THE ROLE OF LOCAL HEMODYNAMICS UPON SHORT-TERM GRAFT HEALING OF POROUS EPTFE IMPLANTED WITHIN A BABOON

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INTRODUCTION

Local hemodynamics and graft ultrastructure impact the healing of implanted expanded polytetrafluoroethylene (ePTFE) vascular grafts. Patent arterial grafts need to exhibit two properties: a flow surface with reduced thrombogenicity and reduced intimal thickening. For well over thirty years, researchers focused their attention upon the healing properties of graft material. Beginning with Wesolowski's 1961 work, researchers concluded that porous synthetic grafts demonstrated improved healing properties (1). Most recently, Clowes documented that ePTFE with a nominal porosity of 60 μm healed at one month with complete endothelial coverage, uniformly distributed pseudointimal growth, and improved intimal stability (2). These results contrasted to what was found when porosity was deceased to an internodal distance of 10 or 30 μm.

Hemodynamic parameters significantly impact graft healing. For native vessels, researchers established that high flow environments inhibit intimal thickening (3-5). For ePTFE grafts, Clowes documented that a high shear environment (24±8 dynes/cm^2) decreased intimal thickening by about 50% and notably decreased smooth muscle cell proliferation and volume (6). Within ePTFE grafts, a high shear environment can reduce already formed intimal area.

While very illustrative, these studies failed to completely answer how the healing properties of ePTFE change with alterations in local hemodyanmics. These studies documented the histologic results of imposing a high shear stress environment under chronic conditions. Grafts frequently fail under either acute or sub-acute conditions; therefore, we wished to investigate how changes in local hemodynamics affect intimal deposition and graft healing under short-term conditions of one month.

METHODS

In this study we utilized two different, porous ePTFE grafts constructed of non-reinforced ePTFE with a 60 μm internodal distance (Impra, Inc., Tempe, AZ). The control graft consisted of a 6-mm internal diameter and a length of 4 cm. The stenotic graft consisted of melding three different segments as detailed by Figure 1. The proximal and distal segments contained a 6-mm internal diameter and a length of 1.25 cm. The middle segment included a 3-mm internal diameter and a length of 1.5 cm.

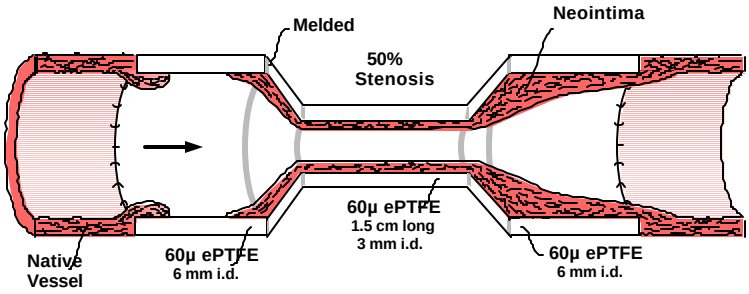

Figure 1

We implanted 15 ePTFE grafts using an end-to-end anastomosis with 6.0 polypropylene sutures within the distal abdominal aortic tree of juvenile male baboons (*papio cyncephalus)* weighing between 17-20 kg. We induced anesthesia with the aid of intramuscular ketamine hydrochloride (10 mg/kg) and continued its effect by inhaled halothane. Both at implantation and at sacrifice, we measured volumetric flow rate using a perivascular flow probe. Two weeks following implantation, we imaged the vascular grafts using a 1.5 T Phillips® MRI. With the use of cardiac gating we measured flow velocity at three separate locations within the graft: upstream, middle throat, downstream. With the aid of gadolinium contrast, we obtained boundary condition information such as coronal views of the entire graft (Figure 2) and axial views using a 2 mm slice thickness along the graft's length (Figure 3). With the aid of Matlab® programming and these axial images we outlined the graft contours. Using the computational fluid mechanics programs, FIDAP® and FLUENT®, we constructed geometric grids and calculated wall shear stress.

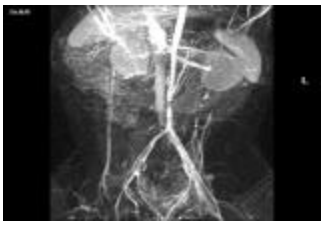

Figure 2

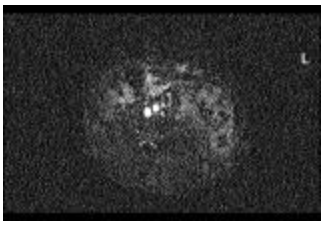

Figure 3

After 1 month we sacrificed the animals by exsanguination. All animals received bromodeoxyuridine (BRDU, 30 mg/kg) at 24, 19, and 12 hours prior to sacrifice. Before excavating the vessels, we administered 5000 units of heparin and 50 mg/kg of evan's blue dye into the arterial circulation. We flushed the vessels in vivo using normal saline at body temperature and a pH of 7.4 followed by pressure perfusion with 10% buffered formalin. Figure 4 describes this assembly. Next, we immersion fixed the grafts in 10 % buffered formalin for at least 24 hours. When we wished to perform scanning electron microscopy upon the harvested graft sections, we changed the fixative solution to 2.5% glutaraldehyde in 0.1 mol/L phosphate buffered saline (PBS). Next, we divided the grafts into 5 mm circular cross-sections, embedded the tissue in paraffin, and microtomed the blocks into 5 μm sections. For one of the respective control and stenotic models, we removed a longitudinal section 5 mm in width to perform scanning electron microscopy (SEM) using a Topcon-ISI DS-130 operated with a LaB6 electron source at 9 kV. We segmented all longitudinal sections into divisions 1 cm in length. All other graft sections underwent staining with Hematoxylin and Eosin (H&E). We performed immunohistochemistry by exposing the slides to antibodies directed against BRDU, smooth muscle α-actin, the endothelial form of c-NOS, and vWF.

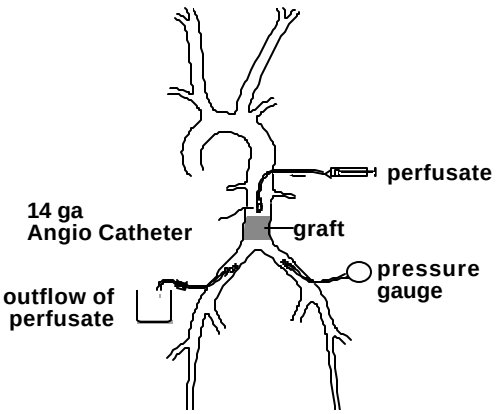

Figure 4

We performed morphometric analysis of all graft sections. We analyzed all slides using a microscope with a 1x objective that connected to a digital camera that captured all images into a TIF format. Using the Image-Pro® Plus Image Analysis software, we measured the average intimal thickness (mm) and the intimal area (mm^2) of all captured images. We also measured the empty volume of graft unoccupied by ePTFE material or the so-called void fraction.

We performed SEM upon two specimens of each graft. Using the Image-Pro® Plus software, we measured the internodal distance, void fraction, and graft thickness of the examined graft material.

RESULTS AND DISCUSSIONS

Analysis of SEM results demonstrated no difference in internodal distance, graft thickness, and void fraction between any of the four detailed graft sections (p-value >> 0.05). The Evan's blue dye and the immunostaining with the monoclonal antibody to vWF demonstrated complete healing along the grafts' lumenal surface.

We quantified the average intimal thickness of the six harvested control grafts. At the proximal and distal ends, transanastomotic ingrowth from neighboring native tissue resulted in pannus deposition. As we moved away from the anastomotic ends, the average intimal thickness decreased, reaching a nadir at the 1.0 cm axial length mark.

We detailed the average intimal thickness of the six harvested stenotic grafts. Once again, at the proximal and distal ends, we saw pannus formation resulting from transanastomotic ingrowth. As we moved away from the proximal and distal ends, the intimal thickness decreased until we reached the stenotic throat or the midpoint of the 4 cm graft. At the stenotic throat the intimal thickness exceeded the values seen either just proximal or distal. Furthermore, at the mid point the average intimal deposition for the stenotic graft was significantly larger than the corresponding location within the control grafts (p-value < 0.05). Using the MRI contour information we constructed geometric grids of the implanted vascular grafts (Figure 5 and Figure 6). At the stenotic throat shear stress values increased by 8 fold, furnishing a strong hemodynamic influence to inhibit an increase in intimal formation. Since the material remain unaltered at the stenotic throat, transmural ingrowth occurred in lieu of the high shear environment. In conclusion these results outlined that changes in local hemodynamics do not exert as pervasive an effect upon early graft healing.

Figure 5

Figure 6

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