CHARACTERIZATION OF COLLAGEN PRODUCTION AND ACTIVE REMODELING PRESENT IN ENGINEERED ARTERIES CREATED BY DIRECTED REMODELING

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INTRODUCTION

There is a sizable unmet demand for effective small-diameter vascular prostheses for use in coronary bypass surgery. Currently, the best replacements for occluded arteries are autologous arteries, which have a cumulative patency rate of up to 93% after 5 years [1], however their availability is limited. Donor veins of appropriate dimensions are more available and frequently used despite substantially lower patency (45% after 5 years) [1]. To address this shortage, many groups have created tissue-engineered blood vessels (TEBVs) using various techniques that grossly resemble native vessels, but in animal studies, their performance was inferior to that of autologous veins [2]. Roughly 50% of TEBVs had decreased patency in 1 month, compared with 19% of vein grafts in 1 year [3]. We propose that intact autologous arteries, not the cells derived from them, may be an attractive starting point for engineered arteries, capitalizing on the ability of intact arteries to grow and remodel in response to changes in their mechanical environment.

Previous results showed that arteries from neonatal pigs (5 kg) were able to elongate 100% in 9 days, retaining a 65% increase upon removal from the system, but they were not viable [4]. Viability improved when using arteries from juvenile (30 kg) and adolescent (100 kg) pigs, while retained elongation (20%) was possible only with juveniles [5]. Special emphasis is made on characterizing the major components of this length and mass increase, such as collagen.

MATERIALS AND METHODS

The steady flow perfusion system consisted of a peristaltic pump, compliance chamber, artery chamber, and reservoir, all connected using Tygon laboratory tubing. Pressure was determined in real time with transducers upstream and downstream from the artery and acquired using a LabView. Gas exchange was provided to both the artery chamber and reservoir via 5% CO_2 bubbling chambers. The entire system, except for the pump, was maintained in a dark, 37^oC environment. All components were sterilized and assembled sterilely. For some experiments the steady flow system was modified to sustain pulsatile pressure with control over mean pressure and amplitude.

Arteries from juvenile pigs (30-kg) were harvested by cardiothoracic surgeons at the Children's Hospital of Philadelphia and transported in ice-cold culture medium (Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin), while arteries from adolescent arteries were obtained from freshly sacrificed animals from a local abattoir. Upon arrival, arteries were cleaned of excess adventitia and connective tissue, and samples were taken for histology and mechanical evaluation. Arteries were installed into the artery chamber by cannulation of the artery onto stainless steel rods via silk sutures, where the outer diameters of the rod and artery roughly matched.

The initial extension ratio (λ) was determined for each sample (1.47 ± 0.03), and each artery was installed at this length. The chamber was then filled with culture medium until the artery was submerged (~200 mL), connected to the perfusion system containing ~500 mL of culture medium and perfused using ~10% of in vivo flow rate. These conditions improved viability in neonatal and adolescent arteries perfused at fixed length [6]. Arteries were acutely stretched 8.3%/day from day 2 to 7, and held at constant length otherwise. Control juvenile arteries were perfused at physiological length for 9 days.

Samples from select freshly harvested and cultured arteries were evaluated using an Instron 5543 system at the McKay Orthopedic Research Lab (Dr Louis Soslowsky). Samples were tested at room temperature while hydrated with Ca free PBS.

RESULTS

A total of 14 carotid arteries from juvenile pigs were perfused and either elongated (n = 8, "elongated arteries"), or used as controls (n = 6). The average elongation attained within the system was 48.1 ± 2.8%, and 20.5 ± 3.3% upon removal as seen in figure 1 below. None of the control arteries retained a length increase. One artery was removed from the perfusion system on day 7 due to a leak. One artery failed on day 3 for technical reasons, while one artery failed on day 4 for no apparent reason. One control was removed on day 7.

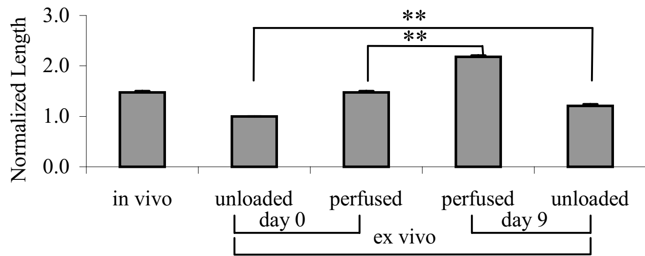

Figure 1. Juvenile artery length (n = 6, ** p<0.005))

The wall thickness of control arteries was ~40% less than elongated and fresh arteries ($p < 0.005$), whereas elongated arteries were similar to freshly harvested specimens. This decrease is consistent with in vivo experiments that showed a direct relationship between decreased transmural pressure and arterial thickness [6]. These data presented indicate that increased axial strain is sufficient to retain arterial thickness even in conditions that normally cause a substantial loss.

The wet weight of elongated arteries increased 39.9 ± 18.4% after elongation, while the dry/wet weight ratio and collagen content per dry weight remained similar for all three cases. Overall collagen content increased 34% in elongated arteries, while controls saw no increase.

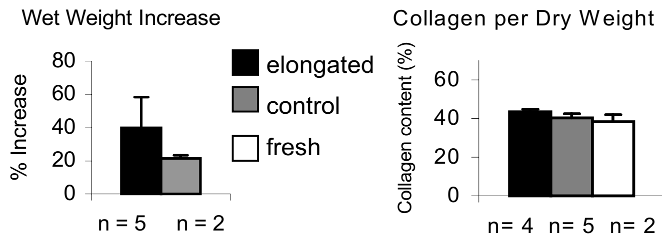

Figure 2. Juvenile artery wet weight and collagen content

Staining of select elongated and fresh juvenile arteries for elastin was performed using the Elastic stain (Sigma), and also autofluorescence. Elongated arteries showed substantial elastin content, which was somewhat fragmented and less organized than fresh samples. Studies are underway to quantify elastin content for all elongated, control and fresh arteries, as well as assessing elastin alignment and arrangement.

Immediately prior to removal from the perfusion system on day 9, three elongated arteries were tested for vasoactive response to norepinephrine (NE) and sodium nitroprusside (SNP), by measuring the pressure drop across the arteries over time. Addition of NE (1×10^{-6} M (n = 2) or 1×10^{-4} M (n = 1)), caused a pressure increase of 52.7 ± 30.3 mmHg in 14.8 ± 4.1 minutes. Addition of SNP (1×10^{-4} M) caused a decrease of 38.6 ± 16.0 mmHg in 6.8 ± 0.4 minutes.

Three arteries from adolescent arteries were elongated 50% under similar conditions but all three failed prior to completion. Other arteries failed when attempted to elongate 100% in 9 days (n = 1) or 27 days (n = 3). Three arteries remained viable after 33% elongation in 9 days (n = 1) or 27 days (n = 2), but retained no length increase.

The cellularity, structure and viability of freshly harvested, control, and elongated juvenile and adolescent arteries were similar, as assessed in histological sections stained with H & E, PCNA and TUNEL. The majority of arteries had good endothelial coverage, while two elongated and one control artery were mostly denuded. TUNEL staining of fresh, elongated and control samples revealed minimal cell death, unless the artery was denuded. This was consistent with previous findings that denuded arteries experienced progressive cell death, irrespective of elongation procedures [7]. Viability was retained as shown by an MTT index of 1.49 ± 0.25 for elongated arteries, and 1.34 ± 0.36 for controls, where the index is the ratio of relative mitochondrial activity of cultured to fresh arteries.

The ultimate stress and strain properties were determined for juvenile arteries as shown below. While not significant, there was a substantial decrease in circumferential stress for elongated arteries.

	Fresh arteries	Elongated arteries	Control arteries
AXIAL	(n = 9)	(n = 4)	(n = 5)
Ultimate stress (MPa)	1.41 ± 0.13	1.39 ± 0.21	2.11 ± 0.10
Ultimate strain (%)	94.1 ± 7.67	121 ± 12.9	115 ± 9.53
CIRCUMFERENTIAL	(n = 3)	(n = 4)	
Ultimate stress (MPa)	1.98 ± 0.46	0.87 ± 0.09	N.A.
Ultimate strain (%)	106 ± 3.70	89.9 ± 23.6	N.A.

Table 1. Juvenile artery ultimate mechanical properties

DISCUSSION

To better assess the phenomena present in elongated arteries, further work is being performed to identify and quantify molecular markers of remodeling, such as MMP2 & 9 (both cleave collagen I), using RT-PCR and staining techniques. Additional studies are planned to use gene arrays and cluster analysis to analyze the response of the arteries to the changes in axial strain and mechanical stimuli (pressure, flow rate). Studies are also underway to investigate extracellular matrix components, such as glycosaminoglycans (GAG) and DNA content to quantify and assess the observed tissue growth in elongated arteries.

We have performed preliminary experiments using a more active mechanical environment, specifically in vivo pulsatile pressure (~100 ± 20 mmHg) and viscosity (~4 cP) with increased flow rate (~100 ml/min). Our hypothesis is that a more mechanically active environment will decrease the negative remodeling associated with reduced flow and pressure. While initial results show that the arteries are viable and histologically similar to fresh arteries, further testing is required to determine how the remodeling compares to previous work.

Further studies are being performed to compare the method by which arteries are elongated. Current techniques use a manual step loading approach, which is likely non-physiological. A prototype has been built to continuously elongate arteries including quantification and feedback of axial force. The prototype is being evaluated to investigate the differences in the remodeling response between continuous strain rate, step increases in strain, as well as constant force elongation.

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