# *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 previously studied tissues and materials. In this study, umbilical arteries were subjected to longitudinal tension tests. These samples yielded a range of data showing a non-linear, viscoelasitc response to mechanical loading. In analyzing the pre and post test observations, and comparing the findings to published data on similar studies, the observed elastic moduli will provide insight into the nature and methodology of testing the human umbilical cord.

## *1. 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 viscoelasitc 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. A component of both the Wharton's jelly and umbilical vessels' extracellular matrix called elastin accounts for the majority of the low load mechanical response, while a more structural component called collagen is responsible for the material behavior at high strain rates.

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. However, John Martin's work was primarily focused on stress-relaxation tests which yield useful information about the viscoelasitc properties of the arteries. The aim of this study was to investigate the histological properties, and images of umbilical cords and the responses of the arteries to tensile tests in an effort to compare mechanical properties with known properties of previously studied materials and to investigate the differences in healthy and abnormal cords.

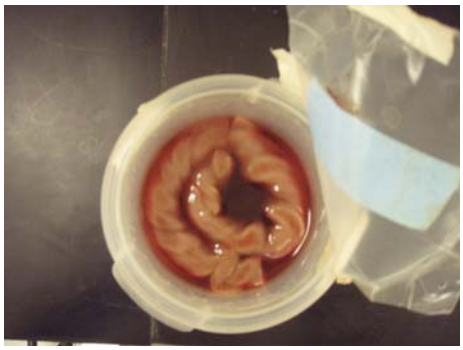
## ***2. Methods/Materials***:

### *2.1 Sample Acquisition*:

Umbilical Cords and accompanying placentas were gathered from Boulder Community Hospital within 24 hours of delivery. This effort required coordination between all members of the Ferguson Lab as well as the nursing staff at Boulder Community Hospital to ensure the safety of the patients and swift transportation of umbilical tissues. These tissues were transported on ice and placed in a refrigerator immediately in order to prevent degradation and subsequent compromise of biological integrity.

### *2.2 Sample Preparation*

Prior to testing, the cord was dissected into three separate sections. These were labeled as proximal, medial and distal sections of the cord. From each section, two umbilical arteries were removed at lengths of 30 mm, and cut longitudinally to create a flat length of arterial tissue. In addition, a longitudinal and radial sample was taken (< 5mm) of the artery, as well as a cross sectional sample of the whole cord (~25mm) for histological analysis. A second cross sectional section was taken for a chemical staining and freeze fracture preparation for SEM imaging.



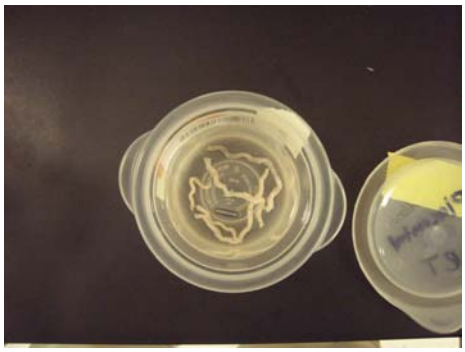


*Figure 2.2.1 (left)- Umbilical cord in PBS before dissection*
*Figure 2.2.2(right)- Umbilical cord arteries ready for testing*

### *2.3 Testing*

2.3 a) Mechanical Arteriole Testing

Flat sections of arterial tissue 30 mm long were subjected to tensile testing using a 150 N load cell on an Instron Insight machine. The tensile test used a spring loaded polymer grip with an adjustable thumb screw tightening system, and was performed in an environmental chamber containing a constant temperature (36.5 ± 5 C) PBS bath. The test protocol was implemented using Testworks software and used 10 cycles to preload and subsequently test the sample to failure. The data from these tests was exported, and graphed and analyzed using Matlab.

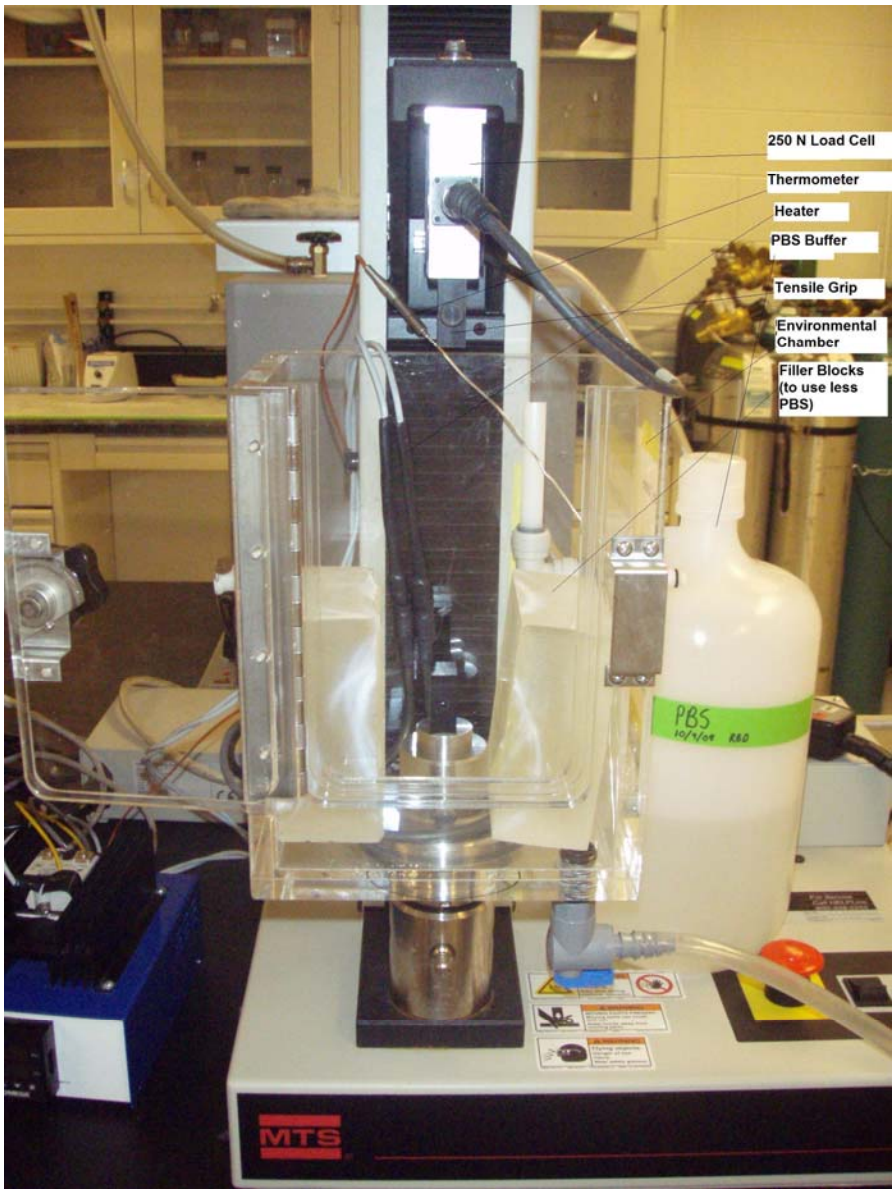



*Figure 2.3.1- Mechanical testing apparatus*

2.3 b) Histological Testing

Radial and longitudinal arterial samples as well as whole cord sections of the cord were sent to Premier Labs in Longmont CO for analysis. Slides were examined using a light microscope, and imaged using Labview software.

*Figure 2.3.2 Histological slides from Premier Labs*

2.3 c) SEM Imaging

Cross sectional samples were prepared for SEM imaging using a maceration staining process (Murakami 1978). This process begins washing the samples after dissection in .1M phosphate buffer, followed by 48 hours of immersion in a 2.5% glutaraldehyde at room temperature. Upon removal, the samples are washed in running tap water for two hours, and then incubated in a solution of 2N NaOH at 25C for 10 days. Finally, the specimens are washed in distilled water until they reached a transparent state (usually about 12 days). The samples are then stained using 1% tannic acid and 1.3% osmium tetroxide. Finally, the samples are dehydrated in graded ethanols, freeze fractured in liquid nitrogen and critical point dried in CO2.

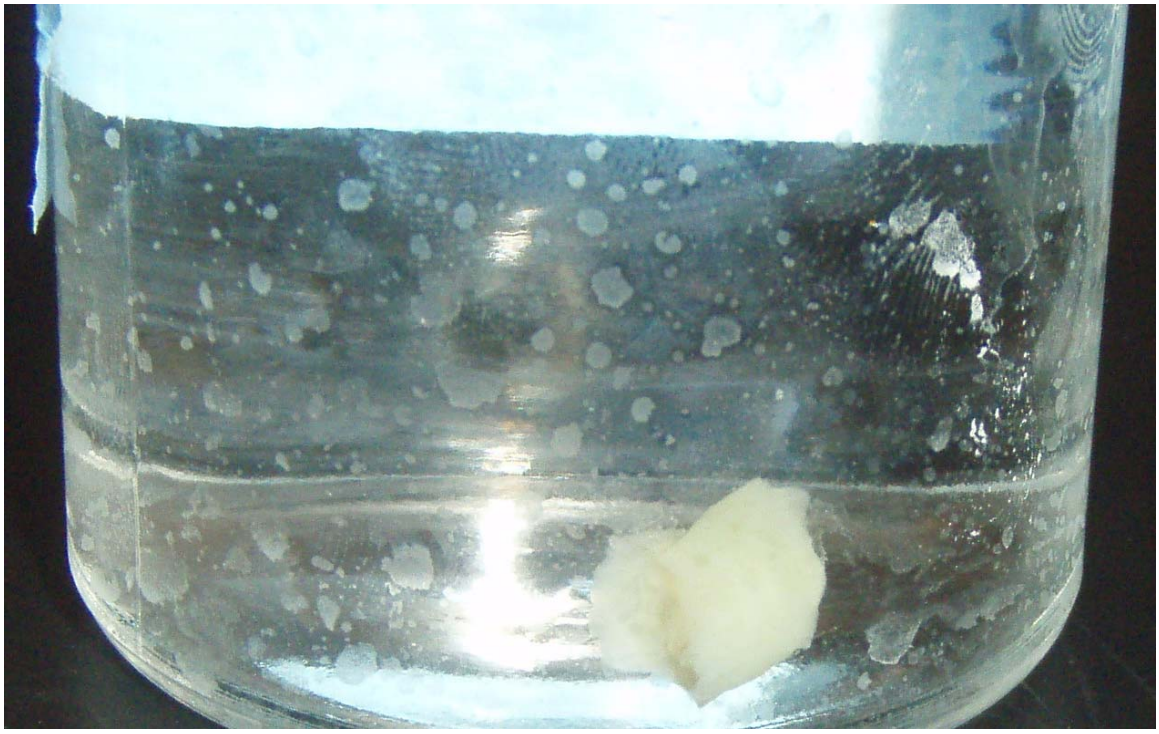


*Figure 2.3.3- A macerated cross sectional sample ready for staining*

## *3. Contributions and Results*

### *3.1 Contributions*

As a member of the team working on this project it was my responsibility to collect and transport umbilical samples from Boulder community Hospital, dissect and prepare samples for mechanical and histological testing, macerate and stain SEM samples, execute tensile testing and data exportation, and transport histological samples and slides to and from Premier Labs. Furthermore, I designed and fabricated a fixture for longitudinal whole cord compression, and although it was not used in the arterial tissue study, I hope to oversee implementation and usage of the fixture to gather more information on the mechanical response of the cord as a whole.

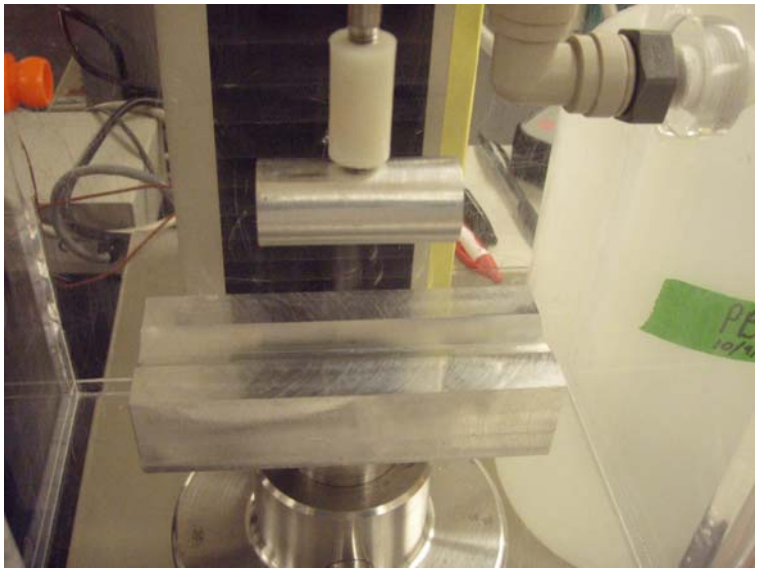


*Figure 3.1.1- Fixture for longitudinal testing of the cord*

## *4. Discussion:*

### *4.1 Conclusion*

As data collection for this project is still on going, a definitive statement relating the findings from the histological investigations, mechanical testing and microscopy cannot yet be made. However, there are several graduate and undergraduate students continuing data analysis, as well as histological examination and SEM imaging, and as more cords are delivered, the comprehensive analysis of each cord will become a more pressing matter for the member of the research team.

### *4.2 Future Work*

Although analyzing the human umbilical cord has proven difficult, Professor Ferguson's group will remain dedicated to its biomechanical characterization. New umbilical cords are delivered weekly, and as testing methods and equipment become more advanced, valuable information is not far from reach. Multiple graduate students in Professor Ferguson's lab will continue this line of research and with a quickly growing sample size should be able to publish a paper detailing the comprehensive results and their significance in the near future.

## *5. References:*

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