Targeted imaging of matrix metalloproteinase activity in the evaluation of remodeling tissue-engineered vascular grafts implanted in a growing lamb model

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Objectives: The clinical translation of tissue-engineered vascular grafts has been demonstrated in children. The remodeling of biodegradable, cell-seeded scaffolds to functional neovessels has been partially attributed to matrix metalloproteinases. Noninvasive assessment of matrix metalloproteinase activity can indicate graft remodeling and elucidate the progression of neovessel formation. Therefore, matrix metalloproteinase activity was evaluated in grafts implanted in lambs using in vivo and ex vivo hybrid imaging. Graft growth and remodeling was quantified using in vivo x-ray computed tomography angiography.

Methods: Cell-seeded and unseeded scaffolds were implanted in 5 lambs as inferior vena cava interposition grafts. At 2 and 6 months after implantation, in vivo angiography was used to assess graft morphology. In vivo and ex vivo single photon emission tomography/computed tomography imaging was performed with a radiolabeled compound targeting matrix metalloproteinase activity at 6 months. The neotissue was examined at 6 months using qualitative histologic and immunohistochemical staining and quantitative biochemical analysis.

Results: The seeded grafts demonstrated significant luminal and longitudinal growth from 2 to 6 months. In vivo imaging revealed subjectively greater matrix metalloproteinase activity in grafts versus native tissue. Ex vivo imaging confirmed a quantitative increase in matrix metalloproteinase activity and demonstrated greater activity in unseeded versus seeded grafts. The glycosaminoglycan content was increased in seeded grafts versus unseeded grafts, without significant differences in collagen content.

Conclusions: Matrix metalloproteinase activity remained elevated in tissue-engineered grafts 6 months after implantation and could indicate remodeling. Optimization of in vivo imaging to noninvasively evaluate matrix metalloproteinase activity could assist in the serial assessment of vascular graft remodeling. (J Thorac Cardiovasc Surg 2014;148:2227-33)

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Our research team developed the first tissue-engineered vascular graft (TEVG) to be used in humans[1] and applied this technology in a clinical trial for congenital heart surgery.[2] We are currently conducting the first Food and Drug Administration–approved clinical trial examining the safety and efficacy of TEVG implantation in children within the United States.[3] The TEVGs were constructed with autologous bone marrow mononuclear cells (BM-MNCs) seeded onto a biodegradable scaffold[4] and demonstrated growth potential in vivo,[5] making the grafts ideally suited for application in infants and children.

The transition of a cell-seeded scaffold to a neovessel is a process characterized by scaffold degradation as a result of hydrolysis, cellular infiltration, and extracellular matrix (ECM) deposition and remodeling.[6] Matrix metalloproteinases (MMPs) are enzymes thought to play important roles in tissue homeostasis and functional growth and might contribute significantly to ECM remodeling during neovessel formation.[6,7] Specifically, MMP-2 and -9 are basement membrane-degrading MMPs that can play critical roles in ECM remodeling. Previous postmortem examinations of TEVGs implanted in a murine model have demonstrated progressively greater MMP-2 expression during a 4-week course after TEVG implantation,

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Abbreviations and Acronyms

BM-MNC = bone marrow mononuclear cell
CT = computed tomography
ECM = extracellular matrix
IVC = inferior vena cava
MMP = matrix metalloproteinase
SPECT = single photon emission CT
^{99m}Tc = technetium-99m
TEVG = tissue engineered vascular graft

and MMP-9 expression peaked at 1 week after surgery and had significantly decreased by 4 weeks.[6] The elevated MMP-2 expression within a murine model of TEVG is consistent with the postmortem findings of Cummings and colleagues,[7] who observed elevated MMP-2 expression in a lamb model of TEVG implantation; however, MMP-9 expression also remained elevated for 80 weeks after implantation. These results suggest that MMPs could play a role in the remodeling of TEVGs and that this process could be prolonged for many months after implantation.

The current clinical imaging techniques for the serial assessment of TEVG remodeling have focused on evaluation of the morphologic changes using x-ray computed tomography (CT) and magnetic resonance angiography[2,5]; however, these approaches cannot provide information related to the underlying mechanisms responsible for the ongoing neovessel formation. Targeted imaging of MMP activity has been previously demonstrated in animal models of atherosclerosis,[8-12] vascular remodeling,[13,14] and myocardial infarction[15,16] using single photon emission CT (SPECT) and magnetic resonance approaches. Additionally, near-infrared fluorescence imaging has indicated progressive remodeling in a murine model of TEVG implantation that was associated with qualitative elevations in MMP-2 and -9 activity.[17] The technetium-99m (^{99m}Tc)-labeled tracer, ^{99m}Tc-RP805, is a broad-spectrum MMP-targeted compound used for SPECT/CT imaging that can localize to the site of ECM remodeling and provide an opportunity to serially assess the progression of TEVG remodeling and the formation of functional neotissue. Therefore, we hypothesized that ^{99m}Tc-RP805 SPECT/CT imaging of MMP activity could complement standard noninvasive imaging approaches and might serve as a useful tool for serial assessment of neovessel formation. To test this hypothesis, we evaluated the feasibility of in vivo and ex vivo SPECT/CT imaging of MMP activity within TEVGs in a clinically relevant large animal model 6 months after TEVG implantation. We also evaluated the serial changes in TEVG morphology using CT angiography.

METHODS

Graft Scaffold

Scaffolds were constructed using a polyglycolic acid nonwoven sheet coated with a 50:50 copolymer solution of poly (L-lactic acid-co-ε-caprolactone; Gunze Corp, Tokyo, Japan), as previously described.[2] Before seeding, all grafts had a measured luminal diameter of 12 mm, wall thickness of 1.5 mm, and length of 20 mm (Figure 1, *A*). Graft porosity was examined using scanning electron microscopy (model XL-30; FEI Co, Hillsboro, Ore).

Bone Marrow-Derived Cell Isolation

Autologous BM-MNCs were isolated from the iliac crest or femoral head of 3 juvenile Dover lambs into a heparinized syringe (100 U/mL), diluted 1:4 in phosphate-buffered saline, and passed through a 100-μm filter to remove the fat and bone fragments. The bone marrow–phosphate-buffered saline solution was added to Histopaque-1077 (Sigma-Aldrich, St Louis, Mo) for density centrifugation at 1500 rpm for 30 minutes. The BM-MNCs were isolated and subsequently washed with phosphate-buffered saline and centrifuged (1500 rpm for 10 min) 2 more times. This cell isolation procedure was performed for each of the 3 lambs that were implanted with the cell-seeded scaffolds.

Scaffold Seeding

The scaffolds were added to a sterile vacuum seeding setup, as previously described.[18] Pressure of 50 mm Hg was applied to the system, thereby vacuum seeding the BM-MNCs onto the scaffolds. After seeding, the scaffolds were placed in autologous serum and incubated for 24 hours (37°C, 5% carbon dioxide, 95% relative humidity, 760 mm Hg). A sample of each graft was stained with Lee's methylene blue on glycol methacrylate-fixed tissue to quantify the number of attached cells.

Graft Implantation

TEVGs were implanted as inferior vena cava (IVC) interposition grafts (3 seeded scaffolds; 2 unseeded scaffolds) in 5 juvenile Dover lambs (implantation weight, 22.4 ± 2.3 kg). The lambs were sedated with intramuscular acepromazine (0.05 mg/kg), followed by intravenous diazepam (0.2 mg/kg) and ketamine (2.75 mg/kg). Anesthesia was maintained throughout surgery with 1% to 5% isoflurane and intravenous propofol (25 μg/kg/min). Perioperative cefazolin (22 mg/kg) was administered. A right thoracotomy was performed through the seventh intercostal space. After isolation of the IVC and dissection of the phrenic nerve, heparin (100 IU/kg) was administered intravenously. The IVC was then clamped for 5 minutes and unclamped for 2 minutes; this process was repeated 3 times for adequate conditioning. Next, a 2-cm TEVG was anastomosed (proximally and then distally) using running monofilament 5-0 suture. Radiopaque markers were placed at the anastomoses (Figure 1, *E*). Fibrin sealant (Tisseel; Baxter International, Deerfield, Ill) was used for hemostasis. A bupivacaine (Marcaine; Hospira, Lake Forest, Ill) nerve block was given for intercostal nerves 5 to 9. Layered closure was performed, and the chest tube was removed in the operating room. Postoperatively, the lambs were treated with fentanyl patches and flunixin meglumine (Banamine; Merck Animal Health, Whitehouse Station, NJ) for analgesia. No postoperative antiplatelets or anticoagulants were given. The Institutional Animal Care and Use Committee at Yale University approved the use of the lambs and all procedures. All the lambs received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health, Animal Welfare Act, and Animal Welfare Regulations.

CT Angiography Imaging and Analysis

In vivo, 64-slice, x-ray CT angiography with iodinated contrast (350 mgI/mL; Omnipaque; GE Healthcare, Little Chalfont, UK) was performed