Automated Dissection of Human Umbilical Vein for Use in Cardiovascular Tissue Engineering

J Daniel, Koki Abe, and Peter McFetridge[*]

Department of Chemical, Biological, and Materials Engineering and University of Oklahoma Bioengineering Center

**: Corresponding Author (pmcfetridge@ou.edu)*

Introduction:

A mechanically sound and compliant biomaterial with superior cell adhesion and remodeling capabilities would be an ideal tissue engineering scaffold. Although processed ex vivo tissues have many of these desired qualities they have seen limited use primarily due to the lack of physical uniformity and tediousness, time consuming preparation methodologies. In this investigation, a novel method for preparing the human umbilical vein (HUV) for use as a thin walled acellular vascular scaffold is described. By automating the dissection method, a uniform vessel with defined wall thicknesses and consistent mechanical properties has been developed. In addition to improved product quality, the dissection process is reduced to approximately 2% of the time required for manual dissection.

Methods:

Fresh human umbilical cords (HUC) were harvested from full-term human placentas. Using scalpel and forceps, mucous connective tissue surrounding the vein was excised until a thickness of 600 – 900 μm was achieved, termed manually dissected HUV (mHUV). Automatically dissected HUV (aHUV) required a stainless steel mandrel to be inserted through the vein lumen to retain the vessels tubular shape during the excision procedure. Sections were progressively frozen to a temperature of -80 °C (2.5°C/min),[1] then cut to a thickness of 750 μm using a rotary high-speed lathe (≤ 2.5 minutes). A representative decellularization process (1% SDS for 12 hours followed by 75% ethanol for 12 hours) was used to determine the effect of processing on the aHUV's mechanical properties ('decellularized HUV' (dHUV)). Burst pressure and compliance were measured by progressive inflation of the vessel until rupture, whilst recording the change in vessel diameter ($\Delta d/d$) over 80 to 120 mmHg.[2] Uniaxial tensile testing was used to determine stress-strain relationships, Young's modulus, and yield stress. Cell adhesion was assessed using primary human vascular smooth muscle cells (hVSMC) isolated from human umbilical arteries as previously described[3] and seeded onto the ablumenal surface of the dHUV and cultured for 7 days. All data sets were calculated from at least three independent veins, each in triplicate. Student's t-test was used to evaluate comparative values and determine statistical significance. Error bars indicate one standard deviation (SD) from the mean. Unless otherwise stated statistical significance is shown at $p < 0.05$.

Results:

Using the high-speed rotary cutting technique required the cord (supported on an appropriate mandrel) to be frozen to increase 'hardness.' Freezing to -80 ºC on a stainless steel 316 tube

(6mm OD; 4mm ID) showed a burst pressure of 1082.0 ± 113.4 mmHg, significantly higher than mHUV segments ($p = 0.01$). After decellularization, mean burst pressure values decreased to 972.8 ± 133.8 mmHg. Compliance ($\Delta d/d$) values for the mHUV, aHUV, and dHUV were 5.7 ± 2.1, 4.6 ± 1.2, and 5.7 ± 1.3 %, respectively. No statistical difference was found in both the yield stress and the Young's modulus between the mHUV and the aHUV samples ($p \leq 0.05$). However, a statistically significant difference was found between the mHUV, aHUV, and the dHUV samples with the dHUV displaying a more elastic property. Suture holding capacity was determined to be 171.78 ± 53.52, 207.45 ± 13.69, and 224.95 ± 15.01 g for mHUV, aHUV, and dHUV, respectively showing a significant difference between all sets ($p < 0.01$). Unlike liquid N_2 prepared samples, no macroscopic or microscopic fractures (at 15000 x magnification) were noted for the aHUV when prepared at –80°C on a tubular mandrel. Histological sections displayed no intact endogenous cell nuclei after the decellualiztion process. hVSMC seeded onto the ablumenal surface and cultured for 7 days demonstrated cellular attachment and migration into the acellular tissue.

Discussion:

The potential advantages of using ex vivo blood vessels as scaffolds for guided organ regeneration are clear. By providing an ideal chemical and physical environment, cellular adhesion and remodeling, improved biological integration and function are more likely to occur. The HUV has a number of important properties that have shown promise as an acellular vascular scaffold, such as being biologic in origin, an allograft material, and its vascular derivation provides improved mechanical compliance and presents surfaces that are conducive to cellular attachment and subsequent remodeling by vascular cells.[4-6] The HUV shows promise for several vascular reconstructive applications with appropriate lengths and diameters.

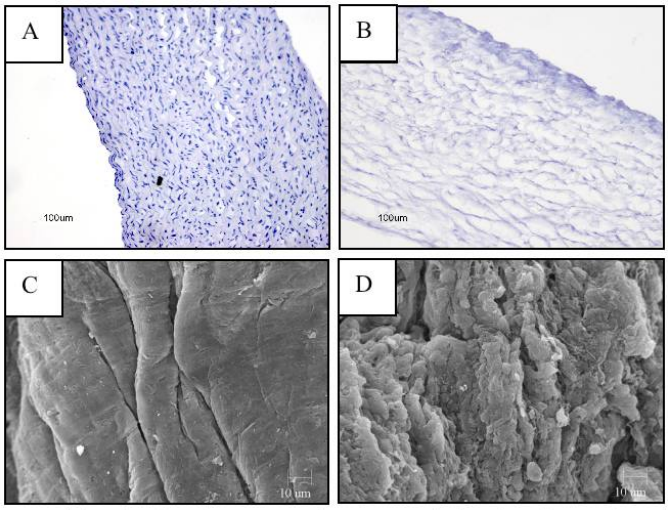

Figure 1: (A) control (unprocessed) HUV, showing neonatal EC and VSMC cell populations. (B) the decellularized vessel showing the absence of whole cells. (C) SEM of decellularized vessels lumenal surface showing the removal of the EC lining and no visible damage to the vessel surface from the processing steps. (D) SEM of decellularized vessels ablumenal surface showing the more fibrous structure of the cut surface.

The aHUV with burst pressures exceeding 1000 mmHg provides significant redundancy above arterial pressure, and is comparable to the canine ex vivo model of 931 mmHg.[7] Similarly the processed aHUV retain sutures under applied force greater than PGA scaffolds that are in current use as vascular grafts.[8,9] The biphasic stress-strain relationship and mechanical compliance has been retained and is in the same order as native arteries.[2]

Preliminary assessment of cell compatibility has shown hVSMC adhesion, maintenance, and migration toward the lumenal surface over a 7 day culture period, suggesting a rapid remodeling potential of this material. Further investigation is required to determine the rates of remodeling and the mechanical stability of the Human Umbilical Vein Scaffold (HUVS) over time. Although the HUVS developed in this study is aimed primarily at vascular reconstructive surgery, its use in other applications has not been discounted.

We have shown the potential of an automated dissection method to eliminate tedious, error prone manual dissection of this ex vivo material, where high throughput production with improved safety is possible. The HUV bioscaffold has shown potential as a vascular matrix in vascular tissue engineering applications where the problems of thrombosis, degradation and neointimal hyperplasia may be minimised through improved biological integration.[10]

References:

1. Oegema TR, Jr., Deloria LB*, et al.*: A simple cryopreservation method for the maintenance of cell viability and mechanical integrity of a cultured cartilage analog. *Cryobiology* 40: 370-5, 2000.
2. Roeder R, Wolfe J*, et al.*: Compliance, elastic modulus, and burst pressure of small-intestine submucosa (SIS), small-diameter vascular grafts. *Journal of Biomedical Materials Research* 47: 65-70, 1999.
3. Kadner A, Zund G*, et al.*: Human umbilical cord cells for cardiovascular tissue engineering: a comparative study. *European Journal of Cardio-Thoracic Surgery* 25: 635-41, 2004.
4. McFetridge PS: *Tissue engineering small diameter vascular grafts (PhD thesis)*. Bath, University of Bath, 2002.
5. McFetridge PS, Daniel JW*, et al.*: Preparation of Porcine Carotid Arteries for Vascular Tissue Engineering Applications. *Journal of Biomedical Materials Research* (in press), 2004.
6. Teebken OE, Bader A*, et al.*: Tissue engineering of vascular grafts: Human cell seeding of decellularised porcine matrix. *European Journal of Vascular and Endovascular Surgery* 19: 381-386, 2000.
7. Huynh T, Abraham G*, et al.*: Remodeling of an acellular collagen graft into a physiologically responsive neovessel. *Nature Biotechnology* 17: 1083-6, 1999.
8. Niklason LE, Gao J*, et al.*: Functional arteries grown in vitro. *Science* 284, 1999.
9. Hoerstrup SP, Zund G*, et al.*: Tissue engineering of small caliber vascular grafts. *European Journal of Cardio-Thoracic Surgery* 20: 164-9, 2001.
10. Dohmen PM, Ozaki S*, et al.*: Tissue engineering of an auto-xenograft pulmonary heart valve. *Asian Cardiovasc Thorac Ann* 10: 25-30, 2002.