Tissue Engineering

Development of the Human Umbilical Vein Scaffold for Cardiovascular Tissue Engineering Applications

Joel Daniel, Koki Abe, and Peter S. McFetridge

Biologic function and the mechanical performance of vascular grafting materials are important predictors of graft patency. As such, "functional" materials that improve biologic integration and function have become increasingly sought after. An important alternative to synthetic materials is the use of biomaterials derived from *ex vivo* tissues that retain significant biologic and mechanical function. Unfortunately, inconsistent mechanical properties that result from tedious, time consuming, manual dissection methods have reduced the potential usefulness of many of these materials. We describe the preparation of the human umbilical vein (HUV) for use as an acellular, three-dimensional, vascular scaffold using a novel, automated dissection methodology. The goal of this investigation was to determine the effectiveness of the auto-dissection methodology to yield an *ex vivo* biomaterial with improved uniformity and reduced variance. Mechanical properties, including burst pressure, compliance, uniaxial tension testing, and suture holding capacity, were assessed to determine the suitability of the HUV scaffold for vascular tissue engineering applications. The automated methodology results in a tubular scaffold with significantly reduced sample to sample variation, requiring significantly less time to excise the vein from the umbilical cord than manual dissection methods. Short-term analysis of the interactions between primary human vascular smooth muscle cells and fibroblasts HUV scaffold have shown an excellent potential for cellular integration by native cellular remodeling processes. Our work has shown that the HUV scaffold is mechanically sound, uniform, and maintains its biphasic stress-strain relationship throughout tissue processing. By maintaining the mechanical properties of the native blood vessels, in concert with promising cellular interactions, the HUV scaffold may lead to improved grafts for vascular reconstructive surgeries. *ASAIO Journal* 2005; 51:252–261.

To replicate the success of autologous arterial transplants, a successful prosthetic must integrate and function in a similar manner to natural arteries. It is the failure of current small diameter prosthetics to integrate appropriately with recipient tissue that initiates a number of unfavorable biologic interactions cumulating in thrombotic and hyperplastic responses leading to graft failure.[1] To improve the host-graft interaction, both a competent endothelium, to serve at the blood-graft interface, and a fully developed, biocompatible vascular wall populated with vascular smooth muscle cells (VSMC) should be present. The logic behind this approach is clear: grafts frequently fail because of poor functional integration. To improve function, a biologic component must be present. It follows that if the biologic component is more comprehensive then improved biologic function is more likely to occur. As neither a functional vessel wall nor an endothelium will spontaneously develop in adult humans (at an appreciable rate), tissue engineering offers a unique methodology where replacement neovessels can be grown *in vitro*.[2–5] By incorporating functional cell lineages into three-dimensional scaffolds or blood vessel templates, improved biologic function can be achieved to minimize intrinsic host repair or defense mechanisms that would otherwise lead to graft failure.

A key component of this process is the choice of three-dimensional scaffold with which tissue growth is guided. The ideal vascular scaffold would be biocompatible, have mechanical properties that replicate native blood vessels, and have the capacity to guide, support, and maintain cellular function. The list of scaffold materials continues to grow with both permanent[6] and biodegradable synthetics.[7,8] An important alternative to these materials is the use of *ex vivo* derived materials that can retain significant biologic and mechanical functions.[9–13] Significant progress has been made to extract immunogenic components from these materials to reduce their immune impact.[12,13] The clinical use of collagen hydrogels in cosmetic surgery and the small intestinal submucosa (SIS) have validated the use of these materials.[14–16] Although *ex vivo* tissue processing is an effective means to reduce the immunogenic load, mass transfer limitations of thicker and larger organs are likely to reduce processing efficiency. A distinct and important advantage of vascular derived scaffolds is that the physical and chemical environment is inherently more conducive to cell adhesion and native remodeling processes than many synthetic alternatives. For example, cell adhesion is enhanced because of endogenous RGD adhesion sequences present in the amino acid sequence collagen within extracellular matrix (ECM)[17]. Further, the retention of native like mechanical properties (compliance matching) that has been shown to be an important predictor of graft success.[18–20] However, compared with many synthetic polymers, processed *ex vivo* materials often lack mechanical uniformity, consistency,

From the School of Chemical, Biological and Materials Engineering, and the University of Oklahoma Bioengineering Center, University of Oklahoma, Norman, Oklahoma.

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Correspondence: Dr. Peter S. McFetridge, School of Chemical, Biological and Materials Engineering, University of Oklahoma, 100 East Boyd street, Norman, Oklahoma, U.S.A.

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and composition and can be restrictive in their final shape and structure.

The human umbilical vein (HUV) has a comprehensive clinical history as a glutaraldehyde fixed bypass graft.[21–26] However, time consuming and error prone manual dissection procedures can result in a lack of mechanical uniformity, limiting the use of this material as a stand alone scaffold (without additional support) for tissue engineering applications. The HUV has a number of properties that show promise as an acellular, three-dimensional vascular scaffold. It has similar structure and form to natural arteries to more closely replicate arterial compliance; its allograft origin reduces the risk of interspecies viral contamination (although disease transmission within species remains a risk factor), and because of its vascular derivation, it presents surfaces that are conducive to cellular attachment and subsequent remodeling processes.[11,27–29] With lengths that can exceed 500 mm and internal diameters from 4–6 mm, the HUV is appropriate for several vascular reconstructive applications.

In the present report, a novel approach to prepare the HUV as a processed acellular scaffold for guided vascular tissue regeneration is presented. The development and use of an automated dissection methodology that isolates the HUV from the umbilical cord with "dialed in" precision is detailed. This methodology reduces the dissection time frame from 1–2 hours for manual dissection to 2–3 minutes, yielding a material with significantly improved mechanical properties. Precision dissection results in uniform wall thicknesses that can be dialed in to a desired thickness from approximately 250–1,000 μm, allowing the option of retaining only the intima and media, or reducing the cutting depth to incorporate the hyaluronic acid rich mucous connective tissue that surrounds the vein.[30–35] This approach significantly reduces mechanical variation and takes advantage of the HUV natural form and function. Comparative analysis between manual, automated dissected, and decellularized HUV has been conducted to assess the suitability of this material as a three-dimensional scaffold for tissue engineering applications.

Materials and Methods

Preparation and Dissection of the Human Umbilical Vein

Fresh human umbilical cords were harvested from full term human placentas collected from the Delivery Suite at the Norman Regional Hospital, Norman, OK. Cords were stored within 10 minutes of delivery at 5°C for no more than 24 hours until preparation for experimental use. Before dissection, the cords were cleaned, rinsed to remove residual blood, and cut to an initial length of 80 mm. After tissue treatments, 10 mm was discarded from each end to eliminate end effects, bringing the final length used for analytic purposes to 60 mm.

Manual Dissection

A 200 mm long × 6 mm outside diameter (OD) glass mandrel was inserted through the vein's lumen to guide the manual dissection process (**Figure 1A**). Using a standard scalpel and forceps, the arteries and mucous connective tissue that surround the vein were progressively excised (as uniformly as possibly), until a thickness of approximately 750 μm was achieved. These samples were designated "manually dissected human umbilical vein" (mHUV) (**Figure 1B**).

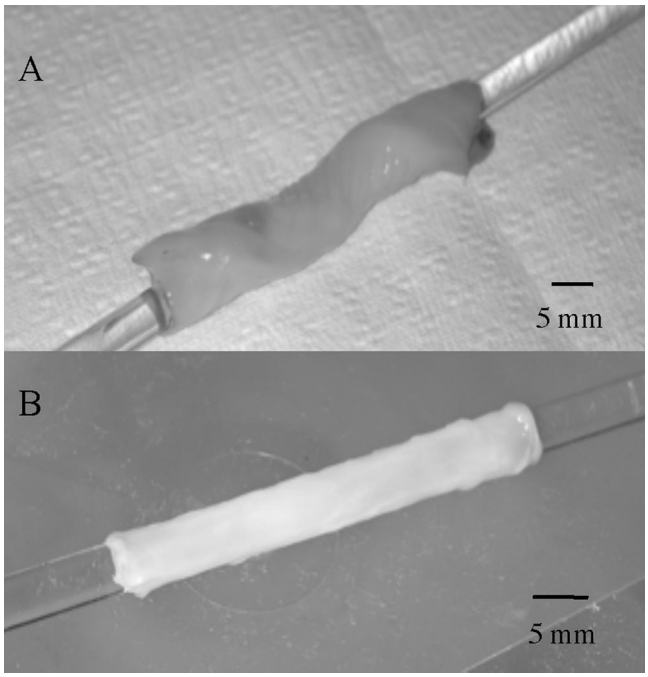

Figure 1. Manual dissection. (A) Section of the human umbilical cord with a glass mandrel inserted through the veins lumen. (B) The manually dissected umbilical vein. The nonuniform nature of the manual dissected vessel can be seen along the periphery of the vessel.

Automated Dissection

A number of different methodologies were assessed to optimize the automated dissection process, including the following: cutting temperatures (−20°C, −80°C, and −196°C) and mandrel specifications (solid 316 stainless steel rod, 6 mm OD × 180 mm L, and 316 stainless steel tube, 4 mm ID, 6 mm OD × 180 mm L). In a similar fashion to the manual dissection method, the automated method required a stainless steel mandrel (6 mm OD, as discussed previously) to be inserted through the vein lumen to straighten the vessel and retain its tubular shape during the excision procedure. The cord was then unwound to reduce the spiraling structure of the vein and then tensioned longitudinally. The mounted cord was then secured at each end using 4 mm nylon zip ties to minimize torque induced slippage during dissection. The accuracy of the procedure relied upon the vein being in close, uniform contact with the mandrel, where raised or buckled sections were minimized to reduce variation in scaffold wall thickness (**Figure 2A**). All sections were progressively frozen within a sealed Styrofoam container at a rate of 2.5°C/min.[36] Vessels frozen to −20°C and −80°C were maintained at their terminal temperatures for a minimum of 12 hours to ensure a uniform temperature throughout the vessel wall. Freezing vessels to −196°C required progressive freezing to −80°C (as discussed previously) then plunging into liquid nitrogen.

Mounted, frozen vessels were removed from frozen storage immediately before inserting the cord/mandrel into the lathe

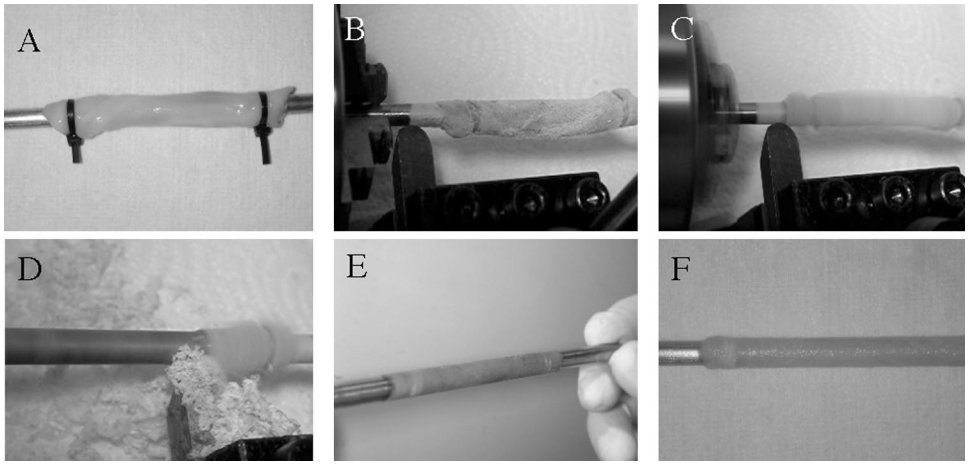

Figure 2. Autodissection methodology. Whole human umbilical cord is mounted on a stainless steel mandrel (A), then frozen to predetermined temperature. The cord is then placed in a modified lathe (B), and the lathe is engaged with the cutting tool transversing the section (C and D), leaving a smooth and uniform scaffold (E). The frozen section is then thawed and decellularized (F).

(Central Machinery, Mod 33647, China). Once the mandrel/cord was secured in the lathe, the rotational speed was set to 2,900 rpm and then engaged. Using a high speed, steel cutting tool, designed for cutting soft materials, the cutting depth was set to 750 μm and the automatic drive engaged at a rate of 5 mm/second until the cutting tool had transversed the cord (**Figure 2, B–D**). Sections dissected in this manner were termed "automatically dissected HUV" (aHUV) sections. Sections were then stored at −20°C until required (minimum 4 hours). The time frame from removal from the −80°C freezer to the completion of dissection and storage was less than or equal to 2.5 minutes. Sections were thawed by immersion in double distilled water at 5°C at 5°C for 1 hour.[36–38]

Decellularization

A representative decellularization process was used to determine the effect of processing upon the HUV's mechanical properties. Because of the poor results of the −20°C and −196°C prepared segments, only vessels prepared at −80°C were decellularized. Glass rods (3 mm OD) were inserted through the vessel lumen to improve dispersion of the treatment solution through the lumen (4–6 mm ID) during decellularization process. Each aHUV segment was immersed in a 25 ml solution of 1% (w/v) sodium dodecyl sulfate (SDS) for 12 hours and then rinsed (three times) in phosphate buffered saline (PBS, Gibco Life Technologies, Grand Island, NY). Sections were then washed for 12 hours in 25 ml of 75% (v/v) ethanol to remove the amphiphilic surfactant molecule and aid lipid extraction.[11] Sections were then washed (three times) in 25 ml of PBS for 1 hour before use. These samples were designated decellularized human umbilical veins (dHUV).

Mechanical Analysis

Samples were categorized into three groups: manually dissected HUV (mHUV), automatically dissected HUV (aHUV), and decellularized HUV (dHUV). Each group was composed of nine samples: three separate cords with three data points obtained from each cord (n = 9). As mentioned previously, to eliminate end effects, 10 mm was removed from each end of each sample before mechanical analysis, leaving a total length of 60 mm.[13]

Burst Pressure and Compliance

Burst pressure and compliance were measured by progressive inflation of the vessel until rupture while simultaneously recording the change in vessel diameter. The ends of each vessel section were attached to stainless steel adapters (4 mm ID, 6 mm OD × 60 mm L), and connected into a circuit of heavily walled silicone tubing (4.76 mm ID, 7.94 mm OD). A modified syringe pump was then attached to one end of the tubing, with a pressure transducer (Master Test Type 220–4s, Mash Instrument Company, Skokie, IL) attached to the distal end to monitor the change in pressure. The syringe pump injected double distilled water into the circuit at a rate of 5 ml/min until vessel rupture. Vessel diameter and pressure were recorded over time using a SVHS video recording system. Analog data were then converted to digital format and analyzed using MetaVue software (Universal Imaging Corp., Version 5.0r1, Downingtown, PA).

Compliance is defined as[13,39]

$$\frac{\Delta d}{d\Delta P}$$

Because of the dynamic nature of blood vessel mechanics, where the compliance value is dependent upon the pressure range, the change in diameter ($\Delta d/d$) was assessed over a physiologic pressure range (80–120 mm Hg).[39] At the point of vessel rupture, the final pressure was recorded to determine the burst pressure for the vessel.

Stress-Strain Testing

A uniaxial tensile testing rig (United Testing Systems, Inc., Model SSTM-2K, Flint, MI) was used for all stress-strain analyses to determine the stress-strain relationship, Young's modulus, and yield stress. Circular vein samples were cut to 5 mm wide ringlets and loaded onto the machine using stainless steel L-hooks.[11,13] Samples were preloaded to a stress of 0.005 N at a rate of 5 mm/min.[11,40] Using the same extension rate of 5 mm/min, samples were stressed until failure.[11]

Suture Holding Capacity

Suture holding capacity was assessed by applying uniaxial stress to the sutured samples (United Testing Systems, Inc.,

Model SSTM-2K, Flint, MI). Vein sections were cut longitudinally to form a 15 mm wide × 80 mm long sheet. A single sterile 3–0 braided silk suture (Henry Schein, Melville, NY) was passed through one end of the tissue section 2 mm below the cut edge, with the other attached to the test rig.[41] Samples were preloaded to 0.005 N stress (5 mm/min). Data were then recorded at an extension rate of 125 mm/min until tissue failure.[41]

SEM analysis

Samples of the luminal and the abluminal surfaces of the aHUV were gently washed with PBS three times for 5 minutes each, then fixed in 1% (v/v) glutaraldehyde (Sigma, St. Louis, MO) for 4 hours. Samples were then washed in PBS (three times) for 5 minutes each. This was followed by a treatment of 1% osmium in PBS for 2 hours. Samples were washed and dehydrated in graded ethanol treatments (30%, 50%, 70%, 90%, 95%, and 100%, v/v) for 10 minutes each, then critical point dried (Autosamdri-814, Tousimis, Rockville, Maryland) and gold sputtered (Hummer IV). Samples were analyzed using a JEOL LSM-880 Scanning Electron Microscopy (SEM).

Primary Human Cell Isolation and Culture

Mixed populations of primary human fibroblasts and vascular smooth muscle cells (hVSMC) were isolated as previously described by the explant method from human umbilical arteries.[42] Cultures were maintained with Dulbecco's Modified Eagle's Medium containing sodium pyruvate, L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco Life Technologies, Grand Island, NY), and 10% fetal calf serum (Atlanta Biologicals, Norcross, GA). All cells were maintained at 37°C in a 5% CO_2 environment and used between passages 3 and 5.

Cell Attachment Studies

Mixed populations of primary human (neonatal) fibroblasts and smooth muscle cells (HVSMC) were seeded onto abluminal surface of the dHUV using a collagen hydrogel seeding method. Briefly, bovine collagen gels (Cell Prime 100, Cohesion, CA) were made up to a concentration of 1.5 mg/ml and inoculated with 1×10^6 of the fibroblast/HVSMC mixed cell population. The solution was inoculated into abluminal void of the vascular bioreactor and allowed to polymerize at 37°C for 1 hour. After 3 days culture, gels contracted off the inner wall of the glass bioreactor onto the scaffold to a final cell density of approximately 3,000 cells/mm^2. Vascular bioreactors consisted of glass cylinders with ports on each end for luminal flow and two ports on the shell side for abluminal flow. Flow of media was performed in a two circuit (luminal and abluminal) process flow system. Luminal flow was maintained at 10 ml/min during gel polymerization (1 hour) and then progressively increased by 25 ml/min (each 6 hours) until a flow rate of 110 ml/min. was achieved. Cultures were maintained for a total of 7 days at 37°C, 5% CO_2.

Histology and Microscopy

HUV samples prepared for sectioning were fixed in 2% formal saline before paraffin embedding and sectioning. Samples seeded with hVSMC on the abluminal surface were stained with hematoxylin (Richard-Allan Scientific, Kalamazoo, MI) using standard protocols and sectioned to assess cell migration into the scaffold.

Statistics

Data sets were calculated from at least three independent experiments, each in triplicate (n = 9), unless otherwise stated. Statistical significance was determined using analysis of variance (ANOVA) with Tukey HSD test. Significance was set at $p < 0.05$. Data are graphically represented using box plots. The box section represents the mean ± 1 SD, the dark line within the box represents the median value, and the lines extending outside the box represent the data range (minimum to maximum) of recorded values.

Results

SEM Surface Analysis

SEM images of the dHUV luminal and abluminal surfaces were shown to be free of whole cells, although debris fragments are noted. The luminal surface (**Figure 3**) displays the typical convoluted basement membrane (A), with grooves observed perpendicular to the longitudinal direction of the vein (B). These were hypothesized to result from rotational slippage of the HUV on the outer surface of the mandrel during the automated dissection process. The structure of the type I collagen abluminal surface shown in **Figure 4** is significantly different from that of the type IV collagen typical of basement membranes on the luminal surface (**Figure 3**). The fibrous (cell free) collagen fibers of the abluminal surface are detailed in the inset in **Figure 4**.

High Speed Rotary Dissection

Results from burst pressures analyses varied significantly depending upon the freezing temperature of the umbilical cord, mandrel configuration, and the method of mounting cord

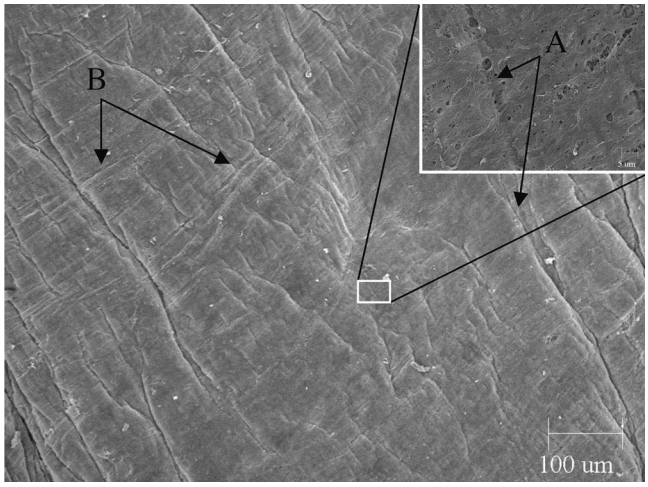

Figure 3. Scanning electron microscopy (SEM) images of luminal surface. The convoluted structure of the luminal surface maintains is maintained after the automated dissection procedure (A). Striations are observed in the direction of cutting (B), with no visual cracking on the lumen.

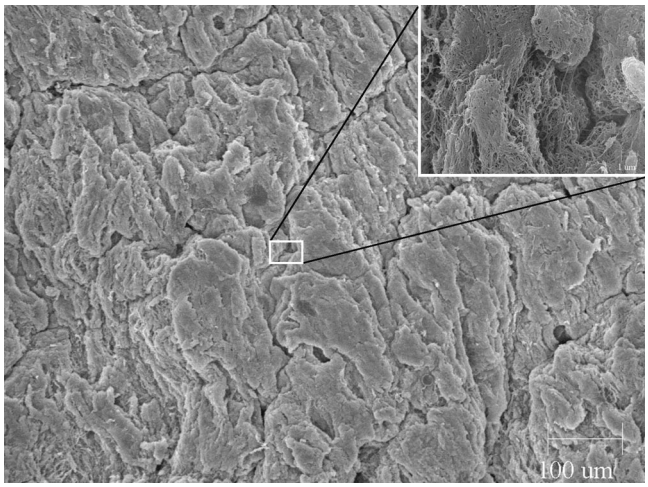

Figure 4. Scanning electron microscopy (SEM) images of the abluminal surface. Displaying the ablumenal surface after the auto-dissection process; no evidence of vessel fracture was noted.

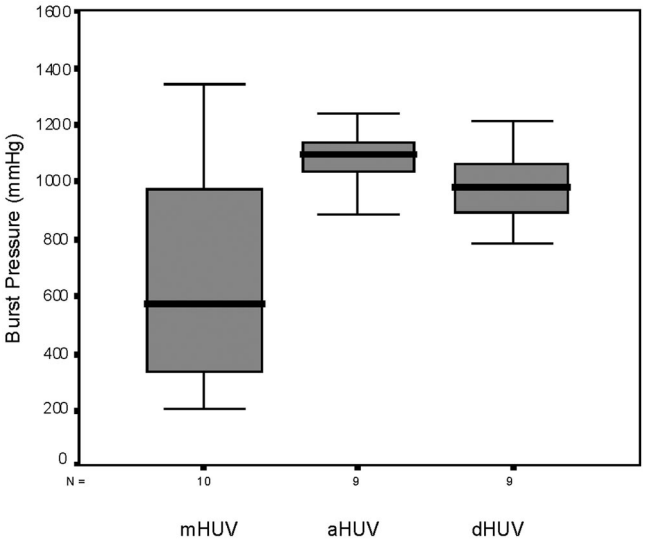

Figure 5. Burst pressure. Mean values aHUV burst pressures were significantly higher than that of the mHUV, with the extended bars representing the data range, shaded region the standard deviation, and the heavy marker in the shaded region the data median. aHUV, automatically dissected human umbilical vein; mHUV, manually dissected human umbilical vein.

onto mandrel. Freezing was required to increase vessel hardness and to allow a uniform dissection. A balance between tearing the tissue (not hard enough) and fracturing the tissue (too brittle) was essential. The high speed, rotary cutting technique required the cord (supported on an appropriate mandrel) to be in close contact with the underlying mandrel. Small gaps caused by the vein's twisted morphology resulted in nonuniform wall thickness (data not shown). Later sections were longitudinally stretched over the mandrel and secured using nylon zip ties to minimize gaps between the mandrel and luminal surface. Of the freezing temperatures assessed (−20, −80, and −196°C), cords frozen to a final temperature of −20°C were too soft for cutting, resulting in tearing by the cutting tool tearing rather than precise excision. Because of the tearing action and resulting lack of tissue uniformity, this protocol was rejected. Freezing in liquid nitrogen (whether direct plunging or progressively by previous freezing to −80°C) resulted in gross fracturing and complete loss of containment (data not shown). This was attributed to collagen being prestressed over the mandrel and passing below its glass transition temperature.[38] This method was also rejected from further analysis. Only sections frozen to −80°C before excision maintained their gross mechanical attributes, allowing further analysis.

Burst Pressure

Using a stainless steel (SS) tube (4 mm ID, 6 mm OD) as the support mandrel, with vessels frozen to −80°C, burst pressure results were 1,082.0 ± 113.4 mm Hg, significantly higher than 699.2 ± 399.1 mm Hg for mHUV segments ($p = 0.01$). After decellularization, mean burst pressure values decreased to 972.8 ± 133.8 mm Hg. The use of the mechanical dissection procedure shows a reduction in sample to sample mechanical variation compared with manual dissection (**Figure 5**).

Compliance

The relationship between vessel expansion and applied internal pressure was assessed. No statistical difference in compliance values ($\Delta d/d$) was found between the mHUV (5.7 ± 2.1%), aHUV (4.6 ± 1.2%), and the dHUV (5.7 ± 1.3%) over the pressure range 80–120 mm Hg (**Figure 6**). Similar to burst pressure results, a reduction in sample variation is noted between manual and automated dissection procedures.

Stress-Strain Analysis

Data from stress strain analysis were used to determine yield stress and Young's modulus. No significant difference was found in the yield stress between the mHUV and the aHUV samples, with yield values at 1.31 ± 0.67 and 1.55 ± 0.28 MPa, respectively. However, a significant reduction in yield stress was found with the dHUV samples to a value of 0.81 ±

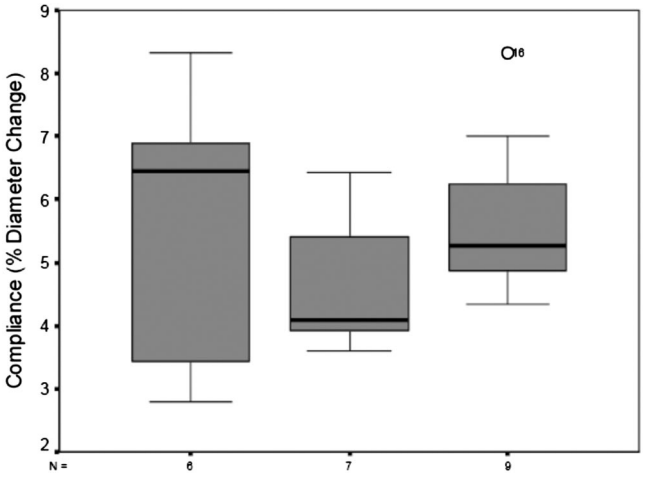

Figure 6. Compliance. No significant difference is associated between dissection methodologies, but range and variance are greatly reduced with automated dissection.

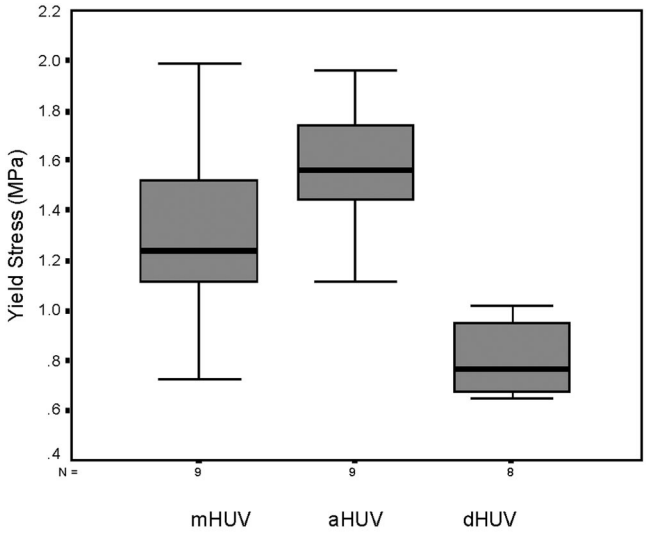

Figure 7. Yield stress. No significant change in the yield stress was observed in dissection methodologies. However, a reduction was found with the decellularization method used.

0.15 MPa (**Figure 7**). Young's moduli of aHUV showed a significant increase from the mHUV sections, with values for the mHUV and the aHUV being 0.63 ± 0.24 and 0.97 ± 0.16 MPa, respectively. The dHUV displayed lower modulus value of 0.45 ± 0.075 MPa that was not significantly different from the mHUV (**Figure 8**). Representative stress-strain curves (**Figure 9**) show a general increase in strain at the point of failure as the vessels are progressively treated. In all cases, the vessels have retained the biphasic nature of natural blood vessels.

Suture Holding Capacity

The suture holding capacity of each group (mHUV, aHUV, and dHUV) was determined by progressively applying force until suture failure. Manually dissected sections of the vein (mHUV) were found to have significantly lower failure rates at 1.68 ± 0.52 N, compared with the autodissection method (aHUV) at 2.03 ± 0.13 N and the autodissected and decellularized sections (dHUV) at 2.21 ± 0.15 N (**Figure 10**).

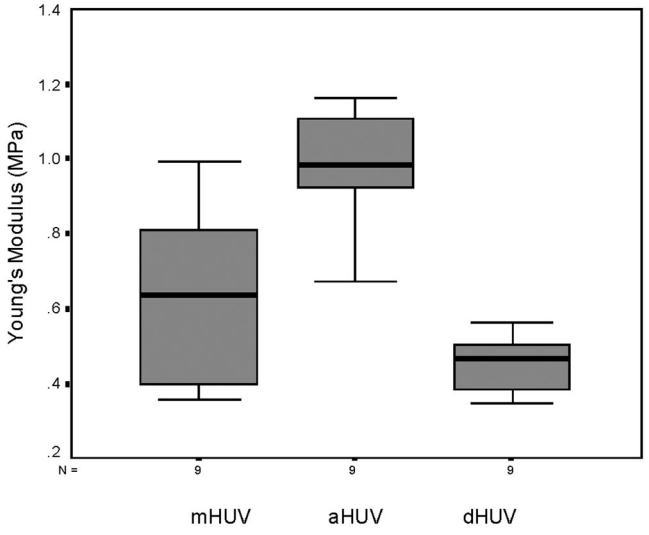

Figure 8. Young's Modulus, a measure of elasticity, is significantly higher with the autodissection methodology.

Histology

After the decellularization and washing processes, analysis of hematoxylin stained sections displayed no intact endogenous cell nuclei; however, some disruption to the ECM fibers was observed. Unlike liquid N_2 treated samples, no gross fractures were noted for the aHUV when prepared at −80°C on a tubular mandrel (**Figure 11A**, manual dissected control) (**Figure 11B**, postautomated dissection and decellularization). The hVSMC seeded to a final density of 3,000 cells/mm^2 onto the abluminal surface and cultured over a 7 day period demonstrated cellular attachment and migration into the acellular tissue as shown in **Figure 11C**.

Discussion

A small diameter, vascular bypass grafting material that is biocompatible, with appropriate mechanical properties, and resistant to thrombotic and hyperplastic responses is yet to be found. The use of *ex vivo* blood vessels as scaffolds for guided organ regeneration aims to provide an ideal chemical and physical environment that promotes biologic function and integration. However, the use of these materials does necessitate a degree of tissue processing to stabilize, sterilize, and prevent chronic foreign body responses.[9,43] Two approaches have generally been taken: 1) cross-linking and 2) removal of host epitopes by decellularization. The tanning, or glutaraldehyde treatment that improves long-term stability and reduces immune reactivity, does so by forming chemical cross-links in the ECM that stabilize the structure and create a barrier for cellular infiltration.[44] The inherent drawbacks of "fixed" or "cross-linked" materials are that they often retain cytotoxic compounds from the cross-linking[45] and are generally incapable of cellular remodeling. As such, these materials remain physiologically inert, behaving much like synthetic materials that cannot respond to changes in their environment.[46] The glutaraldehyde tanned human umbilical vein graft developed by Dardik *et al.*[21,23,25,47] is an effective alternative to poorly performing current synthetic materials for small diameter vascular reconstructions. However, like other cross-linked and permanent synthetic materials, it cannot remodel to form a functional vessel.

To avoid cross-linking, the material must be tolerated by the recipient's immune system and withstand prolonged exposure to the stresses of *in vivo* arterial hemodynamics. As such, the success of *ex vivo* materials is dependent upon the scaffold of choice and the preimplantation processing methodologies to ensure longevity, immunologic acceptance, and graft sterility. The number of methodologies used to prepare *ex vivo* materials is rapidly expanding; these include osmotic shock,[48,49] acids,[49,50] bases,[51] detergents,[52–55] enzymes,[29,55–58] and solvents,[51,56,59–61] with numerous tissues and organs being decellularized, including vascular,[29,44,50,62,63] bladder,[49,64] cardiac valves,[54,58,65] and others.[66,67] Several of these studies have shown cells migrating into and populating the matrix material, indicating that cross-linking is not necessarily a vital

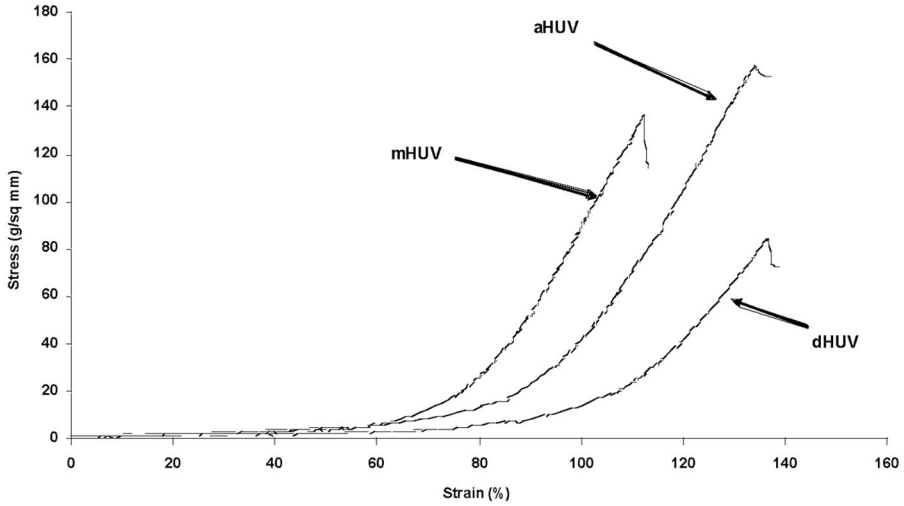

Figure 9. Representative stress-strain analysis. The biphasic stress-strain relationship seen in native blood vessels is retained throughout tissue processing, although a shift in strain is noted as vessels are processed.

step. However, Courtman *et al.*[44] found that, despite decellularization, immunogenic proteins remained localized within the media of the vascular graft (not the graft periphery), concluding that immunogenic proteins arise from proteins associated with the distinct extracellular arterial matrix. Although this explanation is plausible, mass transfer limitations increase as tissue size increases, resulting in a reduced efficiency of the extraction procedures to remove immunogenic residues that lie in the center of the matrix. The development of technologies to limit mass transfer effects using pressure gradients during tissue processing may improve extraction procedures and thus prove valuable in the development of acellular scaffolds.

During development of the automated dissection procedure, parameters such as mandrel size, type, and composition, temperature (freezing, cutting, and thawing), cord tension and twisting, and the shape and rotational speed of the cutting tool during dissection all required optimization to minimize damage to the vessel. It was essential that the diameter of the mandrel be sufficiently large to stretch the vessel circumferentially to avoid variation in cutting depth, while avoiding overstretching and potential fracturing during the freezing process (data not shown). Further, because of the spiraling anatomy of the vein within the cord, it was necessary to unwind and longitudinally tension the cord to secure the vein in the correct position. Failure to do so resulted in significant variation in mechanical properties.

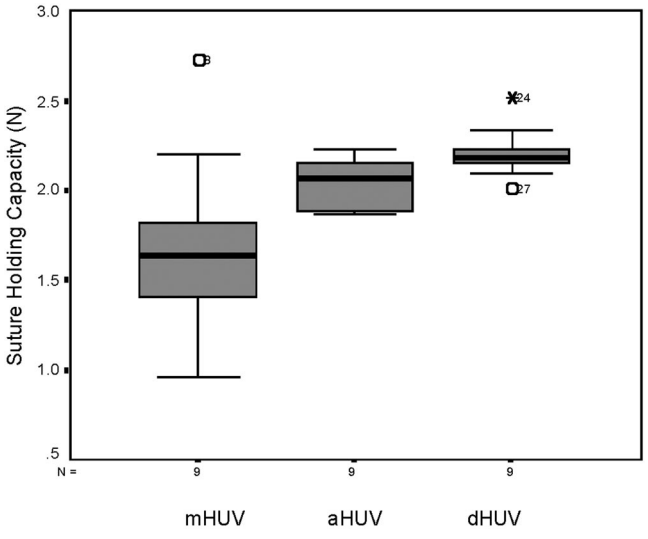

Figure 10. Suture holding capacity. mHUV were found to have significantly lower failure rates at 1.68 ± 0.52 N, compared with the aHUV at 2.03 ± 0.13 N and the autodissected and dHUV at 2.21 ± 0.15 N. aHUV, automatically dissected human umbilical vein; mHUV, manually dissected human umbilical vein; dHUV, decellularized human umbilical vein.

Thermal expansion of the mandrel during the freezing and thawing process was also considered as a potential cause of applied stress to the scaffold. If the mandrel expands or contracts at a rate that is not supportive of the tissue, fracturing or loss of support will result. Although stainless steel has a low coefficient of expansion *and* is unlikely to result in significant fracturing because of expansion or contraction, this is a material specific issue and should be considered if alternative materials are sought.

Mechanical redundancy is critical for long-term resilience to physiologic stresses.[68] The aHUV with a burst pressure of 1,082 ± 113.4 mm Hg has a suitable level of redundancy, although long-term stability is yet to be determined. These results are similar to the canine *ex vivo* collagen with 931 mm Hg,[69] although considerably lower than the porcine small intestinal submucosa (SIS) graft with 3,517 mm Hg.[39] The ability of the processed HUV scaffold (HUVS) to retain sutures under applied force was shown to be greater than comparable polyglycolic acid (PGA) scaffolds deemed acceptable for vascular grafts.[7,10]

After dissection of surrounding matrix, both the aHUV and the processed HUVS have retained mechanical compliance values in the same order as native arteries and preserved the biphasic stress-strain relationship associated with natural blood vessels.[39] Appropriate compliance matching between host artery and prosthetic graft is important to prevent arterial hypertrophy caused by increased local stress at anastomoses.[20] As such, the ideal graft will have a compliance value similar to that of the original vessel. Vessel compliance decreases dra-

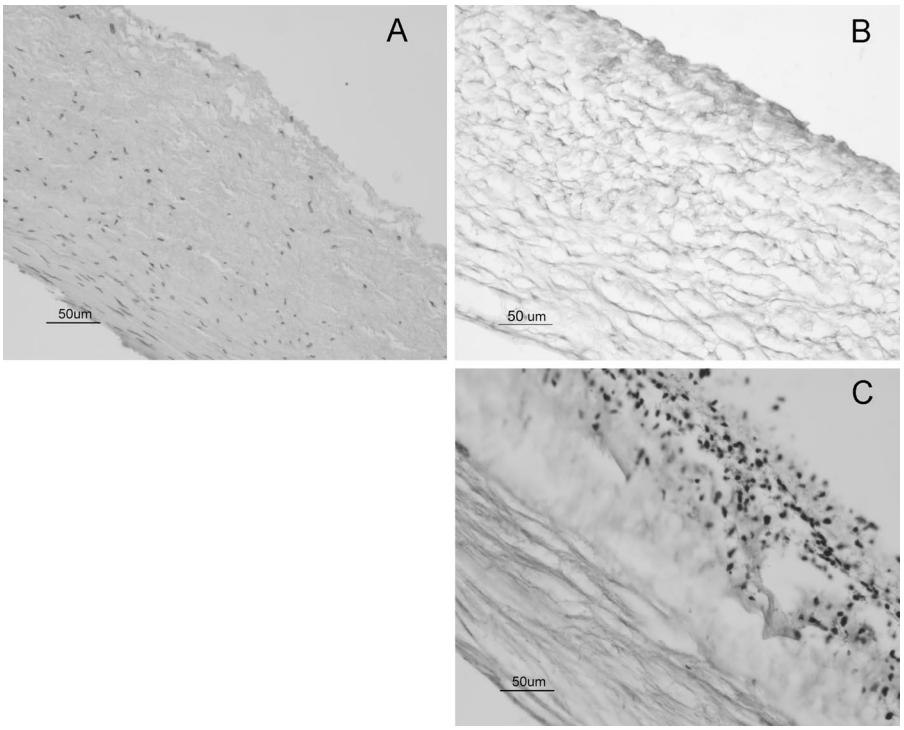

Figure 11. Histologic images. H&E stained images of (A) The native umbilical vein (untreated), (B) the decellularized umbilical vein, and (C) primary human SMC seeded and cultured for 7 days on the abluminal surface showing cellular attachment and migration. H&E, hematoxylin and eosin; SMC, smooth muscle cells.

matically with age, with a loss of up to 60% compliance between ages 30–90, adding to the variability of compliance matching within the expected age range of the patient.[20] Synthetic based polymers often have significantly lower compliance values compared with natural vessels,[18,19] with a compliance value of 0.64% for polytetrafluoroethylene (PTFE).[70] Arndt *et al.*[71] reported a compliance value of 14.7% for the human carotid under normal arterial pressure. Similar to the HUV (5.7 ± 1.3%), the *ex vivo* porcine SIS has a compliance value of 4.6%.[39] Compliance values for PTFE are two orders of magnitude lower than the human carotid, whereas *ex vivo* materials are more comparable to natural, "unprocessed" blood vessels.

An important consideration with regard to compliance and scaffold choice is the change in luminal surface area through diastolic and systolic pressures. Although the diameter changes, the surface area of natural blood vessels does not significantly increase as the cardiac cycle progresses. This is because the naturally convoluted basement membrane (BM) allows the vessel to expand and contract without excessive stretching. By not overly stretching the BM, the adhered endothelium can maintain a competent lining. Recent evidence has shown the importance of mechanically matching the prosthetic with the patient's vessel to minimize hyperplastic responses.[20] As such, synthetics will be designed with increased compliance. If these vessels are designed to promote the development of a competent endothelium and generate native like compliance through the cardiac cycle, a convoluted luminal surface will be necessary to prevent overstretching of adhered cells. If adhered cells do in fact overstretch, then the surface of the prosthetic will contact blood clotting agents during systolic pressures increasing the potential for thrombosis formation and eventual failure.

SDS and ethanol were chosen as representative mechanisms to decellularize the HUV to assess its capacity to undergo tissue processing without altering the vessel's mechanical (or gross biologic) attributes. Although a distinct morphologic change in ECM structure was noted, no significant difference was found in mechanical compliance, burst pressure, or suture retention between the aHUV and the dHUV. Under these conditions, the decellularized HUV scaffold (HUVS) has demonstrated appropriate mechanical characteristics for use as a degradable scaffold in tissue engineering applications. The HUVS has been prepared as a remodelable scaffold, and as such, long-term culture will be required to determine degradation rates and the remodeling and mechanical stability over time.

Our goal has been to develop a biocompatible, cell adhesive, tubular material that has improved mechanical uniformity for vascular tissue engineering applications. By preparing the HUVS with a minimal wall thickness, it is envisioned that seeded VSMC and endothelial cells (EC) (on their respective surfaces) will be within cell to cell communication range (~250 μm),[72] as soon as possible after seeding to speed graft development. Preliminary assessment of cell attachment has shown hVSMC adhesion and maintenance over 7 day culture periods. Further, hVSMC have shown the capacity to migrate from the abluminal surface toward the luminal surface. The ability of hVSMC to migrate through the dense concentric layers of the ECM suggests a rapid remodeling potential of this material. This is not only advantageous, because cellular remodeling optimizes the physical properties, but will promote biologic function and minimize degradation from host bodily fluids.[73,74] As such, we believe that the modified HUVS has the necessary properties for use as a scaffold for small diameter prosthetic grafts. Although the HUVS developed in this study is aimed primarily at vascular reconstructive surgery, its use in other applications, such as vascular access, tissue engineering

the urethra, or as a small tissue patch for wound repair, has not been discounted.

The use of the umbilical cord requires that it be available as a donated tissue source and that ethical consent be obtained. As previously mentioned, the umbilical vein is currently used as a tanned grafting material, confirming commercial availability.[21,23–25,75] As tissue banking expands through increased public awareness, the umbilical cord and other tissues will become more widely available.

This investigation describes a novel preparation and mechanical analysis of the HUV to produce a tubular scaffold for guided tissue regeneration. We have shown the potential of an automated dissection method to eliminate tedious, error prone manual dissection methodologies, where high throughput production with improved safety is possible. Using the tissue engineering methodology, the HUVS has shown potential as a vascular scaffold where the problems of thrombosis, degradation, and neointimal hyperplasia may be minimized through improved biologic integration.[76,77]

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