# A Simple Apparatus and Process to Produce Novel Elastin Biomaterials

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Elastin is the extracellular matrix component responsible for elastic recoil of tissues such as blood vessels, lung, and skin. In our efforts to produce biomaterials for tissue repair, an apparatus and method for processing hot alkali derived porcine carotid elastin was developed. The apparatus and method was designed to remove water and condense the elastin fibrous network to improve mechanical strength and handling of NaOH extracted elastin. To achieve this, a sample holder and process was devised to cure elastin conduits in humidity, and temperature controlled environment in a nitrogen gas enriched atmosphere. Our efforts resulted in a novel biomaterial with significantly improved mechanical properties compared to non-cure processed, hot alkali isolated elastin.

## Introduction

The extraction of soluble a-elastin using hot alkali treatment was first described in 1901[1]. In the 40's and 50's this method was further developed to remove cells and collagen leaving intact elastin fibers [2, 3]. Subsequently, gentler methods to extract the elastin fibers using enzymes such as trypsin, or collagenase were investigated but the success of these procedures are dependent upon enzyme lot [4, 5, 6, 7, 8]. Pure elastin fibers have less mechanical strength than the native vessel because it lacks the collagen that provides strength and durability [9]. Furthermore, investigators found that the 3-dimensional architecture of the elastin fiber structure swells and shrinks depending upon the percent hydration [10, 11]. Additionally it was demonstrated that percent hydration and process method changes the auto fluorescence of elastin as reflected in fluorescent and confocal imaging [12].

In this study, the hot alkali extraction method was revisited and optimized around a specific set of physical characteristics and mechanical test parameters. The hot alkali extraction process typically resulted in a pure elastin fibrous matrix that is mechanically fragile and fully hydrated. Therefore, an apparatus and method for manipulating the mechanical properties of pure elastin conduits by controlled dehydration was developed.

To examine the elastin biomaterial fibers, scanning electron microscopy (SEM) was used. To quantify the biomaterial performance, methods to test the sample for durability, fatigue, strength, and percent water content were employed.

## Materials and Methods

The Elastin Heterograft 1 (EH1) conduit was produced by extracting a 3-5 cm length of intact porcine carotid artery. Arteries retrieved from the abattoir were trimmed of adherent fat and placed into 80% ethanol solution to extract lipids. Collagen and cells were extracted using hot alkali and mild ultra sonication method developed by Crissman [6]. This process yielded a pure elastin matrix as confirmed by amino acid analysis. The resultant elastin

conduits were processed for histology and stained to determine elastin purity, fiber size, orientation, and fragmentation. Specifically, H&E was performed to detect remaining cells, nuclei or cellular debris post extraction. VVG, Trichrome and Movat were utilized to detect extracellular matrix proteins including elastin, collagen, and fibrin.

To produce the Elastin Heterograft Cured (EH1C), the EH1 samples were tied onto nylon barb connectors. The connections were leak tested and subsequently couple to a manifold assembled from DIG drip irrigation system components, including 2-adjustable six zone drip heads, each of which may be adjusted to any flow rate between 3.7 - 74 liter/hr, 1/2" riser adaptor with 1/4" barb, 2-1/2" riser adapters and silicone tubing as shown in figure 1. The manifold was attached to a circulation pump (Tetra-Tec Air Pump 200) with silicone tubing to circulate humidified air through the lumen from each end simultaneously as shown in figure 1. This slightly expanded the sample. Humidified nitrogen rich air was circulated through the lumen of the samples from either end simultaneously to control the dehydration rate and ensure even curing. Oxygen was replaced with nitrogen in the humidified glove box chamber to reduce the potential for oxidation reactions. Flow was measured and standardized for all samples with a flow meter prior to curing. The samples were left to cure for 8 to 16 hours as illustrated in figure 2.

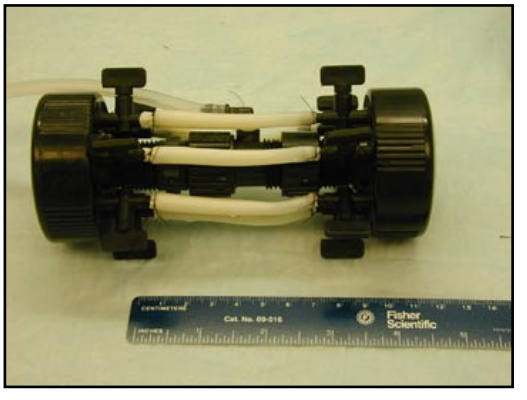


**Figure 1: Elastin conduits mounted on curing manifolds**

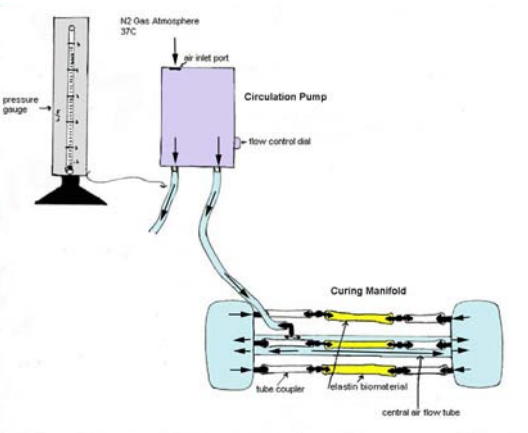


**Figure 2: Diagram illustrating curing manifolds attached to circulation pump and pressure gauge with air-flow path indicated with black arrows**

### *Material Testing*

### *Hydration*

Water content analysis was performed using a CompuTrac Vapor Pro water vapor analyzer. Water content was determined for each EH1C (Elastin Heterograft 1 Cured) pull test sample. Small millimeter ring samples (1-3mm width) were cut from each EH1C sample, weighed, and placed in a clean and dry sample jar with a rubber sealed lid. Via a small hole-punch in the rubber cap, the Vapor Pro measures the percent water content of the sample based on water content and mass of the sample.

### *Tensile Testing*

EH1 and EH1C vessels were cut into dumbbell shaped specimens in the longitudinal direction with a dumbbell cutter as shown in figure 3. The specimens' gauge length was 5 mm with 3 mm grip ends. The thickness and cross-sectional gage length were measured and recorded.

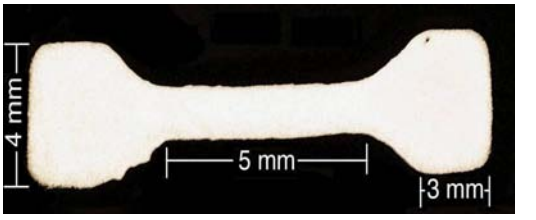


**Figure 3: Typical dumbbell shaped specimen**

The Chatillon Vitrodyne V1000 system with a 500 g load cell was used for mechanical testing. Samples were tested for stress and strain in tension at a crosshead speed of 5 mm/s until failure. The EH1Cs were rehydrated for 15 minutes before testing. These specimens were kept hydrated with saline during the test in ambient temperature. Force and displacement measurements were acquired at 0.1 s intervals. Engineering stress (force/cross-sectional area, F/Acs) and strain (change in length/original length, $\Delta L/L_o$) were calculated and plotted (fig. 4). Linear regression of the slope in the stress-strain plots was used to calculate the elastic modulus (stress/strain, $\sigma/\varepsilon$). Peak stress and strain were taken as ultimate tensile strength and strain.

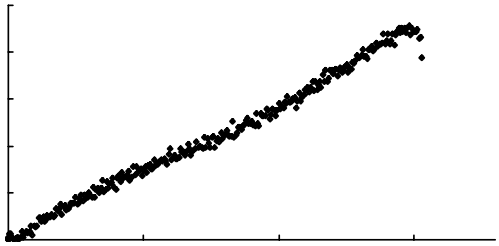

**Figure 4: Stress-strain plot example of a hydrated EH1C sample**

Ultimate tensile strength, ultimate strain, and elastic modulus were analyzed by using a two-tailed unpaired student's t-test to determine any statistically significant differences between the groups.

### *EnduraTech fatigue testing*

To test the endurance of the EH1C'c under physiological conditions, we utilize the Endovascular Stent and Graft Tester (Series 9100, EnduraTech Corporation). Speakers create a sine wave to pulse vessels at a designated frequency, either at 1 Hz (one pulse per second) or at an accelerated rate of 30 Hz. An external pressure head creates a physiological pressure of 120/80. A laser measured vessel diameter during cycling. Enduratech data was acquired over 2 weeks for all frequencies.

### *Histology*

Histology was performed at the Armed Forces Institute of Pathology. Movat, VVG, and H&E stains were employed to determine decellularization and fiber degradation. SEM was performed at Lewis and Clark College using previously established methods.

## Results

### *Histology results*

Hematoxylin and eosin staining results indicated that all cell and cellular debris including nuclei was removed.

Results of SEM imaging of EH1 and EH1C samples show that the elastin fibers of EH1C samples compacted due to curing as compared to a non-cured sample (fig. 5). Additionally, under epi-fluorescent and confocal microscopy, it was observed that the auto-fluorescence of cured elastin is altered as compared to non-cured elastin (data not shown).

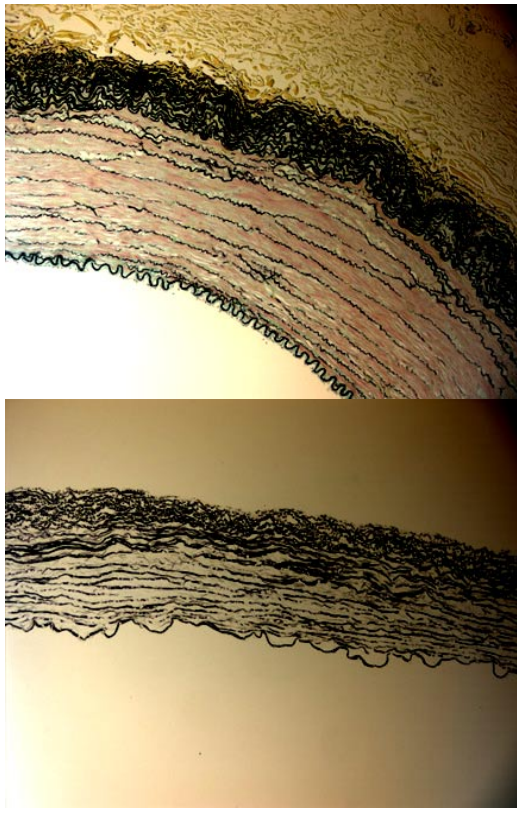

**Figure 5: Porcine carotid artery pre (top) and post (bottom) extraction. Movat stain Elastin stains black**

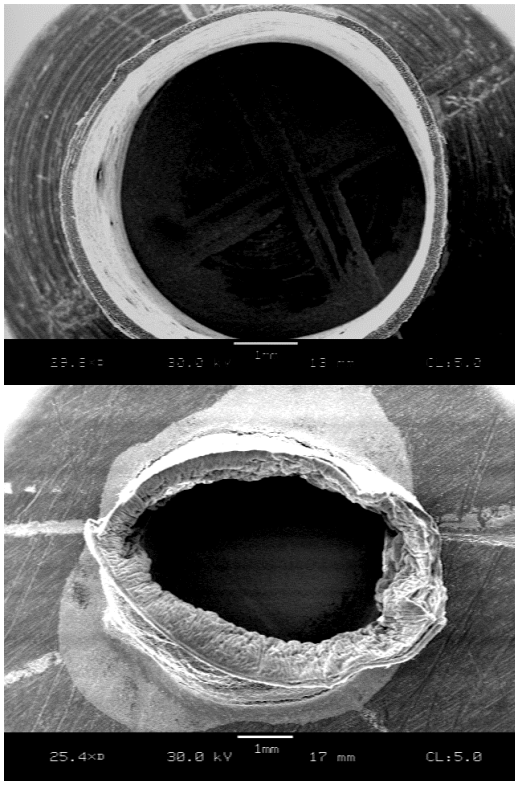


**Figure 6: SEM top down view of cross section of distal portion of uncured elastin conduit EH1 (left). SEM top down view of cross section of distal portion of cured elastin conduit EH1C (right). Note the reduction in wall thickness caused by compaction of the elastic fibers indicated by the arrows. Bar 1 mm**

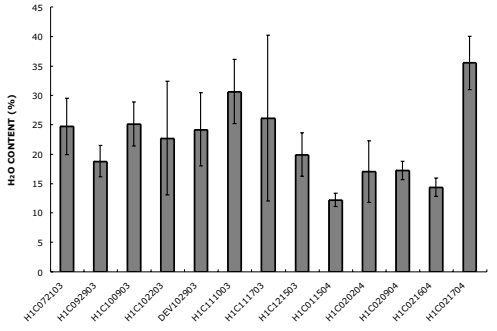


**Figure 7 Percent water content of 13 batches of cured elastin conduits**

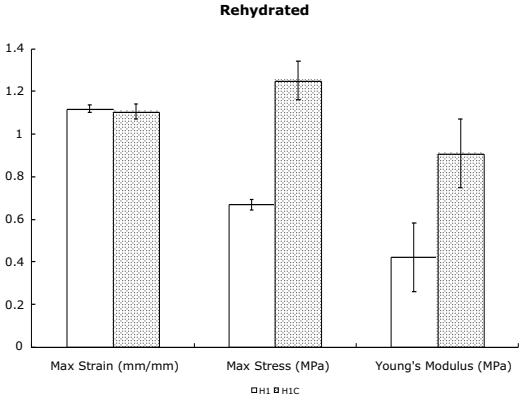


**Figure 8 Ultimate strain, stress and Young's modulus data of cured EH1C (grey bar) and non-cured EH1 elastin (white bar). Although the maximum strain remains unchanged, curing significantly increases the maximum stress and Young's modulus ($p<0.01$).**

### *Mechanical test results*

The percent water content for the cured elastin heterograft samples ranged from 12.2 to 30.6 for all batches tested as shown in figure 7.

The ultimate stress, strain and Young's modulus of non-cured elastin and cured elastin is plotted in figure 8.

The ultimate strain, ultimate tensile stress, and Young's modulus of the non-cured elastin were 1.1±0.02, 0.7±0.03 MPa, and 0.4±0.16 MPa, respectively and cured samples were 1.1±0.03, 1.25±0.09, and 0.91±0.16. Cured samples were re-hydrated prior to testing and there was an average increase of 45% in ultimate tensile strength and 46% increase in elastic modulus compared to non-cured elastin. There were significant differences ($p<0.01$) between the cured and non-cured elastin with the ultimate tensile strength and Young's modulus. No significant differences ($p>0.05$) were found with the ultimate strain. Finally, EH1Cs were fatigue tested with the Enduratech system up to 1.4 million cycles at 1Hz and over 35 million cycles at 30 Hz at a pressure of 120/80 mmHg. Fluid loss was measured at 1 Hz and found that while the three vessels initially lost fluid at a rate of over 21ml/hr/vessel, after 3 days the loss was down to 12.7ml/hr/vessel. No difference in the fatigue profile of the EH1Cs tested at 1 Hz or 30 Hz was found, and none of the vessels burst during testing.

## Discussion

One advantage of using drip irrigation system components is that they are designed to be used with water, thus are suitable for a humid environment and do not rust or corrode. Another advantage of this design is that 6 samples may be mounted onto each manifold and cured simultaneously, thus reducing variability in sample processing. A single pump can process two manifolds each for 12 samples total. Four pumps have been utilized with 2 manifolds each. Thus, 48 samples per run were processed. The entire apparatus was sterilized using Sterrad sterilization and can withstand many sterilization cycles.

In our efforts to produce a pure elastin conduit, we have developed a method and process that resulted in a biomaterial with distinct physical properties as reflected in the SEM images and mechanical test results. Histological data indicate that all cellular debris was removed with the digestion process and there was a significant change in the fiber structure of the elastin due to the curing process as compared to uncured elastin. The fibers of the cured elastin were much more compact and there appeared to be less space between individual fibers. Further image analysis may help to quantify this difference in fiber arrangement. Results from the mechanical testing data indicated that the curing process improves the ultimate stress of the elastin as demonstrated both with and without sample rehydration. Our ultimate stress results were comparable to Fung's results of 0.3 to 0.6 MPa [13]. Additionally, the results of the ultimate strain data, 1.1 for both rehydrated cured and uncured samples are comparable to that reported by Lillie's group of 1.2. Lillie's group tested rings of aortic elastin circumferentially whereas we tested carotid elastin longitudinally [11].

There is inherent variability between biological samples as well as within each sample that contributes to differences in mechanical strength. Furthermore, the variability in the mechanical data of the cured elastin as compared to the non-cured elastin is reflected in the percent water content. The percent water content of the cured elastin varies within batches and between batches demonstrating the need to correlate the mechanical properties to the percent hydration of the samples. More data will be collected to correlate mechanical properties to percent hydration and sample process parameters will be fine tuned for improved reproducibility. Enduratech tests demonstrated that samples tested at 120/80 mmHg held for 35 million cycles. Fatigue testing of elastin demonstrated that cured elastin withstands physiologic pressures. Furthermore, the EH1C conduits have been tested in a rabbit model for urethra repair and as a scaffold for a tissue-engineered artery [14]. Results from these preliminary experiments indicate the potential uses of this biomaterial.

## Conclusion

We have developed a process that removes water from extracted elastin conduits to produce a pure elastin biomaterial with improved handling characteristics. Our unique biomaterial processing apparatus was designed to improve the material properties and handling characteristics of biomaterial conduits by controlled dehydration. This novel apparatus provides a simple means to process biomaterials for applications where a small diameter tube is required such as vascular or urethra repair.

Synopses: We have developed a process that removes water from NaOH extracted elastin conduits to produce a pure elastin biomaterial with improved handling characteristics.

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