Studies on the Human Umbilical Artery in a Rat Xenograft Model

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Purpose

The saphenous vein graft (SVG) has served as primary bypass conduit in cardiac surgery as well as lower extremity revascularization for over 50 years. However, the scarcity of viable autologous SVG remains a limiting factor. We propose that human umbilical arteries (HUA) could address this limitation for patients with ischemia, particularly since the size of the HUA is very well suited to CABG applications. We have developed a strategy to shield transplanted HUA from the host immune system using a rat xenograft model. We hypothesized that the remodeled vessel would be essentially identical to a natural artery. In this continuation of our studies, we have analyzed the process of rebuilding an artery on the implanted, decellularized HUA scaffold

Materials and Methods

Following an IRB/IACUC-approved model in which HUA segments are grafted into rats via infra-renal inter-positional aorta grafts, we studied HUA subjected to various treatments prior to implantation that were intended to extend patency. This reverse xenograft model accelerates the rate of failure due to the extreme difference in species biomarkers. We have performed HUA grafts on 24 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 HUAs, histological and immunofluorescence (IF) protocols are being followed to assess the cellular and structural elements of the remodeling process over time. Anti-rat α-actin (contractile vascular smooth muscle cells (SMC)), anti-rat Von Willebrand Factor (VWF, for endothelial cells), anti-rat Desmin (myofibroblast-like synthetic SMC), and anti-rat CD68 (macrophages, monocytes, and macrophage-like synthetic SMC) are being used in conjunction with secondary antibodies conjugated with Alexa Fluor to determine the presence and relative quantity of their respective antigens. This research is ongoing.

Data

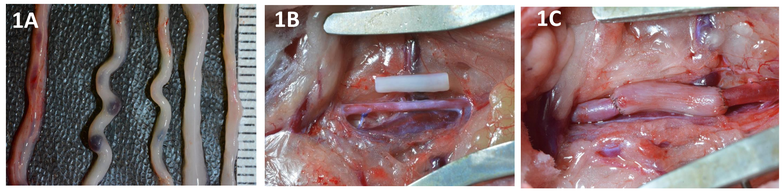

Figure 1. Preparation and Surgical Implantation of HUA.
A) HUA isolated from umbilical cords. **B)** Exposure of abdominal aorta and preparation of HUA for implantation. **C)** HUA inserted into abdominal aorta. As part of the experimental protocol, the HUA can be either untreated or exposed to various treatment regimens prior to implantation.

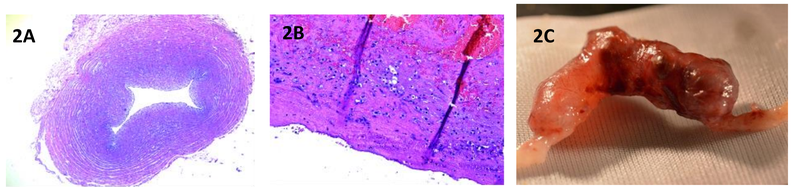

Figure 2. Untreated HUA. **A)** Normal, untreated HUA. H&E stained, 5X magnification. **B)** Histology of normal, untreated HUA after 6 weeks implantation. Note the thickening of the adventitia and media, with none of the open space in the tunica adventitia that is evident in 2A. C) Untreated HUA excised after 6 weeks implantation with evident deterioration.

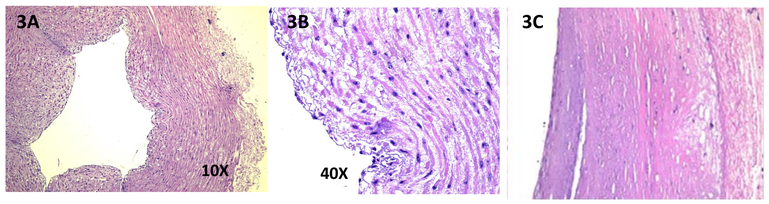

Figure 3. A) and B) Decellularized, unimplanted HUA. These images reveal the impact of our pretreatment on HUA histology. C) Histology of pretreated HUA after 6 months implantation. Note the recellularization and layered structure, similar to that seen in **2A**.

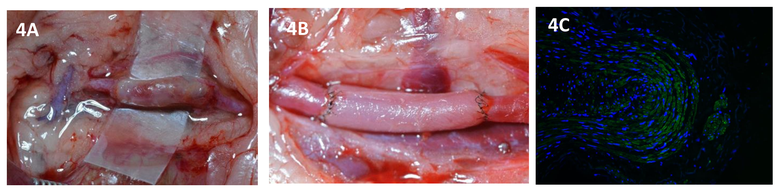

Figure 4. A) and B) Pretreated, implanted HUA at 3 months. These images reveal the impact of our pretreatment on HUA graft survival and patency. Compare these images to the untreated 6 week implant shown in figure **2C**. **C)** Immunofluorescent stain of 3 month implanted HUA, stained to reveal rat α-actin, which stains contractile vascular smooth muscle cells, indicating the vessel has restored the arterial character it possessed before pretreatment and implantation.

Results

Our data support the anticipated immune response to HUA implantation. Rats receiving control HUA (no pretreatment) exhibit inflammatory stenosis as early as 2 weeks post-grafting. All pretreated vessels demonstrate enhanced luminal patency and improved histological appearance compared to controls out to 9 months, with minimal intimal hyperplasia. The decellularized scaffold that is implanted becomes rapidly repopulated with cells from the host, forming a vessel that has a normal gross vascular architecture. The ongoing research challenge is to determine whether the remodeled vessel has a "natural" cellular structure or whether the cells have occupied niches they in which they would not normally be found.

Preliminary analysis revealed that the treated HUAs were rich in alpha actin and VWF and expressed little to no CD68, suggesting a muted immune response as anticipated. However, untreated HUA expressed little alpha actin and VWF, and expressed much CD68, indicating a robust immune response to the untreated UA was attacked by the rat's immune system. This rendered the untreated grafts unable to successfully remodel, leading to the premature failure.

Conclusion:

We are attempting to validate the hypothesis that pretreating HUA segments will allow them to retain patency and exhibit improved histological and physiological performance when implanted into rats, compared to untreated controls. 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 holds significant promise in potentially enhancing allograft bypass patency in both cardiac and lower extremity ischemia.

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