLA-based x-ray marked scaffolds were successfully implanted interventionally (**Figure 2**) into the common carotid arteries (CCAs). No acute stent thromboses were occurred.

During preparation, stent implantation and stent explantation, all pigs were hemodynamic stable with a mean heart rate of 82 ± 10 bpm and a mean arterial pressure of 60 ± 8 mmHg. No physiological changes were observed in blood parameters between stent implantation and stent explantation.

Figure 2: Implantation procedure during angiography showing early dilatation of a PLLA-based polymer scaffold (left) with x-ray marked (arrows) compared with implanted bare-metal stent (right) in situ.

All scaffolds were patent on histological analysis without any signs of excessive recoiling or collapse in the absorbable polymer and permanent BMS stented group after 4 weeks. In all H&E slices the deployed stent cross section was intact, showing a circular shape (**Figure 3**).

Figure 3: Representative histological cross sections 4 weeks after implantation of (left) a bare-metal stent, and (right) a PLLA-based polymer scaffold (H&E, original magnification 12.5). Size bar length = 1mm.

Figure 4 presents vascular injury and inflammatory response of stents implanted into common carotid arteries of pigs after 4 weeks follow-up. **Table 1** presents geometric parameters of scaffolds in stented porcine common carotid arteries after 4 weeks of stent implantation. No differences

between proximal and distal section were detected in all H&E slices.

Figure 4: Vascular injury and inflammatory response of stents (mean $\pm$ SD) implanted into CCAs of pigs after 4 weeks follow-up.

Table 1: Morphometric parameters of stents (mean $\pm$ SD) implanted into CCAs of pigs after 4 weeks follow-up.

	BMS	Polymer	P-value
External elastic lamina area (mm ²)	19.14 $\pm$ 1.23	13.91 $\pm$ 1.81	$\leq$ 0.001
Internal elastic lamina area (mm ²)	16.44 $\pm$ 0.97	10.79 $\pm$ 1.123	$\leq$ 0.001
Lumen area (mm ²)	15.17 $\pm$ 0.96	6.41 $\pm$ 0.83	$\leq$ 0.001
Neointimal area (mm ²)	1.28 $\pm$ 0.13	4.38 $\pm$ 0.72	$\leq$ 0.001
Stent area (mm ²)	1.76 $\pm$ 0.13	3.90 $\pm$ 0.39	$\leq$ 0.001
Stenosis (%)	7.80 $\pm$ 0.88	40.52 $\pm$ 5.01	0.002
Neointimal thickness (mm)	0.03 $\pm$ 0.03	0.12 $\pm$ 0.11	0.002
Medial thickness (mm)	0.09 $\pm$ 0.01	0.17 $\pm$ 0.06	0.007

4 Discussion

Routinely statins are essential component of drug management of vascular patients. A new dual loaded absorbable scaffold design with a luminal and abluminal drug coating would be desirable.

Reducing strut thickness could help improve performance and a goal of future developments is to reduce strut thickness without sacrificing the required radial force.

In the end we need a very good clinical outcome in acute- and middle-term follow-up periods, including very low rate of early thrombotic occlusion and restenosis.

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