Biomechanical Characterization of the Human Umbilical Cord

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Abstract: The human umbilical cord is a complex structure crucial to the development of a fetus. Connecting the fetus to the placenta, the cord encases umbilical vessels, and is responsible for the transportation of nutrients and oxygen to the fetus. In order to provide a reliable pathway, the cord must be constructed in such a way to absorb mechanical loading due to fetal movements and uterine contractions. In order to understand the response of the cord to such mechanical loading, it is necessary to observe the behavior of the individual composite components of the cord as well as the behavior of the cord as a whole. The aim of this study is to compare findings on the mechanical properties of components of the cord to the behavior of the cord as a whole. In this study, small (~1 mm tall) cross-sections of umbilical cord tissue were compressed in unconfined compression. And describe results here in 2-3 sentences. Then put in 1-2 sentences of discussion/conclusions

Introduction

Cord-related pathologies result in the fetal deaths of 2 in every 1000 genetically normal fetuses (Collins, 2004). These deaths are often times the result of abnormalities such as stiff, lean, short or knotted cords (Collins 2004). In order to understand the implications of these abnormalities and of cord compression, researchers must understand the mechanical response of specific components of the umbilical cord. Despite the importance of such an understanding, there are less than 10 publications dealing with the biomechanics of the human umbilical cord; in comparison, there are thousands of publications dealing with the biomechanics of other human tissues such as bone and cartilage.

Biomechanical deficits in tissues of the human umbilical cord (HUC) likely lead to an increased risk for umbilical cord accidents in utero. These accidents are particularly dangerous because the HUC is a crucial lifeline early in human development, carrying oxygenated blood and nutrients to the fetus. In order to prevent this lifeline from being compromised, a supportive tissue called Wharton's Jelly (WJ) adjusts for the majority of mechanical loading in the cord. Primarily regarded as collagen network, WJ is very similar to articular cartilage found in knees. However, where articular cartilage possesses a well organized network designed to absorb compressive forces in one direction, WJ presents itself as a much more loosely organized network in which many canicular spaces radiate outward from the vessel to absorb compressive forces in any direction [Vizza, 1996]. Within these spaces and throughout the WJ, glycosaminoglycan (GAG) chains, covalently bonded to core proteins, form a proteoglycan matrix. Charges found on the GAG chains allow the tissue to absorb water and act as a sponge-like cushion and thereby act in a compression resistant manner. In addition, the umbilical vein and arteries contribute to the resistivity of the cord, and due to the fact that the vessels are primarily made up of endothelial cells and structures [Goigel, 2005], have a different elastic modulus, or stiffness associated with them, and must be examined independently of the WJ. Furthermore, the vessels were shown to exhibit different mechanical properties under physiological pressure, creating an even more complex analysis of the cord.

In addition to the myriad of anatomical complexity, the materials themselves also demonstrate varying behaviors across different applied loads. Both umbilical vessels as well as the WJ exhibit nonlinear stress strain response as strain increasing [Pennati, 2001]. This viscoelastic behavior yields two elastic moduli: an E_{Low} that represents the low load stiffness of the material, and an E_{High} that is dominant during high load tests. While the driver for this difference is not well understood, it is clear that the cord is complex in nature and extensive analysis of the cord as well as its components is required.

Although scarce, research regarding the mechanical testing of component tissues of the HUC has been done. Pennati and his research group have provided an initial estimate of the low load and high load moduli for WJ, and UV in tension. In addition, a member of Professor Ferguson's group, John Martin, has finished his work on the tensile modulus of Umbilical Arteries. To provide a point of comparison, my testing involved the lateral compression of the cord as a whole. My analysis will assess the accuracy of an engineering principal known as the Rule of Mixtures, by which the modulus of a composite material is determined by the volume fraction and associated modulus of its components.

2. Methods/Materials:

2.1 Sample Acquisition:

Umbilical Cords and accompanying placentas were gathered from a local midwife service within 24 hours of delivery. These tissues were transported on ice and frozen immediately in order to prevent degradation and subsequent compromise of biological integrity. 36 hours prior to dissection, the tissue began defrosting in Phosphate Buffered Solution. Once the tissue was completely thawed, the placenta and cord were separated, and the placenta discarded in a biological waste container. The cord was then cut into three sections: one closes to the fetus, a middle section, and one furthest from the fetus. Finally, the cord was stored in 70% ethanol until testing was possible. This effectively dehydrated the cord and prevented any degradation of tissues while in storage.

2.2 Sample Preparation

Prior to testing, the three sections of cord were rehydrated in distilled water for 30 minutes. This hydration enables the WJ to return to physiologically relevant stiffness, and ensures the validity of the WJ modulus. 10 mm thick samples from each section were cut, avoiding regions of heavy coiling or discoloration. The approximate diameter and thickness of each sample were measured before attaching it to a glass microscope slide to prevent floating in the test chamber (see figure 2.2.1). Finally, the slide and sample were placed in the test chamber and filled it with distilled water.

2.3 Testing

In order to accurately test the mechanical response of the tissue, a custom made, unconfined compression fixture for use on the MTS Insight II (Figure 1) was created. This fixture allows for isolated saturation of the tissue being tested, as well as independent temperature control of the bath as a whole. The set up is shown below (See Fig. 2.3.1). The environmental chamber was filled with tap water, and the internal bath with distilled water. Although these two solutions do not approximate physiological conditions, they maintained hydration for the tissue to provide results using a 'first approximation' approach. An external heater/thermometer monitored and controlled the temperature of the internal bath water by heating the water in the environmental chamber to physiological temperature (37.5 +/- .5 degrees Celsius). The cross head of the upper platen was lowered until a small resistance (.15 N) was detected by the load cell, at which point the "extension" of the platen was set to 0.

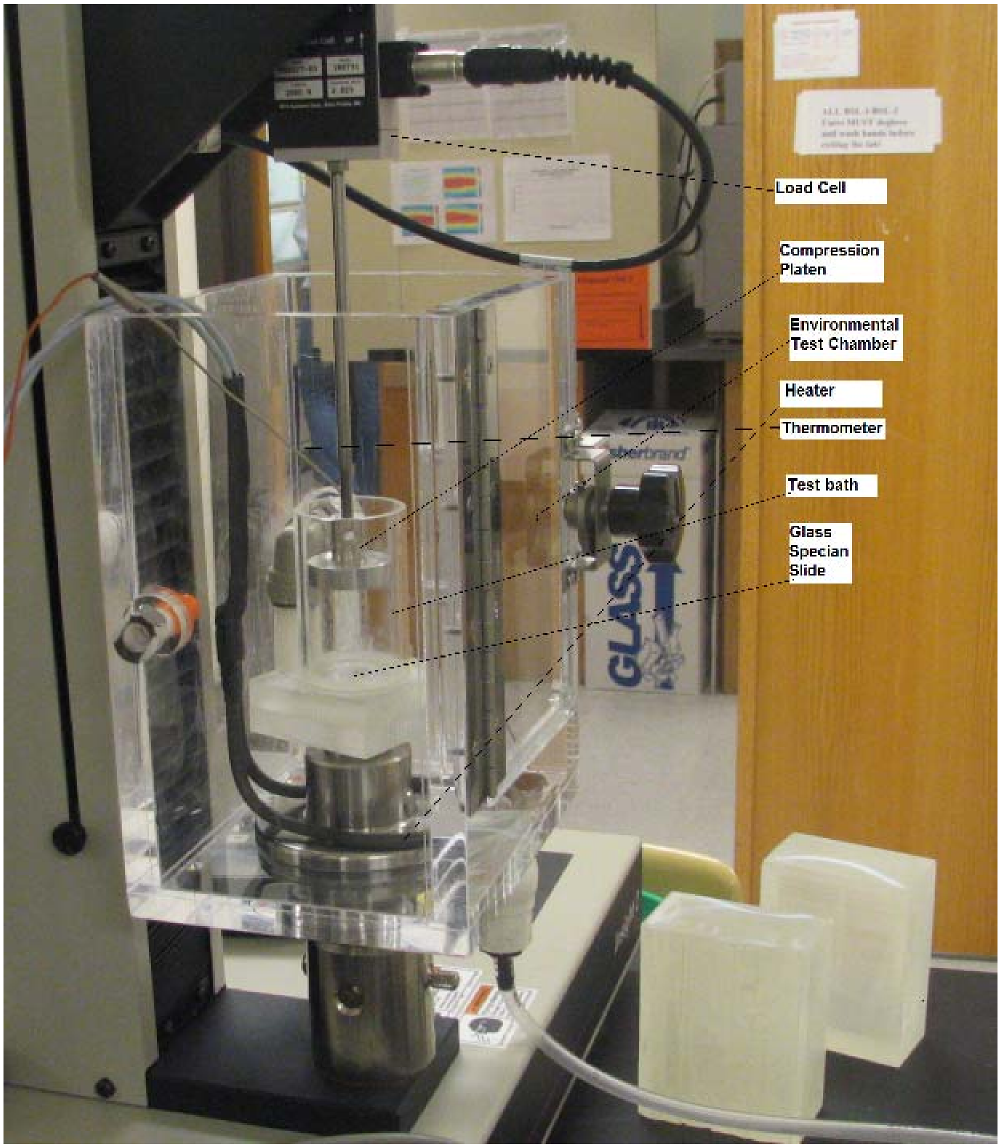

Figure 1. Mechanical testing setup showing the compression fixture within the MTS Insight II environmental chamber.

In unconfined compression testing, a strain rate of .06 mm/mm was used. This rate matched the strain rate used in tensile tests of umbilical cord tissues in the literature [Penatti , 2001] and stopped testing at a final strain of 0.5 %

3. Results:

3.1 Observations:

For many of the samples (1, 3, and 4) a high coiling index was observed prior to testing (See appendix). In addition, some samples (1, 4, and 5) were taken from regions containing discoloration of the cord, likely due to the formation of blood clots within the vessels. During the tests, sample 2 deformed normally until high load, at which point the sample buckled along the coils of the cord. Samples 3 and 5 buckled along the coils of the cord almost immediately. Following completion of the testing on sample 4, it was noted that this sample deformed at an angle that was likely due to poor centering of the specimen. Overall, sample 2 exhibited the most optimal behavior during compression testing with no apparent buckling during the test. For these reasons, sample 2 was analyzed to find the composite cord modulus.

3.2 Graphical Results

The load used to attain the strain in each sample was measured and recorded, and engineering stress was computed by dividing the load by the area of the sample for each data point. A graph comparing the stress and strain behaviors of each sample was then created using Microsoft Excel (Figure 2). This graph is shown below.

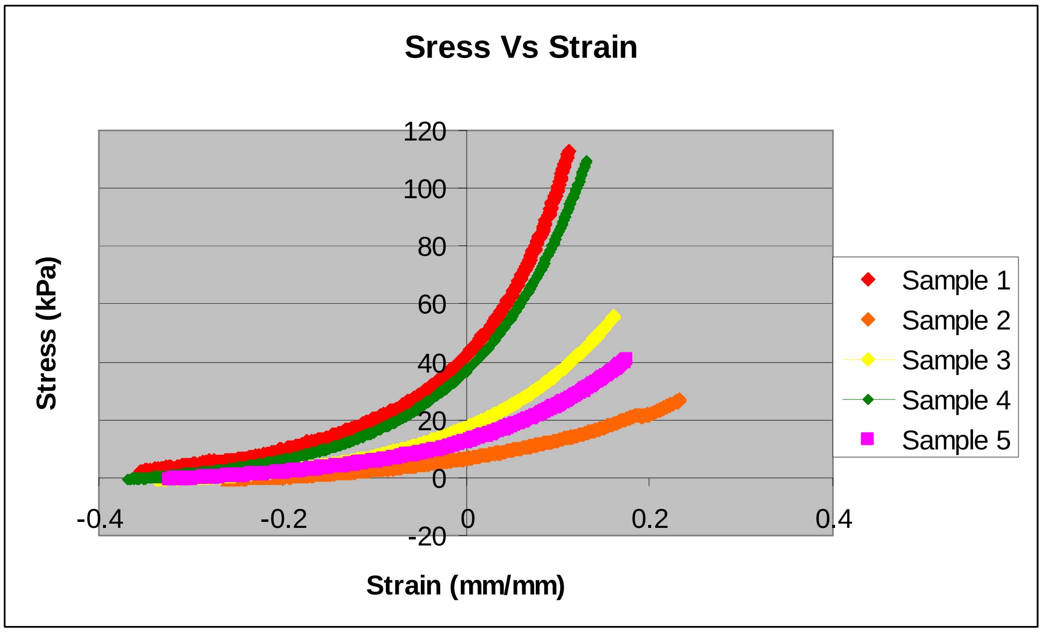

Figure 2. Plot of stress versus strain for each unconfined compression test. These data show the range of responses from each of the five samples that were tested.

3.3 Modulus Calculation

In order to compute the measured modulus for the composite cord, the low load regions of sample 2 were analyzed. The modulus of a given material can be calculated by finding the slope of the stress train curve for the material. This slope, in the low regions – also called E_{low}, is best approximated using a linear regression model (Figure 3).

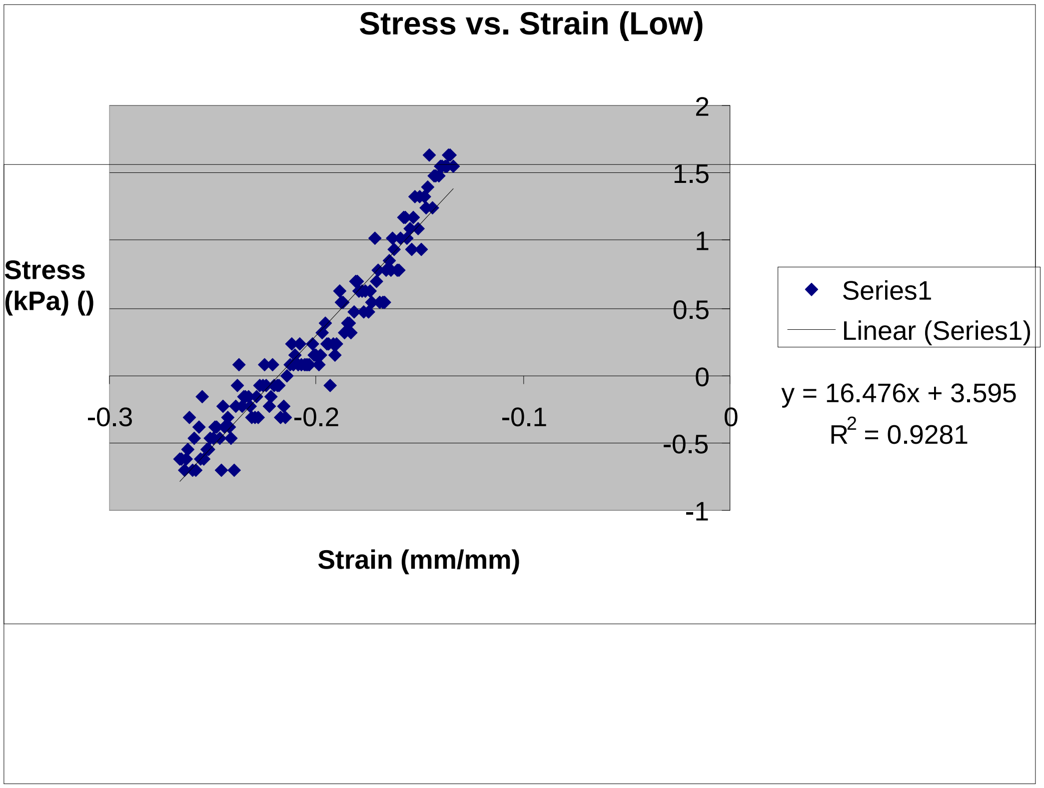

Figure 3: Plot of Low Modulus (E_{low}) of sample 2

The graph displays an equation with a slope of 16.47 and an R^2 value of .928, indicating that the modulus of the composite is 16.47 kPa, and that the line accounts for 93% of the data.

3.4 Rule of Mixtures

The Rule of Mixtures states that the modulus of a fiber re-enforced matrix composite will be the volume fraction of the matrix multiplied by the modulus of the matrix, added to the volume fraction of the fiber multiplied by the modulus of the fiber. In applying this principle to the umbilical cord, I will determine the approximate volume fraction of each of the component of the cord (WJ, UV, UA) and multiply each volume fraction by its respective modulus, given by Penatti [Penatti, 2001] and research done by graduate student John Martin of the University of Colorado [Martin, 2008]. This process is shown below. The Composite Modulus E_{low}= 0.6*.04+2*.014*.05+.91*.9 = **0.85 MPa.**

Table 1. Listing of the published values for umbilical tissue physical dimensions and modulus [Penatti, 2001 and Martin, 2008]. Volume fraction was calculated by dividing the component volume by the composite volume of the cord, where volume is calculated by $Vm=(Pi*Dm^2/4)*T$, where Vm is the measured volume, Dm is the measured Diameter, and T is the sample thickness (10mm).

Tissue Type	**Measured Diameter**	**Volume (mm^3)**	**Volume Fraction**	**Given Modulus (MPa)**

	(mm)			Low, High
Umbilical Vein	2.75	5.94	.04	.60
Umbilical Artery (x2)	1.84	2.66	.05	.014
Wharton's Jelly	?	117.56	.91	.90
Composite (total)	12.81	128.82	1	N/A

4. Discussion:

4.1 Sources of error

The most prevalent source of error for this study could be the source to which I am comparing my measured modulus [Penatti, 2001]. While the initial modulus (E_{low}) should be the same in both tension and compression, no scientific validation of the moduli presented in the limited volume of literature has been done and subtle differences in the methodology and results for testing in tension and compression could arise. Additionally the anatomy of the umbilical cord results in inherent testing difficulties. The diameter of the cord varies throughout its length, and although the umbilical veins and arteries generally remain the same, the volume fraction of the Wharton's Jelly is not consistent throughout the cord. Finally a phenomenon common to umbilical cords, known as coiling, as measured via the 'coiling index', likely causes stress concentrations along coils (See Appendix for image). Due to the looping nature of these coils, stress that would normally be oriented perpendicularly to the surface of the cord are transmitted into shear, or twisting force on the cord. This behavior is seen in the second sample's mechanical behavior (Figure 4), as the "dip" in strain is seen to be a buckling of one such coil, transmitting the normal force torsionally along the sample.

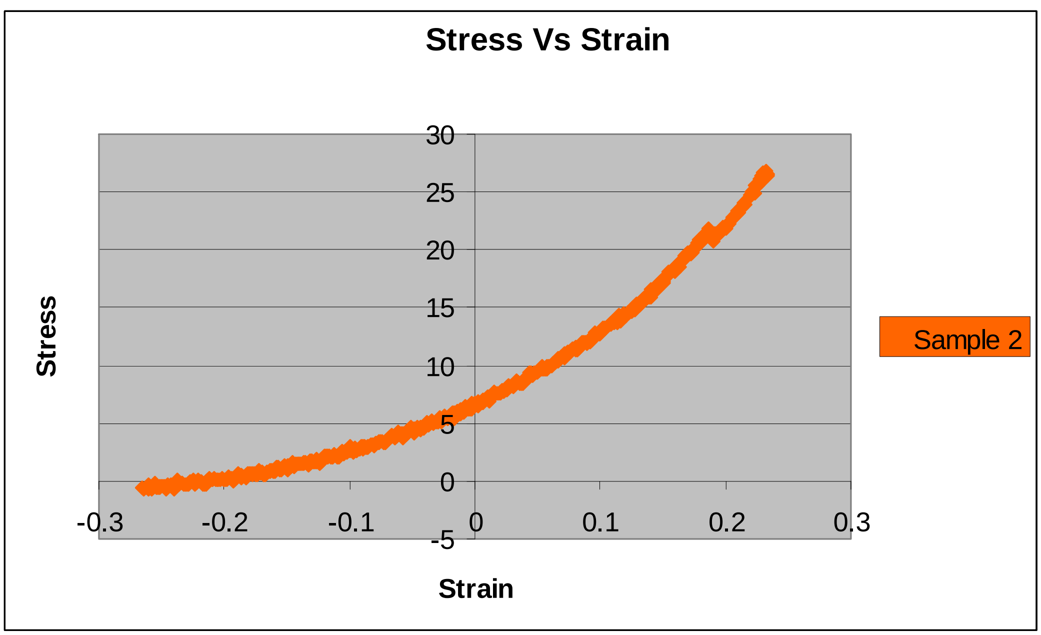

Figure 4:

4.2 Conclusion

Through analysis of previously measured values for elastic moduli of components of the cord, we can see that the modulus measured by lateral compression of the cord as a whole did not match the modulus predicted by the Rule of Mixtures. The modulus predicted was roughly an order of magnitude higher than the modulus measured in experiment, which is likely due to the inherent difficulty of testing the composite cord laterally and the errors that accompany it. However, this research has shown us the different behaviors of samples in accordance to the number of coils present in the cord, and will help our group to develop more efficient and accurate methods of measuring the composite modulus of the cord.

4.3 Future Work

Although the lateral compression of the human umbilical cord has proven difficult, Professor Ferguson's group will remain dedicated to its biomechanical characterization. New umbilical cords are delivered and frozen weekly, and as testing methods and equipment become more advanced, valuable information is not far from reach. Localized indentation testing is still on-going, headed by several graduate students in Professor Ferguson's lab. These tests will provide a regional map of the "stiffness" associated with different parts of the cord, and will serve to validate the published values for moduli of component tissues in the cord. Additionally, compression testing of the cord as a whole will continue to be investigated. One possibility is to longitudinally test the cord, providing insight into the reaction of the cord to everyday fetal movements and uterine contractions. I am confident that this research will yield new and useful information regarding the umbilical cord, and will continue my work during the summer of 2008.

5. References:

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6. Appendix:

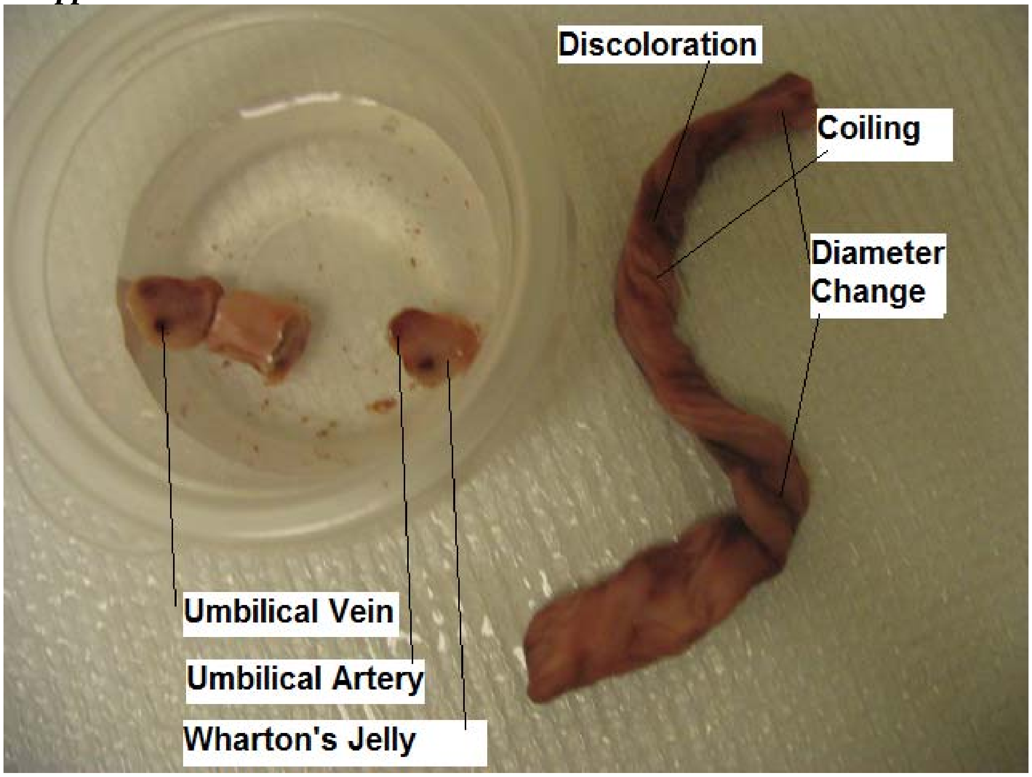

Figure 5. Anatomy of an abnormal cord; a hypertensive, coiled, discolored cord and its anatomy. For this reason, a large number of composite samples are unusable.