TISSUE-ENGINEERED HUMAN ACELLULAR BLOOD VESSELS FOR CORONARY ARTERY BYPASS GRAFTING

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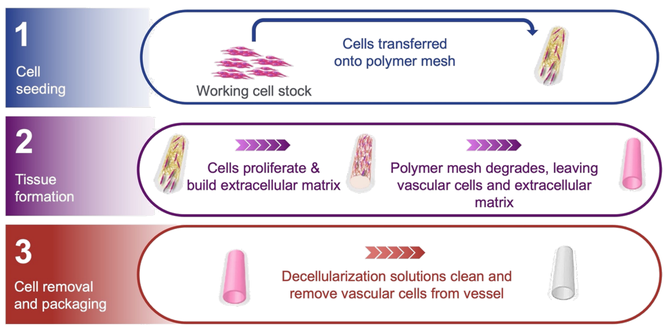
INTRODUCTION

Coronary artery disease (CAD) is the narrowing of the major blood vessels of the heart with symptoms ranging from angina to myocardial infarction and death. Treatment includes medical therapy and procedures such as percutaneous coronary interventions (PCI) or coronary artery bypass grafting (CABG). CABG is among the most common surgeries performed in the US (~400,000 surgeries per year)[1] and has been shown to improve quality of life as well as survival. The primary conduits are left internal mammary artery (LIMA) and saphenous vein, which is used in 80-90% of CABG in the US. SVG patency at 1 year is reported to be as low as 75%, primarily due to thrombosis or neo-intimal hyperplasia.[2] Additionally, SVG harvest can result in surgical wound infection at the harvest site potentially leading to prolonged hospital stay, need for re-vascularization and limb-loss.[3] Therefore, an unmet need exists for an advanced, readily available CABG conduit for the treatment of CAD.

The **Human Acellular Vessel (HAV)**, developed at Humacyte, Inc., is an investigational tissue-engineered blood vessel consisting of human extracellular matrix (ECM) proteins. The HAV is created by culturing human vascular cells within a biodegradable scaffold under biochemical and biomechanical stimulation. The resulting tissue is then decellularized to yield a mechanically robust and non-immunogenic HAV (**Fig 1**).[4] The HAV has been shown to be remodeled by the recipient's own cells to closely resemble native vasculature.[5] In this study, we used a small diameter (3.5mm) HAV as a conduit for CABG in a baboon surgical model and investigated the in-vivo remodeling of the human HAV in the baboon for up to 6 months.

SMALL DIAMETER HUMAN ACELLULAR VESSELS (HAVS)

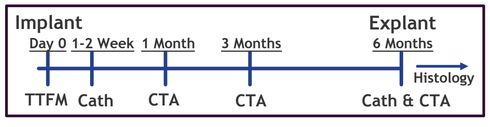


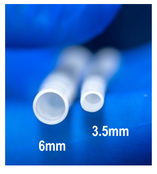
▲ **Figure 1. Schematic of HAV production**

EXPERIMENTAL DESIGN

Small diameter (3.5mm) HAVs were produced at Humacyte, Inc. by scaling down our investigational 6mm HAV platform. Healthy adult male adult baboons (*Papio anubis / Papio cynocephalus*, n=7, 26.8 – 37.0kg) were acquired from Texas Biomedical (San Antonio, TX). Non-immunosuppressed baboons were surgically implanted with a 3.5mm HAV as a CABG conduit under cardiopulmonary bypass using standard surgical techniques. The bypassed native coronary was ligated proximal to the HAV anastomosis and flow through the HAV was confirmed by transit time flowmetry (TTFM). Baboons were given aspirin (3.5mg/kg) and clopidogrel (0.2-0.5 mg/kg) daily throughout the study.

▼**Figure 2. Experimental Timeline**





DEVELOPMENT OF A BABOON SURGICAL MODEL OF CABG

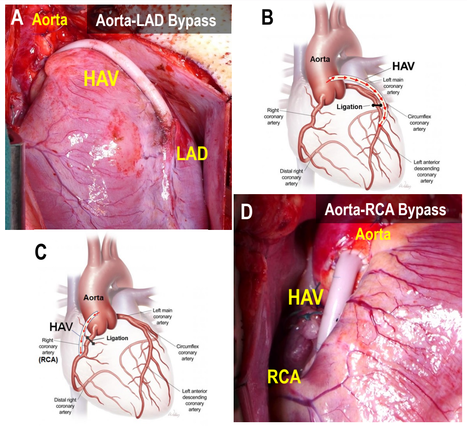


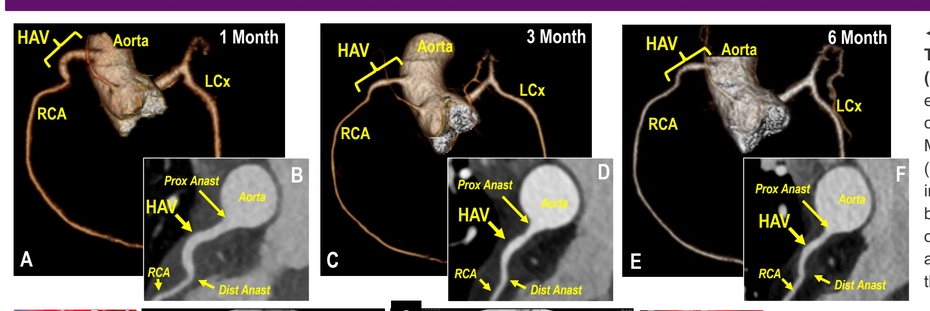
Table 1. Outcomes of 3.5mm HAV CABG Conduits in Baboons

Animal	Weight (kg)	Configuration	HAV Length (cm)	TTFM (mL/min)	Implant Duration	Outcome
Bb 1	30.2	Aorta-LAD	10.0	5	2 Weeks	Occluded
Bb 2	30.0	Aorta-LAD	7.3	20	9 Weeks	Occluded
Bb 3	28.1	Aorta-RCA	7.5	17	0 Days	(Surgical Complication)
Bb 4	26.8	Aorta-RCA	3.1	15	6 Weeks	Occluded
Bb 5	31.5	Aorta-RCA	3.2	34	27 Weeks	Patent
Bb 6	30.5	Aorta-RCA	3.5	19	23 Weeks	Patent
Bb 7	37.0	Aorta-RCA	2.0	25	17 Weeks*	Patent*

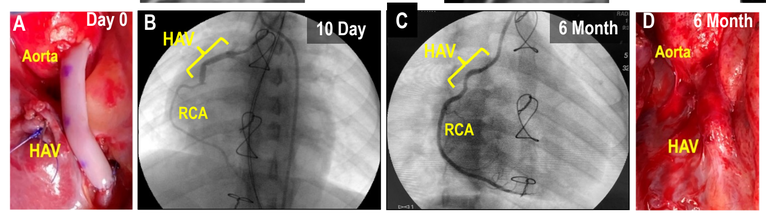
**In-life phase ongoing, planned duration: 6 Months*

◄ **Figure 3. CABG Surgical Model Development.** Baboon (Bb) 1 and Bb2 were implanted with an aorta-LAD HAV bypass (A, B) which was occluded shortly post-op due to size-mismatch. Animals Bb3-7 were implanted with an aorta-RCA HAV bypass (C, D) which resulted in improved success and patency out to 6 Months.

IN VIVO LONGITUDINAL EVALUATION OF IMPLANTED HAV CABG CONDUITS



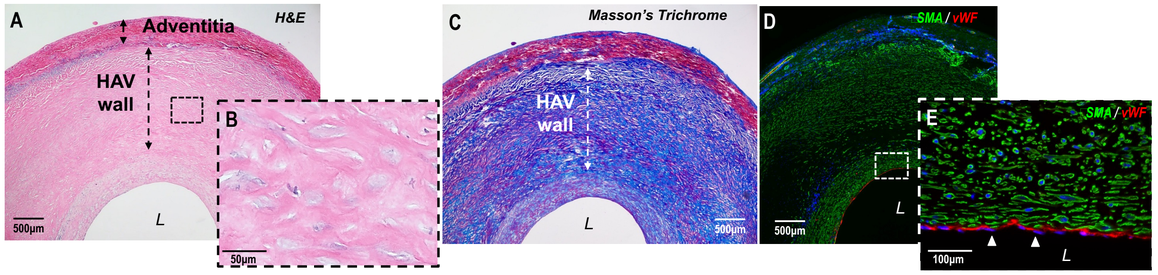
◄ **Figure 4. Computed Tomography Angiography (CTA) 3D Reconstruction** evaluation of HAV CABG conduits at 1 Month (A, B), 3 Months (C, D) and 6 Months (E, F) post-implant. Non-invasive imaging of implanted baboons showed no dilation or significant stenosis of aorta-RCA HAV conduits through 6 Months.



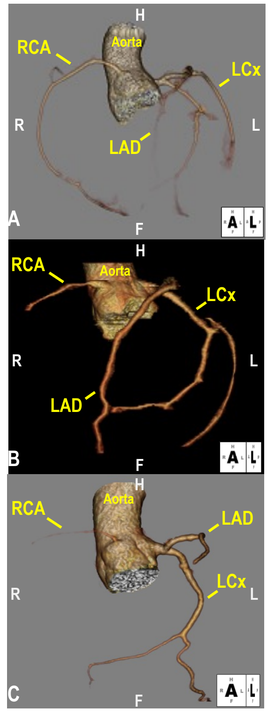
◄ **Figure 5. Following Surgical Implantation** of Aorta-RCA HAV conduits on Day 0 (A), HAVs were evaluated by left heart catheterization at 1-2 Weeks (B) and 6 Months (C) prior to HAV explant (D). Catheter angiography imaging at 6 Months showed patent HAV CABG conduits in animals Bb5 and Bb6. Explanted HAVs were found to be well incorporated to host tissue.

HOST REMODELING OF HAV CABG CONDUITS

▼ **Figure 6. Histological evaluation of host remodeling**. HAVs explanted at 6 Months from animals Bb5, Bb6 were stained by H&E (A, B), by Masson's Trichrome (C), for α-smooth muscle actin (αSMA, D, E) and von Willebrand Factor (vWF, D, E). Formation of a neo-adventitia and infiltration of host αSMA⁺ cells were observed throughout explanted HAVs. The presence of endothelial cells expressing vWF (white triangles, E) on the lumen were observed in 6-Month explants.



BABOON CORONARY ANATOMY VARIABILITY



To date there is only one previous report of the use of baboons in a surgical model of CABG.[6,7] Zilla et al. successfully grafted autologous saphenous veins SVGs to healthy baboon (*Papio ursinus*) coronary arteries in an aorta-LAD configuration for up to 24 weeks (n=8 and n=18). During surgical implantation of our first two animals (Bb1, Bb2), the baboon LAD was found to be significantly smaller in diameter (~1.0 – 1.5 mm), much thinner walled, and with poor distal runoff compared to humans (2.5 – 4.0 mm).

The RCAs in Bbs 3-7 were considerably larger (~2.0mm) and allowed for the long-term implantation of the 3.5 mm HAV in a clinically relevant configuration. The subsequent n=5 animals were implanted with a 3.5 mm HAV in an aorta-RCA configuration, which resulted in improved patency. Pre-operative CTA imaging revealed a varying baboon coronary anatomy with some animals exhibiting a larger RCA, LAD, or LCx artery. Additionally, n=2 animals not included in the study were found to have congenital malformations (dextrocardia and coronary fistulas) at pre-operative screening.

◄ **Figure 7. Variability in Baboon Coronary Vascular Anatomy** was observed by pre-operative CTA imaging. Baboons were imaged before CABG to determine optimum distal target. Animals were shown to have right-dominant (A) and left-dominant coronaries with LAD- (B) and LCx-dominant (C) presentations.

SUMMARY

- Model development was challenging due to variability in coronary anatomy and HAV to host artery size-mismatch, but aorta-RCA configuration was most appropriate.
- The 3.5 mm HAV was successfully used as a conduit in a baboon surgical model of CABG with sustained patency at 6 months.
- The HAV repopulated with host endothelial, smooth muscle, and neo-adventitial cells that remodeled the HAV conduit during the 6-month implantation.

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DISCLOSURE INFORMATION

These results are from a pre-clinical study. The authors employed at Humacyte, Inc. (RDK, KMN, LEN, JHL, and APK) own stock or stock options in Humacyte, Inc.