Published in final edited form as:

J Vasc Interv Radiol. 2010 January ; 21(1): 108–115. doi:10.1016/j.jvir.2009.09.024.

Evolution of shear stress, protein expression, and vessel area in an animal model of arterial dilatation in hemodialysis grafts

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Abstract

Purpose—To evaluate the wall shear stress, protein expression of matrix metalloproteinases-2 (MMP-2), -9 (MMP-9), and the inhibitors (tissue inhibitor of matrix metalloproteinases-1 (TIMP-1), and -2 (TIMP-2)), and vessel area over time in a porcine model for hemodialysis polytetrafluoroethylene (PTFE) grafts.

Materials and methods—In 21 pigs, subtotal renal infarction was performed and 28 days later, a PTFE graft was placed to connect the carotid artery to the ipsilateral jugular vein. Phase contrast MR was used to measure blood flow and vessel area at 1, 3, 7, and 14 days after graft placement. Wall shear stress was estimated from Poiseuille's law. Animals were sacrificed at day 3 (N=7), day 7 (N=7), and day 14 (N=7) and expression of MMP-2, MMP-9, TIMP-1, and TIMP-2 were determined at the grafted and control arteries.

Results—The mean wall shear stress of the grafted artery was higher than the control artery at all time points ($P < 0.05$). It peaked by day 3 and decreased by days 7–14 as the vessel area nearly doubled. By days 7–14, there was a significant increase in active MMP-2 followed by a significant increase in pro and active MMP-9 by day 14 ($P < 0.05$, grafted artery versus control). TIMP-1 expression peaked by day 7 and then decreased while TIMP-2 expression was decreased at days 7–14.

Conclusions—The wall shear stress of the grafted artery peaks by day 3 with increased MMP-2 activity by days 7–14 followed by pro and active MMP-9 by day 14 and the vessel area nearly doubled.

Introduction

In 1893, Thoma observed that arterial diameter increases in response to elevated blood flow resulting in reducing wall shear stress to normal levels [1,2]. As expected, this type of vascular enlargement occurs in high flow arteries supplying patent hemodialysis grafts. The clinical relevance of this hemodialysis access failure is quite significant since more than 200,000 patients in the United States have end-stage renal disease requiring hemodialysis with expanded

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polytetrafluoroethylene (PTFE) grafts placed for dialysis access [3]. Aneurysm formation of the artery supplying the hemodialysis vascular access can occur because of the high blood flow. The true incidence of arterial aneurysm formation is not known and is estimated to be less than 1% and several studies have described surgical and endovascular treatment of these aneurysms [4,5]. Treatment of these aneurysms is important as they can rupture or result in embolization of thrombus to the distal vessels resulting in ischemia.

There are several mechanisms that may potentially contribute to early arterial injury which results in initiation of the signaling pathways that may promote aneurysm formation including hemodynamic factors such as high wall shear stress [6,7]. Wall shear stress is the dragging force of the blood on the vessel wall (endothelium) and subsequently it regulates expression of many proteins implicated in aneurysm formation including matrix metalloproteinase-2 and -9 (MMP-2 and MMP-9) [6,8,9]. Recently, there have been several reports describing the use of phase contrast magnetic resonance angiography with magnetic resonance imaging to determine blood flow and luminal vessel area in order to estimate wall shear stress [10–14]. Finally, several studies have examined the role of wall shear stress and matrix metalloproteinase expression in aneurysm formation; however there have been no studies to date examining the role of this in animal models with abnormal kidney function [6–9]

Our overall hypothesis is that arterial dilatation is in part related to changes in wall shear stress that results in increased activation of MMP-2 and MMP-9. The aim of this study was to determine the temporal changes in wall shear stress, blood flow, and luminal vessel area using magnetic resonance imaging with phase contrast magnetic resonance angiography (MRI/PC MRA), and the expression of MMP-2, MMP-9 and tissue inhibitor of matrix metalloproteinases-1 and -2 (TIMP-1 and TIMP-2) at the grafted artery after PTFE graft placement in a porcine model of hemodialysis graft failure. Hemodialysis grafts are used in patients with advanced renal failure and the regulation of the matrix metalloproteinases and their inhibitors may be markedly different in the uremic milieu [15,16]. Therefore, we performed these studies using a porcine model of renal insufficiency [14,17–20].

Materials and methods

Institutional Animal Care and Use Committee approval was obtained prior to performing any procedures. Housing and handling of the animals was performed in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals revised in 2000 [21].

Anesthesia

Prior to all procedures including the MRI imaging studies, the animals were fasted for 12 h. They were initially anesthetized with a combination of 5 mg/kg tiletamine hydrochloride (50 mg/mL, Fort Dodge Animal Health, Fort Dodge, IA) and zolazepam hydrochloride (50 mg/mL, Fort Dodge Animal Health), 2 mg/kg xylazine (Bayer), and 0.06 mg/kg glycopyrrolate (Bayer) given intramuscularly. To induce additional anesthesia, an intravenous fluid line was placed in an ear vein and zolazepam hydrochloride (5 mg/kg) was used. The animals were intubated and placed on a positive-pressure ventilator delivering oxygen (3–5 mL/kg) and isoflurane (1%–3%, Halocarbon Products Corporation, River Edge, New Jersey 07661).

Creation of chronic renal insufficiency by renal artery embolization

Renal insufficiency was induced in 21 castrated juvenile male domestic pigs (40–50 kg, Larson Products, Sargeant, MN) by total embolization of the left kidney and partial embolization of the right kidney using polyvinyl acrylate (PVA) particles as described elsewhere [14,17–20]. The embolization procedure was standardized so that the left kidney was totally embolized and either the upper or lower artery of the right kidney was embolized. Typically, there was a single

renal artery supplying each kidney with one upper and one lower polar branch with each polar branch having 2 to 3 branches. The smaller of the two polar branches was embolized.

Six F sheaths were placed in the right femoral artery and the left renal artery was selected by using a 5F tapered angled glide catheter (Boston Scientific, Natick, MA). Through this catheter, 150 to 250- μ m polyvinyl acrylide (PVA) particles (PVA Contour, Boston Scientific, Boston, MA) were infused until the left renal artery was completely occluded. Next, either the right upper or lower pole artery was selected and embolized completely in a similar fashion. The sheath was removed and hemostasis was obtained by manual compression. Each pig was treated for 5 days with antibiotics to prevent infection. Blood urea nitrogen (BUN) and creatinine were determined prior to embolization and at the time of graft placement by removing 10-mL of blood from a peripheral vein. The blood urea nitrogen (BUN) and creatinine were determined prior to embolization, at the time of graft placement, and at time of sacrifice.

Polytetrafluoroethylene graft placement

Twenty eight days later, polytetrafluoroethylene (PTFE) loop grafts (4-mm diameter by 7-cm long, Gore, Flagstaff, AZ) were placed from either the right or left carotid artery to the ipsilateral jugular vein (Fig. 1) and the contralateral vessels were surgically isolated and not clamped to serve as controls [14,17–20]. The animals were sacrificed at 3 days (N=7), 7 days (N=7), and 14 days (N=7) after graft placement. Plavix (75 mg by mouth, Bristol-Myers Squibb/Sanofi Pharmaceuticals Partnership, Bridgewater, NJ) was started the night before and given daily until the animal was sacrificed.

Cine phase-contrast magnetic resonance imaging and contrast-enhanced magnetic resonance angiography (PC MRI/MRA)

To assess hemodynamic changes and vascular remodeling of the grafted artery and control vessels, MRI imaging study was performed serially in all 21 animals one day after graft placement and prior to sacrifice at days 3 (N=7), 7 (N=7), and 14 (N=7) following graft placement [12,22]. Animals were anesthetized for the procedure as described previously.

All MR examinations were performed using a Signa CVi 1.5 Tesla system (GE Medical Systems, Milwaukee, WI) with a torso-phased array coil positioned over the upper chest. After an initial 3-plane localizing scan, a test bolus of gadopentetate dimeglumine (1–2 mL) was given (Magnevist; Berlex Laboratories, Wayne, NJ), followed by a 20-mL saline flush injected at 3 mL per second. A single slice overlying the thoracic aorta was scanned repeatedly about once per second and the time delay was noted between the injection of the contrast medium and its arrival in the aorta.

Gadolinium-enhanced 3-dimensional (3-D) MRA was performed using the following scan parameters: TR 5 ms, TE 1.7 ms, flip angle 35°, 0.75 excitations, 0.75 phase field of views (FOVs), 62.5-kHz receiver bandwidth, elliptic-centric phase-encoding, 256 $\times$ 192 scan matrix, and 20 cm $\times$ 15 cm FOV, giving an in-plane resolution of 0.78 mm $\times$ 0.78 mm. Thirty to 40 sections (1.2 mm to 1.4 mm) were obtained with 50% overlapping reconstruction in the z-direction. The scan time was 20 seconds to 25 seconds. Contrast medium (30 mL) was injected at 3 mL per second, followed by a 20-mL saline flush at the same rate. An appropriate scan delay derived from the test bolus sequence was chosen to ensure that the acquisition of the central portion of k-space corresponded with peak arterial enhancement.

The 2-dimensional cine phase-contrast MRI sequences were conducted immediately after the 3-D MRA. Acquisitions were positioned perpendicular to the appropriate vessels at locations selected from maximum-intensity projection images and reformatted images from the 3-D MRA as follow: 1) grafted artery 2 cm proximal to the arterial anastomosis and 2) contralateral

control artery. Scan variables were TR 13.2 ms, TE 4.9 ms, flip angle 30°, 1 excitation, receiver bandwidth 15.6 kHz, 256 × 224 matrix, and 14-cm FOV, for an in-plane resolution of 0.55 mm × 0.62 mm. Slice thickness was 5 mm. The velocity-encoding gradient was set to 100 cm per second, unless aliasing was identified on initial acquisitions. Electrocardiogram triggering was provided by a peripheral pulse oximeter. Acquisition times generally were 20 to 30 seconds. Imaging planes (slices) were placed perpendicular to the artery with positioning guided by the MRA images. Using retrospective gating and view sharing, the images were reconstructed to 20 evenly spaced time points in the cardiac cycle. Segmented k-space acquisition produced 8 views per segment. Quantitative flow information was obtained only in the direction perpendicular to the slice. All flow calculations were made on an Advantix Windows workstation (Cardiac and Flow Analysis Tools, AW Release 4.0; GE Medical Systems). The flow measurements were repeated three times within 5-mm of each other at the artery-to-graft anastomosis and the averaged values from these three measurements were used. The flow, velocity, and area measurements were obtained on the grafted vessels and on the contralateral non-grafted vessels at 20 different phases of the cardiac cycle using the flow analysis program. The location of the control vessels was at the same location as the grafted vessels. The following parameters were calculated from the MRI data: wall shear stress and Reynolds number (Re).

Vessel harvesting

The grafted artery and control vessels were harvested and snap frozen in liquid nitrogen and stored at -80 °C for subsequent Western blotting and zymography [14,17–20].

Reynolds number (Re)

Reynolds number allows one to determine if the blood flow is turbulent. A Re of less than 2,000 generally indicates the lack of turbulence [24]. The Re was calculated as $Re = (r \langle v \rangle d) / \mu$, where r is vessel diameter, $\langle v \rangle$ is average velocity of blood, d is density of blood, and μ is viscosity. Viscosity and density of blood were estimated as 0.00345 N s m⁻² and 1000 kg/L respectively. At every point measured within the grafted artery, the Re was less than 2000 and thus, laminar flow was assumed to exist for all wall shear stress calculations [12].

Calculation of wall shear stress

The average wall shear stress throughout the cardiac cycle was determined using Poiseuille's law. For steady laminar flow in a tube, the wall shear stress in Newtons/m² may be calculated directly from the average velocity ($\langle v \rangle$) in meters per second with the formula wall shear stress = $8 \langle v \rangle \mu / r$, where μ is the viscosity (0.00345 N s m⁻²) and r is the tube diameter in meters [12].

Western Blot and SDS-PAGE Zymography

Matrix regulatory protein expression were assessed with Western blotting and zymography on whole tissue lysate using 100 micrograms of protein (tissue) [14,22]. Antibodies and antisera used included: TIMP-1 (R & D Systems, Minneapolis, MN, Clone # 63515, Mouse IgG2B) and TIMP-2 (R & D Systems, Clone # 127711, Mouse IgG1).

Statistical methods

The luminal vessel area was obtained from the phase-contrast MRI with MRA and averaged for each time point for the same group of animals (i.e. D3, D7, and D14) for the grafted and control arteries. Values are expressed as mean ± standard deviation. Analysis of variance (ANOVA) was used first to compare the means across the three groups of animals (i.e. D3, D7, and D14). If the ANOVA F-test P-value was statistically significant ($P < 0.05$) or showed a trend toward significance, pair wise t-tests were performed to compare each group to every other group. Paired t tests were used to compare vessels (grafted artery and control artery)

within animals for each group (D3, D7, or D14 sacrifice). A *P* value of .05 or less was considered statistically significant. SAS version 9, (SAS Institute Inc., Cary, N.C.) was used for statistical analyses.

Results

Surgical outcomes

Twenty-one pigs underwent placement of 21 (4-mm by 7-cm PTFE grafts)- 2 grafts on the left and 19 grafts on the right with the contralateral vessels serving as controls (Fig. 1). There were no complications as a result of the embolization procedure other than the expected induction of renal insufficiency. The BUN and creatinine prior to induction of renal insufficiency was 8.36 ± 2.01 mg/dL and 1.27 ± 0.18 mg/dL, respectively. The BUN and creatinine at time of graft placement was 17.1 ± 9.12 mg/dL ($P < 0.05$ when compared to pre) and 2.17 ± 0.57 mg/dL ($P < 0.05$ when compared to pre), respectively.

Luminal vessel area, blood flow, and wall shear stress measurements by MRI at the grafted and control arteries

MRI was performed to determine the luminal vessel area and blood flow of the vessels over time in the animals. The accuracy of blood flow measurements in 5-mm diameter vessels to be 0.6–4.4% for blood flow rates of 315 and 540 mL/min [23]. The intrareviewer coefficient of variability for blood flow was low between three different radiologists when interpreting the images and therefore only one radiologist interpreted the blood flow images for the present study [23]. The Reynolds number at all time points was less than 2000 and therefore we assumed that the blood flow was not turbulent. At all time points, the wall shear stress of the grafted artery was significantly higher than the control artery (1.2 ± 0.4 N/m², $P < 0.05$). The mean wall shear stress at the grafted artery was 3.5 ± 2.3 N/m² by day 1, increased to 4.9 ± 1.9 N/m² by day 3, subsequently decreased to 2.7 ± 1.2 N/m² by day 7, and was 1.9 ± 0.6 N/m² by day 14 (Fig. 2). The luminal vessel area of the control artery was 27.7 ± 2.6 mm². The luminal vessel area of the grafted artery was 25.7 ± 12.3 mm² by day 1, decreased to 18.9 ± 2.6 mm² by day 3, subsequently increased to 35.9 ± 14.0 mm² by day 7, and was 52.2 ± 9.3 mm² by day 14 ($P < 0.05$, day 14 vs. control artery). Similarly, at all time points, the blood flow through the grafted artery was significantly higher than the control artery (357 ± 19 , $P < 0.05$). The blood flow was 1227 ± 385 mL/min by day 1, decreased to 904 ± 241 by day 3, increased to 1168 ± 461 by day 7, and was 1604 ± 297 by day 14. Overall, these results indicate that the mean wall shear stress is significantly higher in the grafted artery than the control artery at all time points and peaks by day 3 and by day 14 there is a significant increase in luminal vessel area of the grafted artery when compared to the control artery. At all time points, blood flow is significantly higher in the grafted artery when compared to the control artery.

Protein expression of MMP-2, MMP-9, TIMP-1, and TIMP-2 the grafted and control arteries at day 3, day 7, and day 14 after graft placement

Because shear stress is known to regulate expression of MMP-2 and MMP-9, protein expression was determined by zymography and Western blot on both the grafted and control arteries to determine the temporal relationship of MMP-2, MMP-9, TIMP-1, and TIMP-2 at day 3, day 7 and day 14. Scanning densitometry values from the immunoblots or zymography obtained from protein samples of the grafted artery were divided by the control artery for each time point (Figs. 3–7). There was no difference in the expression of pro MMP-2 between the grafted and control arteries. By days 7–14, there was a significant increase in active MMP-2 at the grafted artery when compared to control arteries (Fig. 3). By day 14, there was a significant increase in pro MMP-9 and active MMP-9 at the grafted artery when compared to control arteries ($P < 0.05$, Figs. 4 and 5). By day 7, TIMP-1 was significantly higher ($P < 0.05$) at the grafted artery when compared to the control artery ($P < 0.05$, Fig. 6). By days 7–14,

TIMP-2 was significantly higher in the control artery when compared to the grafted artery ($P < 0.05$, Fig. 7). Overall, these results show that there is early activation of MMP-2 by days 7–14, followed by increased expression of both pro and active MMP-9 at day 14 with significant increase in TIMP-1 by day 7. There was a significant decrease in the expression of TIMP-2 by days 7–14 in the grafted arteries when compared to control arteries.

Discussion

The mechanisms underlying aneurysm formation of the artery supplying the hemodialysis vascular access have not been well defined. It has been hypothesized that hemodynamic factors such as changes in wall shear stress contribute to arterial aneurysms formation [6,7]. Subsequently, these changes are felt to result in increased expression of MMP-2 and MMP-9 which are implicated in aneurysm formation. In the present study, we employed a porcine model of chronic renal insufficiency in which we placed arteriovenous PTFE grafts. At all time points, the mean wall shear stress was significantly higher at the grafted artery when compared to the control artery and it peaked by day 3. This was associated with significantly higher amounts of active MMP-2 by day 7–14, followed by increased expression of both pro and active MMP-9 by day 14 with significant increase in TIMP-1 by day 7. There was a significant decrease in the expression of TIMP-2 by days 7–14 in the grafted arteries when compared to control arteries.

Wall shear stress, which represents the dragging force exerted by blood flow on the endothelium, regulates the expression of many proteins including MMP-2 and MMP-9 [6,8,9,25], raising the possibility that it may be a key factor in initiating arterial aneurysm formation involving hemodialysis vascular access. Consequently, it is important to understand the temporal changes that occur in wall shear stress after placement of an arteriovenous graft, and how these relate to aneurysm formation. Previous studies have estimated wall shear stress by directly measuring blood flow and velocity by using ultrasound [25,26] or MRI [10,12,13]. In these studies, the mean wall shear stress at the arterial anastomosis of arteriovenous fistulas decreased over time while the mean vessel area increased [26,27]. Furthermore, computational fluid dynamic modeling has been performed at the grafted artery of the vein-to-graft anastomosis which has shown similar findings [28–30]. In pigs with 4-mm diameter by 2-cm long (straight) PTFE grafts placed from the iliac artery to iliac vein, the mean wall shear stress of the grafted artery peaked by day 3 (3.6 N/m^2) and decreased by 50% at days 7–14 after graft placement [26,27]. The mean luminal vessel area and blood flow increased over time and remained higher than the control artery. Both of these studies have several limitations including a small number of animals with no serial evaluation of the changes in wall shear stress. In the present study, despite having different anatomy of the grafts, we observed similar findings with significant increased mean wall shear stress by day 3 which decreased by days 7–14 because the mean luminal vessel area of the grafted artery increases and subsequently the wall shear stress decreases.

Wall shear stress regulates the expression of many proteins including MMP-2 and MMP-9 [6,8,9]. We examined the expression of MMPs and their inhibitors at the grafted artery when compared to control artery [31]. The role of MMP-2 and MMP-9 has been described previously in abdominal aortic aneurysm (AAA) formation. In patients with AAA, increased expression of MMP-9 in the serum has been observed [32]. Furthermore, targeted gene deletion of MMP-9 has been shown to decrease AAA formation in experimental animal model [33]. A recent study performed in experimental animal model of aneurysm formation in rats has shown that administering doxycycline, a matrix metalloproteinase inhibitor, can reduce the formation of the aneurysm [34,35]. These experimental studies were all performed in animal models of normal kidney function. In a similar study performed in patients with hemodialysis vascular access, the effect of doxycycline administration on aneurysm formation were investigated

retrospectively and associated with decreased aneurysm formation of the artery supplying hemodialysis vascular access [36]. In the present study, we observed significantly increased expression of active MMP-2 by days 7–14 followed by increased expression of pro and active MMP-9 at the grafted artery when compared to the control artery. This increased expression corresponded with increased luminal vessel area of the grafted artery which nearly doubled when compared to the control artery by day 14. By day 7, TIMP-1 which is an inhibitor of MMP-2 began to increase while TIMP-2 which is an inhibitor of MMP-9 decreased at days 7–14.

One important aspect of our current study is that the experiments were conducted in pigs with renal insufficiency thus modeling the clinical scenario in a more relevant fashion. Previous experimental studies that have examined mechanisms of arteriovenous graft stenosis have predominantly been performed in animals with normal kidney function which does not simulate the advanced renal failure which may markedly alter the regulation of the targeted proteins [22,31,37,38]. For instance, renal insufficiency is associated with increased oxidative stress, which has been associated with increased levels of MMPs; raising the possibility that wall shear stress may increase MMP levels to even a greater degree in uremic patients [15,16]. In the present study, we created renal insufficiency with subtotal renal infarction causing chronic kidney disease prior to the placement of the grafts [14]. In these animals, there was significantly increased BUN and creatinine prior to graft placement when compared to baseline values, and the characteristics of their arteriovenous grafts are consistent with that observed in clinical specimens.

There are several limitations to the present study which need to be discussed. First, we assumed that the arteries were circular when we were calculating the diameter from the area. Second, we assumed that blood behaved as a Newtonian fluid with no slip, boundary conditions when calculating the wall shear stress. The duration of the renal insufficiency was 6-weeks. Finally, venous aneurysm formation is more common than arterial aneurysm in patients with hemodialysis grafts.

In conclusion, in the present study, we used MRI with MRA to estimate wall shear stress and the subsequent vascular remodeling changes in arteries supplying hemodialysis grafts. The findings from the present study add to the current knowledge of the hemodynamic parameters and vascular remodeling changes which occur in early arterial dilatation in hemodialysis grafts. After placement of PTFE graft, there is a significantly higher wall shear stress at the grafted artery. We hypothesize that the early increases (day 3) in wall shear stress results in increased expression of active MMP-2 followed by increased expression of pro and active MMP-9. Understanding the role of these proteins in arterial dilatation in hemodialysis grafts can help improve outcomes in patients.

References

1. Kamiya A, Togawa T. Adaptive regulation of wall shear stress to flow change in the canine carotid artery. *Am J Physiol* 1980;239:H14–H21. [PubMed: 7396013]
2. Langille BL, Bendeck MP, Keeley FW. Adaptations of carotid arteries of young and mature rabbits to reduced carotid blood flow. *Am J Physiol* 1989;256:H931–H939. [PubMed: 2705563]
3. Collins AJ, Kasiske B, Herzog C, et al. Excerpts from the United States Renal Data System 2003 annual data report: atlas of end-stage renal disease in the United States. *Am J Kidney Dis* 2003;42:A5–A7. [PubMed: 14655179]
4. Murphy GJ, White SA, Nicholson ML. Vascular access for haemodialysis. *Br J Surg* 2000;87:1300–1315. [PubMed: 11044154]
5. Karabay O, Yetkin U, Silistreli E, Uskent H, Onol H, Acikel U. Surgical management of giant aneurysms complicating arteriovenous fistulae. *J Int Med Res* 2004;32:214–217. [PubMed: 15080026]

6. Bassiouny HS, Song RH, Hong XF, Singh A, Kocharyan H, Glagov S. Flow regulation of 72-kD collagenase IV (MMP-2) after experimental arterial injury. *Circulation* 1998;98:157–163. [PubMed: 9679722]
7. Nakahashi TK, Hoshina K, Tsao PS, et al. Flow loading induces macrophage antioxidative gene expression in experimental aneurysms. *Arterioscler Thromb Vasc Biol* 2002;22:2017–2022. [PubMed: 12482828]
8. Shay-Salit A, Shushy M, Wolfowitz E, et al. VEGF receptor 2 and the adherens junction as a mechanical transducer in vascular endothelial cells. *PNAS* 2002;99:9462–9467. [PubMed: 12080144]
9. Wang Y, Chang J, Li Y-C, Li Y-S, Shyy JY-J, Chien S. Shear stress and VEGF activate IKK via the Flk-1/Cbl/Akt signaling pathway. *Am J Physiol Heart Circ Physiol* 2004;286:H685–H692. [PubMed: 14551058]
10. Box FMA, van der Grond J, de Craen AJM, et al. Pravastatin decreases wall shear stress and blood velocity in the internal carotid artery without affecting flow volume: results from the PROSPER MRI study. *Stroke* 2007;38:1374–1376. [PubMed: 17332443]
11. Marshall I, Zhao S, Papathanasopoulou P, Hoskins P, Xu XY. MRI and CFD studies of pulsatile flow in healthy and stenosed carotid bifurcation models. *Journal of Biomechanics* 2004;37:679–687. [PubMed: 15046997]
12. Misra S, Woodrum D, Homburger J, et al. Assessment of wall shear stress changes in arteries and veins of arteriovenous polytetrafluoroethylene grafts using magnetic resonance imaging. *Cardiovasc Intervent Radiol* 2006;29:624–629. [PubMed: 16729233]
13. Oshinski J, Ku D, Mukundan SJ, Loth F, Pettigrew R. Determination of wall shear stress in the aorta with the use of MR phase velocity mapping. *J Magn Reson Imaging* 1995;5:640–647. [PubMed: 8748480]
14. Misra S, Fu AA, Puggioni A, et al. Increased shear stress with up regulation of VEGF-A and its receptors and MMP-2, MMP-9, and TIMP-1 in venous stenosis of hemodialysis grafts. *Am J Physiol Heart Circ Physiol* 2008;294:H2219–H2230. [PubMed: 18326810]
15. Pawlak K, Pawlak D, Mysliwiec M. Circulating beta-chemokines and matrix metalloproteinase-9/tissue inhibitor of metalloproteinase-1 system in hemodialyzed patients--role of oxidative stress. *Cytokine* 2005;31:18–24. [PubMed: 15896974]
16. Pawlak K, Pawlak D, Mysliwiec M. Serum matrix metalloproteinase-2 and increased oxidative stress are associated with carotid atherosclerosis in hemodialyzed patients. *Atherosclerosis* 2007;190:199–204. [PubMed: 16510149]
17. Misra S, Fu AA, Anderson J, et al. Fetuin-A expression in early venous stenosis formation in a porcine model of hemodialysis graft failure. *J Vasc Interv Radiol* 2008;19:1477–1482.
18. Misra S, Fu AA, Puggioni A, et al. Proteomic profiling in early venous stenosis formation in a porcine model of hemodialysis graft. *J Vasc Interv Radiol* 2009;20:241–251. [PubMed: 19028119]
19. Misra S, Gordon JD, Fu AA, et al. The porcine remnant kidney model of chronic renal insufficiency. *J Surg Res* 2006;135:370–379. [PubMed: 16815448]
20. Hughes D, Fu AA, Puggioni A, et al. Adventitial transplantation of blood outgrowth endothelial cells in porcine haemodialysis grafts alleviates hypoxia and decreases neointimal proliferation through a matrix metalloproteinase-9-mediated pathway--a pilot study. *Nephrol Dial Transplant* 2009;24:85–96. [PubMed: 18786975]
21. Washington, DC: Government print office; 1996. Committee on care and use of laboratory animals of the institute of laboratory animal resources.
22. Misra S, Doherty MG, Woodrum D, et al. Adventitial remodeling with increased matrix metalloproteinase-2 activity in a porcine arteriovenous polytetrafluoroethylene grafts. *Kidney Int* 2005;68:2890–2900. [PubMed: 16316367]
23. King BF, Torres VE, Brummer ME, et al. Magnetic resonance measurements of renal blood flow as a marker of disease severity in autosomal-dominant polycystic kidney disease1. 2003;64:2214–2221.
24. Yellin E. Laminar-turbulent transition process in pulsatile flow. *Circ Res* 1966;19:791–804. [PubMed: 5917851]
25. Wentzel JJ, Kloet J, Andhyiswara I, et al. Shear-stress and wall-stress regulation of vascular remodeling after balloon angioplasty : effect of matrix metalloproteinase inhibition. *Circulation* 2001;104:91–96. [PubMed: 11435344]

26. Fillinger M, Reinitz E, Schwartz R, et al. Graft geometry and venous intimal-medial hyperplasia in arteriovenous loop grafts. *J Vasc Surg* 1990;11:556–566. [PubMed: 2182916]
27. Haruguchi H, Teraoka S. Intimal hyperplasia and hemodynamic factors in arterial bypass and arteriovenous grafts: a review. *Journal of Artificial Organs* 2003;6:227–335. [PubMed: 14691664]
28. Keynton R, Evancho M, Sims R, Rodway N, Gobin A, Rittgers S. Intimal hyperplasia and wall shear in arterial bypass graft distal anastomoses: an in vivo model study. *J Biomech Eng* 2001;123:464–473. [PubMed: 11601732]
29. Krueger U, Zanow J, Scholz H. Computational fluid dynamics and vascular access. *Artificial Organs* 2002;26:571–575. [PubMed: 12081514]
30. Van Tricht I, De Wachter D, Tordoir J, Verdonck P. Comparison of the hemodynamics in 6 mm and 4–7 mm hemodialysis grafts by means of CFD. *Journal of Biomechanics* 2006;39:226–236. [PubMed: 16321624]
31. Chan C-Y, Chen Y-S, Ma M-C, Chen C-F. Remodeling of experimental arteriovenous fistula with increased matrix metalloproteinase expression in rats. *Journal of Vascular Surgery* 2007;45:804–811. [PubMed: 17398390]
32. McMillan WD, Pearce WH. Increased plasma levels of metalloproteinase-9 are associated with abdominal aortic aneurysms. *J Vasc Surg* 1999;29:122–127. discussion 127–129. [PubMed: 9882796]
33. Pyo R, Lee JK, Shipley JM, et al. Targeted gene disruption of matrix metalloproteinase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms. *The Journal of clinical investigation* 2000;105:1641–1649. [PubMed: 10841523]
34. Kaito K, Urayama H, Watanabe G. Doxycycline treatment in a model of early abdominal aortic aneurysm. *Surg Today* 2003;33:426–433. [PubMed: 12768368]
35. Abbruzzese TA, Guzman RJ, Martin RL, Yee C, Zarins CK, Dalman RL. Matrix metalloproteinase inhibition limits arterial enlargements in a rodent arteriovenous fistula model. *Surgery* 1998;124:328–334. discussion 34–35. [PubMed: 9706156]
36. Diskin C, Stokes TJ, Dansby LM, Radcliff L, Carter TB. Doxycycline may reduce the incidence of aneurysms in haemodialysis vascular accesses. *Nephrol Dial Transplant* 2005;20:959–961. [PubMed: 15741211]
37. Johnson MS, McLennan G, Lalka SG, Whitfield RM, Dreesen RG. The porcine hemodialysis access model. *J Vasc Interv Radiol* 2001;12:969–977. [PubMed: 11487678]
38. Kelly B, Melhem M, Zhang J, et al. Perivascular paclitaxel wraps block arteriovenous graft stenosis in a pig model. *Nephrol Dial Transplant* 2006;21:2425–2431. [PubMed: 16822794]

Fig. 1.

Upper panel is magnetic resonance imaging with phase contrast magnetic resonance angiography (MRI with PC MRA) performed at day 14. Left lower panel is the phase contrast MRA showing the direction of the blood vessels in opposite direction as depicted by black in the grafted artery and white in the control artery. Right lower panel is the magnitude of the blood flow in the same vessels.

Fig. 2.

MRI measurements of the artery-to-graft anastomosis at day 1, day 3, day 7, and day 14. The wall shear stress calculated from the MRI data. At all time points, the mean wall shear stress of the grafted artery was significantly higher ($P < 0.05$) than the control artery. By day 3, the wall shear stress is significantly higher than days 7 and 14 ($P < 0.05$). The mean wall shear stress of the control artery was $1.2 \pm 0.4 \text{ N/m}^2$. Data are mean $\pm$ SD.

Fig. 3.

Upper graph representing the appropriate protein band of pro MMP-2 by zymography. GA is the grafted artery and CA is the control artery. Lower graph is semiquantitative analysis performed at day 3, 7, and 14. The normalized density of pro MMP-2 at the grafted artery was significantly higher than the control artery by day 14. (*) is a significantly higher value ($P<0.05$). Data are mean $\pm$ SD.

Fig. 4.

Upper graph representing the appropriate protein band of MMP-9 by zymography. **GA** is the grafted artery and **CA** is the control artery. Lower graph is semiquantitative analysis performed at day 3, 7, and 14. The normalized density of MMP-9 at the grafted artery was significantly higher than the control artery by day 14. (*) is a significantly higher value ($P < 0.05$). Data are mean $\pm$ SD.

Fig. 5.

Upper graph representing the appropriate protein band of MMP-9 by zymography. **GA** is the grafted artery and **CA** is the control artery. Lower graph is semiquantitative analysis performed at day 3, 7, and 14. The normalized density of MMP-9 at the grafted artery was significantly higher than the control artery by day 14. (*) is a significantly higher value ($P < 0.05$). Data are mean $\pm$ SD.

Fig. 6.

Upper graph representing the appropriate protein band of TIMP-1 by Western blot. **GA** is the grafted artery and **CA** is the control artery. Graph representing appropriate band for IgG from Western blot for protein loading. Lower graph is semiquantitative analysis performed at day 3, 7, and 14. The normalized density of TIMP-1 at the grafted artery was significantly higher than the control artery by day 7. (*) is a significantly higher value ($P < 0.05$). Data are mean $\pm$ SD.

Fig. 7.

Upper graph representing the appropriate protein band of TIMP-2 by Western blot. **GA** is the grafted artery and **CA** is the control artery. Graph representing appropriate band for IgG from Western blot for protein loading. Lower graph is semiquantitative analysis performed at day 3, 7, and 14. The normalized density of TIMP-2 was significantly lower (*) at days 7–14 when compared to control artery. (*) is a significantly lower value ($P < 0.05$). Data are mean $\pm$ SD.