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Research paper

Effects of different application parameters on penetration characteristics and arterial vessel wall integrity after local nanoparticle delivery using a porous balloon catheter

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Abstract

Catheter-based local delivery of drug loaded nanoparticles agents offers a potential therapeutic approach to reducing restenosis. However, high delivery pressures and large volumes of infusates may cause severe vascular damage and increase intimal thickening. Therefore, we investigated the penetration pattern and vessel wall integrity of fluorescence-labelled nanoparticles (217 nm in diameter) into the non-atherosclerotic aorta abdominalis of New Zealand white rabbits in dependence of the volume (2.5 and 5 ml) and concentration (0.5 and 1 mg/ml) of the nanoparticle suspension, as well as the infusion pressure (2 and 4 atm) using a channelled balloon catheter (SCIMED REMEDY™ model RC 20/2.5). The location and penetration characteristics of nanoparticles in the arterial vessel wall were visualized using confocal laser scanning microscopy and transmission electron microscopy (TEM).

Catheter design and infusion pressure form a radial particle stream through intima and media into the adventitial layer of the aorta abdominalis. Infusion pressures of 4 atm in combination with high particle concentrations lead to effective nanoparticle delivery without severe vessel wall disruptions. Endothelium of the treated vessel segments was slightly affected during catheter insertion showing partly denudation of the innermost cell layer. TEM micrographs underlines transport functional properties of the vasa vasorum inside the vessel wall.

Consequently, local delivery efficiency of nanoparticulate carriers is critically affected by infusion pressure, and concentration of carrier suspensions. These factors need to be taken into consideration for the design of in vivo experiments.

Keywords: Angioplasty; Channelled balloon catheter; Infusion pressure; Local delivery; Nanoparticles

1. Introduction

Interventional procedures such as percutaneous transluminal balloon angioplasty (PTA) or stenting are most frequently accompanied by arterial vessel re-obstruction also known as restenosis. This process is characterized by intimal hyperplasia and vessel remodelling [1–3]. The neointimal formation results from vascular smooth muscle cell (VSMC) migration and proliferation into the media [4] followed by the formation of a new extra cellular matrix [5].

Apart from the application of drug eluting stents for local delivery [6,7], the development of balloon catheter delivery systems [8] allows the infusion of drug-loaded micro- [9–12] or nano-carriers [13–16] to provide a local and sustained drug release at the site of angioplasty. This technique ensures higher drug concentrations than systemic administration [17]. Although recent studies have demonstrated that particles <300 nm easily penetrate the vessel wall and appear, therefore, to be promising carrier systems in restenosis therapy [15,18], their successful application strongly depends on the particular infusion conditions. For instance, the infusion pressure and the volume of the infusate are crucial parameters that may cause severe vascular damage and enhanced intimal thickening [19].

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The present study describes the effects of particle suspension volume, particle concentration, and infusion pressure on the intramural distribution of fluorescence-labelled polystyrene nanoparticles into the non-atherosclerotic vessel wall of the aorta abdominalis in New Zealand white rabbits using a channelled balloon catheter. The aim of this study was to determine how to modulate the infusion parameters for an optimal nanoparticle delivery without severe intramural dissections. Our investigations have shown that the particle migration through the wall tissue is pressure dependent and is characterized by channel-like deposition patterns corresponding to the porous balloon catheter design. The increase of nanoparticle suspension volume could not improve the intramural particle concentration.

2. Materials and methods

2.1. Local delivery catheter

The delivery device, a channelled balloon catheter (SCIMED REMEDY™ model RC 20/2.5, lot 3377794, Boston Scientific, Natick, MA), has been described in detail by Hong [20]. Briefly, it is a three-lumen over-the-wire catheter with separate ports for balloon dilation and local drug delivery, which allows separation of the high inflation pressure for angioplasty from the low pressure desirable for local nanoparticle delivery. The balloon carries 18 channels, with one group of 30-μm diameter pores per channel in a spiral pattern for local delivery. In order to further reduce vessel wall injuries the particle suspension is allowed to leak through the micro pores rather than be propelled by jet streams. The catheter shaft has a diameter of 3.4 French carrying a balloon of 20 mm in length and 2.5 mm in diameter.

2.2. In situ catheterization and local delivery of fluorescence-labelled nanoparticles

The investigation conformed to the guidelines for the care and use of laboratory animals published by the US National Institute of Health ('Principles of Laboratory Animal Care', NIH Publication No. 85-23, revised 1985) and were approved by an external review committee for laboratory animal care.

Four non-atherosclerotic male New Zealand white rabbits weighing 3.5–4 kg were sacrificed using a mixture of embutramide, mebenzonium iodide, and tetracaine hydrochloride. To perform angioplasty and local nanoparticle delivery, the aorta abdominalis was exposed in situ (averaging 20–30 mm in length) and cut longitudinally. The catheter was placed above the aortic bifurcation. The location, quantity, and penetration depth of 217 nm yellow–green labelled polystyrene nanoparticles were evaluated in relationship to particle concentration, injection pressure, and suspension volume of Fluoresbrite™ plain microspheres YG suspended in water (Polyscience, Inc., Warrington, PA). The use of fluorescence-labelled instead of fluorescence-loaded nanoparticles avoids any unintentional dye release that would prevent the exact determination of particle distribution by confocal laser scanning microscopy (CLSM). The experimental set-up is described in Table 1.

Table 1
Experimental set-up for nanoparticle infusion at different settings (particle size: 217 nm)

Animal number	Vessel segment	Infusion pressure (atm)	Suspension volume (ml)	Particle concentration (mg/ml)
1	Distal	2	2	0.25
1	Proximal	4	2	0.25
2	Distal	2	2.5	0.25
2	Proximal	2	5	0.25
3	Distal	2	2	0.5
3	Proximal	2	2	1.0
4	Proximal		No infusion	

In all experiments, the angioplasty balloon was inflated and maintained at a dilating pressure of 8 atm until the entire nanoparticle suspension was infused. A non-treated vessel segment served as negative control. Immediately after the delivery procedure the treated and control vessel segments were excised and fixed in a formalin solution (4% v/v) for CLSM studies or glutaraldehyde (2.7% v/v) in phosphate buffered saline (PBS) 0.1 M, pH 7 for electron microscopy.

2.3. Confocal laser scanning microscopy

Arterial segments, frozen in cooled isopentane, were embedded in Tissue Freezing Medium (Jung, Germany). Cross-sections of 20-μm thickness were cut using a Frigocut 2700 cryo-microtome (Reichert-Jung, Germany) and mounted on SuperFrost plus (Menzel-Glaeser, Germany) glass slides. They were counterstained with 4′6-diamidino-2-phenylindole (DAPI, 1 μg/ml) (Molecular Probes, Leiden, The Netherlands) solution in PBS for 30 min under light exclusion and embedded in PBS/glycerol (2:1 v/v). Localization of fluorescence-labelled nanoparticles was performed using a CLSM (Axiovert, Zeiss CLSM 501, Jena, Germany) equipped with a Zeiss Neofluor 40*/1.3 objective. Excitation wavelengths were 364 nm (long-pass filter [LP] 385 nm) for DAPI and 488 nm (LP 505 nm) for the yellow–green labelled nanoparticles. All confocal images were acquired with the same settings with respect to laser intensity, filter block, and detector gain.

2.4. Transmission electron microscopy

The fixed blood vessels were rinsed three times with 0.15 M PBS, pH 7, for 1 h each and post-fixed for 75 min

using 2% osmium tetroxide solution. Small parts of the vessels were embedded for ultramicrotomy using Durcupan ACM (Fluka, Switzerland), a four component water-soluble embedding medium. After dehydration of the tissues in a water-soluble aliphatic polyepoxide as described elsewhere [18], the dehydrated tissue was placed in a polymerization mixture according to the manufacturer's protocol and left overnight at 4 °C for final mixing and embedding. Polymerization was performed in a freshly prepared mixture of the above composition for 4 days at 42 °C.

Ultrathin sections, approximately 50 nm thick, were obtained using a Leica Ultracut UCT microtome and a diamond knife (Leica Microsystems, Germany). The sections were collected on copper grids covered with a supporting thin collodium/carbon film. Final staining of the sections included the treatment with uranyl acetate for 15 min and with lead citrate for 9 min. Microscopic examinations were carried out in a JEM 3010 transmission electron microscope (Jeol, Japan), equipped with a 2k × 2k slow-scan CCD camera and a LaB_6 cathode, operated at 300 kV.

2.5. Scanning electron microscopy

The inner surface morphology of the blood vessels after catheterization was controlled using both transmission electron microscopy (TEM), as well as SEM observations. The glutaraldehyde fixed specimens were dehydrated using a critical-point drying apparatus Polaron E3000 (Bio-Rad Microscience) after stepwise replacement of the water by acetone, which is than substituted by carbon dioxide. Finally, the dried specimens were coated with a thin gold evaporation layer to prevent charging. Sample characterization was performed by scanning electron microscopy using

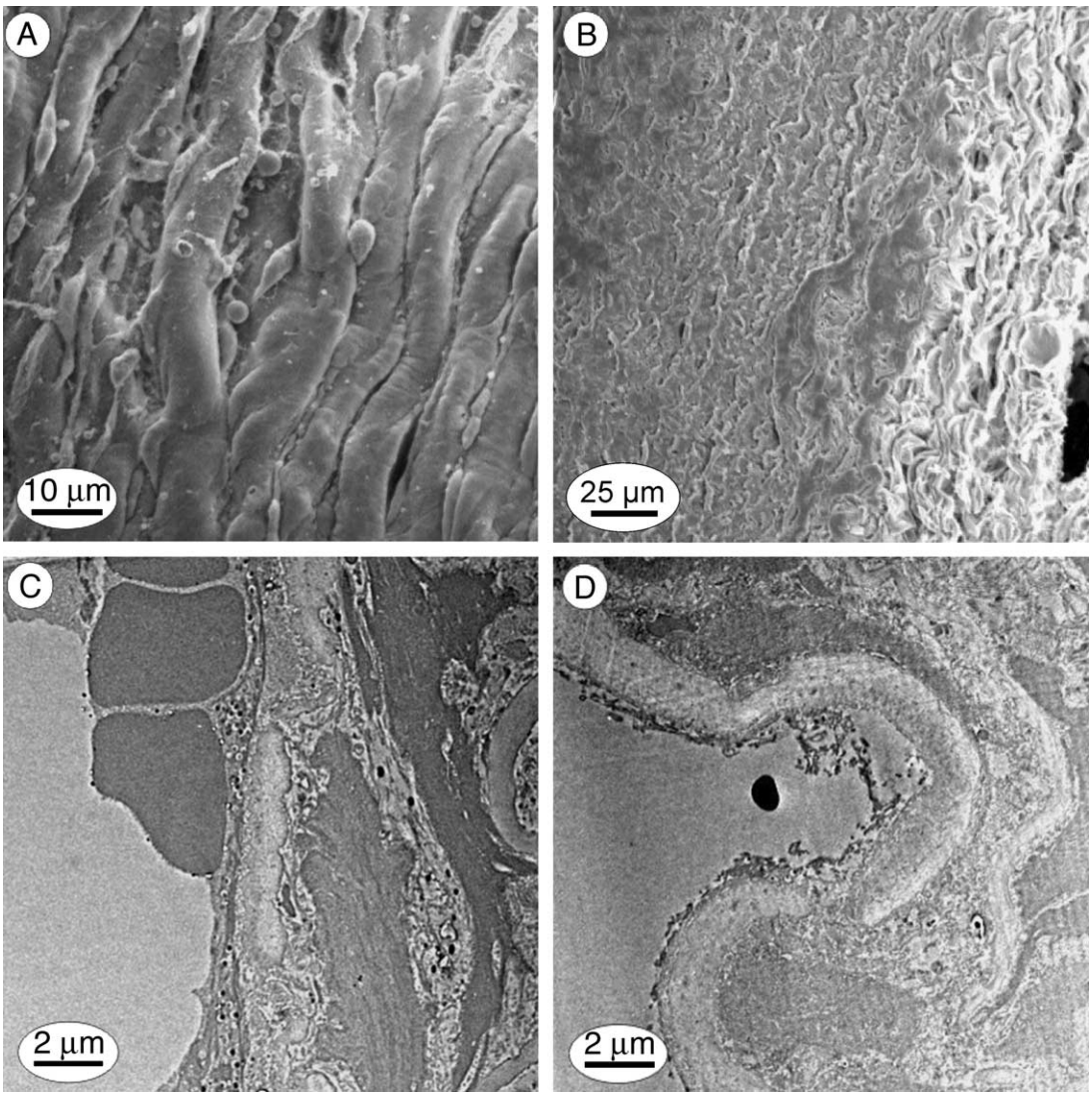

Fig. 1. SEM images (A, B) and TEM images (C, D) of the endothelial layer of the aorta abdominalis. After balloon dilatation and nanoparticle infusion the endothelium appears sporadically intact (A, C). In B and D, the endothelial layer appears to have been completely removed.

a CamScan Series 4 (CamScan, USA) at 20 kV acceleration voltage.

2.6. Semi-quantitative analyses of penetration depth and fluorescent intensity

Selective particle distributions and tissue auto fluorescence complicated the determination of a defined front line of fluorescent nanoparticles. Consequently, the penetration depth was measured semi-quantitatively and graded on a scale from 1 to 3 according to Fram [21]. Briefly, grade 1 described penetration into the inner one-third of the vessel wall, grade 2 into the inner two-third, and grade 3 into the outer one-third of the vessel wall of the aorta abdominalis. The fluorescence intensity was graded in the same manner on a scale from 1 to 3, corresponding to light, moderate, and intense fluorescence.

3. Results

As a result of the catheter insertion into the aorta abdominalis and its removal, as well as catheter dilatation during ballooning, there is always the risk of mechanical injury to the endothelial layer. Generally, the affected vessel segments showed little or no damaging of the endothelium (Fig. 1A and C), although in some selected cases we observed some disturbances of the endothelial layer (Fig. 1B and D). The cross-sections of the treated arteries usually did not show any severe vessel wall disruptions. In CLSM images, the light green auto fluorescence, at an excitation wavelength of 488 nm, as seen in cross-sections of control experiments without nanoparticle infusion was well distinguishable from the blue DAPI stained nuclei of smooth muscle, endothelial, and adventitial cells, as well as from the nanoparticles themselves (Fig. 2A).

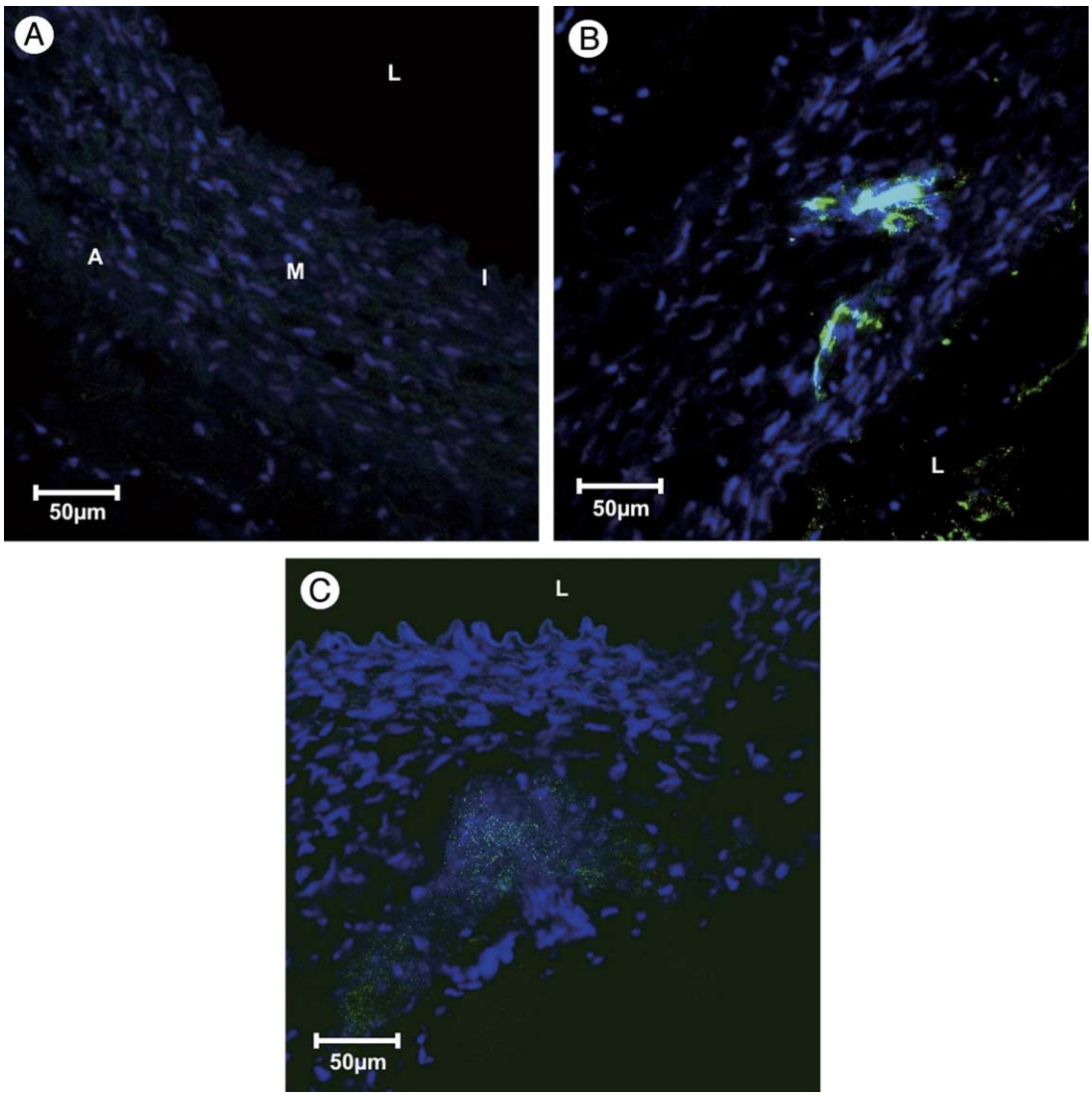

Fig. 2. CLSM analysis of the nanoparticle distribution in the vessel wall of the aorta abdominalis of New Zealand white rabbits: nuclei (blue) stained with DAPI, catheter-delivered yellow–green labelled polystyrene nanoparticles (green). L, M, and A indicate the lumen, media, and adventitia. (A) Balloon-dilated segment of aorta abdominalis: control experiment without nanoparticle infusion. (B) Infusion of 2.5 ml nanoparticle suspension (0.25 mg/ml, 2 atm) in comparison to (C) 5 ml suspension.

Table 2
Infusion characteristics: semi-quantitative analyses of penetration depth and fluorescence intensity

Parameter	Value[a]	Penetration depth[a]	Fluorescence intensity[b]
Infusion pressure (atm)	2/4	2/3	2/3
Particle concentration (mg/ml)	0.5/1	3/3	2/3
Suspension volume (ml)	2.5/5	2/2	2/1

[a] Penetration depth is graded into 1 = inner one-third, 2 = inner two-thirds, 3 = outer one-third of the vessel wall.
[b] Intensity of fluorescence was graded into 1 = light, 2 = moderate, and 3 = intense fluorescence.

One method to enhance drug delivery efficiency is the use of smaller drug-loaded nanoparticles. Further possibilities include the increase of nanoparticle suspension volume, particle concentration, or infusion pressure. Our results of semi-quantitative analyses of penetration depth and fluorescence intensity after delivery of 217 nm-sized fluorescence-labelled nanoparticles in dependence of the latter parameters are shown in Table 2.

The increase of suspension volume from 2.5 to 5 ml did not lead to a higher fluorescence activity in several analysed cross-sections from treated vessel segments as derived from a comparison of Fig. 2B and C. Only light fluorescence in the intimal and medial layer could be observed. The capacity of the vessel tissue appears to be low for large volumes of drug solutions or drug-carrier suspensions.

In contrast, a remarkable increase in nanoparticle uptake was observed as a consequence of an increased infusion pressure from 2 to 4 atm (Fig. 3A and B). In a number of cases, the nanoparticles poured in a funnel-shaped manner through the intimal layer into the medial tissue as shown by CLSM in Fig. 3B, as well as by the TEM images in Fig. 3C. Occasionally, the formation of a particle cloud with high

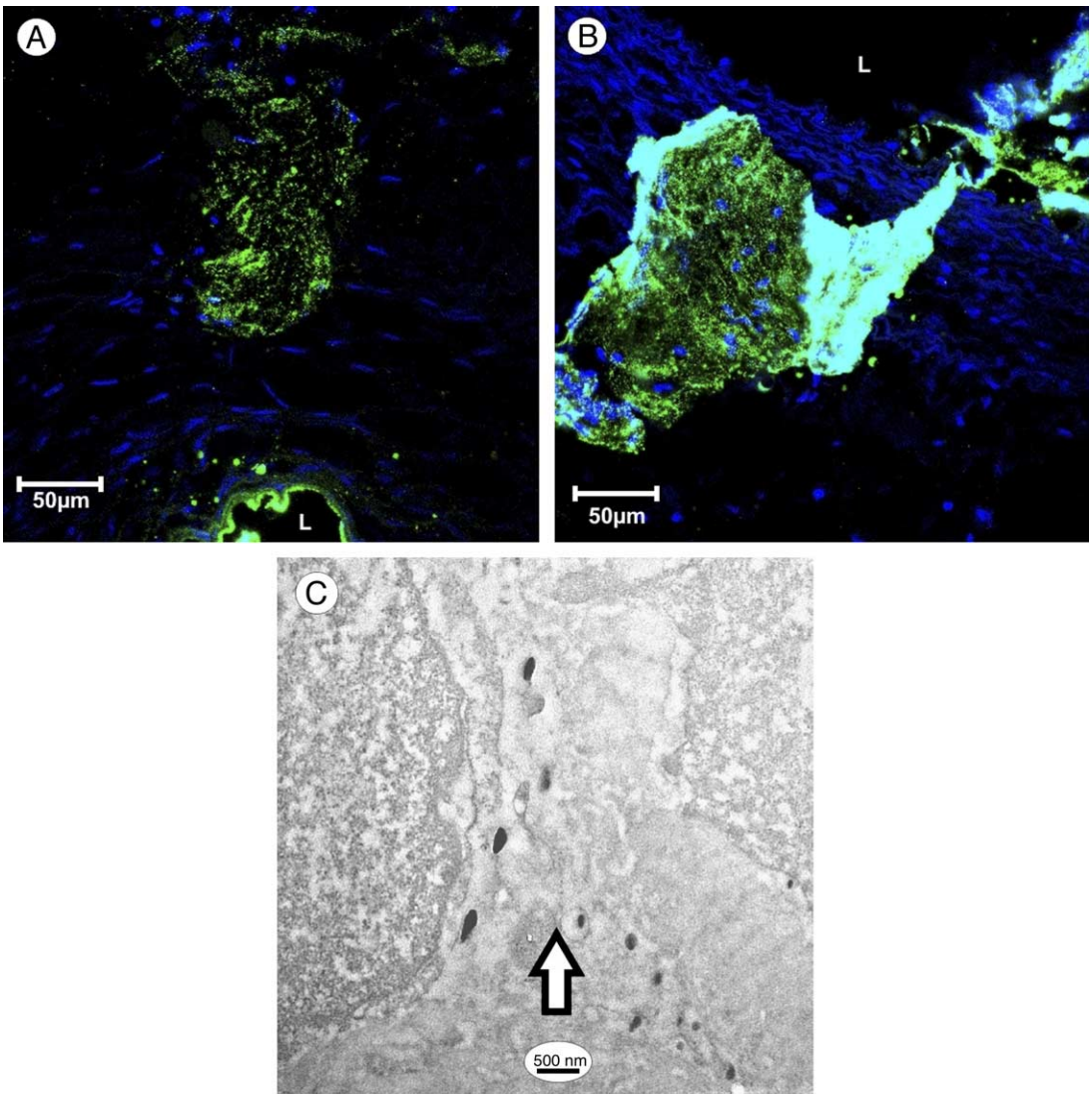

Fig. 3. CLSM images of the nanoparticle distribution in the vessel wall after infusion at (A) 2 and (B) 4 atm. (B) shows a radial channel-like particle influx into the vessel wall with a 'cloudy' particle accumulation spreading over the intimal medial and adventitial layer. (C) transmission electron micrograph of funnel-shaped particle deposition in the medial layer.

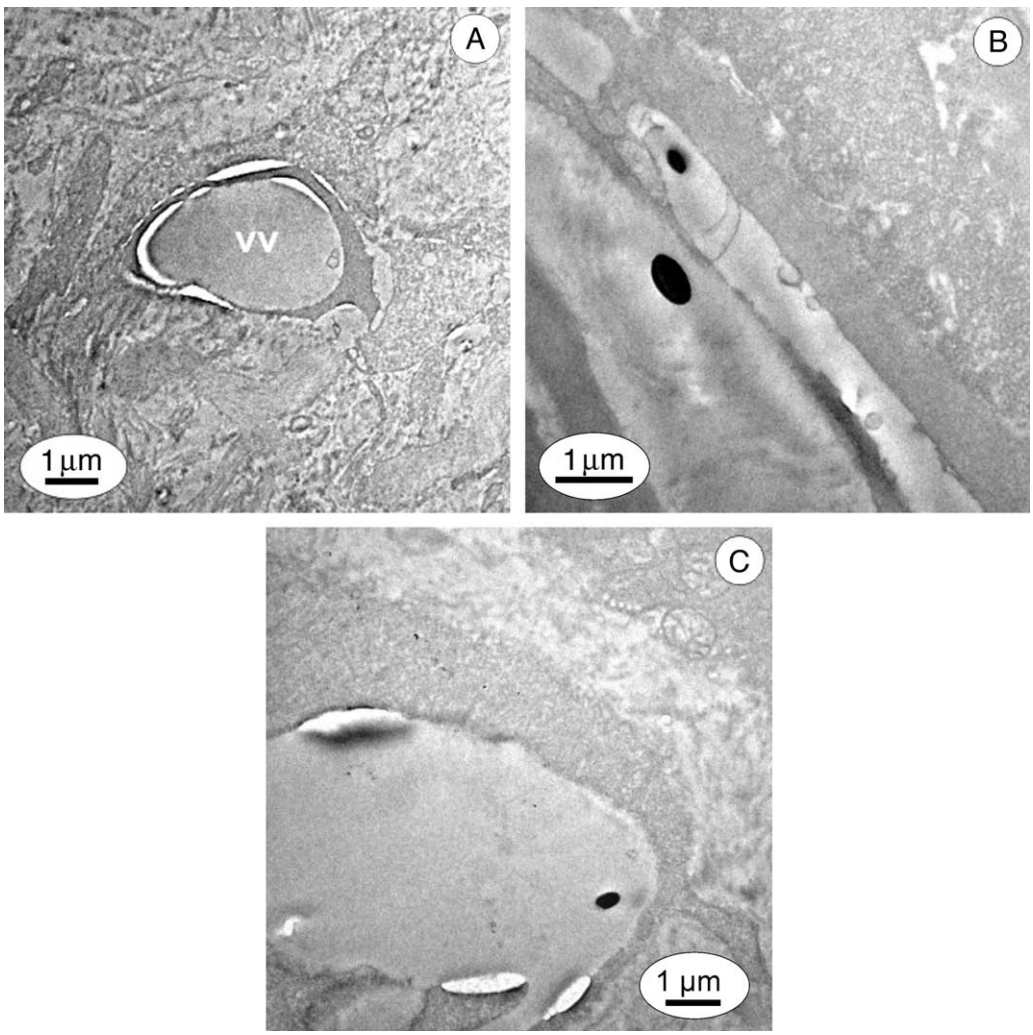

Fig. 4. Transmission electron micrographs of a vasa vasorum (A–C) with nanoparticle deposition displayed in (B) and (C).

fluorescence activity extending up to the adventitial region of the vessel wall could be observed (Fig. 3B). At the interface between the arterial tissue and the lumen, the particles seemed to stream back into the luminal area and build particle clusters. The channel-like particle streams at the infusion site have a diameter of about 25 µm which correlates with the pore diameter of the balloon catheter.

Apart from the depicted penetration characteristics of the nanoparticles, which tend to cross the successive layers of the vessel wall preferably along infusion-induced diffusion channels, other possible transport pathways are revealed in Fig. 4. The TEM micrographs show cross-sections (Fig. 4A and C) and longitudinal sections (Fig. 4B) of the vasa vasora with and without nanoparticle incorporations. These examples are evidence for the transport functional properties of the vasa vasorum.

Moreover, in Fig. 5 an extensive fluorescence signal over all vessel wall layers is shown with a substantially higher intensity at an increased particle concentration of 1 mg/ml (Fig. 5B) compared to concentrations of 0.5 mg/ml (Fig. 5A).

4. Discussion

Although the microporous design of balloon catheters was effective for the local infusion of anti-restenotic drugs [19], the intramural drug delivery is considered to be associated with additional vessel trauma, such as endothelial abrasion, disruptions of intima or elastica lamina, and dissections of the arterial vessel wall. Recent studies have described a direct relationship between the damage of arterial vessels and infusion pressure of local delivery during PTA [22,23]. Wolinsky and Lin found that the infused dye entered such crevices and that the disruptions were indeed induced by the angioplasty procedure [24]. The injuries in the arterial vessel wall may also have been caused by high pressure jet streams from the application pores of the perforated balloon catheter [25,26]. Furthermore, vessel wall disruptions and intimal hyperplasia were more prominent at higher inflation pressures during balloon angioplasty [27], as well as at higher infusion pressures during catheter-based nanoparticle application [28].

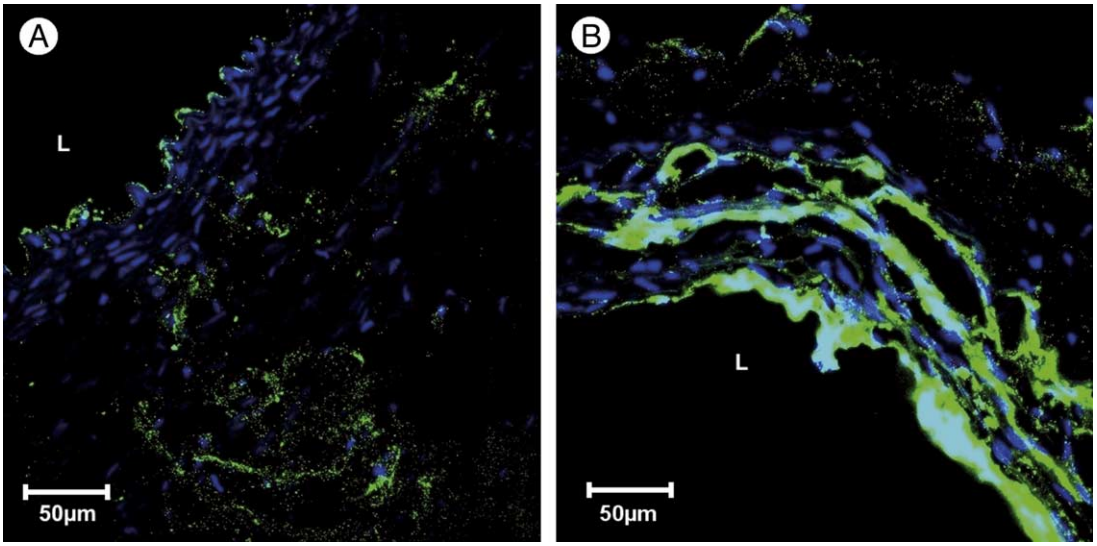

Fig. 5. CLSM images of arterial vessel segments infused with nanoparticle suspensions of (A) 0.5 mg particles per ml and (B) 1.0 mg particles per ml.

Despite some occasional endothelial abrasion caused by catheter intervention, the cross-sections of the present study did not reveal any noticeable dissections of the vessel wall at infusion pressures of 2 and 4 atm or at higher fluid volumes. These observations are in agreement with the findings of Gunn, who showed that local delivery of oligonucleotides for antisense therapy caused no evident vessel wall disruptions at infusion pressures between 1 and 6 atm [29].

Whereas some researchers did not observe any significant differences in the delivery efficiency of drug solutions [30] or fluorescent-labelled microparticles [11] at increasing infusion pressures, Fram and co-workers have shown that the penetration depth of horseradish peroxidase was directly related to the infusion pressure [21]. Our results also demonstrate an enhancement of particle deposition at the delivery site with increasing pressure. On the other hand, however, in the present study the evaluation of the depth of vessel wall penetration revealed no differences at infusion pressures of 2 or 4 atm. The fluorescence-labelled nanoparticles were visible in all vessel wall layers arranged in a funnel-like manner without vascular damage. Data from a non-atherosclerotic rabbit carotid artery model using a channelled balloon catheter indicated jet injuries which were detected in areas where the application pores directly contacted the vessel wall [26]. We suppose that the described deposition pattern is the result of the observed infusion-induced particle stream from the catheter pores.

It has become evident that fluid volumes of more than 2 ml do not effectively increase the drug delivery efficiency simply because of the low capacity of the treated vessel wall segment. Instead, high fluid volumes may increase the traumatic side effects during local drug delivery with porous balloons considerably, as reported by Herdeg [26]. Consequently, it is reasonable to increase the drug delivery efficiency by using higher concentrated drug or drug-carrier solutions, rather than larger volumes.

Limitations of the present study are the lack of quantitative information about the absolute particle mass delivered to the vessel wall which is an important aspect in the validation of the biological effect of drug loaded particles. Also, when using non-atherosclerotic vessel segments one has to be aware that atherosclerotic plaques may reduce nanoparticle delivery. Finally, this study has not taken any delayed vascular damage, such as neointimal thickening and the associated increase in the SMC proliferation activity, into account.

5. Conclusions

We observed a channel-like particle penetration into the vessel wall of the aorta abdominalis as a function of catheter design; however, we were able to determine that the leaking effect of the channelled balloon seemed to prevent traumatic jet streams to the greatest possible extent. Consequently, the increase of the infusion pressure from 2 to 4 atm promoted nanoparticle delivery without causing severe vessel wall ruptures. The local delivery efficiency of particulate drug carriers depended on different factors such as catheter design, particle size, infusion pressure, and concentration of drug carriers. Whereas an increased particle concentration consequently led to a higher suspension viscosity, increased infusion pressures were required for the delivery of the viscous suspensions, which may cause severe damages of the vessel wall architecture. Therefore, it is extremely important to define well-balanced parameters for particle size, infusion pressure, particle concentration and administrated volume specific to the catheter system used.

Nevertheless, nanoparticulate drug and gene carriers based on biodegradable polymers offer a promising approach for the treatment of restenosis after angioplasty.

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