Engineering Characteristics and Effects of Small-Intestine Submucosa (SIS), Small-Diameter Vascular Grafts

Prepared for

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This report explores the compliance matching, modulus of elasticity, burst pressure, viscoelasticity, and immune response of SIS grafts by evaluating the research of Roeder et al. and suggesting improvements to this research.

ABSTRACT

Tissue engineering today is helping millions of people worldwide combat heart disease through the implantation of vascular grafts. The use of small-intestine submucosa (SIS) in an acellular approach to small-diameter vascular grafts has shown promise in previous experiments. It was analyzed experimentally by outside authors (Roeder et al.) for compliance, modulus of elasticity, and burst pressure and analyzed theoretically here for the same factors plus viscoelasticity and immune response. This study tested for compliance by measuring pressure using fluid-filled pressure transducers while adding fluid volume in controlled increments. In the same process, the drop in pressure over a time period as the graft "adjusts" to the volume rise was recorded. Also, burst pressure was found for various test grafts by connecting a fluid-filled pressure transducer to the grafts and increasing the internal fluid pressure until the grafts rupture. A mathematical model was then developed using graphical analysis and engineering stress and strain equations. It predicted relationships between the graft's internal pressure and diameter and the graft's modulus of elasticity and internal pressure. Maximum, minimum, and mean values for the graft's burst pressure were found, and viscoelasticity was predicted by deriving creep and stress relaxation equations for diameter as a function of pressure and time. Comparisons were drawn between the outside authors' work and the propositions made here. For example, both addressed compliance matching and high burst pressure, but the outside authors did not address viscoelasticity and immune response. Suggestions for improving the work of the outside authors and concurrent reasoning were provided.

INTRODUCTION

Heart disease is responsible for more deaths each year than any other cause. Approximately one in every three people who die did so because of a form of heart disease or complications arising from it. So tissue engineering of vascular grafts is obviously both a worthwhile and necessary endeavor for sustaining life. Tissue engineering, like all engineering disciplines, uses mathematical models to explain and simplify complex natural processes. Because cells are forever changing their characteristics (shape, size, position, etc.), mathematical models are sometimes hard to come by in biology and bioengineering. Still, with appropriate generalizations, models can be constructed that illustrate some of the macroscopic behavior of cellular systems. When working with vascular grafts and blood flow, there are specific cells and engineering principles that should be taken into consideration. Endothelial cells (ECs) line the interior of blood vessels and are subject to shear stress as a result of blood flowing past. Blood vessels can be divided into three regions called the intima, media, and adventitia, which contain varying amounts of collagen and elastin fibers. Collagen fibers, which will be used to a large degree in this discussion, are extremely tough and provide high tensile strength [1]. Elastin fibers, on the other hand, can stretch and recoil like rubber bands [1]. These two types of fibers work in conjunction with each other to give the blood vessel its hoop strength, resisting the internal pressure of the fluid, its modulus of elasticity or stiffness, and its viscoelasticity. Arteries also contain smooth muscle cells in their media that give these blood vessels the special ability to contract without any exterior help.

Several different approaches have been taken previously in the development of vascular grafts. EC-seeded synthetic grafts are the most studied of all tissue engineered applications and require "harvesting" ECs from elsewhere in the patient. These cells are then seeded onto a synthetic material, where bonds develop between the two surfaces, before they are implanted back into the patient [2]. Another development is the collagen-based blood vessel model where vascular cells are placed in a collagen gel matrix prior to implantation [2]. Yet another alternative is the cell self-assembly model where sheets of vascular cells are grown and then rolled into tubes and matured to form a viable graft [2]. Finally, the cell-seeded polymeric scaffold approach is something of a combination of EC-seeded synthetic grafts and the collagen-based model. These vascular graft alternatives have all met with relative success but, of course, have their disadvantages and drawbacks as well.

Perhaps the newest development in vascular tissue engineering is the acellular approach. "Small intestine submucosa (SIS) is cell-free collagen derived from the small intestine by mechanical removal of the mucosal and smooth muscle layers; the result is a cell-free, translucent tissue about 100 μm thick" [3]. Because of its relatively recent discovery, this approach has experimented successfully in various animals but has not been extensively used in humans. Cook Biotech, Inc., a company who holds the majority of patents for the new SIS technology, has stated that the first clinical applications of SIS are not for vascular grafts, but for surgical soft tissue repair, hard-to-heal wounds, and urinary stress incontinence [4]. In fact, the most common use of SIS currently seems to be for ligament replacement in anterior cruciate ligament knee injuries [5,6]. Still, the positive feedback the SIS approach has had in these applications should hopefully lead to success in vascular graft engineering.

METHODS AND MATERIALS

Roeder et al. conducted research testing the engineering characteristics, namely compliance, modulus of elasticity, and burst pressure, of SIS vascular grafts. The grafts used in the experimentation were made from two closely fitting concentric, hand-sutured SIS tubes, the SIS itself provided by Cook Biotech, Inc. and coming from the small intestine of an adult pig [3]. To measure compliance, Roeder et al. slowly filled in increments a fully collapsed graft with either Pepto Bismol or Milk of Magnesia. These fluids were used rather than water because of their higher viscosity, which is closer to that of actual blood. Fluid-filled pressure transducers were used to measure the pressure increase and plot the results versus fluid volume so as to estimate the fluid volume when the vessel was undistended. A mathematical relationship was derived for the range of pressures between 80 and 120 mmHg (taken as typical systolic and diastolic pressure) relating fluid volume to the increase in vessel diameter: $\Delta d/d = 0.5(V_{120}/V_{80} - 1)$ where d is diameter and V is volume [3]. Note that the expression $\Delta d/d$ is used here as the quantitative measure of compliance, making it essentially equal to the strain across the diameter. The modulus of elasticity for the SIS graft was derived further from this compliance relationship, assuming the vessel acts as a thin-walled elastic tube:

$$E = \left(\frac{\Delta P}{\Delta V}\right)\left(\frac{2}{t}\right)\left(\frac{V^3}{\pi L}\right)^{1/2}$$

where t is wall thickness, P is pressure, and L is the length of the vessel [3]. Apparently no actual testing was done to determine the modulus of elasticity, it was simply derived mathematically from known equations and previously determined relationships. Finally, burst pressure was tested through increasing the amount, and thereby the pressure, of nitrogen gas via a nozzle into the graft until it burst. A pressure gauge was used to find the actual pressure measurement at burst time [3].

The methods proposed here challenge some of the approaches taken by Roeder et al. while incorporating a great deal of their basic technique at the same time. Their methods were not by any means inaccurate, but some steps, both major and minor, could be taken to potentially improve the results and findings of the experimentation. Testing for compliance by measuring through fluid-filled pressure transducers while adding fluid volume in controlled increments seems to be a well-planned and highly precise process and no changes to its setup are suggested here. Likewise the calculations leading to the equation for modulus of elasticity are founded upon many years of engineering mechanics research and validation and while cells present much more complicated mechanical characteristics than to other engineering materials, a model with a more accurate fit is very hard to come by and most likely far too complicated to present within the scope of this report. Still, the compliance testing could have been improved if more realistic blood substitutes than Pepto Bismol and Milk of Magnesia had been used. Using a fluid with viscosity equal to that of typical blood instead of rough estimates may have affected the final results. Also, it is quite common that patients looking for artificial blood vessel replacements are doing so as a consequence of hypertension, or high blood pressure. For this reason, a larger operating range or a shifted operating range may be necessary, as opposed to the "typical" range selected by Roeder et al. of 80 to 120 mmHg. Here, a larger and shifted range of pressures from 80 to 140 mmHg is used.

Burst pressure was measured by Roeder et al. using nitrogen gas. Because gases are in a completely different phase from liquids, they exhibit much different behavior, particularly when placed under pressure. Gases are compressible while fluids are relatively incompressible. For this reason, burst pressure testing was conducted anew using a fluid, preferably with a viscosity close to that of average blood as discussed above. A fluid-filled pressure transducer similar to the one used to measure compliance was used to measure pressure, replacing the pressure gauge.

Some additional properties that needed to be measured or quantified in order to effectively evaluate the performance of SIS vascular grafts were their viscoelasticity characteristics and the immune response they may elicit. Data on viscoelasticity was gathered quite simply at the same time as compliance testing. After the amount of fluid present in the graft was incrementally increased, this volume was held constant for a period of time. Since viscoelasticity was present, pressure rose and fell and diameter increased during this time period. More fluid was added only once these two variable appeared to reach their resting (steady) states. The immune response of test subjects to implanted SIS vascular grafts was obviously of great interest. If the body's immune system rejects and/or attacks a graft, failure has occurred. After implantation, the immune system of the subjects (their white blood cell count, etc.) was closely monitored and a general synopsis of both their acute and chronic immune response documented and evaluated.

As mentioned before, engineers use mathematical models to explain and simplify complex natural processes. Roeder et al. provided several equations and relationships to construct such a model, which are briefly stated again here. Compliance was modeled using a plot of fluid pressure versus volume, and eventually a relationship was obtained for volume and diameter. Since diameter is truly the measure of compliance and pressure is truly the variable (what changes) in the blood, this model was easily taken one step further and a relationship for pressure and diameter could be found relatively quickly. The modulus of elasticity was derived from engineering mechanics stress and strain equations and was solved for in terms of pressure and diameter. Using the new relationship derived above from compliance testing, the relation of the modulus of elasticity to pressure was again not too difficult to obtain. Roeder et al. presented the maximum and minimum, as well as mean, value of burst pressure, and as much seemed sufficient. Doctors need to know the limitations of vascular grafts and if the pressure they desire is above the minimum found in testing, a SIS graft may not be an appropriate choice. To model viscoelasticity, diameter and pressure were each be plotted against time for every incremental increase in fluid volume. A general stress relaxation equation was then derived for pressure and a general creep equation for diameter, both as a function of time. In essence, this model of viscoelasticity showed how vessels react to step increases in fluid volume or pressure. Immune response was not quantitatively analyzed in this report and therefore was not matched to a mathematical model.

RESULTS

Figure 1 shows the general pressure versus volume relationship obtained by Roeder et al. for a typical SIS vascular graft. Using a different graph for each different size of graft, the volume measurements for pressures of 80 and 120 mmHg are found and the compliance values are calculated and are displayed in Table 1. Roeder et al. reported the compliance values of the tested 8 mm graft to be almost the same as those for a canine carotid artery [3]. The tested SIS vascular grafts also appeared to be on average 5 times more compliant than the synthetic grafts currently in use today. The modulus of elasticity for various arteries, veins, and the tested SIS grafts are found in Table 2 and show that an average canine carotid artery had a modulus of elasticity of about 6 x 10^6 dynes/cm^2 and a SIS graft has a comparable modulus of elasticity around 16.6 x 10^6 dynes/cm^2 [3]. Burst pressures for the SIS grafts tested by Roeder et al. are found in Table 3 and minimum, maximum, and mean values are quickly found. The burst pressures range from 2069 to 4654 mmHg and are on average 3517 mmHg. This minimum burst pressure was well above the range of systolic pressures, even when hypertension is taken into account. In general, Roeder et al. reported that the burst pressures of remodeled SIS grafts are well known for exceeding the burst pressures of the arteries that they replace [3].

The expected results of the new testing proposed here will not vary much for those parts that were kept in similar fashion to the testing conducted by Roeder et al. A plot much like Figure 1 and a table of values similar to Table 1 was expected from the new compliance testing. However, compliance values did become expectedly larger since V_{140} was used in the equation instead of V_{120}. In fact, using approximate values for V_{140} and V_{80} taken from Figure 1, the compliance was almost twice as large as when V_{120} was used. So it seems compliance matching will be much more difficult in patients with hypertension, which is naturally the case, but here is more significant because it is believed hypertension patients make up a large portion of the market for SIS grafts. The table of new testing values for modulus of elasticity looked similar to Table 2 but presented lower values since the compliance values rose. To explain this further, observe the equation presented by Roeder et al.:

$$E = \frac{stress}{strain} = \frac{Pd/2t}{\Delta d/d} \qquad [3]$$

Modulus of elasticity (E) and compliance ($\Delta d/d$) are inversely related, meaning as elasticity increases, compliance decreases and vice versa. Burst pressures decreased somewhat with the new testing procedures due to the use of fluid in place of gas. The variability in their values was of course still dependent on the different grafts being tested.

The results of the viscoelasticity experimentation were completely new. With the increase of fluid volume, pressure initially increased very rapidly. Then, as the vessel's diameter stretched to reach a new equilibrium under this increased pressure, pressure fell to close to its original value. Figures 2 and 3 show the expected behavior of both of these variables against the same time scale since they occurred simultaneously. The immune response of the body to a newly implanted SIS graft was somewhat hard to theorize. Thankfully, there had been some prior research done on the immune response of mice to SIS implantation. This research reported that SIS grafts caused an acute inflammatory response, followed by tissue remodeling. The authors summarized that their initial results suggested SIS obtained from an adult pig does elicit an immune response but that "the resulting immune response is restricted to the Th2 pathway, consistent with acceptance rather than rejection." So it seems that implanted SIS, while causing initial inflammation, would not elicit a chronic rejection by the immune system.

The results of the mathematical model built on the figures and tables described above. From Figure 1, an approximate mathematical relationship between fluid pressure and fluid volume was obtained. A simple linear regression line through the diastolic to systolic pressure range yielded the following equation: $P = 1000V - 250$. Solving for pressure, substituting this into equation for compliance stated earlier, and simplifying, compliance was written as:

$$\frac{\Delta d}{d} = 0.5\left(\frac{P_{120} - P_{80}}{P_{80} + 250}\right)$$

The relationship between modulus of elasticity and pressure was the same as that derived by Roeder et al. It illustrates how modulus of elasticity increased exponentially with pressure:

$$E = E_0 e^{\alpha P}$$

Without concrete data, only very general relationships could be determined for the viscoelastic nature of the SIS grafts. The relation graphed in Figure 2 was a general stress relaxation equation of the form $P(t) = Pe^{(-\mu/\eta)t} * 1(t)$ where P is the pressure, μ is a viscous (dashpot) constant, η is an elastic (spring) constant, and $1(t)$ is a unit step function. The relation graphed in Figure 3 was a creep function, expressed generally as $d = (1/\mu + t/\eta)P*1(t)$ where d is the vessel diameter and P is the pressure increase. As expected the vessel's diameter was dependent upon the internal pressure while it affected the internal pressure not at all.

CONCLUSION

Agreeably, Roeder et al. highlighted the positive aspects of an SIS graft's compliance matching and high burst pressure. They also did well to express the limitations of their experiments, noting the variance in testing equipment and SIS source material as the two largest limiting factors [3]. The viscoelasticity of the blood vessel replacements was not addressed when it seems that it should have been. Viscoelasticity is very similar to compliance except that it relates pressure and diameter to time and thus would have been a great addition to Roeder et al.'s discussion of SIS graft compliance matching. As discussed earlier, a more appropriate testing fluid, one with viscosity closer to that of blood, could have easily been used. Finally, the immune response of the body to implanted SIS vascular grafts went unmentioned by Roeder et al. due to their strict mechanical approach to their research. Research done outside of their study indicates that there is no current cause for alarm concerning immune response to SIS grafts, but none of these experiments were performed on human subjects.

Many changes to the methods used by Roeder et al. have been suggested throughout this report. In order to improve the vascular graft itself, its viscoelasticity should be made to match that of an artery that it will replace. Certain factors, hormones, and/or proteins may also need to be added in order to minimize complications arising from the response of the body's immune system to new implantation. All in all though, SIS grafts have shown promise in previous testing in animals and, given the success found there, researchers may soon be testing their performance in volunteering human subjects.

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APPENDIX

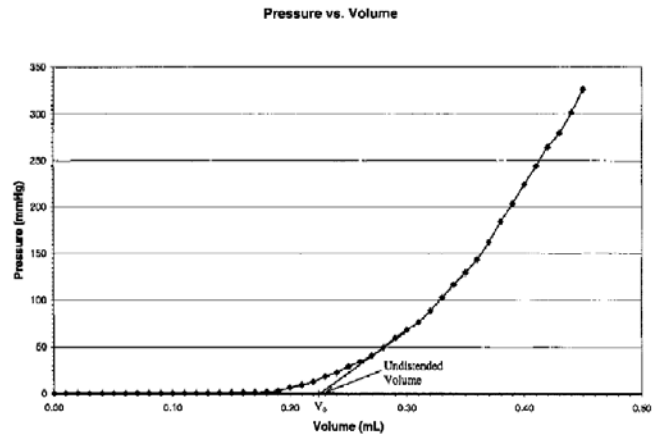

Figure 1: Pressure-volume relationship for a typical SIS vascular graft [3].

Table 1: Compliance for a Pressure Rise from 80 to 120 mmHg [3].

Graft Designation	Diameter mm	$\Delta d/d$
6/18	5.38	0.039
6/29	5.19	0.029
6/30	5.19	0.051
7/16	5.81	0.043
7/29	5.59	0.068
Average	5.43	0.046
8/4A	8.66	0.095
8/4B	8.71	0.072
8/5	8.78	0.094
Average	8.71	0.087

Table 2: Modulus of Elasticity of Vessels, Veins, and SIS Vascular Grafts [3].

Item	P mmHg	Edynes/cm^2	Investigators
DTA (dog)	100	12.7×10^6	Posey (8)
AA (dog)	100	16×10^6	Posey (8)
DTA (dog)	100	4.30×10^6	Bergel (17)
AA (dog)	100	8.90×10^6	Bergel (17)
DTA (dog)	100	12.0×10^6	Nickerson (18)
DTA (dog)	100	$1.2–4 \times 10^6$	Bergel (19)
Abd. A (dog)	Arterial	2.42×10^6	Peterson (20)
DTA (dog) upper	96–124	2.7×10^6	Gow (21)
DTA (dog) mid	87–118	$3.0 \pm 0.33 \times 10^6$	Gow (21)
DTA (dog) lower	101–130	5.7×10^6	Gow (21)
Abd. A (dog)	96–130	$9.8 \pm 1.2 \times 10^6$	Gow (21)
Asc. A (dog)	96	0.76×10^6	Patel (22)
DTA (dog) high	90	0.8×10^6	Patel (22)
DTA (dog) mid	98	1.2×10^6	Patel (22)
DTA (dog) low	93	1.57×10^6	Patel (22)
Abd. A (dog)	86	2.0×10^6	Patel (22)
Asc. A (man)	70	0.76×10^6	Patel (22)
Carotid (dog)	100	6×10^6	Cox, 1975
Carotid (dog)	100	$0.43–2.37 \times 10^6$	Authors
Carotic (man)	97	6.07×10^6	Patel (22)
Carotid (human)	125	0.4×10^6	Arndt (14)
Carotid (human-young)	100	9×10^6	Learoyd (24)
Carotid (dog)	Arterial	3.1×10^6	Peterson (20)
Femoral (dog)	Arterial	3.68×10^6	Peterson (20)
Femoral (dog)	90–130	$12.3 \pm 1.2 \times 10^6$	Gow (21)
Femoral (dog)	100	32×10^6	Learoyd (24)
SIS (pig)	100	8.03×10^6	This study
SIS (pig)	12% strain	16.6×10^6	Authors
SIS (dog)	160	$5.88–12.0 \times 10^6$	Herbert (25)
Vein	100	5.5×10^6	Hinke (26)
Vein (dog jugular)	100	8.5×10^6	Bergel (19)

Table 3: Burst Pressure for SIS Vascular Grafts 5.5 mm in Diameter [3].

	Graft* Length (mm)	Burst Pressure (mmHg)
1	48	4137
2	34.5	3878
3	36	3620
4	44.4	3775
5	47.4	3361
6	35	2844
7	43.4	4654
8	43	3361
9	44	3103
10	46	3879
11	52	3879
12	44.2	3620
13	40.5	3672
14	41.2	2069
15	40	3879
16	42	3361
17	40.8	2586
18	37.7	3879
19	45	3879
20	45.4	3103
21	45	3310
Average	43	3517

*Diameter = 5.5 mm.

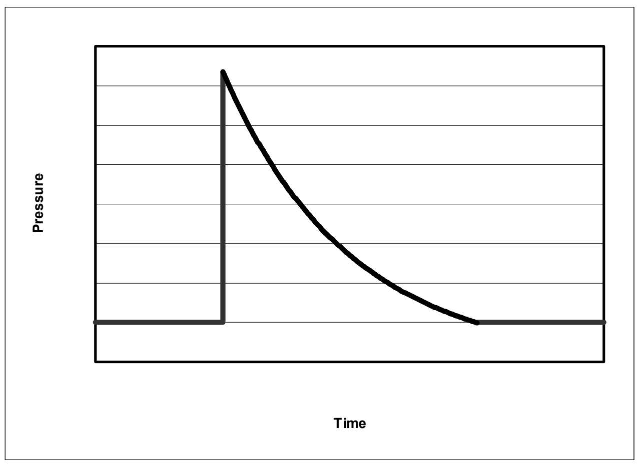

Figure 2: General pressure behavior over time for an incremental increase in fluid volume.

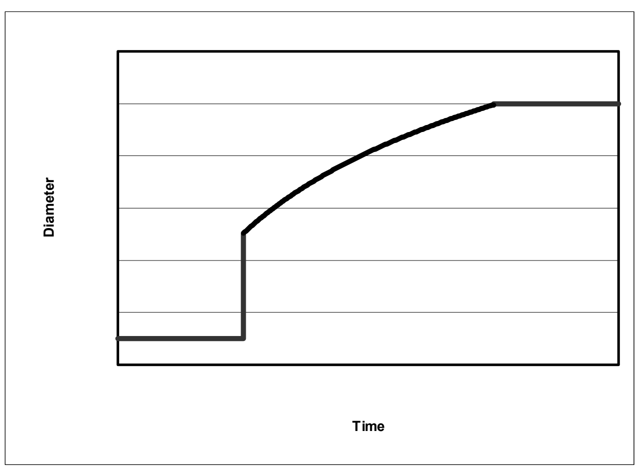

Figure 3: General diameter behavior over time for an incremental increase in fluid volume.