Electromechanical Dynamics of Articular Cartilage as it undergoes Degenerative Changes

by

Eliot H. Frank


Submitted in Partial Fulfillment of the Requirements for the Degree of
Master of Science at the
Massachusetts Institute of Technology
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Abstract

The dynamic mechanical and electrokinetic behavior of articular cartilage is examined during the time course of chemical diffusion and enzymatic digestion within the tissue. Fixed charge provided by the presence of proteoglycan molecules is altered by chemical neutralization as a result of a change in pH or by depolymerization due to enzymatic digestion allowing proteoglycan charge groups to freely diffuse out of the tissue. Although the kinetics of these two processes are widely different, in either case the result is a decrease in net fixed charge, which is shown by rapid decrease in measured dynamic streaming potential. A decrease in dynamic stiffness is observed simultaneously with the decrease in streaming potential.

Cartilage electromechanical response is modeled by a biphasic equation of motion coupled with the equations for hydrogen and enzyme diffusion reaction. The biphasic model predicts a displacement field of frequency dependent finite skin depth in response to the sinusoidal compression employed in the experiments. Using a scan of several frequencies to essentially probe tissue mechanics at varying depths, the progress of slowly diffusing enzymes may be followed.

Dynamic streaming potential is used experimentally as a direct measure of fixed charge within the tissue. Considering the connection between charge density and electromechanical behavior of cartilage, the feasibility and modeling issues of using streaming potential as a diagnostic tool for the early onset of osteoarthritis is examined.

Thesis Supervisor: Alan J. Grodzinsky
Title: Associate Professor of Electrical and Bioengineering
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Chapter I
Introduction

1.1 Cartilage Structure and Electromechanics

Articular cartilage is a specialized connective tissue which covers, protects, and lubricates the articulating surfaces of bone. The mechanical properties of cartilage are essential to the successful function of synovial joints, where the cartilage acts as a load bearing surface. Cartilage must be able to withstand high mechanical stress, during both static and dynamic loading.

Cartilage ceases to maintain its load bearing capabilities in the disease state of osteoarthritis, which is characterized by progressive breakdown of the articular cartilage. Breakdown is seen in intermediate and late stages of the disease as fibrillation of the cartilage leading to bone exposure. The final result of this process is total joint dysfunction and bone destruction. [16]

To understand the events leading to osteoarthritis, cartilage mechanics must be explored and characterized. The unique properties of cartilage which enable it to withstand stress under normal conditions lie in the structure and composition of the tissue.

Cartilage is a biphasic material, composed of interstitial water (60-80%) and a solid collagen matrix containing protein polysaccharide molecules known as proteoglycans. The tissue is maintained by a sparse population of cells (chondrocytes), which are responsible
for the gradual turnover of the collagen proteoglycan matrix. Collagen fibers provide the matrix its basic structural integrity and mechanical stability. The collagen resists any tendency to swell and limits the maximum hydration of the tissue.

Proteoglycans are composed of polysaccharide molecules known as glycosaminoglycans. The glycosaminoglycans (gags) in cartilage are chondroitin sulfate, keratin sulfate, and hyaluronic acid. Chondroitin sulfate and keratin sulfate chains are bound to protein cores to form proteoglycan monomers as illustrated in figure 1.1. These monomers in turn are bound by a link protein to a long backbone of hyaluronic acid to form the proteoglycan aggregates as shown in figure 1.2. Proteoglycan aggregates are intertwined within or possibly bonded to the collagen matrix so as to prevent the proteoglycans from moving relative to the collagen or diffusing out of the tissue [12]. A recent, very detailed electron microscopy study by Poole et al [21] suggests that proteoglycan aggregates are anchored by the hyaluronic acid to collagen fibrils in a regular manner as in figure 1.3. Poole indicates that the aggregates are highly organized within the collagen matrix to a much greater extent than previously recognized [21].

Proteoglycans have been recognized to play an important role in cartilage function, greatly influencing mechanical behavior. Research by Armstrong and Mow [1] has shown a correlation between equilibrium mechanical modulus and gag
Figure 1.1 Glycosaminoglycans and their relationship to proteoglycan monomers
Figure 1.2 Model of proteoglycan aggregate:
HA - hyaluronic acid, CS - chondroitin sulfate,
KS - keratin sulfate (from Muir [19])
Figure 1.3 Proteoglycan aggregates within the collagen matrix as suggested by Poole et al [21]
content in human cartilage. Lipshitz and Glimcher [10] observed increased hydraulic permeability, as evidenced by shorter stress relaxation times, in proteoglycan extracted bovine cartilage as compared to normal samples. Olson [20] reported significant decrease in dynamic stiffness and streaming potential in sinusoidal, uniaxially confined compressional testing of 1/4 inch discs of bovine articular cartilage after removal of 70-75% of the gags by trypsin digestion.

Cartilage matrix fixed charge is suggested as the mediator of proteoglycan influence on the tissue mechanics. At physiological pH, the matrix is negatively charged due to ion dissociation of ionizable side groups. The collagen, being on the whole neutral at physiological pH due to an equal number of ionized carboxyl (-COOH) and amino (-NH$_3$) groups, makes no net contribution to the charge. Net charge is therefore accounted for by the carboxyl, and sulfate (-SO$_3$H) groups present on the gags.

There are two mechanisms involved in cartilage electromechanical transduction. First, electrostatic repulsion between charge groups at the molecular level generates a macroscopic swelling pressure. This swelling pressure is in opposition to compressive forces and is seen effectively to stiffen the material. Thus the material has a higher equilibrium modulus than in the absence of charge. Second, electrokinetic coupling decreases hydraulic permeability. The small hydraulic permeability of cartilage
opposes fluid flow through the tissue brought about by deformation, seen effectively as an increase in dynamic stiffness.

Influence of net fixed charge density through electrokinetic transduction is suggested by streaming potentials induced by fluid flow past the fixed charge of the tissue. Maroudas [11] observed streaming potentials across sections of tissue in response to imposed pressure gradients. Streaming potentials are also seen in response to deformations of the tissue such as step changes in compression or load and steady state sinusoidal compression, which result in fluid flow into and out of the tissue. [8, 20]

The electromechanical coupling is demonstrated in studies where electrostatic forces have been shielded out or neutralized chemically. By increasing Debye shielding through an increase in ionic strength, Lee et al [8] showed that static and dynamic stiffness and dynamic streaming potential in bovine cartilage were markedly decreased. Grimshaw [5] observed a rapid decrease in equilibrium swelling pressure of bovine cartilage when the pH of the tissue was changed to neutralize the fixed charge. Maroudas and Bannon [13] show that equilibrium swelling pressure of cartilage to depend on proteoglycan fixed charge density.

In osteoarthritis, biochemical changes in proteoglycan structure and content have been associated with with degradation of mechanical properties. In osteoarthritic
canine knee cartilage McDevitt and Muir [14] found increased extractability of proteoglycans as well as a decrease in overall gag content and an increase in hydration. Shapiro and Glimcher [23] using a rabbit medial meniscectomy model for osteoarthritis found a decreased amount of gags in cartilage at the site of the meniscectomy compared to that of contralateral control knee and that of sham operated animals. Moskowitz et al [17] in a similar rabbit study found markedly little proteoglycan aggregation in the operated knees compared to control knees.

In the first such study to do so, Hoch et al [6] correlated biochemical changes in gag content with changes in mechanical response in a rabbit meniscectomy model. Hoch found a significant decrease within two weeks after surgery in equilibrium Young's modulus accompanied by a decrease in gag concentration as indicated by uronic acid assay in operated joints versus contralateral and sham operated controls. Hoch further showed that biochemical composition and electromechanical response tracked one another as gag content and modulus increased to normal over the course of six months after meniscectomy.

It is clear that proteoglycans, through their contribution to matrix fixed charge, are essential to normal cartilage behavior. From observations of animal models proteoglycans play an important role in time course of osteoarthritis. Loss of proteoglycans through disaggregation appears implicated in the pathway to osteoarthritis, with
disaggregation of proteoglycans perhaps induced by naturally occurring proteolytic enzymes such as cathepsin B. [24] The disaggregation of proteoglycans in osteoarthritis would allow the proteoglycans to diffuse out [22] and further degrade mechanical properties.

Regarding any process of disaggregation several questions must be answered to understand cartilage degradation. Specifically, how are proteoglycan actually held within the tissue? and what degree of disaggregation is necessary before gags are no longer fixed within the tissue and no longer contribute to cartilage electromechanical behavior?

1.2 Thesis Overview

Attention in this thesis will focus on the role of proteoglycan structure and charge in the electromechanical behavior of cartilage. To experimentally probe cartilage behavior, the basic method will be to alter either the matrix fixed charge or the proteoglycan structure itself and observe changes in electromechanical response. Two sets of experiments are examined: one dealing with chemical neutralization of charge and the other with enzymatic digestion of selected proteoglycan components. Through contrast of chemical and enzyme response, one may be able to highlight specific contributions of proteoglycan aggregate structure, as well as charge, to cartilage behavior.

In the first set of experiments, matrix fixed charge is altered by titrating negative charge groups through a change
in pH. A step change in pH is made by the introduction of 
HCl to the cartilage bathing solution. Hydrogen diffuses 
into the tissue following a chemical diffusion reaction 
process, changing the local pH within the tissue as the 
diffusion progresses. Fixed charge is neutralized, but the 
structure of the proteoglycans is assumed to be unchanged. 
The effects of the resulting transient in charge density in 
material properties are then followed by measurement of 
cartilage dynamic mechanical and streaming potential 
response.

Proteoglycan structure may be altered through the use 
of specific enzymes. Consider the action of two enzymes, 
chondroitinase abc and hyaluronidase. These enzymes affect 
the aggregates in different ways, as diagramed in figure 
1.4. Chondroitinase breaks chondroitin sulfate chains into 
fragments that can be as small as its basic disaccharide 
repeat groups. Thus chondroitin sulfate fragments are 
effectively removed from the proteoglycan monomers, and are 
free to diffuse through the interstitial fluid and out of 
the tissue. The conditions of the experiment and composition 
of the buffer containing the enzyme are such to insure that 
keraatin sulfate groups and hyaluronic acid are left intact.

In contrast, hyaluronidase enzyme depolymerizes the 
hyaluronic acid backbone of the aggregates, breaking the 
proteoglycans into smaller subaggregates or proteoglycan 
monomers containing small amounts of attached hyaluronic 
acid. These subaggregates would still be much larger than
The effect of chondroitinase ABC on proteoglycan aggregates

The effect of hyaluronidase on proteoglycan aggregates

Figure 1.4 Enzymatic degradation of proteoglycans
individual gag chains. Subaggregates may still be large enough to remain entangled in the collagen matrix and not be able to diffuse as freely as the gags.

In the second set of experiments, cartilage is enzymatically degraded using chondroitinase abc. The enzyme is introduced to the cartilage bathing solution and allowed to diffuse into the tissue. In this case the proteoglycan structure is changed: the matrix will contain a certain amount of gag fragments that are unattached to the proteoglycan monomers, and other fragments will have diffused out of the matrix. Both these effects result in a decrease in net matrix fixed charge density. Again these changes with time are followed via dynamic response.

The unattached gag fragments would not be expected to contribute to the electrokinetic response of the tissue, since electrokinetic transduction involves fluid flow relative to fixed charge. Once cleaved from the monomers, these fragments are free to move with the fluid. Thus one would expect an increase in hydraulic permeability and a decrease in streaming potential response, even though the gags may not have had time to diffuse out of tissue and still actually contribute to Donnan osmotic swelling pressure.

The mechanical and streaming potential results are obtained by applying a sinusoidal mechanical excitation over a scan of several frequencies. To give physical insight to the experimental results, a model for the dynamic response
of cartilage to transient changes in material properties is presented. First, the diffusion reaction kinetics of the two experiments are modeled to determine the effect of the transient on fixed charge. The charge profile can then be coupled to the biphasic equations of motion which govern cartilage electromechanics to obtain the total response of the system. The biphasic model predicts that the extent of the mechanical excitation into the tissue, the skin depth, is a function of excitation frequency. In addition, different material properties are excited by different frequency excitations. The frequency response data can thus be used to deduce relative changes in the mechanical modulus, hydraulic permeability, and electrokinetic parameters at various depths into the tissue.
Chapter II

Experimental Response of Cartilage

The goal of the experiments is to characterize cartilage electromechanical response to gradual changes in material properties. Material properties are altered by setting up a diffusion reaction process in the tissue by introducing a chemical or enzyme. The hope is to highlight specific contributions of proteoglycan charge groups to structural, mechanical, and electromechanical behavior.

Rheological characterization of the cartilage is obtained by examination of frequency response to the application of a dynamic mechanical excitation. Dynamic response is studied for several reasons. The imposed displacement gives rise to fluid flow, which implies that hydraulic permeability will contribute to mechanical response as well as mechanical modulus. Fluid flow through the tissue also gives rise to streaming potential, which is an indication of electrokinetic interactions.

Dynamic excitation also sets up within the material a mechanical diffusion wave with a characteristic skin depth dependent on frequency. By employing a scan of several frequencies, tissue mechanics may be probed at varying distances into tissue, and provide information on the progress of the diffusion into tissue.
2.1 Materials and methods

2.1.1 Test chamber

The test chamber, shown in figure 2.1, consists of a 10-15 ml fluid reservoir, constructed of plexiglass. The reservoir is machined to screw into a second plexiglass piece which acted as a mount in the testing apparatus and provided means for an electrode contact. Reservoir and mount are bored to accept a 1/4" diameter electrode. The electrode recessed below the bottom of the chamber to form a depression deep enough to keep a cartilage sample of at least 1 mm thickness in confined cylindrical geometry. A second electrode assembly is suspended in the reservoir fluid directly above the sample. The cartilage is actually compressed by a short piece of porous polyethylene rod placed between the upper electrode and sample. The polyethylene rod acts as a porous platen to allow reservoir fluid to flow freely to and from the cartilage surface. In this apparatus, the porous platen was not bonded to the upper electrode, as was the case in previous studies. [8,20]

The chamber is provided with ports for fluid recirculation. Fluid is recycled constantly by an external peristaltic pump to insure mixing near the cartilage surface and thus reduce the effect of any stagnant layer at the surface. The chamber is also equipped with a heating coil and thermistor to provide constant temperature control by means of a proportional temperature controller. The controller maintains the fluid temperature within plus or
Figure 2.1 Test chamber schematic
minus 1/2 degree of the set point of 20 degrees Celsius. An external heat exchanger in the fluid recirculation path removes excess heat generated by pumping action and environmental sources. The temperature control arrangement is identical to one used by Grimshaw, who found a very strong dependence of modulus with temperature (~ 5%/°C [5]).

2.1.2 Mechanical spectrometer

Experiments are performed in the testing frame of a Dynastat mechanical spectrometer, manufactured by Dynastatics of Troy, New York. The spectrometer consists of a servo position controlled electromechanical actuator. Actuator position is measured by an LVDT which provides both the servo feedback signal and the sample displacement signal. Applied force is measured by a load cell in line with the sample. The test chamber is placed in the jaws of the testing frame, with the chamber itself attached to the actuator and the upper electrode assembly attached to a movable rod in line with the load cell.

The actuator can be prepositioned manually to any desired offset. The spectrometer also has provisions for external excitation input to control actuator displacement. The input is used in the course of experiments to provide the desired sinusoidal excitation to the sample.

2.1.3 Electrodes

Streaming potential is measured by means of a pair of Ag/AgCl electrodes. The electrodes are 1/4" diameter Ag/AgCl pellets manufactured by Annex Research of Santa Anna, CA,
using a thermal deposition process of chloride on scinttered silver. These electrodes were found to have superior offset and mechanical stability than could be obtained through electrolytic plating. The electrodes are mounted on plexiglass holders and epoxied in place so as to prevent leakage of fluid around the pellet onto the electrode contact. Holders were in turn inserted into components of the test chamber. An o-ring between chamber and mount provided a seal against leakage around the lower electrode holder.

For convenience, the upper electrode in contact with the reservoir fluid is defined to be negative. This electrode is grounded to provide a ground path for the fluid and thus prevent the build up of electrostatic charge on the insulating walls of the chamber, which would otherwise act to saturate the streaming potential amplifier.

A Gould Universal differential amplifier was used to amplify and condition the streaming potential signal. The amplifier provided an offset capability to eliminate any offset due to electrode drift or other non-streaming potential such as diffusion potentials. The offset could be adjusted +/- 100 mv while still maintaining a differential gain of 80 dB or more. The amplifier offset feature was modified to allow external control of the offset by a digital to analog converter connected to the computer used to sample experimental data. When the computer sensed that the potential signal was drifting out of range, the offset
was adjusted accordingly.

2.1.4 Signal conditioning

The three signal paths of interest, displacement, load, and streaming potential, are conditioned by four-pole RC filters with a 15.7 Hz cutoff frequency. A Rockland Systems 1022-F Dual Hi/Lo Filter provides the filtering on the displacement and load channels, while the Gould amplifier mentioned above in series combination with a second Gould amplifier is used on the streaming potential channel. The frequency characteristics of the three signal paths must be carefully matched in order to reduce distortion in both magnitude and phase response. Any frequency mismatch will lead to consequent errors in relative phase angle measurements.

2.1.5 Data reduction

Data sampling and reduction is controlled by a Digital Equipment Corporation pdp 11/03 computer. Interconnections between computer and apparatus are sketched in figure 2.2. Displacement, load, and streaming potential signals are monitored by an analog to digital converter (adc). The adc input is buffered by a differential amplifier front end with provision for programmable gain. Signals provided by digital to analog converters are used to balance the differential amplifiers in order to prevent signal offsets from saturating the adc. The adc sampling interval is provided by a timing signal generated by a programmable real time clock (rtc).
Figure 2.2 Schematic of signal path interconnections
The sinusoidal excitation reference to the mechanical spectrometer is supplied by a Rockland Systems 5100 Digital Frequency Synthesizer, slaved to the computer. This arrangement allows program control of excitation frequency selection and initiation of mechanical cycling. Computer control of the excitation is extremely advantageous since it allows the program to optimize the data sampling interval based on the known frequency. The arrangement also provides capability for automated frequency spectrum scan and long term automated testing.

2.1.6 Sample preparation

Intact knee joints from freshly slaughtered 2 year old cattle were obtained from City Packing of Boston, Massachusetts. The medial patellar groove was removed from the knee joints and frozen until ready to use.

When needed the section is thawed and secured in a positionable vise attached to a drill press table. The cartilage surface is aligned perpendicular to the cutting tool. The cartilage is first cut through the surface down to the bone using a 1/4 inch diameter hollow scoring bit. A 3/8 inch diameter hollow cutter is then used to cut a through the cartilage into the subchondral bone.

A cylindrical plug of cartilage and bone is then freed from the rest of the bone and secured in a sledge microtome. The most superficial 40-80 μm is sliced from the articular surface. Then a 600-1000 μm thick section is sliced. A 1/4 inch diameter cartilage plug is obtained along with a
concentric ring of surrounding tissue. The ring is saved to act as a control in later biochemical assays.

2.2 Experimental response of cartilage

2.2.1 Hydrogen diffusion: protocol and results

Cartilage samples, prepared as described above and microtomed to 1000 µm, are placed in the test chamber and allowed to equilibrate in a bathing solution of .01 M NaCl with 50 µg/liter PMSF inhibitor. A 15% offset strain is applied by first adjusting the jaw to jaw distance of the apparatus to the microtomed thickness of the sample and then applying the appropriate displacement. Stress relaxation is then allowed to occur. The sample is preequilibrated in this condition for 2 hours, during which sinusoidal compression testing over the frequency range .005 to 1 Hz is performed.

Hydrochloric acid is then introduced to change the pH of the solution from neutral to the desired final pH value, either the isoelectric point of cartilage (pH 2.6) or pH 3. The sample is continuously tested with a frequency sweep from .01 to 1 Hz until the diffusion process is complete. (Frequency .005 Hz was not used during the hydrogen transient because the time needed to obtain this low frequency data is a considerable fraction of the transient.)

This experimental procedure for hydrogen diffusion was carried out on 3 cartilage samples for which enough HCl was added to reach the isoelectric point (pH 2.6) and 2 samples for pH 3. The data for each of these experiments is shown in figures 2.3 and 2.4.
Figure 2.3(a-f) shows the transient in computed stiffness and measured streaming potential for the three plugs for the pH diffusion from pH 7 to pH 2.6. Note in two of these experiments shown (#4 and #6) an amount of NaOH (equal the amount of HCl added) was added to neutralize the bath after the HCl transient died off in order to demonstrate reversibility of the hydrogen reaction. Response for three frequencies (.01, .1, and 1 Hz) is shown with each response normalized to its initial value prior to the transient. Stiffness decays rapidly to its final value. For comparison with previous data, a decay time, in which the stiffness response falls 1/e of its net excursion, was computed for each of the 3 samples. The decay times were approximately 1000, 1000, and 1200 seconds for sample #4, #5, and #6 respectively. This is compared to the chemically induced stress relaxation time found by Grimshaw [5] of 865 seconds for 685 μm plugs, which becomes 1840 seconds when normalized to 1000 μm. The streaming potential response for the samples is also seen to decay rapidly. (The measured signal actually falls to below the noise level of the data collection apparatus by the end of the transient, since, at the isoelectric point, there is no streaming potential to measure!)

Figure 2.4 shows the transient in computed stiffness and measured streaming potential for the two plugs for the pH diffusion from pH 7 to pH 3. The diffusion transient for pH 3 is notably much longer than for pH 2.6, with the decay
Figure 2.3a Hydrogen diffusion transient response (pH 7 to pH 2.6):
Sample #04: normalized stiffness
Figure 2.3b Hydrogen diffusion transient response (pH 7 to pH 2.6):
Sample #04: normalized streaming potential
Figure 2.3c Hydrogen diffusion transient response (pH 7 to pH 2.6):
Sample #05: normalized stiffness
Figure 2.3d Hydrogen diffusion transient response (pH 7 to pH 2.6):
Sample #05: normalized streaming potential
Figure 2.3e Hydrogen diffusion transient response (pH 7 to pH 2.6): Sample #06: normalized stiffness
Figure 2.3f Hydrogen diffusion transient response (pH 7 to pH 2.6): Sample #06: normalized streaming potential
Figure 2.4a Hydrogen diffusion transient response (pH 7 to pH 3.0):
Sample #41: normalized stiffness
Figure 2.4b Hydrogen diffusion transient response (pH 7 to pH 3.0):
Sample #41: normalized streaming potential
Figure 2.4c Hydrogen diffusion transient response (pH 7 to pH 3.0):
Sample #42: normalized stiffness
Figure 2.4d Hydrogen diffusion transient response (pH 7 to pH 3.0):
Sample #42: normalized streaming potential
time approximately 6800 and 8300 seconds for the two samples. Figures 2.4b,d shows the corresponding transient data for streaming potential. At pH 3, cartilage matrix charge is not completely neutralized, so that at the end of the transient, a measurable streaming potential exists.

2.2.2 Chondroitinase digestion: protocol and results

Cartilage samples are placed in the test chamber and equilibrated in a bathing solution of .05 M Tris-HCl and .06 M NaAC with protease inhibitors (2 mM PMSF, 2 mM EDTA, 10 mM N-ethyl maleimide, 5 mM benzamidine), buffered to pH 8. After testing for 2 to 5 hours, chondroitinase-abc enzyme was introduced to a concentration of .1 units/ml. The sample is continuously tested with a frequency sweep over the range .005 to 1 Hz for 20 to 24 hours (about one complete frequency sweep per hour).

The experimental procedure for chondroitinase digestion was carried out on 8 cartilage samples. Six of the 8 were 640 μm thick samples and used for data smoothing and averaging. The results for these experiments is shown in figures 2.5a,b. Data for each of the 8 individual plugs are included in Appendix A with the smoothed data used to generated figure 2.5 included in Appendix B. Figure 2.5a shows the transient in computed stiffness averaged over the 6 samples. Response for four frequencies (.005, .01, .1, and 1 Hz) is shown again with each normalized to its initial value.

The stiffness response indicates a marked difference
Figure 2.5a Averaged plot of chondroitinase digestion transient response: normalized stiffness
Figure 2.5b Averaged plot of chondroitinase digestion transient response: normalized streaming potential
between high (.1,.1 Hz) and low (.005,.01 Hz) frequency behavior. While the high frequency response decays to about 86 % of its initial value, the low frequency response decays to about 75 %.

Figure 2.5b shows the transient in streaming potential averaged over the 6 samples. For streaming potential, the high frequency response decays to about 76 % of its initial, while the low frequency response decays to about 60 %. The ratios of final to initial response at each frequency are summarized in the following table.

<table>
<thead>
<tr>
<th></th>
<th>plug</th>
<th>0.05</th>
<th>.01</th>
<th>.1</th>
<th>1.</th>
<th>0.005</th>
<th>.01</th>
<th>.1</th>
<th>1.</th>
</tr>
</thead>
<tbody>
<tr>
<td>037</td>
<td>73 %</td>
<td>75 %</td>
<td>85 %</td>
<td>85 %</td>
<td>67 %</td>
<td>65 %</td>
<td>78 %</td>
<td>77 %</td>
<td></td>
</tr>
<tr>
<td>036</td>
<td>75 %</td>
<td>77 %</td>
<td>84 %</td>
<td>87 %</td>
<td>62 %</td>
<td>55 %</td>
<td>74 %</td>
<td>76 %</td>
<td></td>
</tr>
<tr>
<td>035</td>
<td>69 %</td>
<td>71 %</td>
<td>84 %</td>
<td>83 %</td>
<td>56 %</td>
<td>65 %</td>
<td>85 %</td>
<td>84 %</td>
<td></td>
</tr>
<tr>
<td>034</td>
<td>74 %</td>
<td>76 %</td>
<td>85 %</td>
<td>86 %</td>
<td>60 %</td>
<td>56 %</td>
<td>73 %</td>
<td>74 %</td>
<td></td>
</tr>
<tr>
<td>033</td>
<td>84 %</td>
<td>85 %</td>
<td>95 %</td>
<td>97 %</td>
<td>75 %</td>
<td>77 %</td>
<td>89 %</td>
<td>90 %</td>
<td></td>
</tr>
<tr>
<td>031</td>
<td>78 %</td>
<td>77 %</td>
<td>84 %</td>
<td>83 %</td>
<td>45 %</td>
<td>46 %</td>
<td>60 %</td>
<td>66 %</td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>74</td>
<td>76</td>
<td>86</td>
<td>86</td>
<td>59</td>
<td>60</td>
<td>76</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>std</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>9</td>
<td>10</td>
<td>9</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

2.2.3 Controls

Four cartilage samples were run as controls. The procedure for handling control samples is identical to that of the chondroitinase experiment samples, except that no change in bathing solution was made. The samples were tested continuously for 24 hours over the frequency range .005 to 1 Hz. Figures 2.6a,b shows the averaged and smoothed data for the four control samples. Appendix C contains smoothed data plots for the four individual plugs used to generate figure 2.6. No significant change in stiffness or streaming
potential response was observed, which gave assurance that the enzyme experiments could be well characterized and suggests that leach out of proteoglycans does not occur to a significant enough extent to affect the stiffness and potential.

2.2.4 Biochemical and Histological Assays

Uronic acid analyses of enzyme digested and control samples (including sample plug #20-37) are being performed under the supervision of Dr. David Eyre of Children's Hospital, Boston, in the Laboratory for the Study of Skeletal Disorder. Additionally, another series of 8 samples (4 control and 3 treated with enzyme under the testing protocol described and 1 treated in the test chamber but without testing or confined compression with the porous platen) are undergoing staining for gag and sectioning for histological analysis of tissue gag content and distribution by Dr. Adrianne Rogers, Department of Nutrition and Food Science, M.I.T. Results from these studies are expected soon and discussion of results is planned for future publication.
Figure 2.6a Averaged plot of control samples: normalized stiffness
Figure 2.6b Averaged plot of control samples: normalized streaming potential
Chapter III
Electromechanical Dynamics of Cartilage

The electromechanical behavior of cartilage, in response to charge neutralization or enzymatic degradation described in the previous chapter, demonstrates an intimate electromechanochemical coupling of tissue properties. Coupling mechanisms are illustrated in figure 3.1. Thus, in interpreting the information obtained from the experimental data, there are several problems to be dealt with simultaneously. The actual degradation of cartilage is driven by the chemical diffusion processes and reaction kinetics by which a substance enters into and alters the cartilage tissue matrix. The continuum mechanics and electromechanics of cartilage then determine the mechanical and electrical response of the tissue as seen in the experiments. There must also be a description of the coupling which relates the chemical or enzyme reactions to the mechanical properties of the tissue, so that the total response may be considered.

At the outset, diffusion processes are decoupled from tissue mechanics: the effect of mechanical rearrangement or excitation on diffusion is assumed to be small. This is justified when the Peclet number, $\frac{UL}{D}$, the ratio of convective velocity to diffusive velocity, is much less than one, where $U$, $L$, and $D$ are the characteristic velocity, length, and diffusivity respectively of the system. In the sinusoidal steady state excitation employed in the
Figure 3.1 Electromechanochemical coupling mechanisms
experiments, peak displacement rates and therefore fluid velocity, \( U \), is indeed high at the highest frequencies used. However, there is no time average velocity. Over a half cycle of excitation, there is little time for convection and the convection would only occur over the distance of the excitation amplitude (1% of the total thickness). The diffusing substance, over a much longer time course, eventually diffuses the total thickness. Thus the time average contribution of convection to ionic or enzyme transport is assumed to be negligible.

3.1 Diffusion processes

To model chemical or enzymatic effects on cartilage mechanics, the diffusion/reaction process by which a chemical or enzyme enters the tissue must first be described. The processes involved in the two cases are widely different. Chemical diffusion/reaction of hydrogen ions, for example, involves chemical binding of hydrogen with available charge groups while diffusion is taking place. The enzyme diffusion involves an enzyme reaction during diffusion, but the enzyme is not permanently bound to the substrate and thus continues on to diffuse and react further.

3.1.1 Hydrogen diffusion

In the case of the \( \text{H}^+ \) diffusion reaction experiments, hydrogen reacts with the chemical charge groups of the proteoglycans as the diffusion process occurs. The fixed charge groups present in cartilage are amino, sulfate, and
carboxyl groups. Amino groups remain fully ionized into a hydrogen reaction in the pH range of interest of pH 2.5 to 7.0. It is the carboxyl and sulfate groups which react and bind the diffusing hydrogen.

The problem of hydrogen diffusion as a result of large changes in pH has been studied in depth by Grimshaw [5] and is summarized here. The reaction kinetics between $H^+$ and $\text{COO}^-$ and $\text{SO}_3^-$ are governed by statements of chemical equilibrium,

$$\text{COOH} \rightleftharpoons H^+ + \text{COO}^- \quad \text{SO}_3\text{H} \rightleftharpoons H^+ + \text{SO}_3^-$$ (1)

described by equilibrium constants, $K_C$ and $K_S$, given by

$$K_C = \frac{[H^+][\text{COO}^-]}{[\text{COOH}]} \quad K_S = \frac{[H^+][\text{SO}_3^-]}{[\text{SO}_3\text{H}]}$$ (2)

respectively. The number of available carboxyl and sulfate groups is fixed within the tissue

$$n_C = [\text{COOH}] + [\text{COO}^-] \quad n_S = [\text{SO}_3\text{H}] + [\text{SO}_3^-]$$ (3)

where $n_C$ is the total number of carboxyl sites and $n_S$ sulfate sites. Any flux of hydrogen into the material either increases the internal hydrogen concentration or reacts to form COOH or SO$_3$H. Thus diffusion of hydrogen into the material is described by the diffusion equation,

$$\frac{\partial}{\partial t} \left( [H^+] + [\text{COOH}] + [\text{SO}_3\text{H}] \right) = D_H \frac{\partial^2}{\partial z^2} [H^+]$$ (4)

where $D_H$ is the internal hydrogen diffusivity. Using the equilibrium condition, the diffusion equation may be rewritten eliminating $[\text{COOH}]$,

$$\frac{\partial}{\partial t} [H^+] = D_H \left[ 1 + \frac{n_C K_C}{(K_C + [H^+])^2} + \frac{n_S K_S}{(K_S + [H^+])^2} \right]^{-1} \frac{\partial^2}{\partial z^2} [H^+]$$
This nonlinear differential equation must be solved numerically, subject to appropriate boundary conditions.

The diffusion equation is driven by a step change at \( t = 0 \) in the external bath pH. Boundary conditions that must exist at the top surface at \( z=0 \),

\[
[H^+] (z, t=0) = \bar{c}_H^{\text{initial}}
\]

\[
[H^+] (z=0, t>0) = \bar{c}_H^{\text{final}}
\]

where \( \bar{c}_H^{\text{initial}} \) is the initial internal \( H^+ \) concentration and \( \bar{c}_H^{\text{final}} \) is the final concentration when the diffusion transient dies away as \( t \rightarrow \infty \). At the bottom surface at \( z = -\delta \), no diffusion may occur across the boundary, so that,

\[
\frac{\partial}{\partial z} [H^+] = 0 \text{ at } z = -\delta
\]

(6)

Internal \( H^+ \) concentration is found from the external bath pH by assuming Donnan equilibrium of all ions across the boundary,

\[
\frac{c_{\text{NaCl}}}{c_{\text{Na}}} = \frac{c_{\text{HCl}}}{c_H} = \frac{c_{\text{Cl}}}{c_{\text{NaCl}} + c_{\text{HCl}}}
\]

(7)

as well as a requirement of quasineutrality within the material

\[
\rho + F (\bar{c}_H + \bar{c}_\text{Na} - \bar{c}_\text{Cl}) = 0
\]

(8)

where \( \rho \) is the fixed charge density, the sum of the concentration of the fixed charge groups, within the tissue,

\[
\rho = F \left( [\text{NH}_3^+] - [\text{SO}_3^-] - [\text{COO}^-] \right)
\]

(9)
and \( F \) is Faraday's constant. Finding the internal concentration requires knowledge of the bath ionic strength and \( \text{pH} \) (presumably set by the requirements of the experiment) and also of the fixed charge density, \( \rho \), carboxyl site number, \( n_c \), sulfate site number, \( n_s \), and equilibrium constants, \( K_c \) and \( K_s \).

Once the diffusion problem is solved to find \([H^+]\) in time and space within the tissue, the charge density profile, \( \rho(z,t) \), is then determined. [5]

3.1.2 Enzyme kinetics

In dealing with an enzyme diffusion reaction, one must, in general, consider more than one chemical reaction. First, there is the reaction between enzyme and substrate to form an enzyme-substrate complex

\[
E + S \rightarrow ES
\]  
(10)

which can then break down to release the enzyme and form a product,

\[
ES \rightarrow E + P
\]  
(11)

where \( E \) refers to enzyme, \( S \) the substrate, \( ES \) the enzyme-substrate complex, and \( P \) the reaction product. [9] In the case of the enzyme degradation experiments, the enzyme is chondroitinase, the substrate chondroitin sulfate chains, and the product is free chondroitin sulfate fragments of various sizes in solution. It is not yet known for certain whether the chondroitinase enzyme removes disaccharide units from the free ends of chondroitin sulfate chains (as an exo-enzyme) or whether the enzyme also catalyzes internal
linkages as well (as an endo-enzyme) [25]. Hence, the two choices for characterization of [S] are the molar concentration of chains or the molar concentration of disaccharide units.

Each of the above reactions may be characterized by its own reaction rate constant. With $k_{ES}$ the forward association reaction rate constant for the first of the reactions, one may write, under the assumption that (10) is irreversible,

$$\frac{\partial}{\partial t} [ES] = k_{ES} [E] [S]$$

(12)

and for the second, with forward dissociation reaction rate constant $k_{EP}$,

$$\frac{\partial}{\partial t} [ES] = -k_{EP} [ES]$$

(13)

The reverse reaction of (11) is assumed to occur at a negligible rate because of the relatively low concentration of product, [P]. Equations (12) and (13) are appropriately combined to obtain,

$$\frac{\partial}{\partial t} [ES] = k_{ES} [E] [S] - k_{EP} [ES]$$

(14)

For long time steady state $\frac{\partial}{\partial t} = 0$. In an enzyme diffusion reaction, one may be able to assume that a quasi-steady-state will be established at each point in space, if the diffusion or transport of enzyme is very slow compared to the kinetics of the reaction. It is probably justified to assume that the diffusion coefficient or the effective mass transport coefficient is very small in the
case of chondroitinase with its 20,000 MW. The left hand side of the last equation becomes vanishingly small, allowing the right hand side to be arranged into

\[ K_M = \frac{k_{EP}}{k_{ES}} = \frac{[E][S]}{[ES]} \]  

(15)

where \(K_M\) is related to the Michaelis-Menten constant for an enzyme reaction. [9]

To our knowledge, there is no definitive data on the mechanism of transport of chondroitinase in an intact tissue such as cartilage. We will therefore derive a model for this transport assuming diffusion of the enzyme occurs with a well defined diffusivity \(D_{enz}\) within the tissue, and that diffusive transport can be retarded by enzyme reaction with substrate as governed by the diffusion equation

\[ \frac{\partial}{\partial t} \left( \bar{c}_{enz} + [ES] \right) = D_{enz} \frac{\partial^2}{\partial z^2} \bar{c}_{enz} \]  

(16)

where \(\bar{c}_{enz} = [E]\) and \(D_{enz}\) are the internal enzyme concentration and diffusivity respectively. Using the Michaelis-Menten constant (15), the enzyme continuity equation may be rewritten into the form of a nonlinear diffusion reaction equation similar to that derived for hydrogen,

\[ \frac{\partial}{\partial t} \bar{c}_{enz} = D_{enz} \left[ 1 + \frac{[S]}{K_M} \right]^{-1} \frac{\partial^2}{\partial z^2} \bar{c}_{enz} \]  

(17)

which is valid in regimes where the term \(\bar{c}_{enz} \frac{\partial}{\partial t} \frac{[S]}{K_M}\) is small compared to \(\frac{\partial \bar{c}_{enz}}{\partial t} \left( 1 + \frac{[S]}{K_M} \right)\). The enzyme equation (17) contains
two unknowns, $\bar{c}_{\text{enz}}$ and $[S]$, and is therefore coupled to the rate equation for substrate concentration, which is found from (10),

$$\frac{\partial}{\partial t} [S] = -k_{ES} [E] [S]$$  \hspace{1cm} (18)

Driven by a step change in bath concentration of enzyme at $t = 0$, the necessary boundary and initial conditions are

$$\bar{c}_{\text{enz}}(z, t=0) = 0$$  \hspace{1cm} (19)

$$\bar{c}_{\text{enz}}(z=0, t>0^+) = c_{\text{enz}}^{\text{final}}$$  \hspace{1cm} (20)

$$\frac{\partial}{\partial z} \bar{c}_{\text{enz}} = 0 \text{ at } z = -\delta$$  \hspace{1cm} (21)

The initial concentration of substrate is fixed and given by

$$[S] (z, t=0) = [S]^{\text{initial}}$$  \hspace{1cm} (22)

Since a large fraction of fixed charge resides on the chondroitin sulfate substrate, cleavage of chondroitin sulfate disaccharide units or even larger fragments lead to an equivalent reduction of fixed charge. In addition, it is known from previous tests [7] that significant loss of chondroitin sulfate from cartilage occurs over a 24-48 hour digestion when full thickness cartilage discs are bathed in enzyme solution and allowing for extraction from all sides of the discs.

3.2 Dynamic Biphasic Model of Cartilage

The mechanics of cartilage lie in its nature as a biphasic material, composed of interstitial fluid and matrix solid. In this discussion, the biphasic mechanical theory of Kuei, Lai, and Mow [18] will be used to develop a multiple layer model of cartilage. In this development cartilage is
considered to be a linear homogeneous biphasic material, composed of interstitial fluid and solid protein matrix, as described by linearizing the Kuei-Lai-Mow theory.

The multi-layer model will be used to make a piece-wise approximation to a continuous variation in mechanical and electrical parameters. In particular analytic expressions are derived for displacement, stress, and streaming potential for a two layer system. The two layer case is useful for insight and considering limiting behavior of the general multi-layer system.

3.2.1 Mechanical

In a biphasic medium, motion of fluid past the solid matrix results in drag forces which are balanced by stress in the matrix. To find an appropriate equation of motion of the system, we begin by writing the constitutive relation for the total stress in the medium [3],

\[
\sigma_{ij} = 2G(c)\varepsilon_{ij} + \lambda(c)\delta_{ij}\varepsilon_{kk} - \beta(c)\delta_{ij} - (P-\Delta_{II})\delta_{ij} \tag{23}
\]

where
* \(\sigma_{ij}\) = total stress
* \(G\) = shear modulus
* \(\lambda\) = Lame constant
* \(\delta_{ij}\) = Kronecker delta function
* \(\beta\) = chemical stress
* \(P-\Delta_{II}\) = total fluid pressure

In uniaxial confined compression \(\varepsilon_{zz}\) is the only non-zero strain component. Defining compressive strain \(\varepsilon = -\varepsilon_{zz}\) and compressive stress \(\sigma = -\sigma_{zz}\), then (23) becomes
\[ \sigma = (Ne + \beta) + (P - \Delta \Pi) \]  

(24)

where \( M = 2G + \lambda \). Stress balance is then written neglecting inertia as

\[ \frac{\partial}{\partial Z} \sigma = 0 \]  

(25)

Darcy's Law is used to relate fluid flow to the total fluid pressure by the hydraulic permeability \( k_p \),

\[ U = -k_p \nabla (P - \Delta \Pi) \]  

(25)

and \( U \) is related by conservation of mass to the displacement \( u \),

\[ \frac{\partial}{\partial Z} u = -U \]  

(27)

Introducing a linear swelling pressure – strain relation

\[ p = M \varepsilon + \beta \]

then equations (23)-(27) are combined into the normalized equations \[3\]

\[ \frac{\tau_m}{\tau_c} \frac{\partial \zeta}{\partial t} = \frac{M}{\pi^2} \frac{\partial^2 \zeta}{\partial Z^2} + \frac{\tau_m}{\tau_c} \left[ \frac{P - \beta}{M} \frac{\partial M}{\partial a} + \frac{\partial \beta}{\partial c} \right] \frac{\partial c}{\partial t} \]  

(28)

and

\[ \frac{\pi^2}{\tau_c} \frac{\tau_m}{\partial t} = \left( \frac{\partial u}{\partial Z} \frac{\partial M}{\partial c} + \frac{\partial \beta}{\partial c} \right) \frac{\partial c}{\partial Z} + M \frac{\partial^2 u}{\partial Z^2} \]  

(29)

In this thesis, the limit \( \tau_m \ll \tau_c \) applies. With no dynamic excitation, (28) and (29) can be used to find the response to chemically induced uniaxial stress relaxation and creep, respectively \[3\].
For the case of sinusoidal excitation, a moving front model will be derived to describe the time evolution of the dynamic stiffness and streaming potential. In this model the material properties, \( M \), \( \beta \), and \( k_p \), are assumed to be homogeneous in the two regions above and below the front. Therefore in each region, the first term on the right hand side of (29) goes to zero and therefore one can write

\[
\frac{3u}{3t} = \frac{3^2u}{p 3z^2}
\]

which is the desired equation of motion.

Consider a layer of thickness \( \delta \), with imposed displacement at the surfaces, \( z = 0 \) and \( -\delta \), to be a general excitation in the form of a traveling wave,

\[
u_z (z=0) = \text{Re} \{ u^\alpha \exp [j (\omega t - k_y y)] \}
\]

and

\[
u_z (z=-\delta) = \text{Re} \{ u^\beta \exp [j (\omega t - k_y y)] \}
\]

The general displacement solution (in two dimensions) is

\[
u = u_z(z) = \text{Re} \{ \tilde{u}(z) \exp [j (\omega t - k_y y)] \}
\]

with boundary conditions \( \tilde{u}(0) = u^\alpha \) and \( \tilde{u}(-\delta) = u^\beta \).

Substituting into the equation of motion yields

\[
\frac{3^2}{3z^2} \tilde{u} = \frac{j \omega}{D} \tilde{u}
\]

or

\[\left( \frac{3^2}{3z^2} - \gamma^2 \right) \tilde{u} = 0\]

where \( D = k_p M \) is the so-called mechanical diffusivity and

\[\gamma^2 = \frac{j \omega}{D}\]

The real part of \( \gamma \), \( \text{Re} \{ \gamma \} \), is taken to be the reciprocal of skin depth, the effective penetration of the displacement or strain field. Now, by direct substitution, a solution for
\( \tilde{u}(z) \) is

\[
\tilde{u}(z) = \frac{\sinh \gamma (\delta + z)}{\sinh \gamma \delta} u^a - \frac{\sinh \gamma z}{\sinh \gamma \delta} u^b
\]  

(37)

where the superscripts \( \alpha \) and \( \beta \) refer to quantities at the top and bottom surfaces of a homogeneous layer. Referring to a typical sample geometry of figure 3.2 for a single layer, \( u^b \) would be zero and \( u^a \) would be the imposed displacement \( u^0 \),

\[
\tilde{u}(z) = \frac{\sinh \gamma (\delta + z)}{\sinh \gamma \delta} u^0
\]  

(38)

Defining the stress \( T_{zz} \) as that component of \( \sigma \) that is produced by strain,

\[
T_{zz} = \sigma - [\beta - (P - \Delta \Pi)]
\]

(39)

then

\[
T_{zz}(z) = M \frac{\partial \tilde{u}}{\partial z} = M \gamma \frac{\cosh \gamma (\delta + z)}{\sinh \gamma \delta} u^a - M \gamma \frac{\cosh \gamma z}{\sinh \gamma \delta} u^b
\]

For a single layer,

\[
T_{zz}(z) = M \gamma \coth \gamma \delta u^0
\]

(41)

Since the total fluid pressure is zero at \( z = 0 \), the measured stress at \( z = 0 \) is \( T_{zz}(0) \). The effective stiffness of the material is therefore defined as

\[
\Lambda = \frac{T_{zz}(0)}{u^0/\delta} = M \gamma \delta \coth \gamma \delta
\]

(42)

To find effective stress throughout a multiple layer system, a transfer relation can be defined by

\[
\begin{bmatrix}
  T^a_{zz} \\
  T^\beta_{zz}
\end{bmatrix}
= M \gamma
\begin{bmatrix}
  \coth \gamma \delta & -\frac{1}{\sinh \gamma \delta} \\
  -\frac{1}{\sinh \gamma \delta} & \coth \gamma \delta
\end{bmatrix}
\begin{bmatrix}
  u^a \\
  u^\beta
\end{bmatrix}
\]

(43)
Figure 3.2 Sample geometry
The sample is constrained by solid walls to the sides and at $z=-\delta$. A free flow boundary condition exists at $z = 0$. 
which can be written as

\[
\begin{bmatrix}
T^a_{zz} \\
u^a
\end{bmatrix} = \begin{bmatrix}
\cosh \gamma_\delta & M_Y \sinh \gamma_\delta \\
\frac{1}{M_Y} \sinh \gamma_\delta & \cosh \gamma_\delta
\end{bmatrix} \begin{bmatrix}
T^b_{zz} \\
u^b
\end{bmatrix}
\]  

(44)

Now a multi-layer system will be considered. Subscript notation will be used to denote different layers. For a multi-layer system of \( n \) layers, \( u^a_m \) will denote displacement at the \( a \) surface of the \( m \)th layer, \( u^b_m \) displacement at the \( b \) surface of the \( m \)th layer, etcetera. For \( n \) layers, the boundary conditions are at each layer

\[ u^b_m = u^a_{m+1}, \quad T^b_m = T^a_{m+1}, \quad 0 \leq m \leq n-1 \]

and at the top and bottom surfaces

\[ \sigma = T^a_0, \quad u^a_0 = u_0, \quad u^b_n = 0 \]

Therefore

\[
\begin{bmatrix}
T_{zz}(0) \\
u_0
\end{bmatrix} = \begin{bmatrix}
T^a_0 \\
u^a_0
\end{bmatrix} = \begin{bmatrix}
n \\
\Pi_{m=0}^{n} A_m^{[2 \times 2]}
\end{bmatrix} \begin{bmatrix}
T^b_n \\
u^b_n
\end{bmatrix}
\]  

(45)

where \( A_m^{[2 \times 2]} \) is the transfer relation matrix for layer \( m \).

Considering only two layers, 0 and 1, and solving for \( T_{zz}(0) \),

\[
T_{zz}(0) = \frac{M_0}{\delta_0} u_0 \gamma_0 \delta_0 \coth \gamma_0 \delta_0 \frac{M_0}{\delta_0} \gamma_0 \delta_0 \tanh \gamma_0 \delta_0 + \frac{M_1}{\delta_1} \gamma_1 \delta_1 \coth \gamma_1 \delta_1
\]

Expansion of this expression to find the magnitude and phase components could be done at this point, but the
manipulations would be lengthy and physical insight would be lost in the algebraic soup. Instead an intuitive feel for the behavior can be gained by examining the subcomponents associated with each layer with respect to the high and low frequency limits.

At this point it would be useful to consider a "cutoff" frequency \( \omega_c \) such that for frequencies below cutoff the low frequency approximation holds and above cutoff the high frequency approximation holds. A different \( \omega_c \) may in turn be defined for each layer, where below this frequency a linear displacement field exists and above a nonlinear (exponential) displacement field exists. An appropriate \( \omega_m \) for layer \( m \) could be defined where \( \text{Re}\{\gamma_m^\delta_m\} = 1 \) when the skin depth is approximately equal to the thickness of the layer. This frequency roughly corresponds to the inflection point of mechanical phase angle in a single layer model with the material properties of layer \( m \).

First consider the low frequency limit. In this limit the terms \( \gamma_m^\delta_m \to 0 \). These terms appear as arguments of the complex hyperbolic functions coth and tanh. Tanh approaches its argument for small arguments and coth approaches the inverse of its argument,

\[
\lim_{\alpha \to +0} \tanh \alpha = +1 \quad \text{and} \quad \lim_{\alpha \to +0} \coth \alpha = +1/\alpha.
\]

Using these limits in the expression for \( T_{zz}(0) \),

\[
\lim_{\omega \to +0} T_{zz}(0) = \frac{M_0}{\delta_0} u_0 \left( \frac{M_0}{\delta_0} + \frac{M_1}{\delta_1} \right) \to \frac{M_0}{\delta_0} + \frac{M_1}{\delta_1} = \frac{M_0}{\delta_0} \frac{M_1}{\delta_1} u_0 \quad \text{(47)}
\]
For high frequency both tanh and coth terms approach 1 for large arguments. Thus

\[
\lim_{\omega \to \infty} T_{zz}(0) = M_0 \gamma_0 u_0 \frac{M_0 \gamma_0 + M_1 \gamma_1}{M_0 \gamma_0 + M_1 \gamma_1} u_0 = M_0 \gamma_0
\]  

(48)

It is seen that at high frequency only the material properties of the top layer are apparent. Physically this implies that the displacement field is confined to the top layer at high frequency and that the bottom layer is effectively unstrained.

There are two other cases to be examined in the two layer model in addition to the low and high frequency limits. Where \( \omega_{c0} \) is well separated from \( \omega_{c1} \), a transition would be observed for \( \omega \) between the two cutoff frequencies.

In the case where \( \omega_{c0} \ll \omega \ll \omega_{c1} \), terms associated with the top layer approach their high frequency limit while bottom layer terms approach the low frequency limit. Thus

\[
T_{zz}(0) = M_0 \gamma_0 u_0 \frac{M_0 \gamma_0 + \frac{M_1}{\delta_1}}{M_0 \gamma_0 + \frac{M_1}{\delta_1}} u_0 = M_0 \gamma_0
\]  

(49)

Immediately it is seen for \( \omega_{c0} \ll \omega \ll \omega_{c1} \), a nonlinear displacement field exists in the top layer and no displacement field "penetrates" to the bottom layer. Thus the top layer predominates and result is the same as the high frequency approximation.

In the other case for \( \omega_{c1} \ll \omega \ll \omega_{c0} \), the top layer is in the low frequency limit and the bottom layer is in the
high frequency domain:

\[ T_{zz}(0) = \frac{M_0}{\delta_0} u_0 \left( \frac{M_0}{\delta_0} \left( \gamma_0 \delta_0 \right)^2 + M_1 \gamma_1 \right) \frac{u_0}{\delta_0} = \frac{M_0}{\delta_0} \] (50)

Material behavior in this case would appear linear, as in the low frequency limit, but with a different effective modulus than the low frequency limit.

To summarize, the two layer model appears to have 3 distinct limits in the frequency domain as opposed to the single layer which has 2 distinct limits. These limits are written in the following table:

<table>
<thead>
<tr>
<th>frequency</th>
<th>behavior of displacement field</th>
<th>limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \omega &lt;&lt; \omega_{c0}, \omega &lt;&lt; \omega_{c1} )</td>
<td>linear</td>
<td>low frequency</td>
</tr>
<tr>
<td>( \omega_{c0} &lt;&lt; \omega \ll \omega_{c1} )</td>
<td>nonlinear</td>
<td>high frequency</td>
</tr>
<tr>
<td>( \omega_{c0} &gt;&gt; \omega &gt;&gt; \omega_{c1} )</td>
<td>linear</td>
<td>intermediate</td>
</tr>
<tr>
<td>( \omega &gt;&gt; \omega_{c0}, \omega &gt;&gt; \omega_{c1} )</td>
<td>nonlinear</td>
<td>high frequency</td>
</tr>
</tbody>
</table>

Two limits are linear regions, one with an effective composite modulus and one with an effective modulus of the top layer. The other limit is nonlinear with effective modulus of the top layer.

3.2.2 Streaming potential

Streaming potential is incorporated into the mechanical model through a simple linear relationship describing electrokinetic transduction. Fluid velocity and current density is related to voltage and total fluid pressure gradients by
\[
\begin{bmatrix}
U \\
J
\end{bmatrix} =
\begin{bmatrix}
-k_{11} & k_{12} \\
k_{21} & -k_{22}
\end{bmatrix}
\begin{bmatrix}
\frac{\partial (P-\Delta \Pi)}{\partial z} \\
\frac{\partial v}{\partial z}
\end{bmatrix}
\]  
\hspace{1cm} (51)

where \( U \) is fluid velocity, \( J \) current density, \( p \) pressure, and \( v \) voltage [4]. In the open circuit configuration of the proposed experiments \( J = 0 \) and the above relationship may be written eliminating the total fluid pressure gradient term:

\[
U = -\frac{k_p}{k_{21}} k_{22} \frac{\partial v}{\partial z} = -\frac{a}{\partial x} 
\]  
\hspace{1cm} (52)

where \( k_p \) is related to the \( k_{ij} \) by \( k_p = k_{11} - \frac{k_{12} k_{21}}{k_{22}} \). The above may be integrated to find the voltage across a layer in terms of the imposed displacement,

\[
v = \int_{-\delta}^{0} \frac{k_e}{k_p} j \omega u \, dz 
\]  

\[
= \frac{j \omega}{\gamma} \frac{k_e}{k_p} \frac{\cosh \gamma \delta - 1}{\sinh \gamma \delta} (u^a - u^b) 
\]  
\hspace{1cm} (53)

where \( k_e = \frac{k_{21}}{k_{22}} \). For a multi-layer system the total voltage is the sum from all the layers.

3.2.3 Mechanical interface compliance

Previous research by Lee et al [8] has shown that uniaxial confined compression of cartilage using porous platen does not follow predictions of the biphasic theory as presented thus far. Lee et al postulated that interdigitation of the cartilage surface into the pores of the platen causes the cartilage-platen interface to behave
in the manner of a spring. When a compliance term was incorporated into the biphasic theory, Lee et al and Olson [20] showed that the theory with an interface compliance predicted experimental behavior to a significant extent using values of modulus and permeability in agreement with independent experiments and the previous literature.

To summarize Olson, the interface compliance appears as an ideal elastic element in series with the cartilage compliance. Writing the effective stiffness as an expression of the interface stiffness and the cartilage stiffness in series,

$$\Lambda(\omega) = \frac{\Lambda_S \Lambda_C(\omega)}{\Lambda_S + \Lambda_C(\omega)}$$ (54)

where $\Lambda_S$ is the stiffness of the interface "spring" and $\Lambda_C$ is the cartilage stiffness as a function of frequency. For a homogeneous single layer of cartilage,

$$\Lambda_C(\omega) = M \gamma \delta \coth \gamma \delta$$

and

$$\Lambda(\omega) = \frac{\Lambda_S M \gamma \delta \coth \gamma \delta}{\Lambda_S + M \gamma \delta \coth \gamma \delta}$$ (55)

Again the low and high frequency limits are evaluated

$$\lim_{\omega \to 0} \Lambda = \frac{\Lambda_S M}{\Lambda_S + M}$$

$$\lim_{\omega \to \infty} \Lambda = \Lambda_S$$

and again it is observed that for low frequency the result is a series expression for stiffness, while for high
frequency the upper layer dominates. In this case the interface compliance takes the place of an upper layer.

The effect of interface compliance on streaming potential can also be considered. The spring behavior of the interface can be viewed as acting to decrease the effective displacement at the surface of the cartilage. In the expression for streaming potential \( u^a \) becomes

\[
\begin{align*}
  u^a &= u_0 \left( 1 - \frac{A}{A_s} \right) \\
  &= u_0 \frac{A_s}{A_s + M \gamma \delta \coth \gamma \delta}
\end{align*}
\] (56)

and then \( v \) becomes

\[
\begin{align*}
  v &= -\frac{j \omega}{\gamma} \frac{k_e}{k_p} \frac{\cosh \gamma \delta - 1}{\sinh \gamma \delta} u_0 \frac{A_s}{A_s + M \gamma \delta \coth \gamma \delta}
\end{align*}
\] (57)

The high frequency limit reduces to simply

\[
\begin{align*}
  v &= k_e \frac{A_s u_0}{\delta}
\end{align*}
\] (58)

In interpreting the data one must take account how a change in cartilage mechanics affects the interface compliance. Since the compliance is a result of a cartilage and platen interaction, the compliance must be a function of both cartilage and platen mechanics as well as the geometry of the interface. At high frequency, finite hydraulic permeability limits fluid flow, increasing dynamic stiffness and causing the cartilage to appear to be more incompressible. The platen may be displaced into the cartilage instead by shear.

To estimate the effect of the interface, assume a
simple model of a platen surface described by a sinusoid with amplitude $\alpha$ and wave length $\lambda$. The peak surface curvature of the platen is given by

$$R = \frac{\lambda^2}{\pi \alpha}$$

(59)

Timoshenko and Goodier [26] describe the theory of contact between spherical surfaces of two elastic solids. Summarizing, one first finds the radius of contact of the platen impressed into the cartilage at a single peak

$$r_c = \left( \frac{3 R (1-v^2)}{4 E \sigma \lambda^2} \right)^{\frac{1}{3}}$$

(60)

The stiffness of the interface would then be given by

$$\lambda = \frac{4 r_c E \delta}{3 (1-v^2) \lambda^2}$$

(61)

The result predicts an elastic interface with stiffness proportional to the modulus (which for the case of equation (61), the Young's modulus, which can be expressed in terms of $M$ and the Poisson's ratio $\nu$). This conclusion assumes that $\nu$ does not change very much in comparison to $E$, and assumption discussed by Hoch et al [6].

3.3 Mechanochemical front model

The electromechanical model can now be combined with the diffusion models by assuming that material properties, $M$, $k_p$, and $k_e$, are defined as point by point functions of the local chemical conditions throughout the tissue. The transient is viewed as changing the material properties from an initial state of uniform properties to one with gradients
in properties to a final state with once again uniform, but new material properties. This modeling approach has been applied by both Grimshaw [5] and Eisenberg [3] for accurately describing transient swelling behavior in cartilage. The goal now is to extend the approach to sinusoidal steady state response as well.

The diffusion processes described earlier in the chapter define the chemical state of the tissue throughout time and space. The local chemical state is taken to be a function of the local ionic strength and local fixed charge density at some point in the tissue. However, local ionic strength may be expressed in terms of the fixed charge and external bath ionic strength by assuming conditions of quasineutrality and Donnan equilibrium. These assumptions are valid provided that diffusion of the ions, Na\(^+\) and Cl\(^-\), is fast compared to diffusion of hydrogen or enzyme, and that chemical changes to the bath do not perturb the bath ionic strength. [5] Thus in cases where external bath ionic strength is held fixed, local chemical conditions may be formulated in terms of local fixed charge density alone.

3.3.1 Multi-layer approximation

Under these assumptions, the material properties, represented by \( M, k_p, \) and \( k_e \), are written as functions of the fixed charge density in time and space,

\[
M = M(\rho(z,t)) \\
k_p = k_p(\rho(z,t)) \\
k_e = k_e(\rho(z,t))
\]
Solving for the profile of $\rho$ and applying a staircase approximation, one can obtain a staircase approximation for $M$, $k_p$, and $k_e$. Substituting the staircase values into the transfer relation matrices of the multilayer biphasic model, a solution for the electromechanical response is found.

3.3.2 Front approximation

As a first approximation, the charge profile resulting from the diffusion reaction is viewed as a propagating wave. The approximation is appropriate if the profile is extremely steep at some point in space for all time. Then one set of parameters would characterize the material on one side of the front and another set would characterize the other side. The material can then be treated as a two layer system, where the interface between the layers is moving with the charge profile front. For that region where the front has not reached the material parameters are identical with those of the initial conditions for the whole material. Where the front has past, the parameters are changed to those consistent with the new conditions present, that is, the final conditions for the whole material. To find the total response for all time, one would only have to know the material parameters estimated from the material behavior before the start and after the end of the diffusion.

The location of the front can be established by the charge profile given by the diffusion reaction models. Calculating the average charge density,
(a). Step approximation to charge profile

(b). Front approximation to charge profile

(c). Mechanical properties in two layer front model

Figure 3.3 Mechanochemical front model
\[ \rho_{av} = \left[ 0, \frac{\rho(z)}{\delta} \right] \, dz = \frac{1}{\delta} \left\{ \rho_f \lambda(t) + \rho_i \left[ \delta - \lambda(t) \right] \right\} \]  \hspace{1cm} (62)

where \( \lambda(t) \) is the location of the front. \([5]\) Solving for \( \lambda(t) \)

\[ \lambda(t) = \frac{\rho_f - \rho_{av}(t)}{\rho_i - \rho_f} \delta \]  \hspace{1cm} (63)

The various models of cartilage chemistry and mechanics presented in this chapter will be solved and further explored in chapter 4.
Chapter IV
Numerical Methods and Theoretical Results

The mechanochemical coupled problems presented in the previous chapter are now solved. Using a numerical method, we first determine the chemical state of the tissue described by the diffusion reaction equations. The discrete solution is then used to determine how the electromechanical parameters are partitioned in space as a function of time for the two layer or multi-layer biphasic model.

4.1 Crank-Nicolson diffusion solutions

The diffusion reaction equations derived in the previous chapter are in the form of nonlinear differential equations which must be solved numerically. In the numerical solutions presented, the Crank-Nicolson [2] formulation is used and applied to normalized diffusion equations of the form

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial z^2}
\]  

(1)

where the normalizations

\[
z = \frac{z}{\delta} \quad (2)
\]

\[
t = \frac{t}{\delta^2 / D} \quad (3)
\]

have been used. The normalized diffusion coefficient $D$ is determined by the details of the diffusion reaction process and is a function of time and space. In the absence of any reaction process $D$ would simply be 1.

The normalized concentration $C$ is discretized in time and space at points $t = n\Delta t$ and $z = i\Delta z$ for integers $n$ and $i$
respectively. The discrete concentration is then written as

\[ c_i^n = c_i (z = i\Delta z, t = n\Delta t) \]  \(4\)

The discrete derivatives are defined at points \( t = (n+\frac{1}{2})\Delta t \) and \( z = i\Delta z \) by difference equations

\[ \frac{\partial c}{\partial t} = \frac{c_i^{n+1} - c_i^n}{\Delta t} \]  \(5\)

\[ \frac{\partial^2 c}{\partial z^2} = \frac{\Delta^2 (c_i^{n+1}) + \Delta^2 (c_i^n)}{2 (\Delta z)^2} \]  \(6\)

where the discrete 2nd derivative function is defined by the operator

\[ \Delta^2 (c_i^n) = c_{i+1}^n + c_i^n + c_{i-1}^n \]  \(7\)

The normalized diffusion equation can now be transformed into a discrete difference equation

\[ c_i^{n+1} = c_i^n + D_i^n \frac{\Delta t}{2 (\Delta z)^2} \left[ \Delta^2 (c_i^{n+1}) + \Delta^2 (c_i^n) \right] \]  \(8\)

The approximation \( D_i^n = D_{i+\frac{1}{2}}^{n+1} \) has been made in order to make the diffusion equation solvable implicitly. The difference equation may be rewritten in vector form. The spatial concentration vector is written as \( \mathbf{c}^n \), representing a vector of species concentrations at each discrete point in space. The vector difference equation becomes

\[ \mathbf{c}^{n+1} = \mathbf{c}^n + m^n \frac{\Delta t}{2 (\Delta z)^2} \left[ \mathbf{c}^{n+1} + \mathbf{c}^n \right] \]  \(9\)
where the square matrix $M^n$ is the diffusion coefficient matrix, which, for $N$ spatial divisions, is given by

$$
M^n = \begin{bmatrix}
0 & 0 & 0 & 0 & \cdots \\
D_1^n & -2D_1^n & D_1^n & 0 & \cdots \\
0 & D_2^n & -2D_2^n & D_2^n & \cdots \\
\cdots & \cdots & \cdots & \cdots & \cdots \\
0 & 0 & 0 & 0 & \cdots \\
\end{bmatrix} (10)
$$

The vector difference equation can be rewritten and solved for $\tilde{c}^{n+1}$ in terms of $\tilde{c}^n$ by using the identity matrix $I$,

$$
\tilde{c}^{n+1} = \left[ I - \frac{\Delta t}{2 (\Delta z)^2} M^n \right]^{-1} \left[ I + \frac{\Delta t}{2 (\Delta z)^2} M^n \right] \tilde{c}^n (11)
$$

This last equation is the desired implicit solution which may be readily implemented provided $\Delta t$ and $\Delta z$ are sufficiently small to insure the accuracy of the calculations. Because of generality of the Crank-Nicolson method, it may be applied equally well for both the hydrogen and enzyme diffusion.

4.2 Hydrogen diffusion solution

Applying the Crank-Nicolson method to the hydrogen diffusion problem, the hydrogen concentration, radical site numbers, and reaction constants are normalized by

$$
c_H = c_H c_{IEP} (12)
$$

$$
n_c = n_c K_c \quad \quad n_c = n_c K_c (13)
$$

$$
K_C = K_C c_H^{IEP} \quad \quad K_S = K_S c_H^{IEP} (14)
$$
where \( c_{\text{IEP}} \) is the hydrogen concentration at the isoelectric point and which yields the normalized diffusion coefficient

\[
D = \left[ 1 + \frac{n_c}{(1 + \frac{c_H}{K_c})^2} + \frac{n_s}{(1 + \frac{c_H}{K_s})^2} \right]^{-1}
\]  
(15)

The charge density is obtained directly from the auxiliary equation

\[
\rho = \left[ \frac{n_c}{K_c + 1} + \frac{K_c}{K_c + c_H} + \frac{n_s}{K_s + 1} + \frac{K_s}{K_s + c_H} \right] (c_H - 1)
\]  
(16)

where

\[
\rho = \rho_F c_{\text{IEP}}^H
\]  
(17)

The numerical model for hydrogen diffusion is implemented and the auxiliary equation used to find \( \rho \). As stated, the model requires knowledge of \( n_c, pK_c, n_s, pK_s, pH^{\text{IEP}} \), and \( \bar{D}_H \). \( \bar{D}_H \) is related to \( D_{\text{H}}^\infty \), the diffusivity of \( H^+ \) in infinitely dilute solution, by the relation

\[
\bar{D}_H = D_{\text{H}}^\infty \left( \frac{1 - V_p}{1 + V_p} \right)
\]  

where \( V_p \) is the solid volume fraction [5]. Values for these quantities were taken from Grimshaw [5], who used \( n_c = 0.25 \text{M}, pK_c = 3.4, pH^{\text{IEP}} = 2.5 \), and \( D_{\text{H}}^\infty = 9.3 \times 10^{-9} \text{ m}^2/\text{s} \) in a similar numerical solution for hydrogen diffusion, and from Grodzinsky [28] for values of \( n_s = 0.25 \text{M} \) and \( pK_s = 1.5 \). Solutions for \( \rho \) versus \( z \) parameterized by time is shown in figure 4.1 for a transient from \( pH \) 7 to \( pH \) 2.6 and in figure 4.2 for a transient from \( pH \) 7 to \( pH \) 3, as used in the experiments.

4.3 Enzyme diffusion solution

For the enzyme diffusion problem, the appropriate
Figure 4.1 Hydrogen diffusion (ph 7 - pH 2.6) 
Charge density vs space parameterized by time
Figure 4.2 Hydrogen diffusion (pH 7 - pH 3.0)
Charge density vs space parameterized by time
normalizations for concentration and diffusion coefficient are

\[ C_{\text{enz}} = C_E C_{\text{final}} \]  
\[ (18) \]

\[ D = \left[ 1 + \frac{C_{\text{CS}}}{K_M} \right]^{-1} \]  
\[ (19) \]

where \( C_E \) is the normalized enzyme concentration identified with \( c \) in the diffusion equation and \( C_{\text{CS}} \) is the normalized chondroitin sulfate (substrate) concentration given by

\[ [S] = C_{\text{CS}} [S]_{\text{initial}} \]  
\[ (20) \]

and

\[ K_M = K_M [S]_{\text{initial}} \]  
\[ (21) \]

The required substrate concentration is given by the auxiliary equation

\[ \frac{\partial C_{\text{CS}}}{\partial t} = -k_{ES} C_{\text{CS}} C_E \]  
\[ (22) \]

where

\[ k_{ES} = k_{ES} \frac{\delta^2}{D} C_{\text{final}} \]  
\[ (23) \]

The substrate concentration is solved by the difference equation

\[ \frac{C_{\text{CS}}^{n+1} - C_{\text{CS}}^n}{\Delta t} = -k_{ES} \frac{C_{\text{CS}}^{n+1} + C_{\text{CS}}^n}{2} C_E \]  
\[ (24) \]

which may be written in implicit form as

\[ C_{\text{CS}}^{n+1} = \frac{1 - \frac{\Delta t}{2} k_{ES} C_E C_{\text{CS}}^n}{1 + \frac{\Delta t}{2} k_{ES} C_E C_{\text{CS}}^n} C_{\text{CS}}^n \]  
\[ (25) \]
Values for the enzyme reaction constants, $k_{ES}$, $k_{EP}$, and $K_M$, were estimated from Thurston et al [25], who characterized chondroitinase-ABC enzyme activity. Thurston found a $K_M = 0.03$ mM (using Thurston's value of equivalent disaccharide) and $V_{max} = 3 \frac{\text{nmol}}{\text{min}}$ using an enzyme concentration of $0.04 \mu g$ per mL. The value of $V_{max}$ implies that the enzyme present was equivalent to $0.003$ enzyme units. The reaction velocity $V_{max}$ is related to the reaction constants through [9]

$$V_{max} = k_{EP} [E]$$

Therefore

$$k_{EP} = 3 \frac{\text{nmol}}{\text{mL min}} \times \frac{\text{min}}{60 \text{ sec}} \times .003 \text{ units} = 16 \text{ nmol units}^{-1} \text{ sec}^{-1}$$

and

$$k_{ES} = \frac{k_{EP}}{K_M} = \frac{16 \text{ nmol}}{\text{units sec}} \div 0.03 \text{ mM} = 0.5 \text{ mL units}^{-1} \text{ sec}^{-1}$$

Concentration of chondroitin sulfate within the tissue is estimated from biochemical assays of bovine cartilage used by Grimshaw [5]. Grimshaw used a concentration of $0.112$ M for glucuronic acid, which is present on both chondroitin sulfate and hyaluronic acid. Chondroitin sulfate is estimated to be approximately 80-90% of the proteoglycan aggregate weight [19]. Using these figures, the substrate concentration is estimated to be $[S] = 0.1$ M. However, biochemical studies performed to Dr. Tom Koob [7] of CHMC, Boston, characterizing chondroitinase in bovine cartilage indicate that not all the substrate is removable or
accessible by the enzyme. Koob's results show that only about 70% of the chondroitin sulfate can be removed under maximal extraction conditions. Combining these results, the effective substrate concentration used in the model is $[S] = .07 \text{ M}$.

The experimental enzyme concentration used is .1 units ml$^{-1}$. However, no diffusion coefficient is available for chondroitinase in cartilage, though it is certainly reasonable to assume that $D_E << D_H$. Several values of $D_E$ were examined ranging from 100 to 10000 times smaller than $D_H$. In the numerical model, normalized parameters used are $K_M = K_M/[S] = .0004$ and $K_{ES} = 125-12500$ for a $\tau_{norm} = \delta^2/D = 2500-250,000 \text{ sec}$ (strained $\delta=500\mu m$, $D=10^{-10}-10^{-12}$). To find a reasonable value of $D$ in this range, the time predicted by the model for complete digestion of the substrate was compared with results of Koob's characterization of chondroitinase. For an enzyme concentration of .12 units, Koob found approximately 65% loss of chondroitin sulfate as assayed by uronic acid loss in 24 hours for full thickness ($\sim 1\text{ mm}$) plugs. Although the data is not directly comparable because of different geometry, the value of $K_{ES} = 625$ was chosen as the most reasonable, which predicts a time of 30 hours for maximal digestion, defined as the point where the substrate front has passed completely through the tissue as shown in figure 4.6. Using this value of $K_{ES}$, figures 4.3 and 4.4 show plots of enzyme and substrate concentrations respectively versus depth and parameterized by time.
Normalized depth into tissue

Figure 4.3 Enzyme diffusion vs space parameterized by time
Figure 4.4 Substrate concentration vs space parameterized by time
Note that the substrate concentration is an excellent approximation to a front in concentration, much more so than the hydrogen diffusion examples. The location of the front as a function of time as given by equation (3.63) is shown in figure 4.5 for the (pH 7 - pH 3) hydrogen diffusion simulation and in figure 4.6 for the enzyme diffusion simulation.

The diffusion reaction process is viewed as basically one where the reaction retards the diffusion. Mathematically this is expressed as the unitless terms on the right hand side of equations (3.5) and (3.17), which are always less than 1. Physically one would argue that the reaction must be satisfied before further diffusion into the tissue. Hydrogen is initially taken up to neutralize carboxyl and sulfate groups and enzyme is taken up to form complex. The result is a moving front in fixed charge density as shown in the model simulations of figures 4.1, 4.2, and 4.4.

4.4 Total biphasic response solution

The matrix transfer relation equations (3.45) are directly implemented to find the total response of the tissue. Values of material properties ($\Lambda_s$, $M$, $k_p$, $k_e$) are estimated by means of a nonlinear regression analysis of the biphasic model to the initial and final experimental response.

For the (pH 7 - pH 3) hydrogen diffusion experiments, initial and final electromechanical parameters were determined for the 2 samples tested. For sample #41, initial
Figure 4.5 Fixed charge density front vs time for hydrogen diffusion (pH 7 - pH 3)
Figure 4.6 Substrate front location vs time for enzyme diffusion.

Normalized depth into tissue vs time (hours).
and final values obtained are

\[
\begin{align*}
\text{initial} & \\
\Lambda_s &= 13 \text{ MPa} \\
M &= 2.0 \text{ MPa} \\
k_p &= 2.8 \times 10^{-15} \text{ m}^2/\text{Pa} \cdot \text{s} \\
k_e &= 1.6 \times 10^{-8} \text{ v/Pa} \\
\text{final} & \\
\Lambda_s &= 2.7 \text{ MPa} \\
M &= .05 \text{ MPa} \\
k_p &= 1.5 \times 10^{-15} \text{ m}^2/\text{Pa} \cdot \text{s} \\
k_e &= 3.7 \times 10^{-9} \text{ v/Pa}
\end{align*}
\]

and for sample #42, initial and final values are

\[
\begin{align*}
\text{initial} & \\
\Lambda_s &= 17 \text{ MPa} \\
M &= 2.9 \text{ MPa} \\
k_p &= 1.7 \times 10^{-15} \text{ m}^2/\text{Pa} \cdot \text{s} \\
k_e &= 5.8 \times 10^{-9} \text{ v/Pa} \\
\text{final} & \\
\Lambda_s &= 5.5 \text{ MPa} \\
M &= .12 \text{ MPa} \\
k_p &= 0.8 \times 10^{-15} \text{ m}^2/\text{Pa} \cdot \text{s} \\
k_e &= 8.7 \times 10^{-9} \text{ v/Pa}
\end{align*}
\]

For the enzyme degradation experiments, initial and final electromechanical parameters were determined for the 6 samples. Averaged initial and final values obtained are

\[
\begin{align*}
\text{initial} & \\
\Lambda_s &= 11 \text{ MPa} \\
M &= 2.0 \text{ MPa} \\
k_p &= 1.3 \times 10^{-15} \text{ m}^2/\text{Pa} \cdot \text{s} \\
k_e &= 5.1 \times 10^{-9} \text{ v/Pa} \\
\text{final} & \\
\Lambda_s &= 9.5 \text{ MPa} \\
M &= 1.7 \text{ MPa} \\
k_p &= 1.8 \times 10^{-15} \text{ m}^2/\text{Pa} \cdot \text{s} \\
k_e &= 4.6 \times 10^{-9} \text{ v/Pa}
\end{align*}
\]

To model these transitions, the front approximation was implemented using the result from the hydrogen and enzyme diffusion reaction simulations as input to the two layer model. The averaged initial values found provided parameters for the lower layer in the model and final values the upper layer. Using the electromechanical parameters obtained for sample #42 and the (pH 7 - pH 3) hydrogen diffusion simulation, the normalized response as a function of time for 3 frequencies was generated as shown in figure 4.7. Averaged electromechanical parameters for the enzyme
digested plugs were used with the enzyme diffusion simulation to generate normalized response as a function of time for 4 frequencies as shown in figure 4.8.

In the enzyme diffusion stiffness response, figure 4.8a, for each frequency there is a small region where the response appears to plateau. This region occurs when the front passes the skin depth of the tissue for that frequency, which is given by

$$\delta_{\text{skin}} = \frac{1}{\text{Re}(\gamma)} = \left( \frac{2Mk}{\omega} \right)^{1/2}$$  \hspace{1cm} (27)

Figure 4.9 shows the location of the substrate front versus time for the enzyme diffusion (figure 4.6) with the skin depth for frequencies of interest marked to show where the front passes the skin depth. For lower frequencies this occurs later in time and is therefore not very apparent in figure 4.8a. Before the front reaches the skin depth, the excitation is operating in the region $\omega_{c1} < \omega < \omega_{c0}$, as discussed in chapter 3, while after front has passed $\omega_{c1} < \omega_{c0} < \omega$. The corresponding streaming potential response, figure 4.8b, at each frequency displays several inflections. A minimum in streaming potential response occurs at each frequency where again the front passes the skin depth.
Figure 4.7a Total biphasic front model response:
Normalized stiffness vs time for hydrogen diffusion
Figure 4.7b Total biphase front model response: Normalized streaming potential vs time for hydrogen diffusion
Figure 4.8a Total biphasic front model response: Normalized stiffness vs time for enzyme diffusion
Figure 4.8b Total biphasic front model response: Normalized streaming potential vs time for enzyme diffusion.
Figure 4.9 Substrate front location vs time shown with skin depth at 0.005-1 Hz
Chapter V
Discussion and Conclusions

5.1 Major Conclusions Based on Experimental Data

From comparison of the data from control experiments (figure 2.6) with data from experiments involving addition of HCl or chondroitinase abc, it is clear that addition of either reagent causes a significant, monotonic decrease in dynamic stiffness and streaming potential. The fact that stiffness and streaming potential are relatively unchanged during the course of the 24 hour control experiments suggests that artifactual leachout of proteoglycan monomers or fragments does not occur to any great extent. The results of uronic acid analyses will be needed to absolutely confirm this.

Addition of HCl was found to cause a significant decrease in stiffness and streaming potential over a period of several hours (figures 2.3 and 2.4). This is not surprising in view of the fact that addition of HCl in this study was carried out to the point of almost complete charge neutralization. Furthermore, previous studies have revealed a sharp decrease in the static isometric compressive stress after addition of HCl [5].

However, while enzymatic extraction of chondroitin sulfate caused a rapid and significant drop in the dynamic streaming potential (figure 2.5b), we observed a relatively smaller drop in dynamic stiffness (figure 2.5a). In short, the major conclusion is that chondroitinase enzyme, in
breaking chondroitin sulfate into freely diffusing constituent disaccharide units, allows charge groups to no longer contribute to electrokinetic interactions. This is evidenced by rapid decrease in streaming potential and increase in hydraulic permeability, as shown in decrease in dynamic biphasic response. It is possible that cleaved oligosaccharides of CS that have not yet diffused out of the matrix may still contribute to the dynamic stiffness, thus resulting in the relatively smaller decrease in stiffness as compared to streaming potential (figure 2.5b).

The use of dynamic electromechanical behavior to follow a slowly varying transient process is believed shown here to be a powerful tool in the study of biomaterials such as cartilage, where the interaction of fluid and solid are significant contribution to mechanical behavior. Where charge interactions (electrostatic repulsion and electrokinetic transduction) act to modulate mechanical behavior, dynamic streaming potential is a strong indicator of the effect of degradation of matrix charge on material properties. Analysis of the large amount of information acquired through dynamic testing is simplified in the special case where the result of the diffusion reaction process can be modeled by a front in material properties.

5.2 Comparison of Theory With Experimental Results

The initial and final values of \( A_s \), \( M \), \( k_p \), and \( k_e \), obtained by fitting the biphasic model to the data (as presented in section 4.4), show several important trends.
Significantly, for the case of enzyme diffusion, results indicate that the parameters $M$ and $k_e$ do not change as much as $k_p$ appears to change. The theoretical curve fit showed that final values of $M$ and $k_e$ were 85% and 90% of their initial values respectively while final $k_p$ was 140% of its initial value. Thus even though the loss in $M$ was only 15%, the change in $k_p$ is great enough to substantially decrease the dynamic stiffness response especially in the lower part of the frequency spectrum, as indicated by the divergence between the high and low frequency response seen in figure 2.5. The lower frequencies are those where the response is biphasic (as opposed to an elastic response, as with the compliance dominated high frequencies) where fluid flow interactions play a major role (as indicated by peaks in mechanical phase angle at $\sim 0.002-0.008$ Hz [9]).

In contrast, for the case of hydrogen diffusion, $M$ decreases for more than that of the enzyme experiments, to only a few percent of its original value. The permeability $k_p$ appears to decrease to about half its original value, but this decrease is not nearly enough to prevent the significant drop in stiffness seen over the entire frequency range as shown in figures 2.3 and 2.4.

This finding may be an important consequence of the major difference between these two experiments. Namely, enzymatic extraction of chondroitin sulfate in cartilage plugs held at constant thickness results in a loss of mass from the tissue, specifically a loss of molecules known to
impede fluid flow. However, for our experimental conditions, only a fraction of chondroitin sulfate, apparently enough to reduce M by a small amount and to increase $k_p$. However, with the addition of HCl, little or no chondroitin sulfate is removed from the tissue, but rather the net charge of the tissue is reduced to almost zero. Hence the entire contribution of double layer repulsion and osmotic swelling to the equilibrium modulus M is eliminated, consistent with the large decrease in M calculated from the model. The fact that proteoglycan monomers and aggregates are presumably unchanged accept for the ionization state may explain why there is relatively less of an influence on $k_p$. The fact that $k_p$ is seen to decrease in this case cannot be explained on the basis of electrokinetic theory alone since charge neutralization would eliminate the electrokinetic backflow term [28] potentially resulting in a 10-50% increase in $k_p$ depending on offset strain.

The central question at this point vis-à-vis the enzyme degradation is what is actually happening inside the tissue? What are the implications of the observed changes in stiffness and streaming potential response?

One of the hardest questions to answer is whether the enzyme actually gets deep into the tissue. The enzyme does not produce as large a response as the addition of HCl, even though the enzyme might be expected to free almost as many charge groups as is neutralized at pH 3. One possibility is that the enzyme is getting into the tissue unevenly, perhaps
due to stearic hindrance of the porous platen, in which case the enzyme could be entering the tissue predominantly around the outer edge of the plug. This may appear as a more gradual change in material properties contained in the surface than is allowed for in the front model and could explain some of the discrepancies between theory and experiment mentioned. The results of histological studies now in progress will help to answer this question.

With regard to the theoretical response of figures 4.7 and 4.8, the biphasic front model does not fit the trends of the hydrogen and enzyme response of figures 2.4 and 2.5 in the high frequency as well as the low frequency regimes. In particular, the high frequency theoretical data is not as bunched together as seen in figure 2.5 and much of the predicted time course at low time (where the individual frequency responses cross one another) is not seen in the experimental data. Also, the characteristic plateaus in stiffness and inflections in potential predicted by the theory are not apparent in the experimental data. This is disappointing since observation of these events would pinpoint the location of the front with great accuracy. This disagreement could be a function of the width of the sampling window used in the experiments, which, due to the minimum length of time required to obtain the lower frequency data, causes one to miss important events in the higher frequency response. Nevertheless, the model appears to be a reasonable and physical explanation of events in the
lower frequency regimes.

Electromechanical response at higher frequencies is dominated by the interfacial compliance which is interpreted as acting to smooth out and lengthen response at higher frequencies. The surface compliance is difficult to model without making numerous assumptions. The effect of the compliance is to make the higher frequencies appear elastic (as in the low frequency limit) rather than biphasic (as observed in intermediate frequencies), lowering the mechanical phase angle below that predicted by the purely biphasic model [9].

5.3 Future Work

There are major topics suggested by this work for future projects. For answering some of the questions posed in chapter 1, it is important that dynamic testing of cartilage be continued with other selective enzymes such as hyaluronidase or dithiothreitol (dtt, which removes the proteoglycan monomer from the hyaluronic acid backbone). As mentioned in section 1.2, these other enzymes would break up proteoglycan aggregates into products of significant size, subaggregates and monomers, which may still be enmeshed within the tissue. The transient in behavior would be expected to be much different from that of chondroitinase digestion. Key information would be provided about interaction between the collagen matrix and proteoglycans.

The diffusion of reaction product (out of the tissue) has not been modeled here. Although this may be a straight
forward extension of diffusion reaction process presented in chapters 3 and 4, the implicit assumption was that the relatively small disaccharide product would diffuse much faster than chondroitinase enzyme. This would not be the case with another enzyme whose product was on the order of the size of monomers or subaggregates.

Micromodels have been formulated to relate the phenomenological coefficients \( k_{ij} \) of matrix equation 3.46 directly to microscopic structural parameters and molecular surface charge density [3]. It would be valuable to correlate results of these types of transient enzyme or chemical experiments with such models to confirm the functionalities of streaming potential and hydraulic permeability with fixed charge density.

5.3.1 Use of Streaming potential in vivo

Streaming potential is shown to be strongly coupled to mechanical behavior and as such streaming potential is valuable in determining electrokinetic response and following degradation processes involved in enzymatic digestion. One might speculate if use of streaming potential can be extended to in vivo measurement. Such measurement could be in form of an invasive device which could impose some mechanical excitation on cartilage and induce a streaming potential response. Such a device might be of practical use during certain surgical procedures by enabling clinicians to assess the state of the cartilage over time.

A device which builds on the biphasic electromechanics
discussed in chapter 3. A standing wave in applied pressure set up by dual or more stage chamber. As depicted in figure 5.1, for one half wave length, applied total fluid pressure is positive \((P-\Delta P) = +\frac{P}{2}\), while for the next half wave length the pressure would be negative \((P-\Delta P) = -\frac{P}{2}\). Fluid pressure is related to potential by the lower part of matrix equation 3.46, with \(J = 0\),

\[
\nabla \Phi = k_e \nabla (P-\Delta P) \quad (1)
\]

Integrating, the differential voltage would then be given by

\[
\Delta v = k_e p \quad (2)
\]

Thus determination of \(k_e\) involves finding the ratio of the resulting potential to the driving pressure.

5.3.2 Standing wave solution

Another geometry which could be used for in vivo measurements is a standing wave in displacement (figure 5.2). Although a more complicated geometry than the one above, it is direct extension of the model presented in chapter 3 and is presented for completeness.

A standing wave displacement imposed at \(z=0\) may be written in complex exponential form as

\[
u_z(0) = \text{Re} \{u_0 \cos k y \exp j\omega t\} \quad (3)
\]

Using a complex exponential identity, the standing wave may be expressed as the sum of positive and negative traveling waves,
Figure 5.1 Pressure excitation geometry

Figure 5.2 Standing wave displacement geometry
\[ u_z(0) = \text{Re} \left\{ \frac{u_0}{2} \left( \exp j(\omega t - ky) + \exp j(\omega t + ky) \right) \right\} \] (4)

Assuming a linear response, the displacement profile is a superposition of positive and negative traveling wave solutions. A solution to a traveling wave was given in chapter 3, and yields the standing wave solution

\[ u_z(z) = \text{Re} \left\{ \frac{u_0}{2} \left( \frac{\sinh \gamma_+(\delta+z)}{\sinh \gamma_+} e^{-jky} + \frac{\sinh \gamma_-^{(\delta+z)}}{\sinh \gamma_-} e^{jky} \right) e^{j\omega t} \right\} \\
= \text{Re} \left\{ u_0 \frac{\sinh \gamma^{(\delta+z)}}{\sinh \gamma^{\delta}} \cos ky e^{j\omega t} \right\} \] (5)

where \[ \gamma_+ = \gamma_- = \gamma; \quad \gamma^2 = \frac{G}{M} k^2 + \frac{i\omega}{D} \] (6)

as given in chapter 3. The stress is then given by

\[ T_{zz}(0) = \text{Re} \left\{ M \gamma u_0 \coth \gamma^{\delta} \cos ky e^{-j\omega t} \right\} \] (7)

and the potential is given by

\[ v = \frac{j\omega}{\gamma} k e \frac{\cosh \gamma^{\delta}-1}{k \sinh \gamma^{\delta}} u_0 \cos ky \] (8)

The form of the stress and potential response is identical to that given in chapter 3. Thus in vivo streaming potential would be subject to same analysis as that given in chapters 3 and 4.
References


11. Maroudas, A., Muir, H., and Wingham, J., "The


Appendix A

Experimental Response of Individual Samples

Normalized stiffness and streaming potential for the 8 samples treated with chondroitinase enzyme are shown in the following figures.
Figure A.1a Chondroitinase diffusion experimental data: Sample #20: normalized stiffness
Addition of chondroitinase

Normalized Potential

Experiment Time (hours)

File: ehf020.dyn     Date: 18 Oct 82     Time: 9:34 pm

Figure A.1b Chondroitinase diffusion experimental data:
Sample #20: normalized streaming potential
Addition of chondroitinase

Experiment Time (hours)

Normalized Stiffness

File: ehf027.dyn      Date: 14 Nov 82   Time: 3:55 pm

Figure A.2a Chondroitinase diffusion experimental data:
Sample #27: normalized stiffness
File: ehf027.dyn       Date: 14 Nov 82       Time: 3:55 pm

Figure A.2b Chondroitinase diffusion experimental data:
Sample #27: normalized streaming potential
Addition of chondroitinase

Experiment Time (hours)

File: ehf031.dyn  Date: 17 Dec 82  Time: 1:33 pm

Figure A.3a Chondroitinase diffusion experimental data:
Sample #31: normalized stiffness
Addition of chondroitinase

Normalized Potential

Experiment Time (hours)

File: ehf031.dyn    Date: 17 Dec 82    Time: 1:33 pm

Figure A.3b Chondroitinase diffusion experimental data:
Sample #31: normalized streaming potential
Figure A.5a Chondroitinase diffusion experimental data: Sample #34: normalized stiffness
Figure A.5b Chondroitinase diffusion experimental data:
Sample #34: normalized streaming potential

File: ehf034.dyn      Date: 22 Dec 82      Time: 1:13 pm
Figure A.6b Chondroitinase diffusion experimental data:
Sample #35: normalized streaming potential
Figure A.7a Chondroitinase diffusion experimental data:

Sample #36: normalized stiffness

Date: 30 Dec 82
Time: 1:07 pm
Experiment Time (hours):

File: ehf036.dyn

Addition of chondroitinase
Figure A.7b Chondroitinase diffusion experimental data:
Sample #36: normalized streaming potential

File: ehf036.dyn  Date: 30 Dec 82  Time: 1:07 pm
Figure A.8b Chondroitinase diffusion experimental data:
Sample #37: normalized streaming potential
Appendix B
Smoothed Experimental Response

Normalized stiffness and streaming potential for the 8 samples treated with chondroitinase enzyme shown in Appendix A were smoothed using the smoothing algorithm described by Eisenberg [1983] and the results are shown in the following figures. It is the smoothed data of figures B.3-8 which were averaged to produce figures 2.5a,b.
Figure B.1a Smoothed plots of chondroitinase experiments:
Sample #20: normalized stiffness
Figure B.2a Smoothed plots of chondroitinase experiments:
Sample #27: normalized stiffness
Figure B.2b Smoothed plots of chondroitinase experiments:
Sample #27: normalized streaming potential
Figure B.3a Smoothed plots of chondroitinase experiments:
Sample #31: normalized stiffness
Figure B.4a Smoothed plots of chondroitinase experiments:

File: ehf033.dyn
Date: 20 Dec 82
Time: 5:16 pm
Sample #33: normalized stiffness

Addition of chondroitinase

Experiment Time (hours)

1.000
0.9000
0.8000
0.7000
0.6000
0.5000
0.4000
0.3000
0.2000
0.1000
0.0000

1 Hz
.1 Hz
.01 Hz
.005 Hz
Figure B.4b Smoothed plots of chondroitinase experiments:
Sample #33: normalized streaming potential
Figure B.5a Smoothed plots of chondroitinase experiments:
Sample #34: normalized stiffness
Figure B.5b Smoothed plots of chondroitinase experiments:
Sample #34: normalized streaming potential
Figure B.6a Smoothed plots of chondroitinase experiments:
Sample #35: normalized stiffness
Figure B.6b Smoothed plots of chondroitinase experiments:
Sample #35: normalized streaming potential
Figure B.7a Smoothed plots of chondroitinase experiments:
Sample #36: normalized stiffness
Addition of chondroitinase

File: ehf036.dyn    Date: 30 Dec 82    Time: 1:07 pm

Figure B.7b Smoothed plots of chondroitinase experiments:
Sample #36: normalized streaming potential
Figure B.8a Smoothed plots of chondroitinase experiments:
Sample #37: normalized stiffness
Figure B.8b Smoothed plots of chondroitinase experiments:
Sample #37: normalized streaming potential
Appendix C

Experimental Response of Control Samples

Normalized stiffness and streaming potential for the 4 samples presented as controls to the chondroitinase experiments are shown in the following figures. Smoothed data plots (figures C.1-4) were averaged to produce figures 2.6a,b.
Figure C.1b Smoothed plots of control experiments:
Sample #24: normalized streaming potential
Figure C.2a Smoothed plots of control experiments:
Sample #29: normalized stiffness

File: ehf029.dyn      Date: 25 Nov 82      Time: 5:16 pm
Figure C.3a Smoothed plots of control experiments:
Sample #30: normalized stiffness
Figure C.3b Smoothed plots of control experiments:
Sample #30: normalized streaming potential
Figure C.4b Smoothed plots of control experiments:
Sample #32: normalized streaming potential

File: ehf032.dyn     Date: 17 Dec 82     Time: 26:17 pm