A BIOMEDICAL STUDY AND MOLECULAR ANALYSIS OF SOFT TISSUES

by

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Presented to the Faculty of the Honors College of
The University of Texas at Arlington in Partial Fulfillment
of the Requirements
for the Degree of

HONORS BACHELOR OF SCIENCE IN BIOLOGY

THE UNIVERSITY OF TEXAS AT ARLINGTON

December 2005

ABSTRACT

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Publication No. _____

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The University of Texas at Arlington, 2005

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The human body is fundamentally dependent on external soft tissues such as corneal and skin tissues to function. However, these soft tissues, despite being remarkably resilient to damage, can and do fail, especially as individuals age. Most of today's treatments for diseases and conditions resulting from soft tissue failure do not actually cure the problem itself, but only treat the symptoms through invasive surgery and/or implantation of a biomedical device.

A thorough understanding of the physiology of soft tissues and their failure, involving biomechanics at both the macroscopic and microscopic levels, offers much hope for better treatments for soft tissue-related conditions and diseases in the future. Such treatments could cure the diseases at their root, not just alleviate their symptoms.

The aims of this research were to: 1) understand the strengths and shortcomings of the past research done on soft tissues; 2) propose novel theories and lab procedures that will assist researchers to better understand the underlying physiological changes and events causing soft tissue failure; and 3) provide some simulation models to reduce human testing in the future. Hopefully, this research will help deliver a new understanding of soft tissues and will ultimately lead to improved devices and therapies to treat diseases caused by soft tissue failure.

This paper includes three parts. The first is a literature research on past and present research done on the macroscopic biomechanical properties of soft tissues. Different research approaches adopted by various scientists, and the possibility of correlating them to provide a basis for meaningful comparison is discussed. Second, novel theories (or novel approaches) explaining the molecular physiology and molecular biomechanics of soft tissues as they age and fail are proposed. Methods to test these novel theories are also proposed. Laboratory test results related to some of proposed theories in this thesis are presented and discussed. Third, some soft tissue simulation models were designed to hopefully standardize future soft tissue research by reducing the number of variables needing to be measured experimentally in the laboratory. Also, data that needs to be collected before an ideal simulation model can be developed is discussed.

A thorough biomechanical study and molecular analysis of soft tissues is presented in this paper. Although most of the novel theories as well as the simulation models presented in this thesis are applicable to all type of soft tissues, the focus of this thesis is on healthy or prolapsed vaginal tissues. As a final note, research on the biomechanical aspects of the skin and the cornea, based on the novel theories proposed in this thesis, is currently underway at UT Southwestern.

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Chapter 1

History and Introduction

The human body is fundamentally dependent on soft tissues to function. Although soft tissues such as the skin, the cornea, and the vaginal epithelium are remarkably versatile and resilient to constant wear and tear, our bodies' repair mechanisms often do fall short in repairing these soft tissues as we age. When these soft tissues fail, many medical problems can result, from cosmetic blemishes such as wrinkles and loose skin to, more seriously, pelvic organ prolapse (which involves organs such as the bladder or the uterus falling out of their proper place when the vaginal epithelium fails [i.e., prolapses] and stops supporting them) to, even more seriously, opacification of the cornea, which can result in blurred vision and eventually blindness.

Laser surgery to slow the progression of corneal opacification offers only temporary relief. Medical treatments to repair pelvic organ prolapse require painful surgery and often involve permanent implantation of biomedical devices. Preventative drug treatments, methods for early detection and intervention, and "smarter" devices targeted at the root of the problems associated with soft tissue failure will all be possible after we understand the physiology of soft tissues, from their macroscopic biomechanics to their microscopic fiber-level structures and biomechanics. Therefore, the area of molecular biology and biomechanics of soft tissues is of pivotal importance to the understanding of human physiology, aging, and disease, and opens up the development of novel targeted treatments for soft tissue related ailments.

Today, 1.012 billion US dollars are spent each year on the treatment of pelvic organ prolapse alone. (Bhatt 2005) Comparable billions of dollars are spent each year on

the treatment of corneal opacification, cosmetic skin operations, and other soft tissue related problems. Collectively, hundreds of billions of dollars are spent each year on medical devices and treatments related to curing or alleviating medical problems relating to soft tissue aging, prolapse, disease, and/or injury. Judging from the enormous expense in the past in dealing with problems associated with the aging/failure of soft tissues, it is clear that research in this area is of high priority.

Although the specific study of soft tissue biomechanics is relatively new, the closely related discipline of soft tissue biology has actually been investigated and documented in one way or another since at least as early as 3000 BC in ancient Egypt, as evidenced by medical papyri from that time recently unearthed by archaeologists. (Medicine in Ancient Egypt 2000) Since that time, there have been numerous approaches from physics, biology, philosophy, and, of course, religion to understanding soft tissues, their role in our body, and how to fix their related ailments.

Studies on soft tissues in the 20th century mostly focused on biological testing. There was renewed vigor in finding the causes of the soft tissue problems through understanding the structure and function of the tissues. However, the limited equipment and medical knowledge available at that time precluded truly pivotal research from being undertaken.

Chapter 2

Soft Tissue Biomechanics Literature Research

2.a Some Background on Relevant Terminology

In the past, people have focused on studying the elastic properties of soft tissues through analyzing their biomechanical properties. The elastic modulus (**E**), also known as Young's modulus, is a common property used by most people as an indicator of tissue strength in studying the elastic properties of soft tissues. According to the definition of Young's modulus, the linear relationship between the elastic modulus, stress, and strain for a specific specimen can be expressed as $\mathbf{E = \delta / \varepsilon}$. According to the formula, **E**, the elastic modulus, is defined as Stress ($\boldsymbol{\delta}$) per unit Strain ($\boldsymbol{\varepsilon}$), where $\boldsymbol{\delta}$, the stress, is defined as the tensile force (**F**) divided by the cross sectional area (**A**) (i.e., $\boldsymbol{\delta = F/A}$) and $\boldsymbol{\varepsilon}$, the strain, is the ratio of the specimen's change in length ($\mathbf{l_1 - l_0}$) under force to the specimen's original (or un-stretched) length ($\mathbf{l_0}$) (i.e., $\boldsymbol{\varepsilon = (l_1 - l_0) / l_0}$). (Materials by Design 1996) This formula for finding the elastic modulus only works for small stresses which do not irreversibly stretch the material. That is to say, the equations above only work for small stresses within the material's linear elastic region.

Figure 1, below, shows a typical stress-strain curve that is applicable to many materials, including soft tissues. It shows that all materials go through various regions (or zones) when the applied stress increases. At very low stresses, there is a non-linear zone. At slightly higher stresses, there is a linear elastic zone. Most materials function properly

within their linear elastic zones. When stresses large enough to permanently deform a particular material are applied, that material enters the ductile zone. Finally, at even higher stresses, complete failure of the material occurs and it enters the brittle zone. UCS is the maximum stress a material can sustain before it fails completely. (Materials by Design 1996)

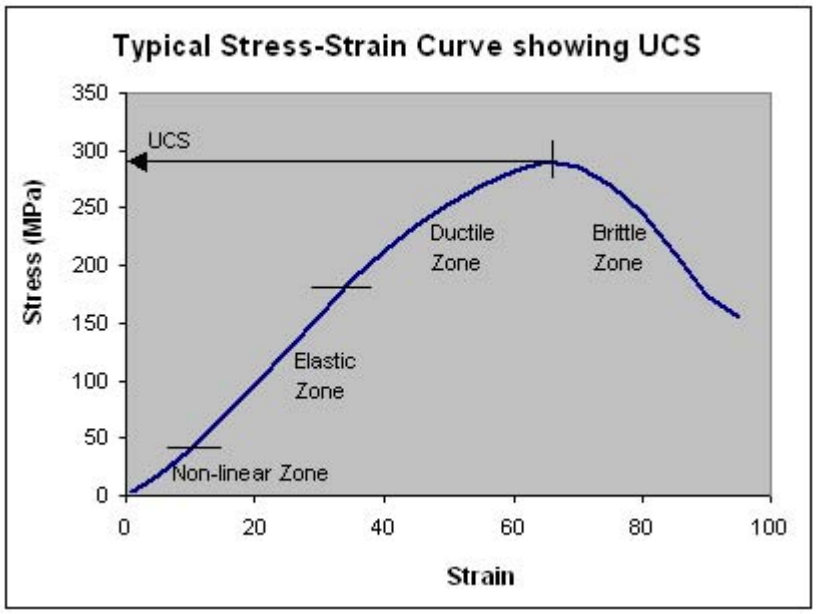

FIGURE 1 – A Typical Stress-Strain Curve showing the Non-Linear Zone, the Linear Elastic Zone, the Ductile Zone, and the Brittle Zone after Failure. UCS represents the maximum stress a material can sustain before it fails completely.

Figure 2, below, shows the linear elastic zone of the curve in Figure 1. The elastic modulus E, which is linearly proportional to the stress and inversely linearly proportional to the strain, is actually the slope of the line as shown in Figure 2.

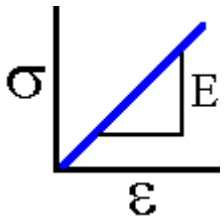

FIGURE 2 – Finding the Elastic Modulus E from the Linear Elastic Region of a Stress-Strain Curve, where the Y Axis represents Stress and the X Axis represents Srain.

Figure 3, below, shows a typical experimental setup for determining the elastic modulus of a sample. Basically, the sample is mounted to two clamps at either end, and a tensile testing machine is attached to the clamps. While the tensile testing machine pulls on the clamps, recording equipment simultaneously calculates and records the sample's strain/stress curve (Materials by Design 1996). Due to concerns over not damaging the tissue in order to obtain the most accurate results, variations of the above basic setup are usually used to meet this special need in actual studies of the elastic properties of vaginal tissues.

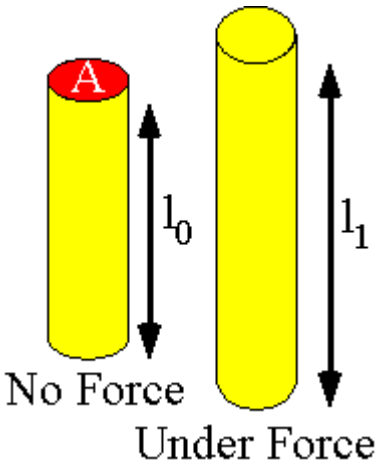

FIGURE 3 – A Typical Experimental Setup for Determining the Elastic Modulus of a Sample, where l_0 represents the initial length of the sample and l_1 represents the stretched length (when the sample is under force).

2.b Soft Tissue Biomechanics Literature Research

2.b.i Cosson et al. (2004)

Relatively little past work has been done in the area of soft tissue biomechanics and properties. Cosson's et al. (2004) study has been a major contribution to this area. Vaginal tissues from 16 post-menopausal patients removed *in vivo* from the posterior vaginal fundus during surgical operations were used in this study. Clamps were attached to both ends of a tissue sample, and a machine pulled on one of the clamps, stretching the tissue, while the other clamp held the tissue in place, allowing measurements of the tissue sample's tensile and bending strengths. The resulting measurements are listed below in Table 1 (Cosson et al. 2004).

TABLE 1 – Measurement of Strength (N) and Elongation (mm) of Vaginal Tissues

Patient	Age	Tensile		Bending	
		Elongation (mm)	Rupture force (N)	Elongation (mm)	Rupture force (N)
1	69	22.67	49.48	6.02	14.48
2	68	14.54	12.61	7.09	21.32
3	42	18.28	15.14	10.95	27.77
4	72	20.40	44.76	14.26	34.52
5	75	22.69	35.82	10.07	40.39
6	63	18.23	29.47	8.18	43.14
7	73	32.62	76.33	20.37	43.24
8	69	23.22	32.90	13.00	51.68
9	62	16.37	25.68	7.88	56.89
10	63	22.03	66.12	6.24	62.97
11	64	42.11	60.84	16.31	76.17
12	69	24.84	72.50	13.62	83.20
13	59	24.92	64.87	12.64	93.43
14	77	19.30	33.44	11.41	105.40
15	65	26.03	44.20	16.37	130.77
16	55	10.68	21.58		
Mean	66.00	23.22	44.28	11.63	59.02
S.D.	8.38	6.84	20.29	4.18	33.02

Cosson et al. (2004) then used a statistical analysis (as shown in Figure 4 below) to interpret the data.

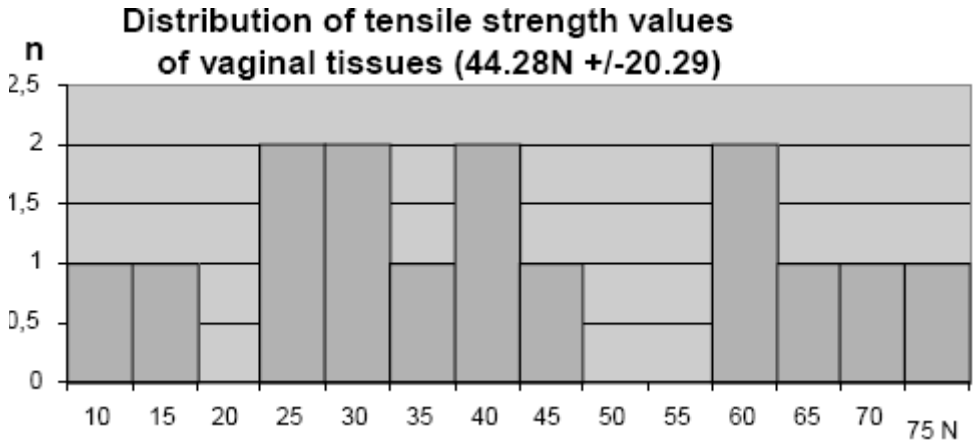

FIGURE 4 – Experimental data on tensile strength values of vaginal tissue samples (Cosson et al. 2004).

From the bar graph in Figure 4, Cosson et al. (2004) observed that the ultimate tensile strengths of vaginal tissues are unpredictable.

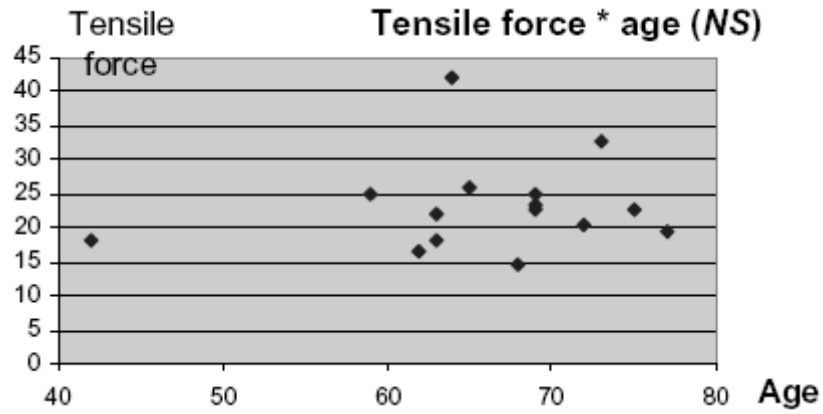

FIGURE 5 – Experimental data on values of rupture force in tensile strength tests in relation to patient age (Cosson et al. 2004).

Cosson et al. (2004) observed that the value of the rupture force in tensile strength tests remains remarkably independent of age (i.e., the strength of the vaginal tissue is unrelated to a person's age).

The previous two observations should not be taken as conclusions, because, as Cosson et al. (2004) noted, the tissue samples obtained were of non-uniform thicknesses, age (the ages of the patients from which tissue samples were obtained ranged from 42 to 77), and condition (the condition of the samples when they were tested were different because the elapsed time between surgical removal and testing of the samples was different for each sample) (Cosson et al. 2004). In addition, the sample size of 16 individuals was relatively low statistically (Cosson et al. 2004).

2.b.ii Goh (2003)

This study was another major contribution to the study of soft tissue properties and biomechanics. In this study, vaginal tissue was collected at the time of surgery in women with symptomatic prolapse (Goh 2003). Tissue from the anterior vaginal wall was obtained and analyzed in 10 postmenopausal and 8 premenopausal women. The age range for postmenopausal women was 51–88 years (mean 69) and that for the premenopausal women was 30–49 years (mean 41). The dimensions of the samples obtained from the anterior vaginal wall were standardized in a cropping method. The aim of this study was to biomechanically assess vaginal tissue from pre- and postmenopausal women with symptomatic prolapse (Goh 2003).

The biomechanical properties of the tissues were measured. The data from Goh's (2003) experiment is shown below in Table 2, and the tissue tensile testing setup is illustrated below in Figures 6 and 7.

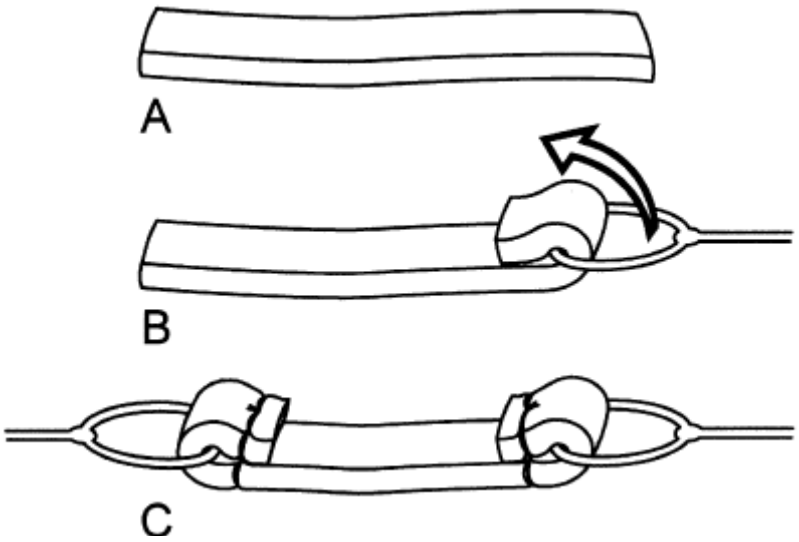

FIGURE 6 – Experimental tissue strength testing setup, showing how the tissue sample was folded over and pulled on by metal rings (Goh 2003).

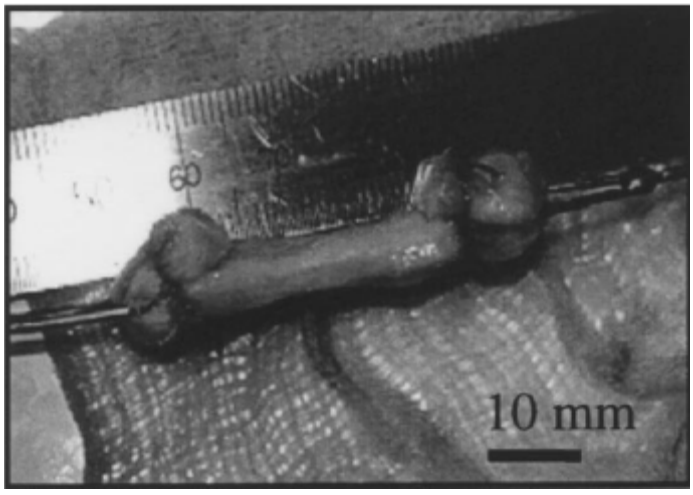

FIGURE 7 – Picture of tissue about to undergo mechanical testing (Goh 2003).

TABLE 2 – Experimental tissue testing results on the premenopausal and postmenopausal vaginal tissue biomechanics, where E represents Elastic Modulus and SD represents Standard Deviation (Goh 2003).

	Premenopausal	Postmenopausal	*P* value
Age (SD) (years)	41.4 (6.6)	69.7 (11.6)	
E (SD) At 0.2 MPa	8.3 (1.09)	9.78 (1.44)	0.025
E (SD) At 0.3 MPa	9.5 (1.3)	11.3 (1.72)	0.022
E (SD) At 0.4 MPa	11.5 (1.51)	14.35 (2.01)	0.006

Figure 6 shows how Goh (2003) mounted vaginal tissue specimens for mechanical testing. The ends of the tissue specimens were folded over and sewn onto the specimen (as shown in Figure 6) and then a hook at either end was put in the folded-over region. Also, only one of the hooks applied force to stretch the tissue sample; the other hook held the other end in place. Table 2 shows calculated elastic moduli from Goh's (2003) results.

The dimensions of the tissue samples were standardized before their mechanical properties were tested (Goh 2003). However, as Goh (2003) noted, the cropping method used to standardize the tissue samples could potentially alter the biomechanical properties of the tissues and ultimately create non-standardized samples (Goh 2003).

This biomechanical analysis of vaginal tissue in pre- and postmenopausal women demonstrated possible age-related changes (Goh 2003). The higher elastic modulus of postmenopausal vaginal tissue indicates that the tissue is stiffer, but does not signify that postmenopausal tissue is weaker than premenopausal vaginal tissue. The higher elastic

modulus in postmenopausal tissue implies that, for a given tension increase, the tissue elongated less. There were no significant differences in other biomechanical parameters (Goh 2003). As in the research of Cosson et al. (2004), the sample size of Goh's (2003) study was relatively small statistically.

2.b.iii Bhatt (2005)

2.b.iii.1 Recent Research

More recently Bhatt (2005) has carried out biomechanical studies on tissue samples from the anterior vaginal wall. In this study, clamps were attached to both ends of the tissue sample, and a machine pulled on one of the clamps, stretching the tissue, while the other clamp held the tissue in place (Bhatt 2005). The aim of this research was to understand the mechanisms of tissue failure through biomechanical and ultrastructural studies. The study involved tissues from three groups – two subject groups and one control group. The samples for the Control group were obtained from 3 patients without cystocele and vaginal prolapse. The samples in Groups 1 and 2 were taken from postmenopausal women undergoing cystocele repair. The 16 samples obtained for Group 1 were stored in unbalanced saline prior to the analysis. The 23 samples obtained for Group 2 were stored in saline-moistened gauze prior to the analysis (Bhatt 2005).

Reliable Young's moduli for vaginal tissues have never been obtained before because tissue sample dimensions and pre-treatment conditions were not standardized. In Bhatt's (2005) research, certain adjustments were made to stabilize tissue dimensions and handling conditions to try to standardize the study. Due to these adjustments,

standardization was assumed and Young's moduli for vaginal tissues were calculated based on the data collected. The results are shown in Table 3, below (Bhatt 2005).

The three experimental groups in Table 3, below, were separated based on different post-excision tissue treatment and transport methods (Bhatt 2005).

TABLE 3 – Calculated Young's Moduli for the Control Group, Group 1, and Group 2 (Bhatt 2005).

Group	Young's moduli (N/mm^2)
Control (n = 3)	10.2 ±3.8
Group 1 (n = 16)	4.4 ±2.8
Group 2 (n = 23)	8.4 ±4.8

However, Bhatt's (2005) research may have had some methodological factors which could have impacted the accuracy of the calculation of Young's moduli. Bhatt (2005) attempted to standardize her experiments by testing all tissue samples within three hours of surgical excision and by trimming the tissue samples to a set of standard dimensions. However, as reported by Goh (2003), such trimming has the potential to disrupt the microscopic fiber-level tissue structure; therefore, it can alter the macroscopic properties of the tissue sample. The age distribution for Group 1 and Group 2 was widespread between 52 and 85 (Bhatt 2005). The tissue thicknesses varied from 1.4 mm to 4.4 mm (Bhatt 2005). In addition, the number of samples used to calculate the Young's moduli was 3 for the Control group, 16 for Group 1, and 23 for Group 2 (Bhatt 2005).

The number of samples used to calculate the Young's moduli for each group was statistically small.

2.b.iii.d Future Research

Skin tissue is especially important in cosmetic applications. A thorough study of the "prolapse" or failure of skin as we age (a property that manifests itself in wrinkles, loose skin, etc.) holds promise in delivering methods by which to "fix" old skin into tight, young skin again.

The cornea, of course, is crucial in vision. Corneal disease is a major health problem especially in older individuals. As the cornea ages, it sometimes becomes opaque, reducing the field of vision significantly. It is postulated that the reason for the opaqueness of the cornea is due to the rearrangement (or actually, misarrangement) of collagen fibers as the tissue ages. An understanding of the molecular mechanisms of this rearrangement, and whether different production mechanisms are responsible for this structural change, promises to play a key role in ultimately curing many such corneal diseases.

Due to their importance, the skin and the cornea were selected for investigation by other researchers at UT Southwestern.

2.b.iv Related Research

New techniques and tools developed by some researchers for application in this area have helped improve the chance of getting more fruitful results for future investigators. Nava and Mazza's (2004) aspiration experiment, and Han's et al. (2003) real-time ultrasound indentation system are among these contributions that I believe will further solidify the foundation for future research in this area.

For example, Nava and Mazza (2004), of the Institute of Mechanical Systems at Zurich, have developed a method known as the "aspiration experiment" for measuring the mechanical properties of soft biological tissues *in vivo*. Currently, they have tested their method on several uterine cervixes. Their method is useful. However, as they said, the limitation of their method is that it only allows *in vivo* testing under "open surgery" conditions (Nava and Mazza 2004). It is impossible to form a control group by obtaining mechanical soft tissue data from completely healthy volunteers who do not medically need any surgery.

Han et al. (2003) have developed a real-time ultrasound indentation system coupled with reverse finite element modeling to measure the *in vivo* properties of soft tissues. Currently, they have tested the technique on healthy human breast tissue (Han et al. 2003). Although Han's et al. (2003) technique shows promise, it needs to be refined to take into account the nonlinear behavior of soft tissue before it can be used in precise studies on soft tissue biomechanics.

2.c Future Directions

As indicated in this paper, the problems associated with past studies of macroscopic biomechanics of soft tissues are numerous, including *in vitro* testing, non-standard dimensions, wide age ranges, and small sample pools, among other irregularities associated with the tools and tissue samples used.

Future experiments on macroscopic biomechanics of soft tissues should preferably be conducted *in vivo* with new tools such as refined aspiration experiments (Nava and Mazza) or refined ultrasound indentation systems (Han et al. 2003). The following points should be taken into account in any future *in vivo* experiments.

1) True control data from healthy volunteers should be collected.
2) Only data obtained from tissues from people with similar ages should be compared.
3) Experiments must be done on sample sizes that allow reliable statistical analysis.

The following points should be taken into account in future *in vitro* experiments, if conducted.

1) Only data obtained from tissue samples taken from individuals with similar ages should be compared.
2) Only data obtained from tissue samples having similar dimensions should be compared.
3) Measurements should be conducted as soon as possible after the tissue samples have been obtained.

4) Tissue samples should be tested exactly at a certain predetermined amount of elapsed time (for example, one hour) after the sample has been obtained.
5) Sample sizes must be large enough to allow definitive statistical testing.

Running standardized tests as mentioned above, combined with the development of accurate simulators, will help usher in a bright future in the study of the biomechanics of soft tissues.

2.d Different Experimental Setups and the Elastic Modulus

Even though experimental approaches for measuring the biomechanical properties of tissue samples (like those adopted by Goh (2003), Cosson et al. (2004), and Bhatt (2005)) may vary, the different experimental setups (including variations on a given basic experimental setup, such as different methods of securing the tissue sample) should not affect the calculated elastic modulus, if the data is used properly in calculating the elastic modulus (i.e., based on the laws of statics and dynamics). The correct elastic modulus can only be obtained when factors pertaining to the specific experimental setup are taken into account, on a case-by-case basis. Variations 1, 2, and 3, below are three examples.

Variation 1 – If the experimental setup is basically to pull the tissue at one end, with the other end firmly secured, the elastic moduli of similar tissue samples taken from the same area in the body should be comparable when calculated from stress/strain data taken at the center of the tissue sample.

Variation 2 – If the experimental setup is basically to pull the tissue at one end, with the other end firmly secured, but the stress/strain data were taken near the

edge of the samples, the correct elastic modulus can only be obtained when complicated edge effects from different experimental methods are quantified and taken into account.

Variation 3 – If stress/strain data were obtained from an experimental setup consisting of pulling on a stitch placed at the center of the sample, with both ends of the tissue sample firmly secured, the correct elastic modulus should be calculated from stress and strain data obtained from the tissue sample at either midline between the stitch and the tissue edge, taking into account that the stress on each side is equal to half of the total applied stress.

Chapter 3

Correlation of Experimental Data

The original goal of doing the literature research was to analyze and correlate the data from different sources in order to obtain a better understanding of the macroscopic biomechanics of vaginal tissue. However, two problems prevent the planned correlation from being done. Use of different methodologies to obtain data made comparisons across studies impossible. These included the use of tissues from different parts of the vagina, a lack of control tissues, and statistically small sample sizes.

Chapter 4

Novel Theories about Tissue Aging and Prolapse

4.a Novel Theories

Past research done on the biomechanics of soft tissues has all been done macroscopically. This chapter will examine the biomechanics of soft tissues from a microscopic perspective. There are many possible microscopic causes for tissue aging and prolapse. One or more of the proposed causes below may actually be the true cause(s). The experimental work proposed after each cause will help to identify whether that cause plays a role in tissue failure or not.

1) MECHANICAL EXTRACELLULAR MATRIX (ECM) DEFICIENCY – Because most of the mechanical strength of the tissue derives from extracellular matrix fibers such as collagen, fibrin, and particularly elastin, overexertion of these fibers could cause tissue failure or prolapse. Electron microscopic examination of these fibers in prolapsed tissues could help determine whether fiber failure is responsible for tissue failure.
2) AUTOGENEIC DEGRADATION – Because most of the mechanical strength of the tissue derives from extracellular matrix fibers such as collagen, fibrin, and particularly elastin, autoimmune degradation of these fibers could cause tissue failure or prolapse. Microscopic counts of the numbers of immune cells such as cytotoxic T lymphocytes (CTLs) in prolapsed tissues placed in cell culture could help determine whether autoimmune degradation of fibers is responsible for tissue failure.

3) CELLULAR SENESCENCE – Because cells are the building blocks of tissues, cellular aging or death (senescence) could cause tissue aging and prolapse. Microscopic counts of the numbers of senescent cells in prolapsed tissues placed in cell culture could help determine whether cellular senescence is responsible for tissue failure.

4) CYTOPLASMIC MEMBRANE RECEPTOR FAILURE – Because receptors such as cellular attachment glycoproteins connect cells to other cells as well as cells to extracellular matrix fibers, receptor failure could cause tissue aging and prolapse. Microscopic or biochemical study of the structures of the receptors in prolapsed tissues and subsequent comparison with the structures of normal receptors could help determine whether receptor failure is responsible for tissue failure.

5) EXTRACELULAR MATRIX (ECM) TURNOVER DEFICIENCY – Because most of the mechanical strength of the tissue derives from extracellular matrix fibers such as collagen, fibrin, and particularly elastin, low levels of these fibers could cause tissue failure or prolapse. All fibers are degraded normally at a slow rate; thus, new fibers are normally synthesized on an ongoing basis. Since these fibers are always made from protein precursors, low production levels of these fibers' precursors could ultimately cause tissue aging and prolapse. If the precursors of collagen, fibrin, elastin, and other important fibers in prolapsed tissues are found to be at low levels (various existing

experimental methods could make this measurement), then a low level of fiber production could be responsible for tissue failure.

6) FIBER MISFOLDING – Because most of the mechanical strength of the tissue derives from extracellular matrix fibers such as collagen, fibrin, and particularly elastin, misfolded fibers could cause tissue failure or prolapse. All fibers are degraded normally at a slow rate; thus, new fibers are normally synthesized on an ongoing basis. Since newly made fibers must be folded properly to function, misfolded fibers could cause tissue failure or prolapse. If the number of chaperones (proteins that help other proteins to fold correctly) for these proteins in prolapsed tissues are found to be at low levels (various existing experimental methods could make this measurement), then a low level of chaperone production could be responsible for tissue failure. Also, if the chaperones in prolapsed tissues have themselves mutated or misfolded (microscopic or biochemical study of the structures of the chaperones in prolapsed tissues and subsequent comparison with the structures of normal chaperones could determine this), they would not be as effective as they were before, and factors causing chaperone damage (ranging from immune responses to drug effects) could be responsible for tissue failure.

Detailed experiments such as those proposed above could determine which of these mechanisms is/are responsible for tissue aging and prolapse as well as the degree to which each mechanism contributes to tissue aging or failure.

4.b Some Experimental Results

Two aspects of the novel theories set out in Section 4.a above have been examined by researchers at UT Southwestern.

1) MECHANICAL EXTRACELLULAR MATRIX (ECM) DEFICIENCY

    a. Microscopic studies indicate that prolapsed tissues, after being stretched, have a loosely packed, irregular, and unorganized, although not necessarily broken, network of collagen fibers with elastic fibers in varying degrees of fragmentation (Bhatt 2005).

    b. Non-stretched prolapsed tissues from the same patient, on the other hand, showed tightly bundled, organized aggregates of collagen and long and un-fragmented elastic fibers (Bhatt 2005).

    c. The mean diameter of the collagen fibers in stretched samples of prolapsed and non-prolapsed tissues was significantly lower than the mean diameter of collagen fibers in the unstretched samples. It was proposed that the collagen fibers unwound and stretched during application of the stretching load, causing the fibers to thin (Bhatt 2005).

It was concluded that fiber failure can cause tissue failure (Bhatt 2005). The experiment proved that applying force to stretch tissues can make the tissue fail and can consequently lead to the failure of the internal fibers. It did not prove that tissue failure or prolapse *in vivo* is actually caused or solely caused by fiber failure.

2) CELLULAR SENESCENSE

Light and electron microscopic studies have shown that prolapsed tissues had a normal stratified epithelium (Bhatt 2005). This observation proved that the prolapsed tissues did not have widespread cellular senescence in the stratified epithelium. It did not prove anything concerning cellular senescence in other parts of the prolapsed tissues.

3) THE ROLE OF ELASTIN

Recent unpublished microscopic work at UT Southwestern indicates that elastin's role appears to be to organize collagen fibers into tight bundles. It was concluded that elastin does not contribute to the overall mechanical strength of vaginal tissues. However, decreasing elastin levels, misfolded elastin, and elastin failure could all potentially lead to the collagen bundles becoming disorganized, which would then lead to tissue failure or prolapse. Therefore, elastin could still indirectly contribute to the overall mechanical strength of vaginal tissues, and thus elastin could still play an important role in tissue aging and prolapse.

The above observations and experiments were carried out on partial aspects of this author's respective theories as set out in Chapter 4.a. More work must be done on other aspects of these theories before their value can be judged. Also, work needs to be done on the other theories described in Chapter 4.a. To conclude, although some data has been collected on the microscopic aspects of tissue biomechanics, more data remain to be gathered to fully understand the microscopic aspects of tissue biomechanics.

Chapter 5

Soft Tissue Simulation Models

5.a Currently Possible Models

Most experimental work in testing biomechanical properties has encountered difficulty in obtaining standardized samples (samples obtained in the recent past have had different sizes, came from different areas, were taken from people of different ages, and were processed at various times after being obtained, among other factors). In addition, taking tissue samples from healthy people to establish a control group is another challenging ethical issue. The problems associated with creating a control group as well as those associated with non-standardized methodologies could be solved if there were equipment capable of non-destructively testing the mechanical properties of tissues *in vivo.* Such equipment has not yet been invented. Therefore, computer modeling of soft tissues is the potential answer to all of the problems mentioned above. However, computer modeling of soft tissues can only provide meaningful predictions/results after the basic nature of soft tissues is understood and some basic information regarding soft tissues is obtained.

The computer simulation tool ANSYS was used to model soft tissues for various experimental setups in this research. Data generated from studies of previous researchers (Bhatt 2005) was used in modeling the soft tissues. However, as noted in previous chapters, this data cannot be considered "standardized." Also, data that would allow the generation of realistic elastic anisotropic models (which would take into account direction- and location-dependent material properties) is not yet available, so the sample simulations presented below were generated with an elastic isotropic model. Thus, the

simulations presented below are only meant to show the simulation model's potential. Only when standardized experimental data becomes available can the simulation model be used to replace some lab work.

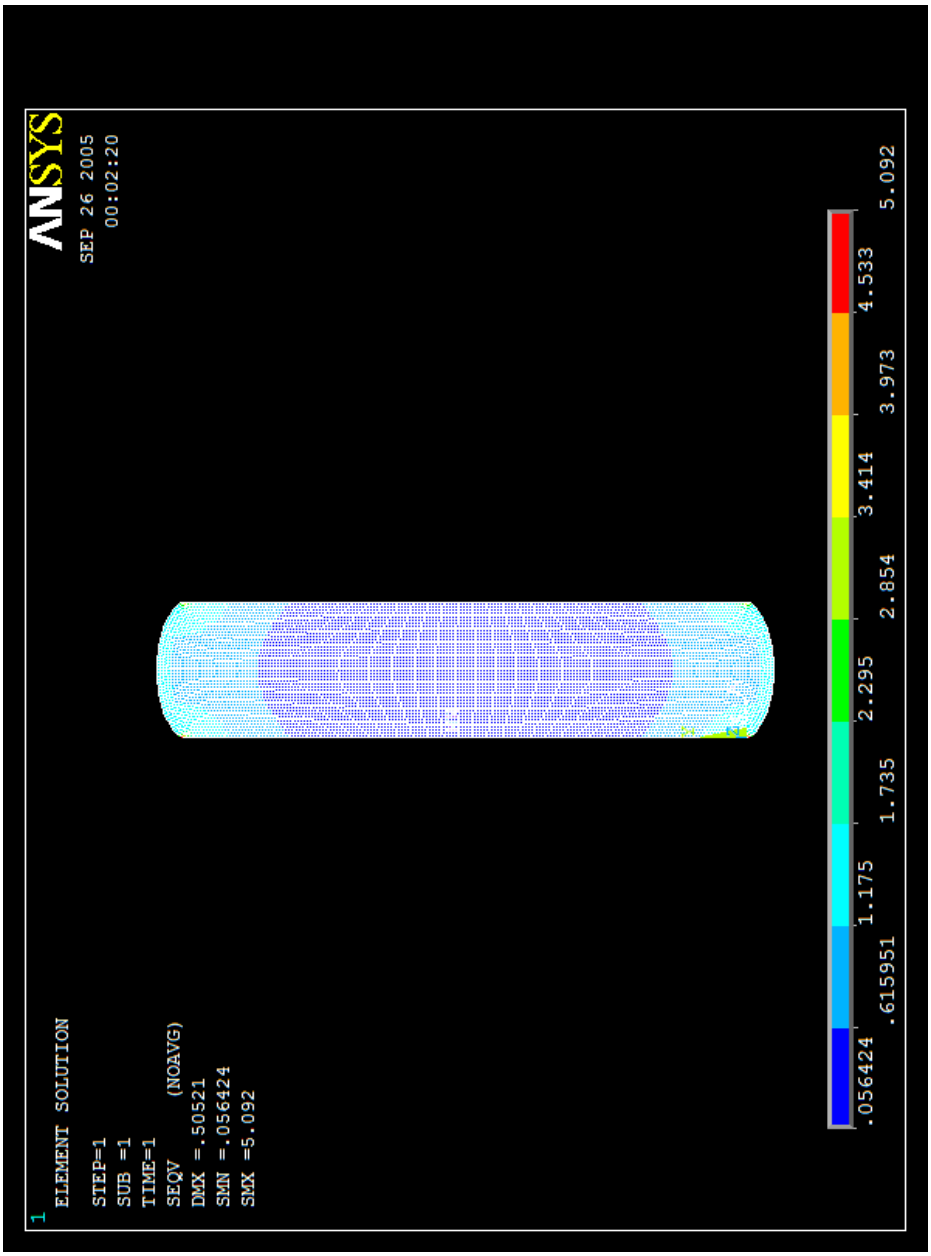

FIGURE 8 – The Structure and Internal Stress Distribution of a Computer-Modeled Soft Tissue under Stress at its Two Ends.

Figure 8 shows the structure of a modeled soft tissue being pulled at both ends. Although this specific experimental approach has never been used in any laboratory, this model shows the stresses that would occur in a real tissue under such conditions. At both ends, a stress of 1.36 N / mm^2 was applied. This stress, according to Bhatt (2005), is in the linear elastic zone for vaginal tissues. In this model, the tissue was given an elastic modulus value of 8.4 N/ mm^2, the average value for vaginal tissues in Group 2 in Bhatt (2005) and a Poisson's ratio of 0.48 which is considered a reasonable value for soft biological tissues (Lakes 1987). A linear isotropic elastic model was used due to lack of available data for anisotropic modeling. For precise results, 100000 quadrilaterals were used to mesh the tissue. The internal stress distribution inside the tissue, which is shown in Figure 8, can be used to help scientists understand how tissues react under stress from two ends. Such information is currently not obtainable from the laboratory. For example, the figure shows that the maximum stress occurs at either end and has a value of 1.735 N / mm^2. It also shows that the maximum deformation of the tissue occurs at about halfway between the midpoint and either end.

Figure 9 shows the structure of a modeled soft tissue being pulled at one end. This is one experimental methodology that has been used in the past (Bhatt 2005). At the end being pulled, a stress of 1.36 N / mm^2 was applied as used in the experimental studies of Bhatt (2005) and which is within the linear elastic zone for vaginal tissues. In this model, the tissue was given an elastic modulus value of 8.4 N / mm^2, the average value for vaginal tissues in Group 2 in Bhatt's (2005) study and a Poisson's ratio of 0.48 which is considered a reasonable value for soft biological tissues (Lakes 1987). A linear isotropic

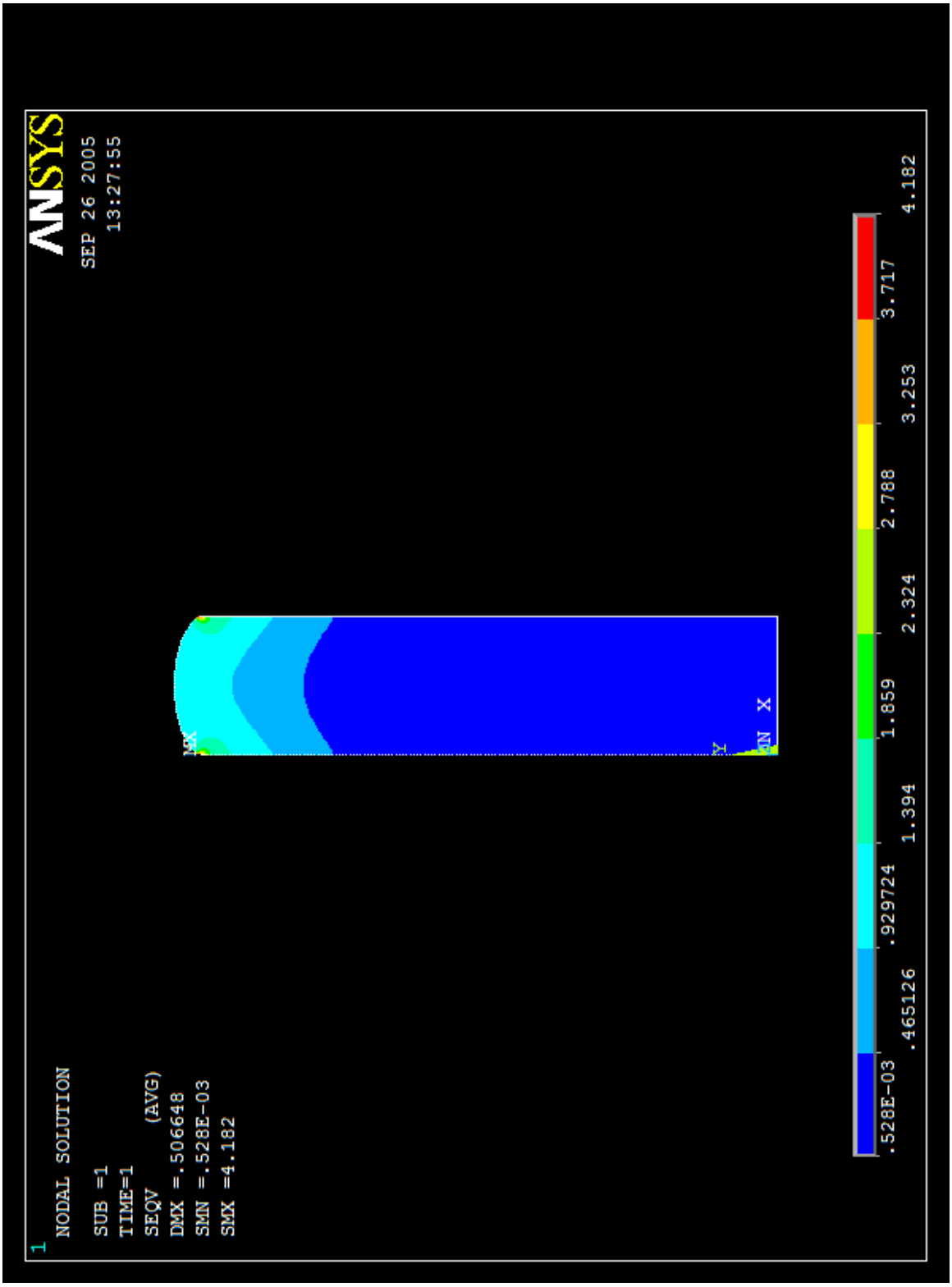

FIGURE 9 – The Structure and Internal Stress Distribution of a Computer-Modeled Soft Tissue under Stress at One End.

elastic model was used due to lack of available data for anisotropic modeling. For precise results, 100000 quadrilaterals were used to mesh the tissue. The internal stress distribution inside the tissue, which is shown in Figure 9, can be used to help scientists understand how tissues react under stress from one end. Such information is currently not obtainable from the laboratory. For example, Figure 9 shows that the maximum stress occurs at the end being pulled and has a value of 1.394 N / mm^2.

Figure 10 shows a perfect direct linear relationship between the maximum internal Von Mises stress and the applied stress, as expected. The applied stress was varied in increments of 0.2 N/mm^2 from the minimum stress within the tissue's linear elastic zone (0.8 N/mm^2) to the tissue's UTS (1.8 N/mm^2), and the maximum internal von Mises stress calculated by the simulation model for each applied stress was recorded. The maximum internal von Mises stress values were obtained from the simulation model at each applied stress. The simulation results at each applied stress are shown in Figures 11 – 16, below. A Young's modulus of 8.4 and a Poisson's ratio of 0.48 were used in the simulations.

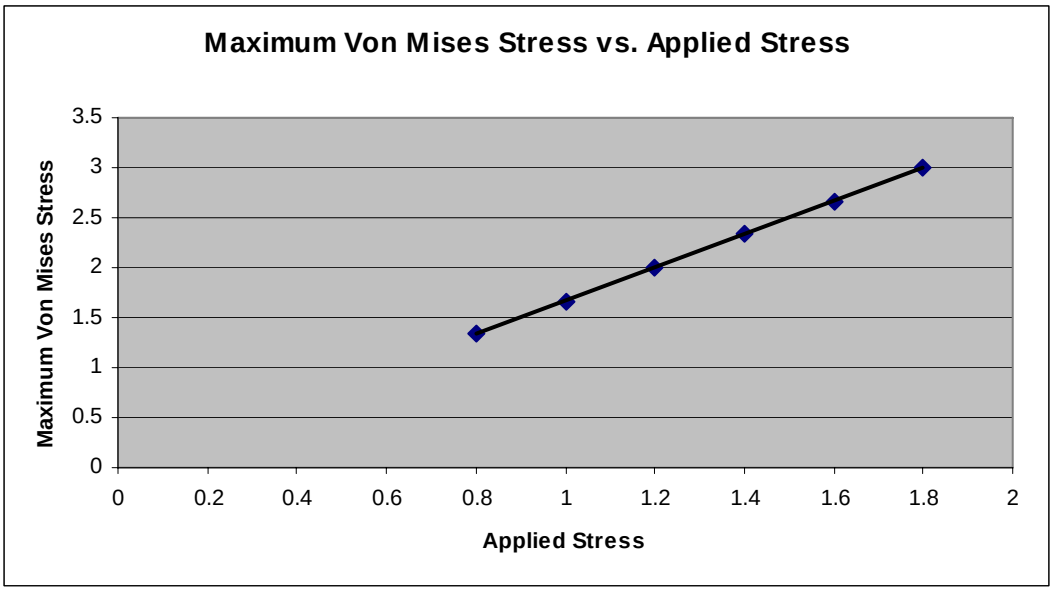

FIGURE 10 – Maximum Internal Von Mises Stress vs. Applied Stress for a Computer-Modeled Soft Tissue under Stress at One End.

Figure 11

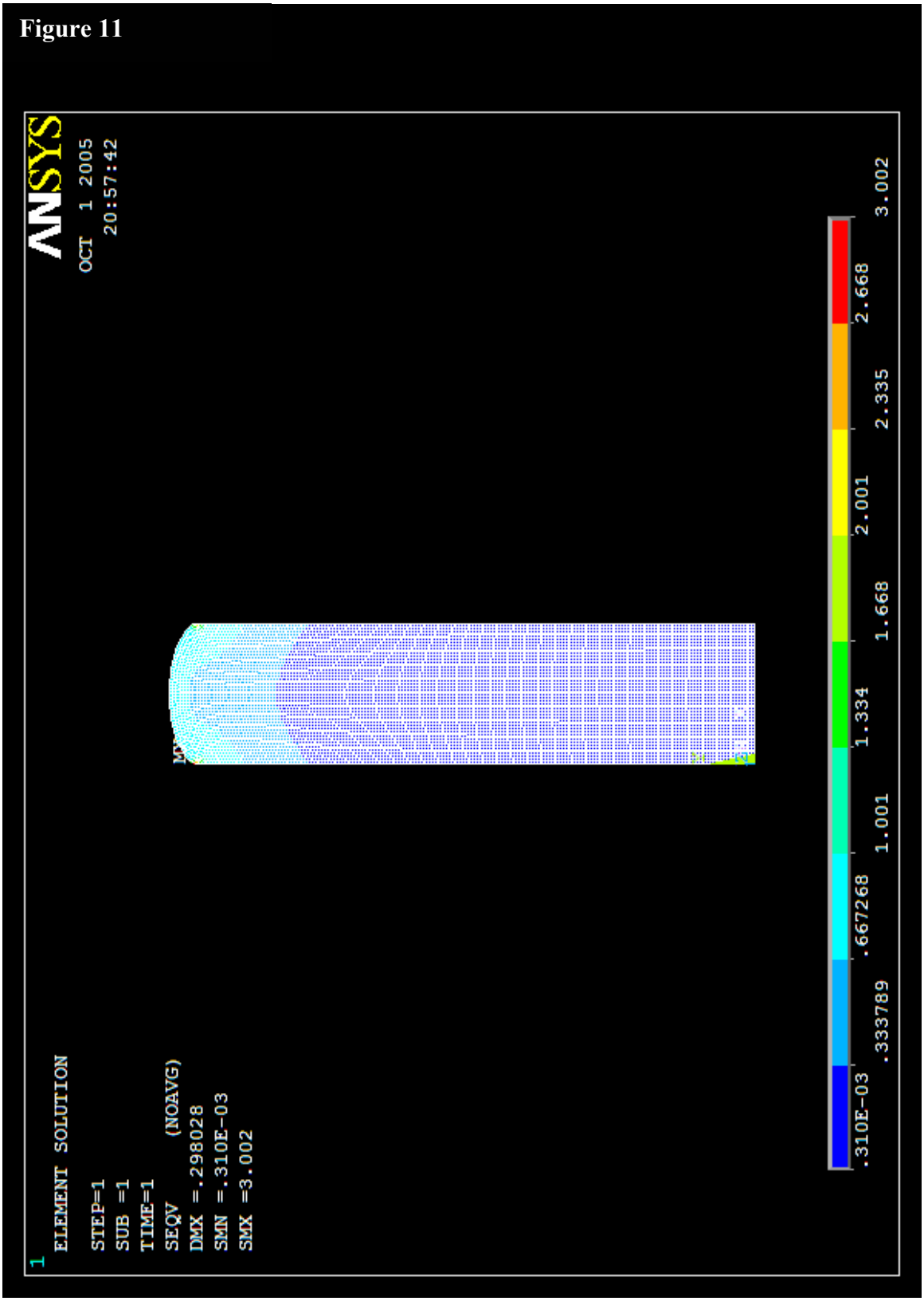
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Figure 12

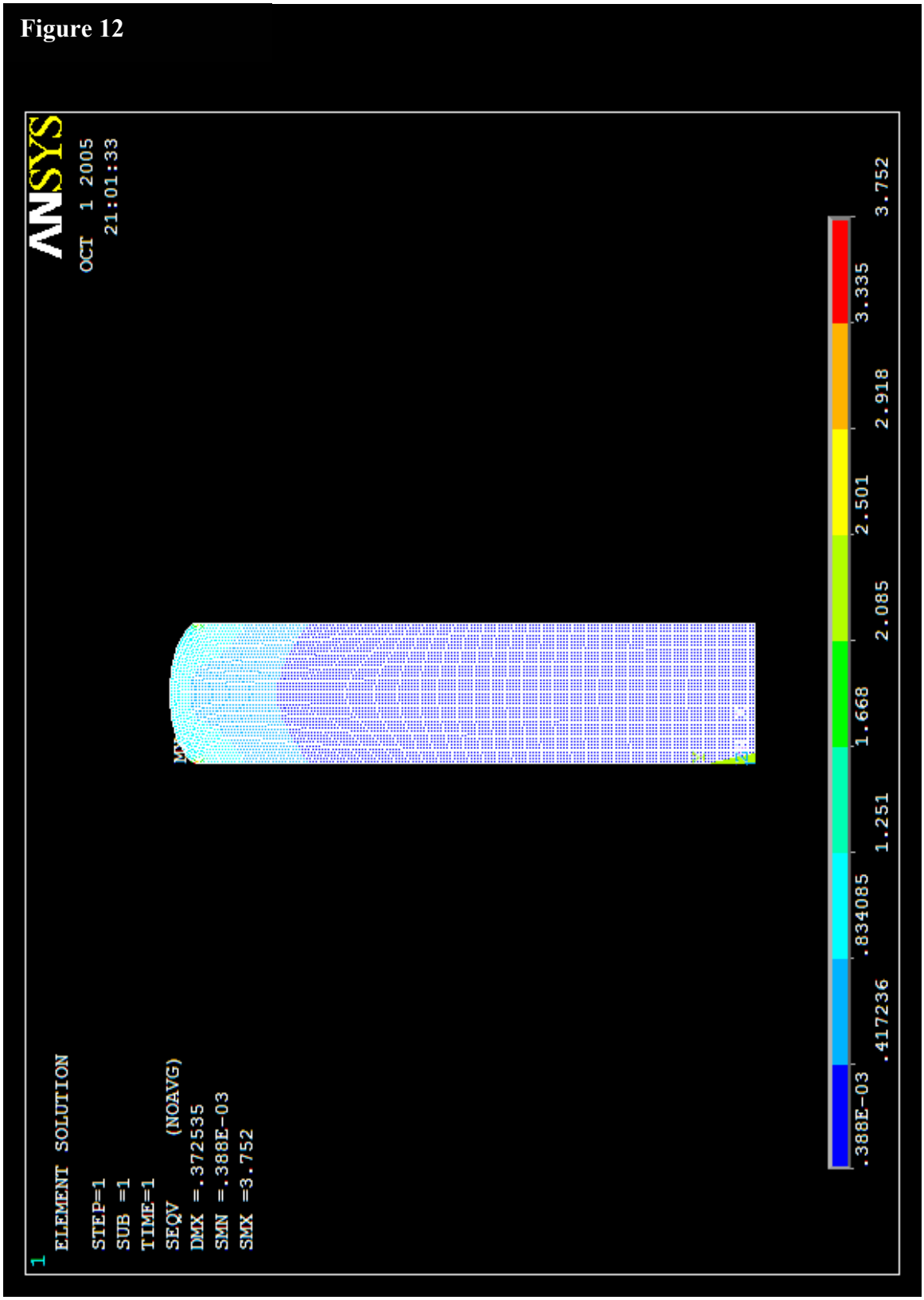
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Figure 13

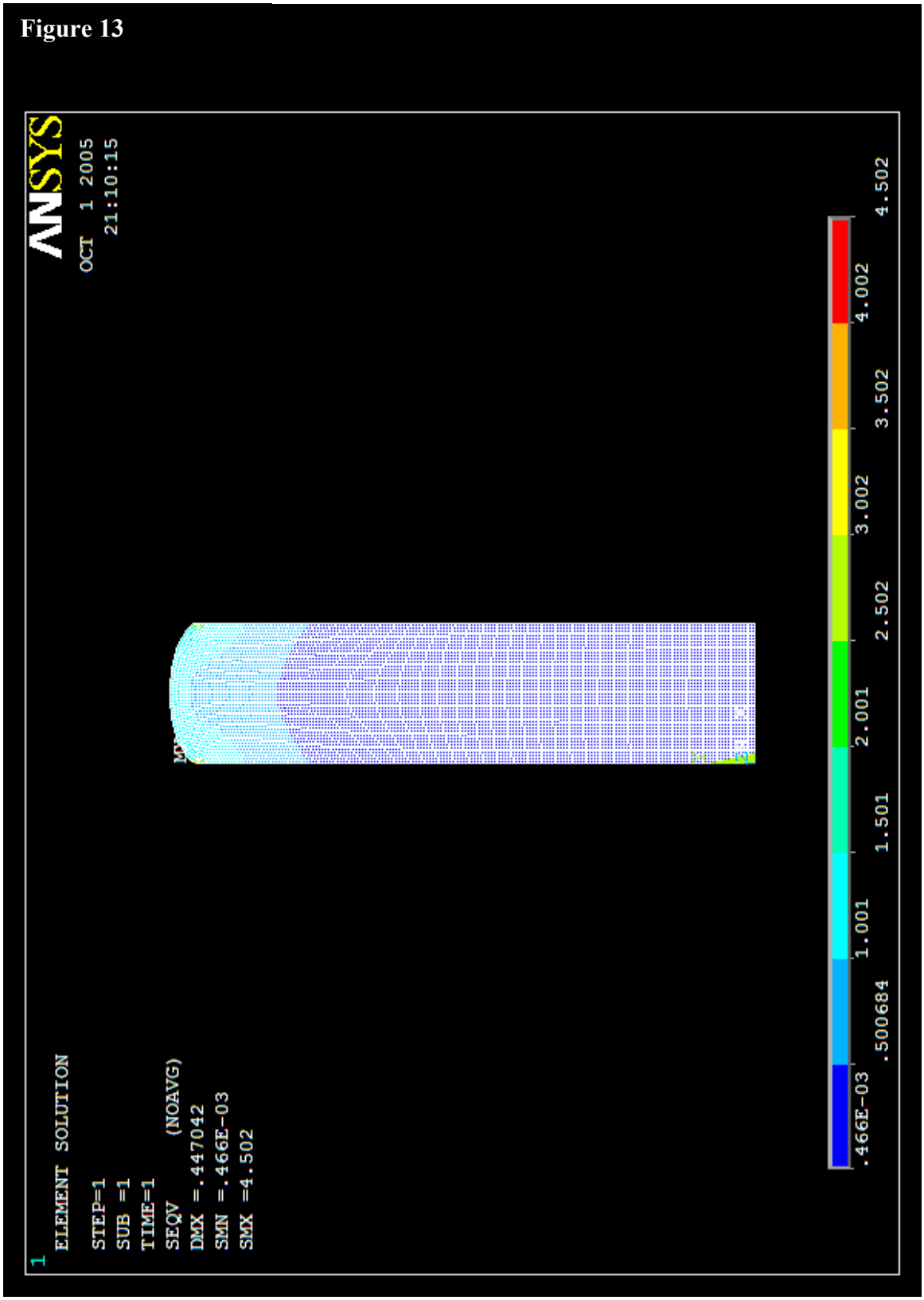
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Figure 14

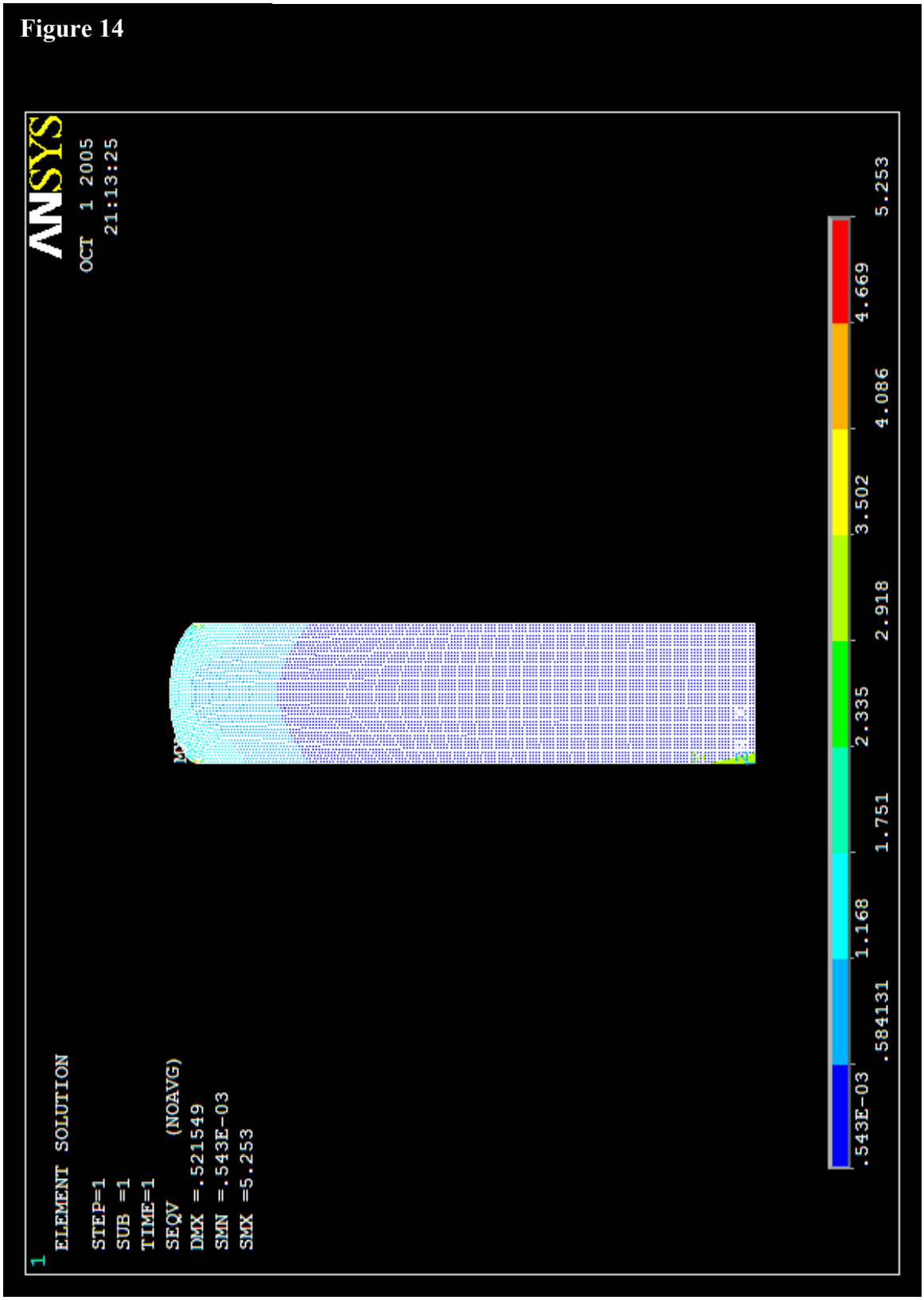
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Figure 15

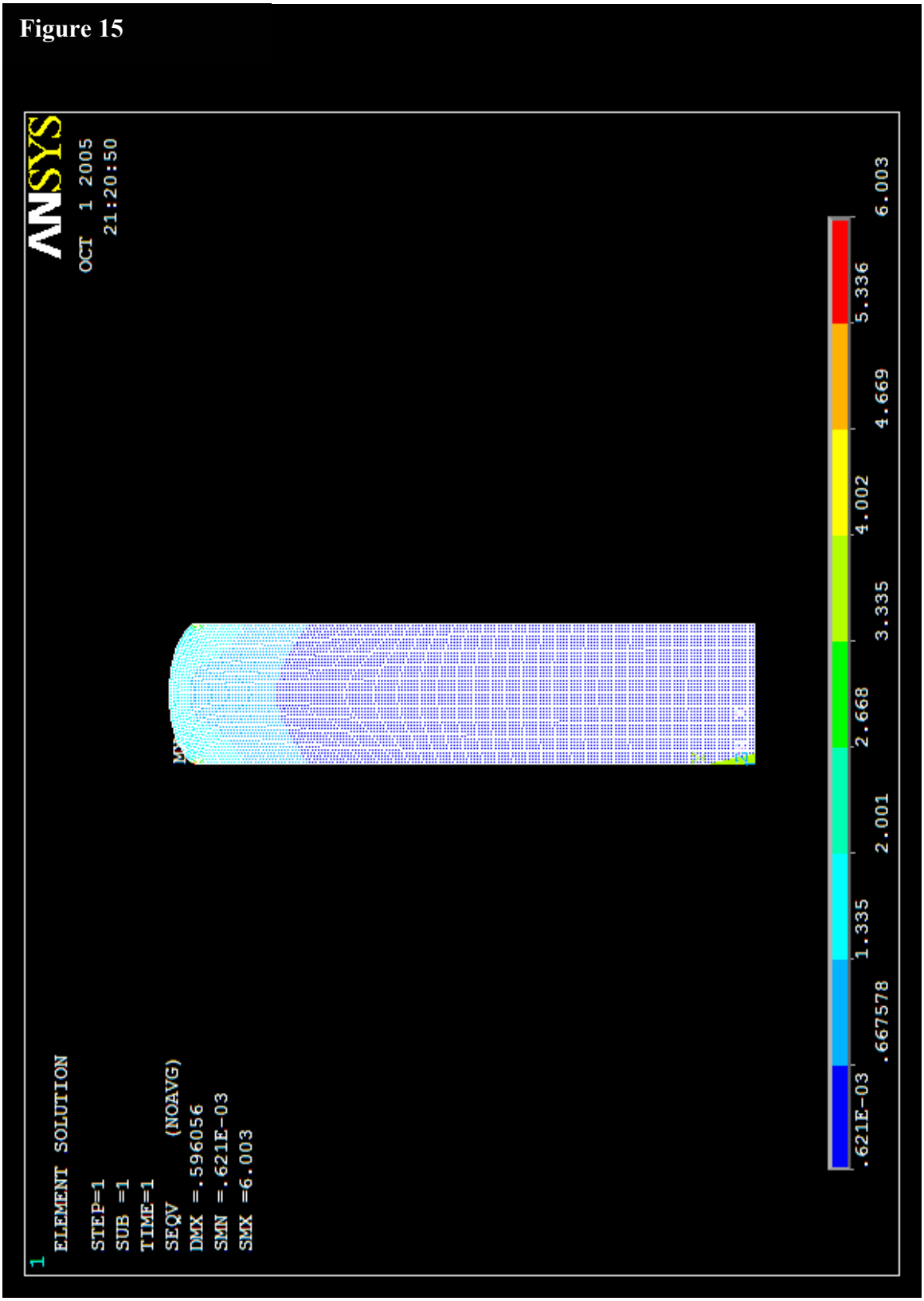
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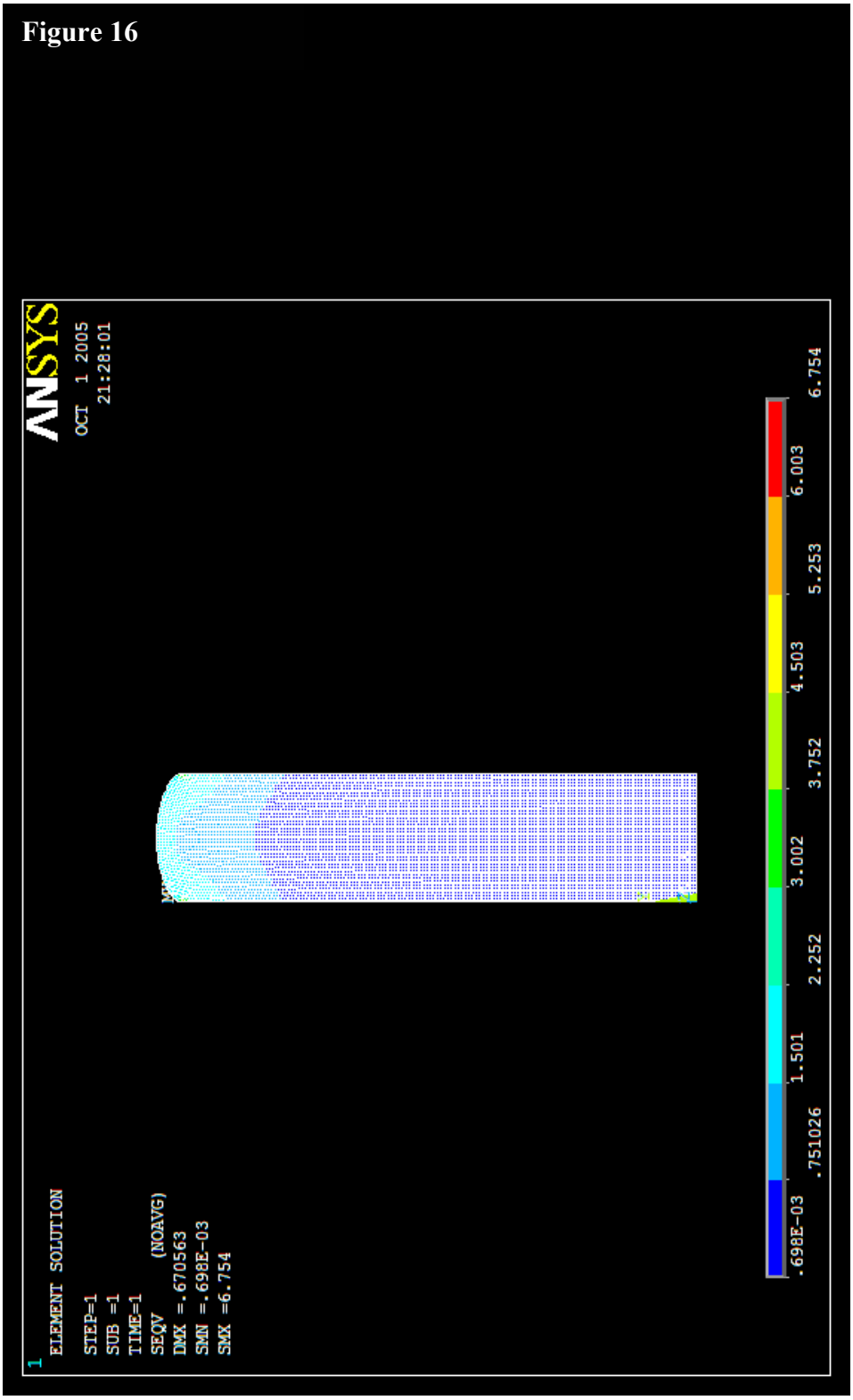
Figure 16



FIGURES 11 – 16 – Simulation Results at Varying Applied Stresses, with Parameters Described in Figure 10, above.

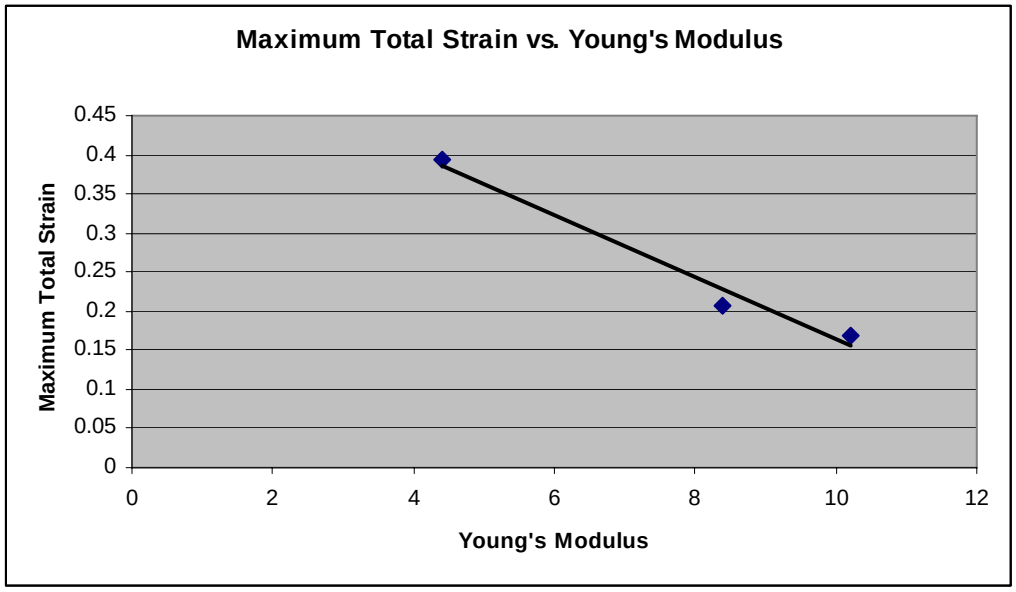


FIGURE 17 – Maximum Internal Total Strain vs. Young's Modulus for Computer-Modeled Soft Tissues under Stress at One End.

Figure 17 shows an inverse linear relationship between Young's modulus and the maximum total strain, as expected. The Young's moduli values used above were the average Young's moduli values for vaginal tissue samples from three groups of subjects in Bhatt's (2005) study. The maximum internal total strain values were obtained from the simulation model for each Young's modulus value used. The simulation results at each Young's modulus value are shown in Figures 18 – 20, below. The applied stress was held constant at 1.36 N/mm^2, and a value of 0.48 for Poisson's ratio was used.

Figure 18

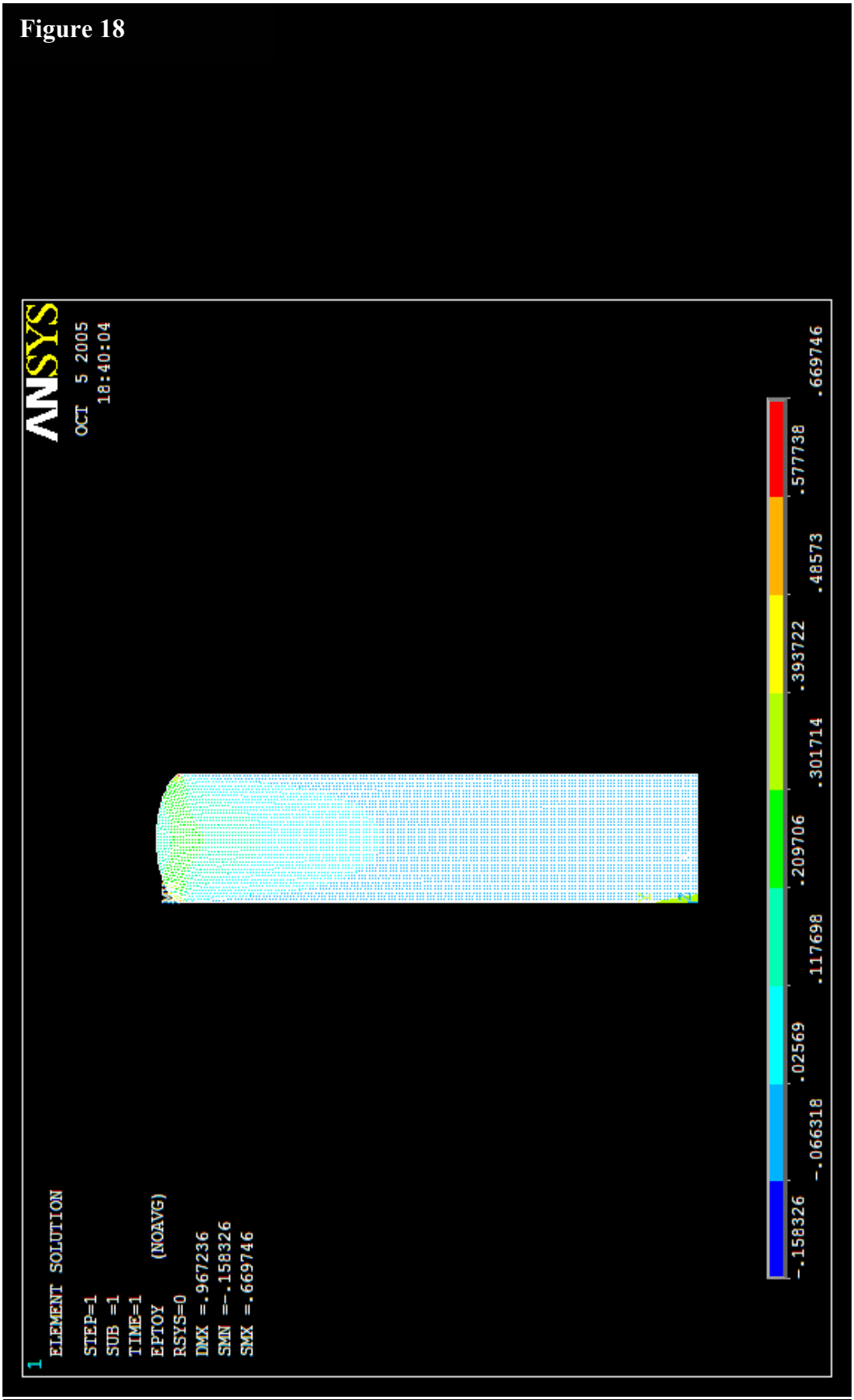

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Figure 19

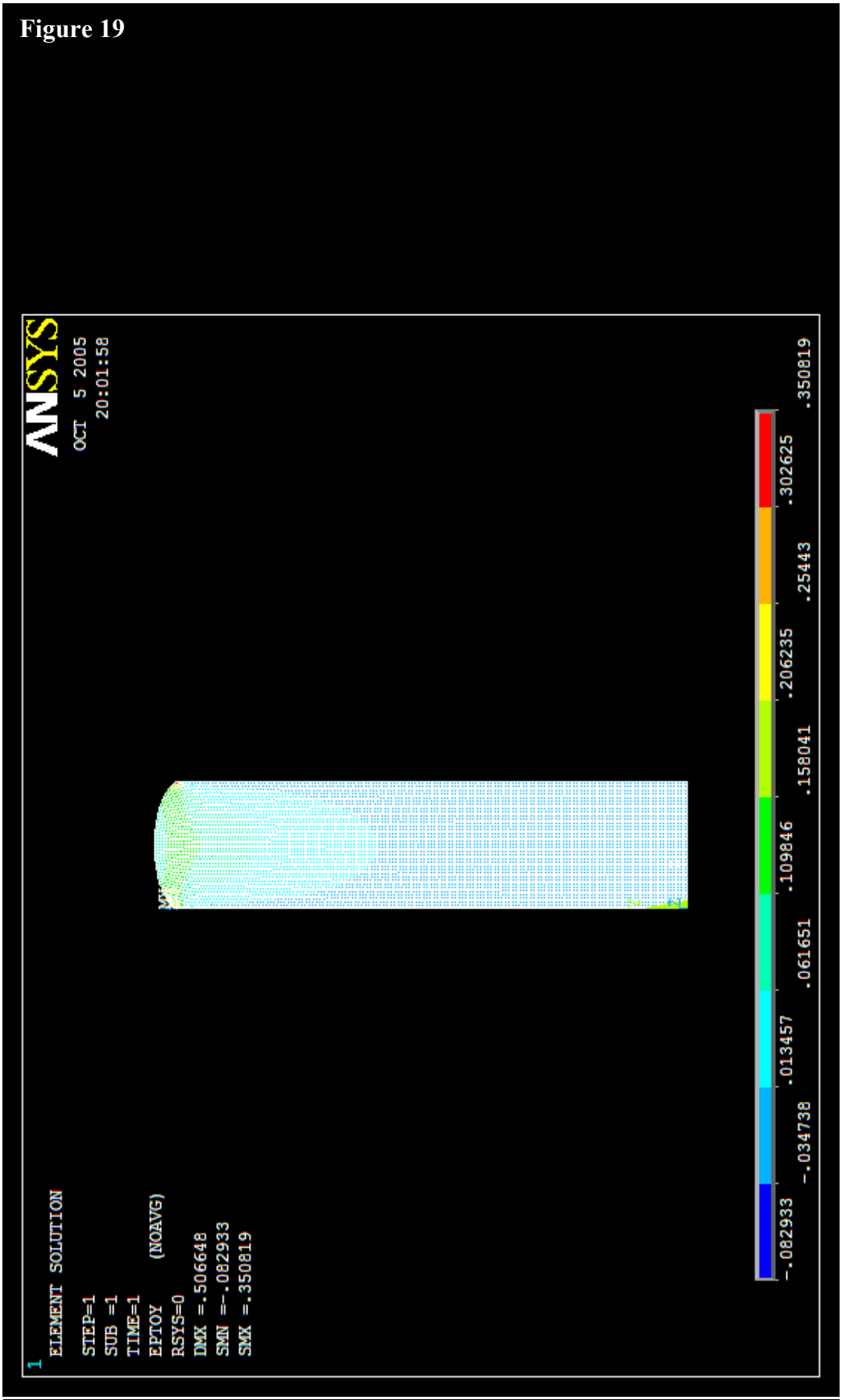
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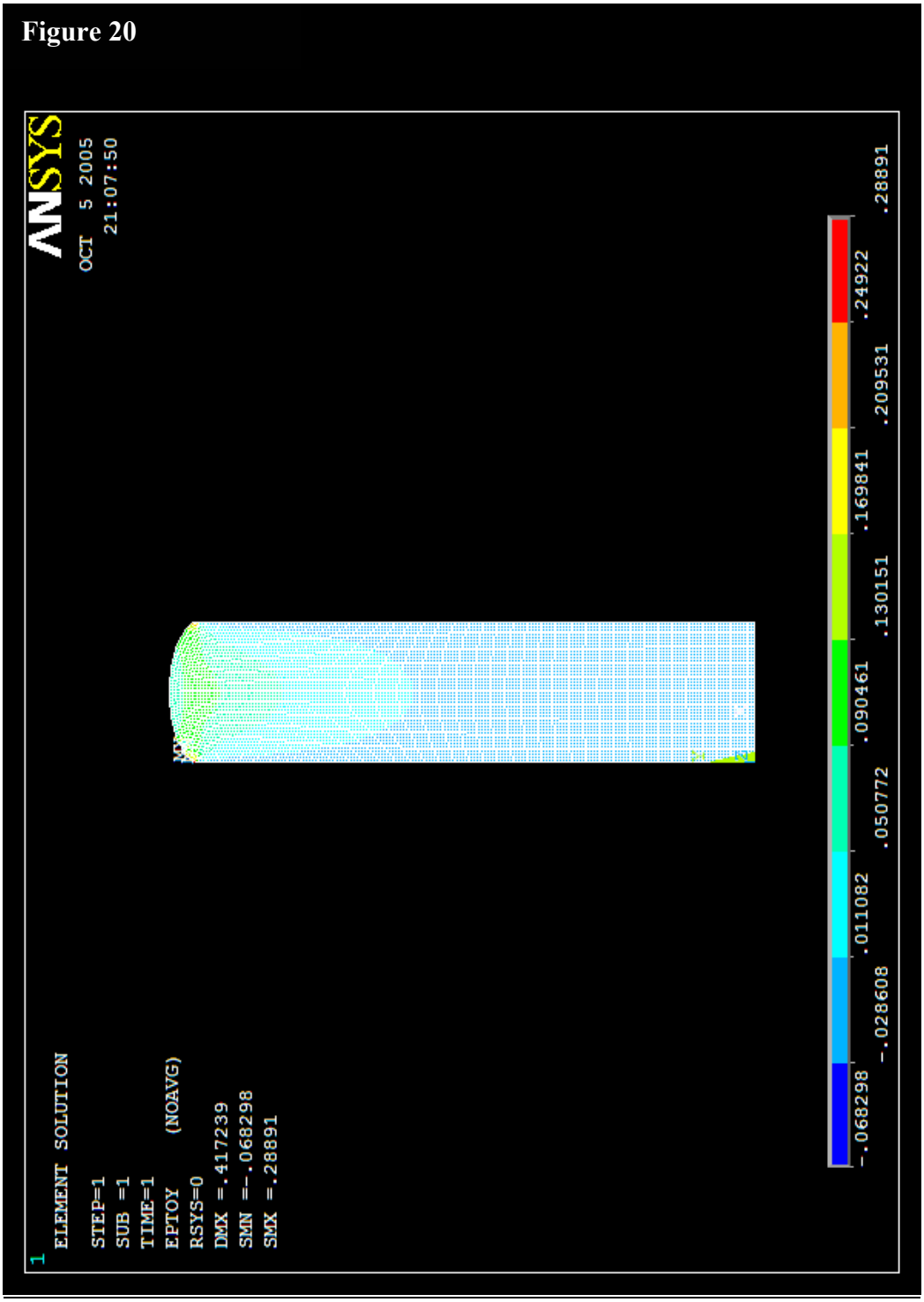
Figure 20



FIGURES 18 – 20 – Simulation Results at Varying Young's moduli, with Parameters Described in Figure 17, above.

5.b Ideal (Future) Models

Firstly, when location-dependent data is available on soft tissues, then elastic anisotropic models can be generated so that direction- and location-dependent tissue properties (studies have shown that soft tissues are really elastic anisotropic materials) can be taken into account.

When more detail is available on the internal structure of soft tissues, higher resolution and accuracy can be brought to these simulation models to make them microscopically accurate. Basically, further work along the lines of the recent research presented in Section 4.b, as well as research regarding the other theories presented in Section 4.a, needs to be completed before accurate simulation models can be developed. The data that needs to be acquired on tissue fibers to be used in a simulation model includes:

1) the types of fibers supporting the tissue;
2) the biomechanical properties of fibers supporting the tissue;
3) the orientations of fibers supporting the tissue; and
4) the dimensions of fibers supporting the tissue.

Additional data on the types and numbers of normal, senescent, and immune cells present, the extracellular matrix proteins, and receptors, and their respective locations and biomechanical properties also need to be gathered. Finally, simulation modeling can help during this process by generating models for postulated internal structures and comparing the results to available microscopic experimental data.

In the future, SolidWorks or other CAD software can be used to draw detailed models of tissues that can later be used in ANSYS or other modeling software. Current models do not need to use such stand-alone CAD drawing software (rather, the CAD capabilities included in software such as ANSYS are sufficient), but ideal future (more complex) models incorporating the actual internal structure of the tissue might need to make use of such software.

Chapter 6

Conclusion and Future Plans

In conclusion, current therapies treating the symptoms of tissue aging and failure make billions of dollars annually, but real cures have yet to be found due to the underlying mechanisms of tissue failure not being completely understood.

In recent times (as described in Chapter 2) more specific studies have been conducted on one of these soft tissues (i.e., vaginal tissues). These groundbreaking studies, however, can serve only a limited role in future scientific analysis due to methodological inconsistencies and complication due to uncontrolled variables. Also, these studies have only involved the macroscopic biomechanics of the studied tissues; few microscopic studies have yet been completed.

Although findings so far appear to show tissue strength to be remarkably inconsistent, a molecular analysis of the underlying microscopic fibers providing tissue strength has a high likelihood of resolving the inconsistencies of results reported in current studies. In fact, nonstandard samples may have caused most of the variation in results of past studies. *In vivo* tests will ultimately be necessary to confirm detailed models for tissue strength. However, human testing is costly and, in the case of *in vivo* testing, often impossible to achieve (as is currently the case). Computer simulation models have the potential of drastically reducing the amount of human testing necessary, and, thus can be very useful tools in this area of research.

To conclude, a complete understanding of soft tissue physiology and biomechanics is essential to the smart design and fabrication of many drugs and biomedical engineering devices to treat the symptoms of and eventually repair tissue

prolapse, injury, and aging. Future comprehensive studies with controlled macroscopic experiments aided by finite element models, as previously discussed, and future studies regarding the theories presented above, should stand a good chance of delivering this understanding. Eventually, it is hoped that this new knowledge will make it possible for improved drugs and treatment options to be available to patients.

REFERENCES

APS Home Page. Fibrous Connective Tissue. University of Guelph Department of Animal and Poultry Science. 10 Nov. 1998. <http://www.aps.uoguelph.ca/~swatland/ch2_3.htm>.

Bhatt, Aradhana. Biomechanical Properties of Anterior Vaginal Wall Tissue in Post-Menopausal Women. Arlington, TX: The University of Texas at Arlington, 2005.

Cosson, Michel, Eric Lambaudie, Malik Boukerrou, Pierre Lobry, Gilles Crepin, and Anne Ego. A biomechanical study of the strength of vaginal tissues. Results on 16 post-menopausal patients presenting with genital prolapse. European Journal of Obstetrics and Gynecology and Reproductive Biology. 112 (2004): 201-5.

Goh, J.T.W. Biomechanical properties of prolapsed vaginal tissue in pre- and postmenopausal women. International Urogynecology Journal 13 (2003): 76-9.

Han, Lianghao, J. Alison Noble, and Michael Burcher. A novel ultrasound indentation system for measuring biomechanical properties of *in vivo* soft tissue. Ultrasound in Medicine and Biology 29.6 (2003): 813-23.

Lakes, Roderic. Foam structures with a negative Poisson's ratio." Science 235 (1987): 1038 – 40.

Materials by Design. Elastic Modulus. Cornell University Department of Materials Science and Engineering. 1996. <http://www.mse.cornell.edu/courses/engri111/modulus.htm>.

Nava, Alessandro and Edoardo Mazza. Measuring the mechanical properties of soft biological tissues i*n-vivo*." Institute of Mechanical Systems. 2004.

The Asclepion. Medicine in Ancient Egypt. Indiana University Bloomington. 19 May 2000. <http://www.indiana.edu/~ancmed/egypt.HTM>.