An Alternative Conduit: Remodeling the Saphenous Allograft

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Purpose

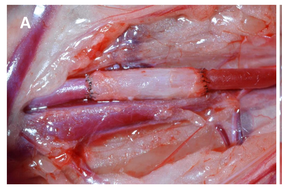
The saphenous vein graft (SVG) has been the conduit of choice for over 50 years, but its lack of availability makes its superior utility unreliable. Because of the paucity of autologous vein grafts and poor results of alternative bypass conduits, we hypothesized vein allografts could be used for patients with limb-threatening ischemia who do not have adequate autologous vein conduit for restorative bypass surgery. Despite substantial improvements in outcomes in the past decade, graft patency and conduit availability remain the 'Achilles' heel' of this procedure. The process we have developed to pretreat the saphenous veins prior to using them for graft procedures provides superior results when compared to allografts and synthetic (PTFE/Dacron) conduits. In this continuation of our previous research, we have analyzed the process of rebuilding an artery on the implanted, decellularized scaffold. We hypothesized that the remodeled vessel would be essentially identical to a natural artery.

Materials and Methods

We have developed an IRB/IACUC-approved model in which human saphenous vein segments are grafted into rats via infra-renal inter-positional aorta grafts, after subjecting the veins to various treatments intended to extend patency. This reverse xenograft model accelerates the rate of failure due to the extreme difference in species biomarkers. We have performed SVGs on over 50 Sprague-Dawley rats, using either no pretreatment, or one of several pretreatment cocktails including various fixatives, decellularizing agents, nucleases, proteases and alcohols, at varying concentrations for periods of time from 1 week to 9 months.

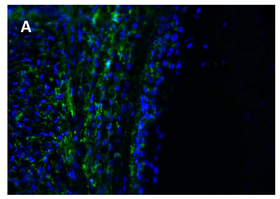
After recovery of implanted SVGs, immunofluorescence (IF) protocols were developed in our lab to assess the cellular and structural makeup of the remodeled SVGs over time. Anti-rat α-Actin, Anti-rat Von Willebrand Factor (VWF), Anti-rat Desmin, and Anti-rat CD68 antibodies were used to determine the presence and relative quantity of their respective antigens. Secondary antibodies conjugated with Alexa Fluor enabled fluorescence analysis of the respective targets. α- Actin is a biomarker for contractile vascular smooth muscle cells (SMC) (currently in progress), VWF fluorescence indicates endothelial cells, Desmin presence indicates myofibroblast-like synthetic SMC, and CD68 presence is indicative of macrophages, monocytes, and macrophage-like synthetic SMC. All images are 20X magnification.

Data



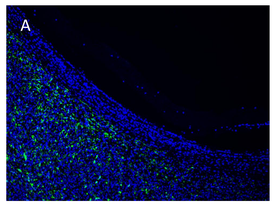
Surgical Field. .
A. Immediately after SV implantation.

B. Three months after SV implantation



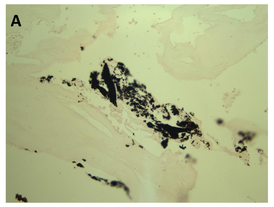
A. Untreated SVG after 2 weeks anti-CD68 Ab (green). Blue stain shows all cell nuclei. Immune cell infiltration is apparent. Lumen is indicated by an arrow on all slides.

B. Treated SVG after 9 months. Anti-CD68 AB. Absence of green stain indicates no immune cell infiltration



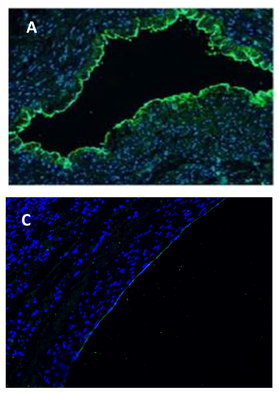
A. Untreated SVG after 2 weeks anti-Desmin Ab (green). Presence of Desmin indicates development of non-arterial character.

B. Treated SVG after 6 months. Anti-Desmin Ab. Absence of Desmin expression indicates the graft is behaving as an artery.



A. Bone tissue stained for the presence of calcium with von Kossa stain. Calcium stains black

B. Treated SVG after 9 months. Stained with von Kossa stain. Calcification is minimal. Indicates a healthy vessel.



A. Normal, untreated, unimplanted SV, stained for von Willebrand factor (VWF), indicative of endothelial cells. This image shows the normal ring of endothelium lining the lumen.

B. Untreated, implanted SVG after 3 months., stained for VWF. This image shows an abnormal distribution of VWF, indicating significant disruption of the vascular architecture.

C. Treated, implanted SVG after 6 months, stained for VWF. This image shows faint staining of VWF, but only surrounding the lumen, where it should be.

D. Treated, implanted SVG after 9 months, stained for VWF. Staining is still faint, but still in the appropriate location for endothelium.

Results

Microscopic analysis revealed that the treated SVGs were rich in α-actin and VWF; and expressed little to no CD68. This was evidence that the graft had not aroused an immune response, even after nine months in the host. Conversely, untreated SVGs expressed little α-actin and WVF, and expressed much CD68, suggesting a powerful response with immune-cell infiltration and an ongoing process of rejection of the graft, with little remodeling in the untreated vessels. Both treated and untreated SVGs expressed moderate amounts of desmin for periods of up to 4 weeks after implantation. Beginning at three months, desmin expression in treated SVGs decreased significantly, indicating that the vessel was developing arterial functionality.

The lack of α- actin expression in the untreated SVGs reveals a lack of contractile vascular smooth muscle cells. The presence of desmin indicates fibroblast-like synthetic (proliferative) SMC, which do not contribute to arterial functionality. The presence of CD68 indicates the cells of the rat innate immune system (monocytes, macrophages, and macrophage-synthetic SMC) have infiltrated the graft, reacting to reject the untreated SVGs, rendering them non-patent and thrombotic.

Conclusion

We have successfully tested the hypothesis that human saphenous vein segments can be subjected to pre-treatment such that when implanted into rats, they will retain greater patency and have improved histological and physiological performance. Immunofluorescence analysis of the remodeling process, as the decellularized implanted scaffold becomes repopulated with cells, suggests that the architecture of the final product greatly resembles a natural artery. This is not the case for vessels that were not pre-treated, which are rapidly destroyed by immunological attack. This research has significant implications for graft patency and retention in clinical applications.

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