Technical Advance

Function and Structure of Pressurized and Perfused Porcine Carotid Arteries

Effects of in Vitro Balloon Angioplasty

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In this report we describe the application of an *in vitro* pressure-perfusion system for study of functional/structural changes after *in vitro* balloon dilation injury. Pig carotid arteries were perfused at P = 100 mm Hg and Q = 100 ml/min, balloon angioplastied (BA), and cultured under these hemodynamic conditions for 4 or 8 days ($n = 5$ BA and 6 controls for each time point). To assess endothelial function, outer diameter changes in response to bradykinin (BK) were measured daily. Remodeling was determined from the shift in pressure-passive diameter relation, as obtained after papaverine addition. Arterial samples were processed for histology. Control arteries showed spontaneous tone, BK-induced relaxation, and inward remodeling that was more pronounced at day 8 (ratio end-to-start passive diameter at P = 100 mm Hg, 0.69 ± 0.04 ; $P < 0.001$) than at day 4 (0.85 ± 0.03 , $P = 0.03$). Intimal hyperplasia was detectable in these control vessels at day 8 with accumulation of α -smooth muscle actin-positive cells around the lumen. Angioplasty caused ruptures and dissections and abolished tone that returned after 5 days of perfusion along with BK-dependent relaxation. No significant inward remodeling or intimal hyperplasia was observed at day 8 after angioplasty. Thus, BA inhibits remodeling, which occurs after *in vitro* perfusion of conductance arteries. (*Am J Pathol* 2003; 163:1743–1750)

Balloon angioplasty (BA) remains an important procedure for recanalization of atherosclerotic lesions.^{1,2} Although already applied clinically for more than 40 years the effects of BA on the behavior of the segments in the

first weeks after intervention are still not clear. It is unclear why a number of lesions show an exaggerated response to injury after BA, possibly resulting in restenosis, whereas others remain patent.^{3,4} Also, the relation between structure and function of an angioplastied arterial segment is incompletely understood.⁵ Thus, study of the architectural and functional consequences of BA on the arterial wall remains necessary.

The application of *in vitro* techniques for study of blood vessels can be helpful in understanding the pathophysiology associated with angioplasty because of the ability to directly and continuously observe the vessels.⁶ Because the importance of arterial hemodynamics on vascular biology has been recognized both in normal physiology and in vascular pathology,^{7–9} the application of hemodynamic stimuli in these *in vitro* models is indispensable.^{6,10} Therefore, pressurized and perfused arterial preparations form a valuable *in vitro* model for studying changes after BA.

In this study we describe a model for the study of arteries after *in vitro* BA. This model allows for perfusion and pressurization of cultured vessels. The setup has been equipped with devices for diameter measurements and interventional procedures. Using this model, we aimed to monitor the function and structure of vessels in the first week after BA. Accordingly, porcine carotid arteries were cultured for 4 or 8 days with a number of vessels receiving *in vitro* BA at the start of culture. Vascular reactivity was assessed daily while arterial mechanics were assessed at the start and the end of the perfusion period. Also, samples were collected for histological analysis.

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Figure 1. A: Photograph of culture chamber with pig carotid artery dilated at day 1 and perfused until day 8. Note the balloon-dilated part in the middle of the arterial segment (**bracket**). **B:** Scheme of perfusion system; **arrows** indicate the direction of flow as set by the pump (Q). Pressure is imposed at P. The whole setup was placed in an incubator at 37°C.

Materials and Methods

Harvest and Preparation

Fifteen female Yorkshire pigs (weighing 19 to 26 kg, 12 to 18 weeks old) were anesthetized and intubated as described previously.¹¹ Both carotid arteries were isolated, and after heparinization (0.1 ml/kg i.v.) were excised and immediately placed in 4°C MOPS-buffered Ringer solution containing (mmol/L): 145.0 NaCl, 4.7 KCl, 1.17 MgSO₄·7 H₂O, 2.0 CaCl₂·2 H₂O, 1.2 NaHPO₄·H₂O, 5.0 glucose, and 2.0 pyruvate, at pH = 7.35 (Sigma, St. Louis, MO). After transfer to the laboratory, arteries were pinned down in a silicone-bottom dish filled with MOPS-buffered Ringer solution containing 1% albumin at 4°C. Arteries were cleaned under a dissection microscope that was placed in a sterile flow hood. From this moment on, all manipulations were performed under sterile conditions.

After cleaning, a segment (~4 to 5 cm) of the artery was transferred to an incubation chamber (Figure 1A) filled with Leibovitz L-15 medium (BioWhittaker, Verviers, Belgium) containing antibiotics [penicillin, streptomycin, and amphotericin B, Gibco, (Grand Island, NY)] and supplemented for 10% with heat-inactivated Australian reprocessed fetal calf serum (BioWhittaker). This solution was used for both the intra- and extravascular environment. A remaining piece of artery, ~5 mm in length was used as a fresh control and stored in 4% formalin for histology.

Of a total of 30 arteries from the 15 pigs, 24 were mounted onto glass cannulas and gently pressurized with a medium-filled syringe to check for possible leaks. Then the vessels were stretched to their *in vivo* length by stretching the vessel to 150% of its unloaded length while gradually increasing pressure to 100 mmHg. The 24 vessels were taken from 15 pigs (one or two vessels per pig). The remaining six arteries (of six different pigs) were used for an unrelated study. After closure of leaks with sutures the cannulation chamber was connected to the organ culture perfusion system.

Culture System

Our culture system was adapted from the system devised by Bardy and colleagues.⁶ It consists of a custom-made three-port glass reservoir connected to the cannulation chamber by PharMed tubing (Masterflex; Cole-Parmer Instruments, Vernon Hill, IL). A roller-pump (Console

Drive, Masterflex; Cole-Parmer Instruments) inserted between the distal end of the artery and the reservoir was used for controlling flow (Figure 1B). Pressure was applied to the reservoir by means of an electronically controlled Venturi valve (T5200-50; Fairchild Co., Winston-Salem, NC). Reservoirs were pressurized at a mean pressure of 100 mmHg resulting in a mean arterial pressure of 91 mmHg. Because of the peristaltic character of the roller pump, pressure pulsated at 90 beats/minute between 82 and 104 mmHg. Flow averaged 100 ml/minute and fluctuated between 28 and 149 ml/minute at mean arterial pressure. A Y-connector was inserted in the circuit proximal to the artery to allow for the introduction of a BA catheter. Separate parts of the system were connected by means of three-way stopcocks for introduction of vasoactive agents and air-free changes of intravascular perfusion fluid. The whole setup was placed in a heat incubator kept at 37°C under humidified air for periods up to 8 days with both the intra- and extravascular medium being changed every day. The perfusion setup was equipped with an imaging system to allow for outer diameter measurements of the artery during the incubation. This imaging system comprises a microcamera setup (MTO-AMC Instrumentation Department), mounted onto a position-controlled frame. Images were captured by a frame grabber (PCI-1409; LabVIEW National Instruments, Austin, TX) controlled by IMAQ software (LabVIEW). After calibration, diameters on the images were measured in Photoshop (version 5.0 LE; Adobe, San Jose, CA).

Culture Protocol and Measurements

Arteries were mounted in the perfusion circuit (day 0) and cultured for 4 or 8 days (both *n* = 12). Every day starting at day 1 (ie, 24 hours after cannulation) diameter measurements were performed before (active diameter) and after bradykinin (BK, 10⁻⁷ mol/L, Sigma) addition. The reaction to BK was used as a measure for endothelial reactivity. Furthermore, at day 1 and the last day of the perfusion period (day 4 or 8), papaverine (PAP, 10⁻⁴ mol/L, Sigma) was added to the perfusate for maximum dilation and the pressure-passive diameter relationship was determined. In a separate series of experiments, these relationships were also determined 30 minutes after start of perfusion with PAP in Ca²⁺-free MOPS solution to determine any extra relaxation. Remodeling was determined from the shift of the passive pressure-diameter

relationships between days 1 and 4 or 8. All diameters were normalized to the passive diameter (mean $\pm$ SEM: 4.80 ± 0.13 mm and 4.95 ± 0.11 mm for 4 and 8 day group, respectively) at day 1 and $P = 100$ mm Hg and expressed as a percentage.

After the determination of the pressure-passive diameter relationship at day 1 and during maintained vasodilation, a BA catheter (Cordis, Miami Lakes, FL) was introduced via the Y-connector into 12 of 24 arteries with the remainder serving as nonangioplastied controls. The balloon was inflated three times for 1 minute with 30-second intervals at 6 bars. Balloon dilation ratio (DR) was defined as the balloon diameter divided by the outer vessel diameter at 100 mm Hg. The diameter of the balloon was measured with sliding calipers after inflation of the balloon with water at 6 bars at room temperature. After dilation, the catheter was withdrawn and perfusion was continued. After the final assessment of the pressure-passive diameter relationship at day 4 or 8, the vessel was taken from the cannulas and fixed in 4% formalin for histological processing.

Histology and Immunohistochemistry

After fixation, arteries were divided into 5-mm segments. All segments were dehydrated and embedded in paraffin. Segments were cut in 5- μ m cross sections and mounted onto glass slides (Starfrost, Burgdorf, Germany). Sections were stained with hematoxylin and eosin (H&E) and elastin von Gieson (EvG).

Immunohistochemistry was performed according to the indirect horseradish-peroxidase method. Slides were preincubated for 30 minutes with 10% normal goat serum, followed by incubation with anti- α -smooth muscle actin (α -SMA) (Sigma) for 60 minutes. For negative controls, the primary antibody was omitted. After rinsing in phosphate-buffered saline (PBS) (2×5 minutes.) slides were incubated with a biotinylated secondary antibody for 30 minutes, rinsed again in PBS (2×5 minutes.) and incubated for another 30 minutes with peroxidase-conjugated Extravidin (Sigma). Peroxidase activity was visualized using 3,3'-diaminobenzidine as substrate.

Light Microscopic Evaluation

All sections were analyzed under a Nikon Eclipse Pol 600 microscope (Nikon Europe, Badhoevedorp, The Netherlands), set at a preset illumination value (with filtering in case of pixel saturation). Images were captured with a Nikon Cool Pix digital camera and analyzed off-line with Scion imaging software (NIH Image). Lumen and external elastic lamina bounded areas as well as total vessel areas were assessed quantitatively in mm² and from these areas the radius of the lumen and the mean thickness of the respective vessel layers were calculated, assuming circular anatomy. Coverage by the endothelium was scored as a percentage while internal elastic lamina ruptures and dissections were counted manually. Intimal hyperplasia was measured at four equidistantly spaced positions and averaged. Staining for α -SMA was evaluated

Figure 2. The active diameter, normalized to the diameter during papaverin addition as a function of day number. Results are shown for the 8-day group, results for the 4-day group are comparable for relevant time points. Pre-PAP, active diameter before relaxation with papaverin; post-PAP, passive diameter after relaxation with papaverin. At all time points after BA, the diameter in the BA group was significantly greater than diameters in the control group: *, $P < 0.05$ control versus BA.

by determining the mean gray value density in the separate vessel layers after background subtraction.¹²

Statistical Analysis

Data are presented as mean $\pm$ SEM. Differences between groups were assessed by unpaired *t*-tests, differences within groups by analysis of variance with Bonferroni posthoc testing. R^2 -value significances were determined with the *F*-statistic. All differences were considered significant at a level of $P < 0.05$.

Results

Basal Tone and Endothelium-Dependent Relaxation

Two vessels were lost during culture because of technical problems. The remaining 22 vessels had developed basal tone after 24 hours of culturing (Figure 2, day 1). Papaverine caused relaxation to by definition 100% (day 1, PAP). In the noninjured group ($n = 12$), vessels redeveloped tone in the course of hours after PAP was washed out ($P < 0.05$). Tone in this group progressed throughout days (decrease in outer diameter post-PAP day 1 versus day 2 and day 2 versus day 3: $P = 0.019$ and $P = 0.035$, respectively) and was maintained for the duration of culture (no significant changes from day 4 onwards). BA ($n = 10$) however completely abolished vessel tone and even induced a significant increase in diameter up to day 4 as compared to the passive diameter immediately after PAP at day 1 (Figure 2). In the first day after BA, vessels remained unresponsive to either endothelin or phenylephrin, as opposed to controls ($n = 2$, data not shown). From day 5 onwards, reductions in active diameter redeveloped and this became significant at day 8 ($P = 0.048$ versus post-PAP-day 1). However, on days 2 to 7 (but not day 8) diameters in the BA group were still larger as compared to the basal diameter in the presence of tone at day 1 before BA (Figure 2). Differ-

Figure 3. Normalized relaxation as a function of number of days of perfusion. Data are superimposed on those of Figure 2. In the control group significant changes in diameter after BK were measured at all time points. In the BA group, significant changes were found only at days 1 (immediately before BA), 7, and 8. Furthermore, at all time points after BA, a significant difference between groups was found: *, $P < 0.05$ control versus BA relaxation. Error bars refer to relaxation data.

ences in comparison with the non-BA control group remained significant up to the end of culture ($P < 0.05$). Reference segments of balloon-dilated arteries showed a reduction in diameter throughout culture comparable to that of control vessels (see the photo in Figure 1A). BA never caused any spasms.

We established the dilation to the endothelium-dependent dilator BK, as shown stacked on top of the normalized active diameter in Figure 3. Noninjured arteries relaxed in response to the endothelium-dependent dilator BK (Figure 3). The relaxation remained significant during culture. However, the diameter after vessel relaxation with BK became progressively smaller during culture in the control group. As indicated below, this reflects inward remodeling rather than a lack of endothelium-dependent dilation.

The balloon-injured arteries had lost all tone up to day 4 and EC function could not be established here. However, as soon as tone started to redevelop (day 5), small responses to BK became apparent again and reached significance from day 7 on.

Remodeling

The above reduction in diameter in the presence of BK in the control group throughout time reflected inward remodeling in this group rather than loss of endothelial function. Indeed, substantial inward remodeling occurred in these control vessels. Thus, in the high-pressure range, reductions in passive diameter in the control group, as determined using PAP, were already present at day 4 (Figure 4, top). At day 8, these changes became significant for all pressures. No extra relaxation was found by perfusing with Ca^{2+} -free MOPS solution (data not shown).

In contrast, in the BA group inward remodeling did on average not occur (Figure 4, bottom). Rather, at 10 mmHg passive diameter had increased at day 4. The increase was reversed at day 8. Although on average no remodeling was found at days 4 and 8 after BA, individual vessels did show remodeling. Interestingly, vessels that were only slightly distended by BA showed a decrease in normalized passive diameter at day 8, as opposed to the highly distended arteries. Indeed, a strong correlation

Figure 4. Pressure-passive diameter relationship at start (day 1), and after 4 or 8 days of perfusion. **Top**, control; **bottom**, BA. #, $P < 0.05$ day 1 versus day 4; *, $P < 0.05$ day 1 versus day 8.

between normalized passive diameter at day 8 and DR was found (Figure 5; $R^2 = 0.73$, $P < 0.05$). The correlation between normalized active diameter (at day 8) and DR was even stronger (Figure 5; $R^2 = 0.93$, $P < 0.01$).

Histology and Immunohistochemistry

Histological sections (H&E and EvG) of control arteries perfused for 4 days indicated an apparently normal artery with a intima bounded by the internal elastic lamina and a cell-rich media bounded by an external elastic lamina and adventitia (Figure 6, A and B), comparable to the fresh controls. The same appearance was found after 8

Figure 5. Normalized passive and active diameter at day 8 of culture versus DR in the BA group.

Figure 6. Photomicrographs of histological sections: **A** and **B**, control day 4; **C** and **D**, control day 8; **E** and **F**, BA day 4; **G** and **H**, BA day 8 (all H&E); **I**: control day 4, anti- α -SMA. **Inset** in **D**: Corresponding EvG stain. **Boxed areas** in **A**, **C**, **E**, and **G** refer to magnifications in **B**, **D**, **F**, and **H**. Scale bars: 500 μ m (**A**, **C**, **E**, **G**); 50 μ m (**B**, **D**, **F**, **H**); 100 μ m (**I**). Note the IH in **D** and the damage induced by BA in **E** (both indicated by **arrows**).

days of perfusion, although little but significant intimal hyperplasia (IH) was detectable (Figure 6, C and D; and Figure 7, top). In the BA group varying degrees of injury, depending on the DR, were observed: loss of most of the endothelial cells (ECs), dissections and other damage to the medial layer and ruptures of the internal elastic lamina (Figure 6; E to H). A significantly lower endothelial coverage and higher number of dissections were found after BA (Table 1).

Image analysis of anti- α -SMA-stained sections revealed no differences in average staining intensity in the media of control, dilated, and fresh vessels (data not shown). However, we did note local spots of intense staining at both the intima-media and the media-adventitia borders in cultured, but not fresh, vessels (Figure 6I). This was also found on the outside of the adventitia. Histomorphometry of perfused arteries indicated no differences in media thickness-lumen ratio between control-perfused and BA vessels. Also, no difference was found in this ratio between vessels harvested at days 4 or 8 (Figure 7, middle). An increased adventitial thickness-lumen ratio was found at day 8 in the control group as compared to the BA vessels (Figure 7, bottom).

Discussion

The purpose of this study was to evaluate the functional and structural properties of *in vitro* pressurized and perfused conductance arteries, and to assess the effect of BA on these properties. The results of this study show that nonangioplastied arteries maintained basal tone and endothelium-dependent dilation. The histological appearance of these vessels was normal after perfusion, although some IH could be detected at day 8. Furthermore, these vessels displayed eutrophic inward remodeling at day 4, which at day 8 became more pronounced and associated with adventitial growth.

BA caused both macroscopic and histological damage to the tissue and a lack of tone in the first days after the procedure. From day 5 onwards, active diameters started to decrease and by that time EC function could again be detected, as judged from the response to BK. These responses, however, remained depressed in comparison with the non-BA controls, in accordance with the low coverage by ECs. After *in vitro* BA, vessels on average did not show inward remodeling up to 8 days.

Figure 7. IH (top) and medial (middle) and adventitial (bottom) thickness lumen ratios of perfused arteries for both groups and time points: *, $P < 0.05$ BA versus control.

Critique of the Perfusion Model

Perfusion models, like the one described in this study and elsewhere,^{6,10} are valuable techniques for the *in vitro* study of vascular structure and function, because they allow the incorporation of vascular hemodynamics. These models also have several attractive advantages compared to *in vivo* models, including the independent control of pressure and flow, the direct observation of diameter, and the ease of recurrently sampling and manipulating the preparation. However, a number of limitations are present in the described model. These include concerns with respect to manual dissection and mechanical loading of the preparation. A further limitation is the replacement of blood by culture medium (Leibovitz L-15) as the perfusion fluid, especially because blood-

borne cells have been recognized to be important in a number of pathophysiological conditions, such as restenosis after BA.^{13,14} Yet, the current model reveals that such blood-borne cells may not be necessary for either inward remodeling or neointima formation, which was observed toward the end of culture.

The replacement of blood by perfusion medium causes another limitation. Because shear stress (τ) during constant flow is linearly related to perfusion fluid viscosity, the smaller viscosity of culture medium in comparison with blood causes a decrease in τ as experienced by the arterial endothelium. Typically, at the start of the experiments τ was ~ 2 to 3 dyn/cm^2 , as estimated from an outer diameter of 4.8 mm and correction for a wall thickness of $300 \mu\text{m}$. After pump perfusion the vessels developed tone, during which τ increased to a more physiological stable value of $\sim 20 \text{ dyn/cm}^2$ (at optical density = 2.7 mm) in the control-perfused vessel. Higher flow rates were not attempted for technical reasons including pressure gradients in the tubing and excessive pulsation frequencies. These would have influenced the otherwise physiologically relevant values for mean arterial pressure and pressure and flow pulsations.

Behavior of Control Vessels

Throughout culture, arteries maintained significant although depressed relaxation responses to BK. This depression can mainly be explained by the constrictive remodeling (Figure 4, top) occurring during perfusion. Thus, at day 8 vessels relaxed from 55.9 to 65.9% of the initial passive diameter on application of BK, whereas full dilation by PAP resulted in a further relaxation to only 67.7% of the initial passive diameter. Interestingly, outer diameters of pressure-perfused arteries appeared to converge at this stage to an absolute value of $\sim 2.7 \text{ mm}$. As indicated above, this outer diameter results in an intraluminal shear stress of $\tau = 20 \text{ dyn/cm}^2$, which approaches the normal arterial shear stress levels found *in vivo*.^{8,15} Based on the proposed relation between shear stress and remodeling,^{7,16,17} the inward remodeling observed in this study could therefore have resulted from the low initial shear stress. Thus, we suggest that the observed behavior reflects the control of shear stress by initial vasoconstriction and later inward remodeling. However, this hypothesis requires further testing.

Thus far, reports of conductance artery remodeling have been described only for *in vivo* models. To the best of our knowledge, the present study is the first to describe conductance artery remodeling *in vitro*. The fast onset of the remodeling response in our perfused nonin-

Table 1. Endothelial Coverage and Number of IEL Ruptures and Dissections

	Coverage [%]		IEL ruptures [no.]		Dissections [no.]	
	Day 4	Day 8	Day 4	Day 8	Day 4	Day 8
Control	97.8 ± 1.6	98.5 ± 1.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
BA	$9.2 \pm 5.9^*$	$9.4 \pm 5.5^*$	2.8 ± 1.2	2.2 ± 1.0	$3.2 \pm 1.0^*$	$2.4 \pm 0.4^*$

* $P < 0.001$ control versus BA. $n = 6$ for control groups (total $n = 12$) and $n = 5$ for BA groups (total $n = 10$).

jured arteries is noteworthy because another study on the time-course of *in vivo* conductance artery remodeling after flow cessation shows a slower onset of remodeling.¹⁸ In that respect, our results are comparable with studies on resistance arteries, which show constrictive remodeling on a shorter time course, both *in vitro*^{19,20} and *in vivo*.^{17,21}

It is interesting to observe that perfused resistance arteries can develop IH *in vitro*, in the absence of immune cells (Figure 6D). A prerequisite for this IH could be the observed accumulation of highly α -SMA-positive cells around the lumen, which has also been described *in vivo*.^{22,23} (Figure 6I). The accumulation of these cells on the outside of the adventitia is probably related to the observed thickening of the tunica adventitia, which has been described for *in vitro* preparations.^{24,25} This outgrowth may be related to surgical damage induction²⁶ or lack of contact inhibition because of removal of (peri)adventitial tissue.

Behavior of BA Arteries

Most studies describing vascular reactivity after arterial injury *in vivo* have used Fogarty balloons to induce injury.^{27–29} These studies are therefore hard to compare with our results. The only *in vivo* study reporting semiacute effects of BA using a percutaneous transluminal angioplasty (PTA) balloon catheter on vascular reactivity demonstrated a lack of tone and reactivity to vasoconstrictors (ergonovine) that remained for 4 weeks.³⁰

The initial loss of basal tone after BA is not surprising taking into account the amount of structural damage induced by the angioplasty procedure. The injury induced by BA was clearly detectable at day 4 (Figure 6, E and F; and Table 1), comparable to injury observed *in vivo* after BA overstretch injury.^{23,31} However, in our *in vitro* model, from day 4 onwards, active diameters started to decrease although decreases became significant only at day 8. Despite recovery of SMC function injury was still detectable on histology at this time (Figure 6, G and H; and Table 1). It thus seems that recovery of contractile function can occur more rapidly than full restoration of the tissue structure.

At day 8, responses to BK also became detectable again. Endothelial coverage was low after BA, and not significantly different between days 4 and 8. Apparently, the few remaining ECs after BA seem to be able to mediate a dilating response to BK. Whether these remaining ECs underwent recovery of function in the first days after BA or were functional all of the time could not be established, because of the lack of SMC contractility in this period. Future studies using this model should therefore incorporate direct measurements of endothelial dilator products. The absence of an increase in endothelial coverage at day 8 indicates that full restoration of endothelial functions is much slower than the redevelopment of tone. In this phase, the vessel seems susceptible for spasm, followed by medial shrinkage, with occurrence of thrombosis being an additional risk in the *in vivo* situation. Strategies to accelerate restoration of the en-

dothelial coverage and function after BA are therefore desired. Whether circulating progenitor cells³² could play a role in such recovery is clearly a subject of substantial interest; the current *in vitro* setup should allow for evaluating their role in future research.

We previously established in studies on resistance vessels that tone is a prerequisite for inward remodeling.^{19,20} Although the current study was not aimed at investigating this relation in conduit vessels, the current data are in accordance with this view. Thus, control non-injured vessels showed an initial tone followed by inward remodeling. In the BA vessels, the absence of remodeling up to day 4 coincided with the lack of tone, while between days 4 and 8 both tone and remodeling started to reoccur. Strikingly, vessels that received BA at a slightly lower DR (Figure 5) displayed the earliest reappearance of tone and at the same time started to remodel.

Clinical Implications and Conclusion

Inward remodeling or shrinkage of vessels after BA is probably a major factor in the recurrence of insufficient perfusion.^{33,34} Preventing such inward remodeling therefore is beneficial. The correlation at day 8 between high DR and inhibition of both redevelopment of tone and inward remodeling (Figure 5) substantiates the bigger-is-better paradigm in interventional cardiology.³⁵ The relation that we found in this study between tone and remodeling points at a crucial role for medial SMC in shrinkage. We suggest establishing interventional procedures for the prolonged inhibition of smooth muscle function, as could be achieved by prolonged, local vasorelaxation.³⁶ Currently, such interventional procedures include radioactive brachytherapy and photodynamic therapy,³⁷ which exert their efficiency by eradication of SMCs. Clearly, such techniques also inhibit any endothelial function that might still exist after BA. It thus becomes critical to evaluate whether indeed ingrowth of new ECs and recovery of their function precedes the repopulation of the media. In this way, regulation of vascular caliber by ECs, among others in response to shear stress, can occur at the moment when functional SMCs become available again. Under this hypothesis, restoration of endothelial function should always precede redevelopment of smooth muscle and fibroblast function.

We aimed to monitor the function and structure of vessels in the first week after BA. This was done because the course of events in this time frame, especially recovery of tone and endothelial function, may well influence IH and inward remodeling in later phases. The actual development of substantial IH was not encountered in our model. This likely relates to the short duration of culture, which may also have been responsible for the observed lack of remodeling after BA.³⁴ Alternatively, limited IH could reflect an incomplete response-to-injury because of the lack of blood-borne components. The current model would allow addressing these questions: the culture period can be extended while inclusion of blood-borne components also seems feasible. Under these

conditions we anticipate IH and remodeling in the damaged vessels to catch up with and even increase beyond the amount of IH and remodeling observed in the controls. In this way the setup could be used to address questions related to the development of IH after BA, as observed in clinical practice.

We conclude that BA inhibits inward remodeling that is observed in noninjured control vessels after *in vitro* perfusion. Furthermore, we anticipate that an optimal result after BA is achieved when this procedure leads to a maximal loss of SMC function in the angioplastied vascular segment.

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