5-2018

The Effect of Elastin Degradation on the Biomechanical Properties of Porcine Aortic Tissue

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An Undergraduate Honors Thesis

in the

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Abstract

The rupture of abdominal aortic aneurysms (AAA) is currently the 13th leading cause of death in the United States [1]. AAA is characterized, in part, by an increase in presence of proteolytic enzymes which degrade the structural proteins collagen and elastin [6]. The goal of this study was to examine the effect that elastin degradation has on the biomechanical properties of aortic tissue. For this experiment, porcine aortic tissue was cut in the circumferential and longitudinal directions. These specimens were exposed to an elastase solution for varying time-intervals and then underwent uniaxial tensile testing. Elastase-treated tissue tested in the longitudinal direction appears to exhibit strain-stiffening behavior at lower strain values when compared to the control. Additionally, elastase exposure appears to lower ultimate tensile strength for these same sample groups. Conclusions cannot be made on elastase's effect on aortic tissue in the circumferential direction as the elastase concentration used was insufficient to degrade the higher number of circumferentially oriented fibers present in arterial tissue.

I. Introduction

Abdominal aortic aneurysms (AAA) can be described as the focal widening of the abdominal aorta, and rupture occurs when wall stress exceeds wall strength at any point [3]. The rupture of AAA is the 13th leading cause of death in the U.S. and has a mortality rate of 90% [1, 2]. Surgical intervention to repair AAA is an invasive procedure that requires an extended hospital stay and is associated with prolonged postoperative pain [1]. Furthermore, most patients with AAA are elderly and experience greater risks when undergoing surgery due to comorbid conditions [1]. Thus, to effectively manage a patient with AAA, physicians must weigh the risk of rupture against those risks associated with surgery [3].

The current criterion for assessing rupture-risk is based on maximal diameter. If a particular aneurysm grows beyond 5.5 cm, surgical intervention is recommended [3]. The 5.5cm criterion is based on the Law of Laplace, which states that for a perfect cylinder or sphere, as radius increases, so does pressure. However, the geometries of AAAs are much more complex than a simple cylinder, displaying a high level of tortuosity. This tortuosity results in complex stress distributions that vary throughout the length of the abdominal aorta [3]. Because of this, the diameter criterion is unreliable and smaller aneurysms sometimes rupture while larger aneurysms frequently do not [4, 5]. Therefore, the need exists for an improved method of assessing rupture-risk so that physicians may make better informed decisions regarding surgery.

Collagen and elastin are the two main structural proteins which comprise the aortic wall. Elastin fragmentation is associated with aneurysm dilatation, while collagen degradation is thought to be primarily responsible for aneurysm rupture [1]. Throughout the progression of most AAAs, the collagen matrix undergoes remodeling in response to increased stress and ECM fragmentation

while the elastin matrix remains degraded [1]. This collagen remodeling varies significantly from patient to patient [1]. These variations in protein degradation and collagen remodeling may indicate why some aneurysms rupture while others remain intact.

AAAs are characterized by an increase in the presence of matrix metalloproteinases (MMP) which degrade collagen and elastin throughout the aorta [6]. While the mechanism is not fully understood, both stress and hypoxia play a role in upregulating the expression of these enzymes [3]. For example, focal stress concentrations result in saccular outpouchings characterized by low procollagen and elastin content [3]. Additionally, elevated stress has been shown to increase matrix metalloproteinase production in smooth muscle cells, fibroblasts, and macrophages present on the aortic wall [7-9]. Hypoxic conditions, imparted by the presence of an intraluminal thrombus, further stimulate the release of MMP-7 and elastase [3]. As stress and hypoxia vary locally throughout any given AAA, so do levels of protein degradation.

The purpose of this study is to elucidate the effect that elastin degradation has on the biomechanical properties of aortic tissue. Porcine aortic tissue samples will be exposed to time-dependent treatments of elastase. These tissues will then undergo uniaxial tensile testing. The specific aims of this experiment are twofold: 1.) demonstrate via stress-strain curves how the biomechanical properties vary as a function of protein degradation, and 2.) generate data which may provide a range of material properties for analysis in modeling programs such as finite element analysis.

II. Materials and methods

Sample preparation

Porcine aortas were obtained, and samples were cut into segments of approximately 80mm x 15mm in both the circumferential and longitudinal directions. Fat tissue was removed with a scalpel. Tissue samples were then frozen until the experiments were run.

Enzymatic degradation

Two aliquots of 20 mg purified elastase having an activity of 8.79 units/mg was added to two centrifuge tubes containing 30 ml HBSS to achieve a concentration of 5.86 units/ml. Additionally, two centrifuge tubes were prepared containing only 30 ml HBSS to serve as a control. Eight samples cut in the circumferential direction were added to one tube containing elastase and to one containing only HBSS. The same was done for samples cut in the longitudinal direction. The centrifuge tubes were then placed on a shaker at 37°C and 245 rpm. At t= 4, 8, 12, and 16 hr, two samples were removed from each centrifuge tube and tested.

Mechanical tensile testing

Uniaxial mechanical tensile testing was performed using Instron technology. Tissue specimens were mounted with approximately 25 mm of tissue length clamped in each grip. Grips were jogged until the specimens were extended but experiencing minimal tension. Length, width, and thickness measurements were taken with electronic calipers. Three measurements were taken

for thickness, and the average was used. Tissue specimens were preconditioned to 20% strain for 20 cycles. The tissues were then lowered into a Biopuls bath heated to 37°C and containing 1X PBS. The mechanical tensile test was then run using Bluehill 3 software. Grips were cleared of any tissue debris between tests. Stress and strain measurements were exported, and stress-strain curves were formed in Excel.

III. Results and Discussion

Uniaxial mechanical tensile testing yielded significantly different results for tissues tested in the longitudinal and circumferential directions. For the longitudinal experimental group, strain-stiffening behavior was observed at much lower strain values when compared to the control. The linear region for all elastase time-exposure groups ended between .17 and .25 mm/mm, while the linear region for the control group ended at approximately .35 mm/mm (Fig.1).

This shift in strain-stiffening is likely caused by the tri-helical nature of collagen. Collagen is composed of three peptide chains which interweave to form a helix. At low strains, the helix is uncrimped, or loose, and provides little resistance to deformation. At higher strains, the helix tightens and an increase in stiffness is observed [11]. Thus, collagen microstructure is responsible for the strain-stiffening behavior characteristic of blood vessel tissue. As elastin is degraded, collagen must begin bearing load at lower strain values. This results in strain-stiffening occurring earlier in the loading cycle.

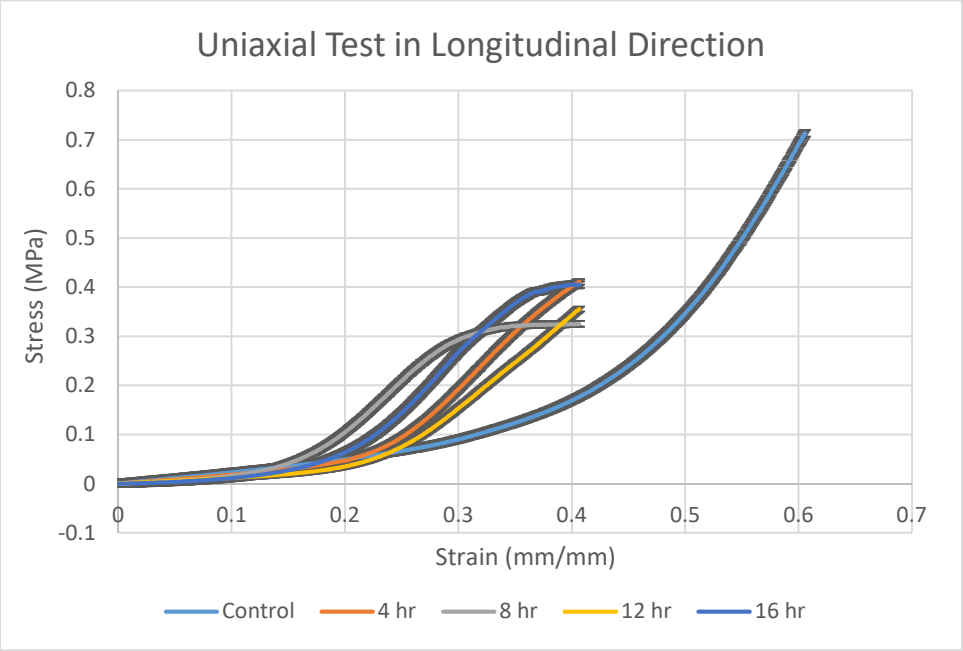

Fig. 1. Uniaxial mechanical tensile test results for elastase-treated porcine aortic tissue tested in the longitudinal direction

It would be expected that higher times of exposure to elastase would result in shorter linear regions; as more elastin fibers are degraded, collagen fibers would begin bearing load at earlier strain values. Thus, the 16 hr treatment should result in the shortest linear region, while the 4 hr treatment should have the longest. However, increasing time exposure did not result in a sequential decrease in linear region length. The 12 hr treatment exhibited the longest linear region, followed, in order, by 4 hr, 16 hr, and 8 hr treatments (Fig. 1). It is possible that these results occurred because too high of an elastase concentration was used, and complete elastin degradation was achieved. The differences in strain-stiffening behavior would then simply be a consequence of natural variances in tissue biomechanical behavior. Increasing sample size while lowering elastase concentration would correct for this and improve the probability of the expected results being observed.

Interestingly, a similar change in strain-stiffening behavior was not observed for elastase-treated tissue tested in the circumferential direction. The linear region for all treatments as well as the control ended at approximately .33 mm/mm (Fig. 2). This absence of change in strain-stiffening behavior could be due to variations in elastin fiber orientation, which has been shown to be a function of depth. The inner arterial media is characterized by an even distribution of circumferentially and longitudinally oriented elastin fibers, while the outer media contains elastin oriented in the longitudinal direction [12]. The bulk of the media, however, is comprised of elastin fibers oriented circumferentially [12]. Thus, it would stand to reason that higher concentrations of elastase solution would be required to adequately degrade the higher number of circumferentially oriented fibers found deep within the arterial wall.

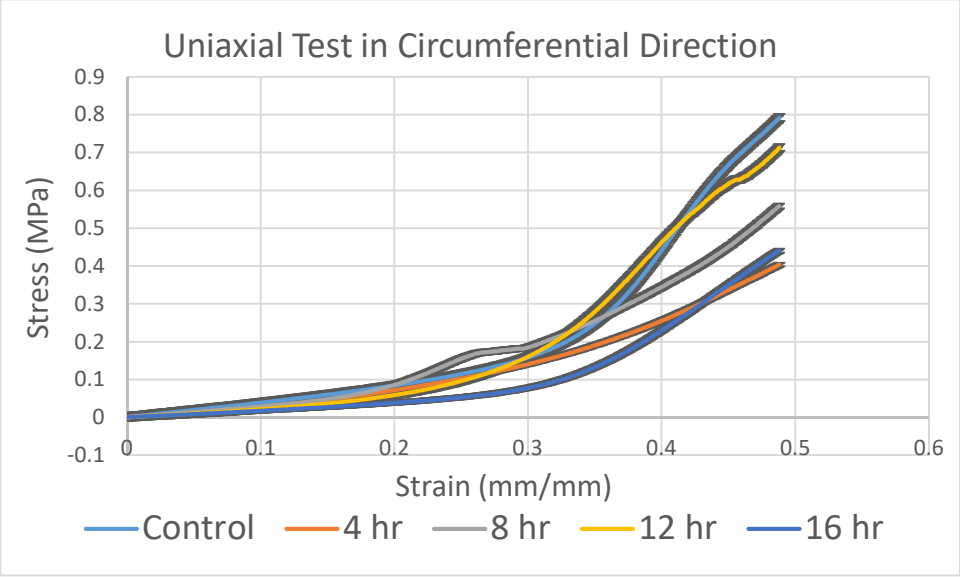

Fig. 2. Uniaxial mechanical test results for elastase-treated porcine aortic tissue tested in the circumferential direction

Ultimate tensile strength data was also collected from tissues tested in the longitudinal and circumferential directions. All four treatment groups appear to have a much lower ultimate tensile

strength than that of the control group (Table 1). The control group's average was approximately 0.7 MPa greater than the 4 hr and 8 hr groups and about 1.06 MPa greater than the 12 hr and 16 hr groups. This decrease in ultimate tensile strength in response to elastin degradation is to be expected. In healthy tissue at high strains, both elastin and collagen fibers are bearing load. As elastin fibers are degraded, the load-bearing ability of the extracellular matrix is partially compromised, and rupture should happen at lower stresses. A two-sample t-test was run. In reference to the control, p values for the 4, 8, 12, and 16 hr treatments were .06, .2, .04, and .02, respectively.

Table 1. Ultimate tensile strength in the longitudinal direction

Sample	Ultimate Tensile Stress
Control 1	1.636
Control 2	1.478
Mean	1.557
4 hr 1	0.928
4 hr 2	0.688
Mean	0.808
8 hr 1	0.410
8 hr 2	1.352
Mean	0.881
12 hr 1	0.633
12 hr 2	0.416
Mean	0.5245
16 hr 1	0.419
16 hr 2	0.480
Mean	0.4495

Ultimate tensile strength data was inconsistent for sample groups tested in the circumferential direction (Table 2). The fact that no change in strain-stiffening was observed for the circumferential experimental group seems to indicate that little elastin degradation occurred. If insufficient elastin degradation was achieved, then little variation between ultimate tensile strengths should be expected. This was not the case, as there was a 1 MPa difference between the control group and the 16 hr treatment group (Table2). Variation between the control, and the 4 hr and 12 hr treatments was also observed, but to a lesser extent. These discrepancies between the different groups likely resulted from natural variance and were magnified due to the small sample size. Another two-sample t-test was run which confirmed this hypothesis. In reference to the control, p values for the 4, 8, 12, and 16 hr treatments were .20, .63, .29, and .10, respectively.

Table 2. Ultimate tensile strength in the longitudinal direction

Sample	Ultimate Tensile Stress
Control 1	1.962
Control 2	1.310
Mean	1.636
4 hr 1	1.311
4 hr 2	0.745
Mean	1.028
8 hr 1	2.022
8 hr 2	1.584
Mean	1.803
12 hr 1	1.633
12 hr 2	0.885
Mean	1.259
16 hr 1	0.675
16 hr 2	0.651
Mean	0.663

It appears that elastin degradation affects strain-stiffening behavior in the longitudinal direction while leaving stiffness unaffected (Fig. 1). This lack of correlation between elastin degradation and tissue stiffness appears to conflict with a study performed by Wilson et al. that examined the relationship between circulating markers of elastolysis (SEP and E-AT) and tissue distensibility. This study found that as markers of elastolysis increased, tissue stiffness decreased (Fig. 3 and 4) [14].

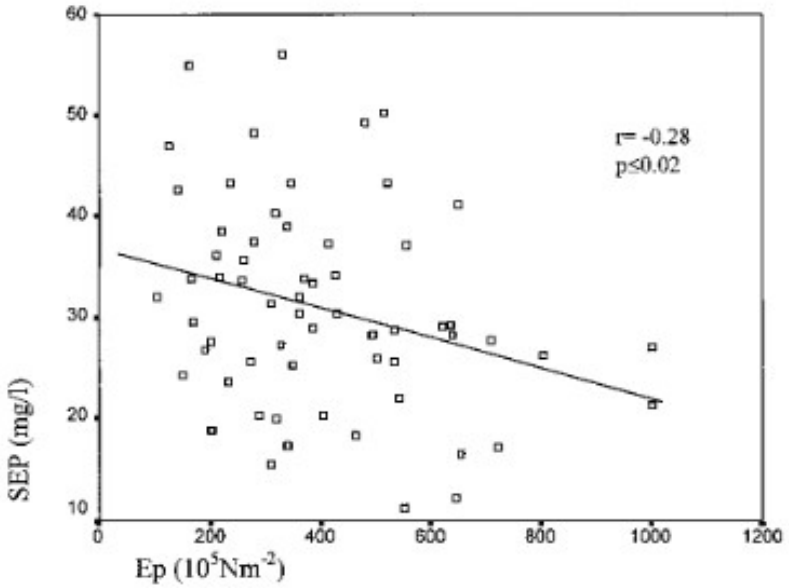

Fig. 3. Relationship between SEP levels and Ep [14]

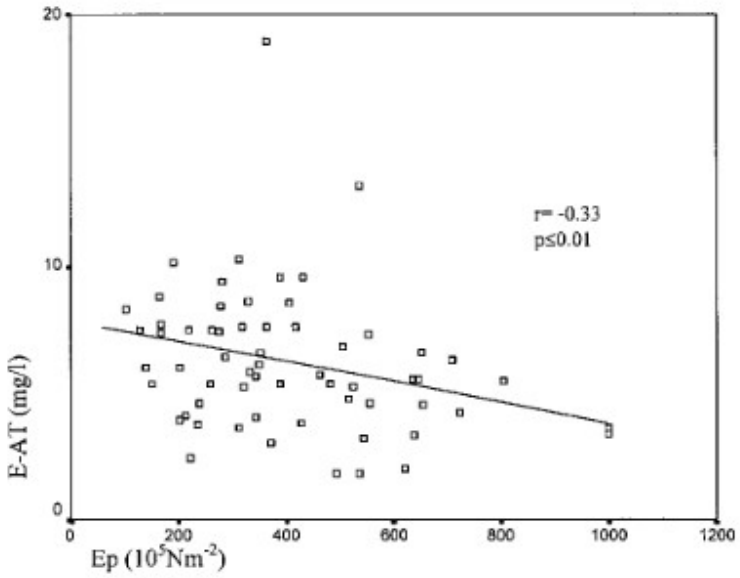

Fig. 4. Relationship between E-AT and Ep [14]

As the stress-strain elastic modulus data was based on in vivo measurements obtained from a 3.5 MHz linear array transducer, its accuracy may be questioned due to the high intra-observer variability associated with this device [15]. This could explain the apparent discrepancy between this study and the one carried out by Wilson et al.

Abdominal aortic aneurysms are further characterized by the degradation of collagen which varies significantly from patient to patient [10]. Alfaori et al. performed an experiment of similar design to this one in which porcine aortic tissue underwent collagenase time-dependent treatments and biaxial mechanical tensile testing. Their results demonstrated an inverse relationship between time of collagenase exposure and tissue strength (Fig. 5) [13]. While it appears that elastin degradation affects strain-stiffening behavior, collagen degradation has been shown to decrease tissue stiffness at higher strain values.

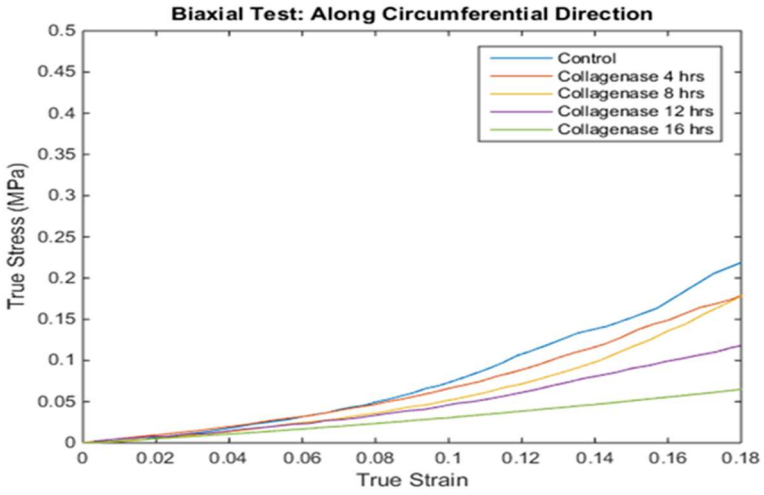

Fig. 5. Biaxial tensile test results for collagenase-treated porcine aortic tissue along the circumferential direction [13]

After biaxial testing, material properties were obtained from this stress-strain data and applied to an abdominal aorta modeled in finite element analysis. Collagen-degraded material properties resulted in higher strain values at lower stresses when compared to those values obtained via the material properties derived from healthy aortic tissue (Fig. 6 and 7) [13].

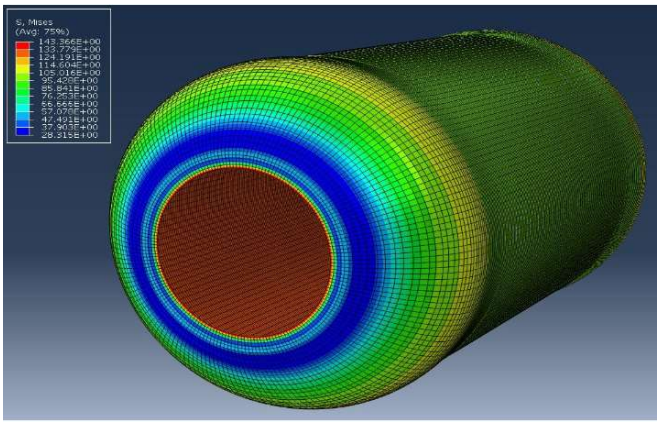

Fig. 6. Stress distributions for healthy aortic tissue modeled in finite element analysis [13]

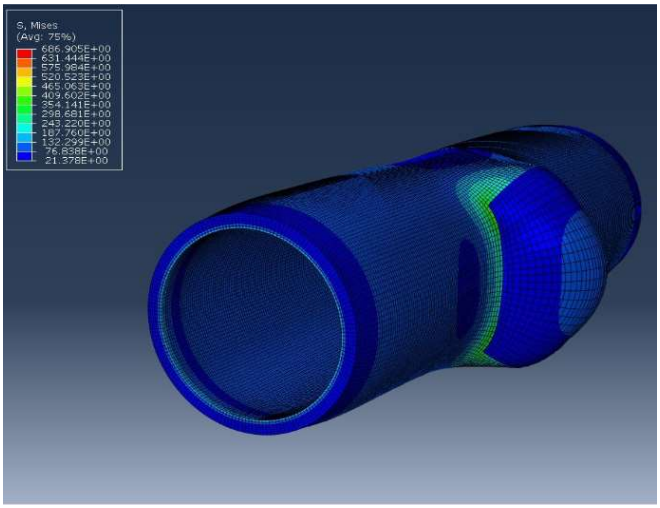

Fig. 7. Stress distributions for aortic tissue containing a collagen-degraded segment modeled in finite element analysis [13]

IV. Conclusion

Abdominal aortic aneurysms are characterized by varying levels of elastin degradation. Elastase-treated porcine aortic tissue tested in the longitudinal direction exhibited strain-stiffening behavior which occurred at lower strain values when compared to the control. This change in strain-stiffening location is most likely a result of previously un-recruited collagen fibers bearing load at lower strain values. Similar results were not observed for tissue tested in the circumferential direction. This lack of change in mechanical behavior could be due to the higher number of circumferentially oriented elastin fibers located in the media of the aortic wall. Longitudinally-tested specimens also appeared to have a lower ultimate tensile strength than the control, however, the results were only statistically significant for the 12 hr and 16 hr treatments. The ultimate tensile strength results were inconsistent for tissue tested in the circumferential direction, which was likely a product of natural variance magnified by the small sample size that was available.

Future research should focus on the combined effect of collagen and elastin degradation on the biomechanical properties of aortic tissue to better mimic in vivo conditions. A potential experiment could involve exposing test groups to varying concentrations of both collagenase and elastase. Uniaxial or biaxial mechanical testing could then be performed and the material properties derived. This data could then be incorporated into a modeling program such as finite element analysis, which can be used to model abdominal aortic aneurysms. The effect that local variations of collagen and elastin degradation has on a given aneurysm's propensity for rupture could then be assessed.

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