Daniel Marous

Mathematical Model of Cartilage Regeneration via Hydrogel
Honors Thesis, Wittenberg University Department of Mathematics

Abstract

Because of the large number of individuals with cartilage problems, whether due to sports injuries or diseases such as arthritis, there is a medical need for effective cartilage regeneration. To assist with the development of a procedure for regeneration, a mathematical model is desirable. A mathematical model is useful because it can allow regeneration times to be calculated for various initial conditions, without having to biologically test those certain conditions in lab. In this project, we present and discuss a mathematical model for cartilage regeneration via hydrogel, a biocompatible scaffolding material. The novel model presented in this work was designed to be more biological in nature than previous models.

Cartilage is a connective tissue that contains special cells (chondrocytes) and is found in joints as well as other places. In a healthy environment, chondrocytes are able to repair minor damage, but more extensive damage can render the cartilage irreparable by natural means. Consequently, researchers are seeking techniques to encourage cartilage regeneration. One possibility for cartilage repair involves injecting the damaged site with hydrogel, a gelatinous substance containing chondrocytes. Our model uses a system of ordinary differential equations (ODE) that illustrates the build-up of extracellular matrix (ECM), the structural material of cartilage, using hydrogel to stimulate repair.

Introduction

The focus of this work is on articular cartilage, which is found only in joints (hips, knees, etc), preventing bone on bone contact (Erggelet *et al.*, 2008). Excluding water, cartilage is comprised of two main elements: specialized cells called chondrocytes and an extracellular matrix (ECM) composed of a framework of proteins and proteoglycans (Lodish *et al.*, 2008, Erggelet *et al.*, 2008). The majority of the tissue is not comprised of

cells; the chondrocytes comprise only 1% of the cartilage volume (Erggelet *et al.*, 2008).

Figure 1 highlights cartilage tissue, scattered with chondrocytes. Note that the

chondrocytes are not in contact with each other, but are dispersed throughout the ECM.

The chondrocytes produce the ECM, which forms most of the non-water volume in

cartilage. (Lodish *et al.*, 2008)

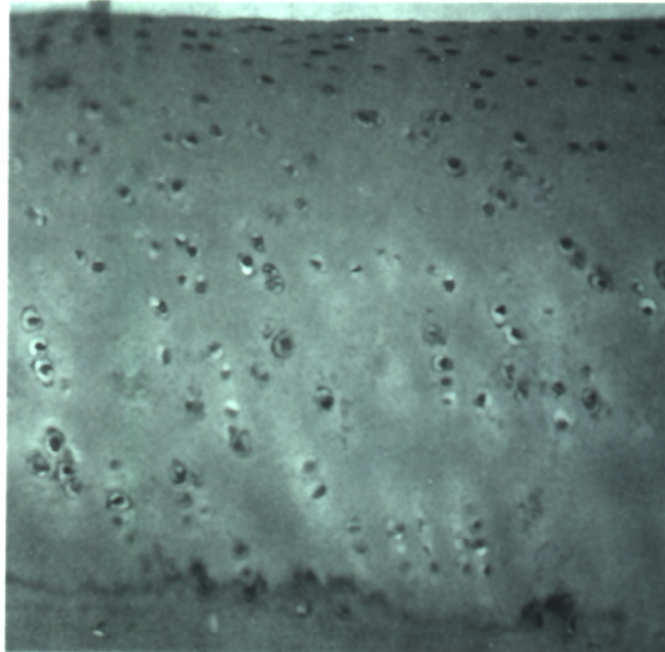Figure 1: Cartilage Tissue

Figure 1 shows cartilage tissue. Note how the chondrocytes (dark spots) are
dispersed throughout the ECM. These cells are responsible for building and maintaining
the ECM. (NCSU REU)

The ECM itself is made of two components, collagen and proteoglycans.

Approximately 60% of the dry weight of cartilage is made of collagen (Erggelet *et al.*,

2008). Collagen forms long, rope-like structures made of amino acid chains (Lodish *et

al.*, 2008). It is these "ropes" that give cartilage its ability to stretch and tolerate sheer

forces. (Erggelet *et al.*, 2008) The collagen molecules themselves are synthesized on

ribosomes attached to the endoplasmic reticulum (ER) (in the chondrocytes) from

individual amino acids. Further processing occurs in the ER and Golgi apparatus, and

collagen strands (called procollagen at this point) are secreted from the cell

(chondrocyte). Once outside the cell, the strands cross-link, forming the collagen

network. (Lodish *et al.*, 2008) Collagen in the ECM is pictured in Figure 2.

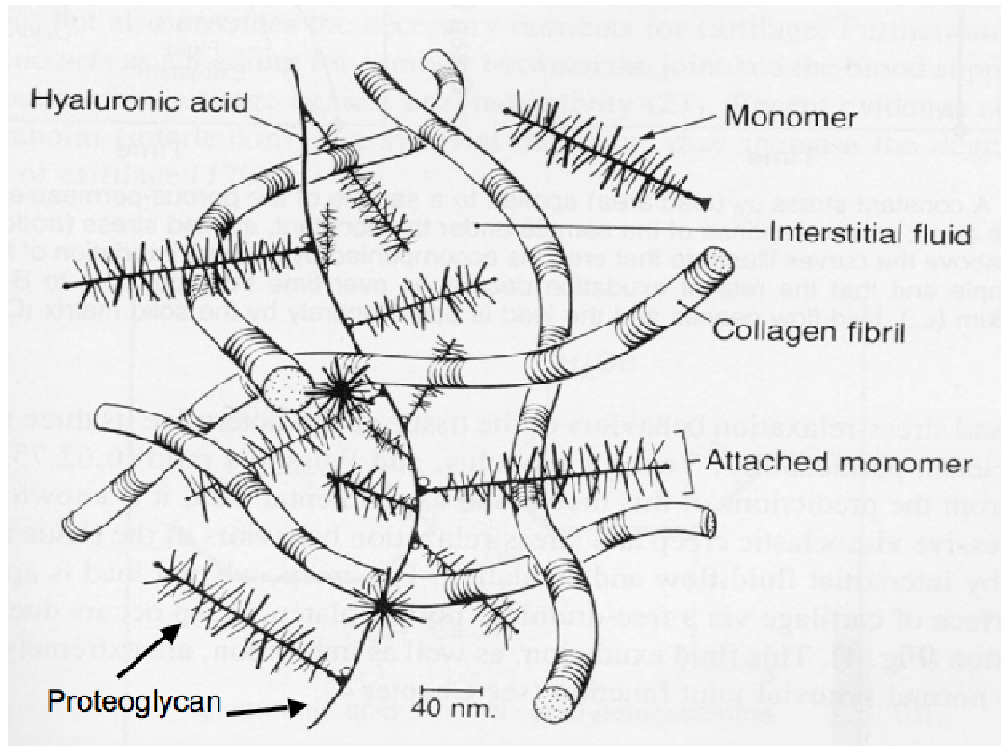Figure 2: Collagen and Proteoglycans in ECM

Figure 2 shows the collagen and proteoglycan strands that comprise ECM. The collagen is responsible for the stretching strength of the cartilage and is pictured as the thicker fibrils. The proteoglycans, which maintain water levels in the cartilage, are the thinner fibrils. (NCSU REU)

The second main component of ECM, the proteoglycans, consist of multiple

subunits. The backbone of the proteoglycan is a polysaccharide called hyaluronan.

Figure 3 highlights the chemical structure (one unit) of hyaluronan.
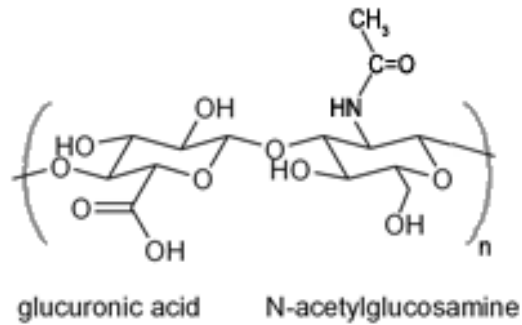
Figure 3: Hyaluronan Structure

Figure 3 shows the chemical structure of one unit of hyaluronan.
Hyaluronan forms the sugar backbone of the proteoglycan molecules.
(http://www.glycosan.com/what_hyaluronan.html)

Hyaluronan is made by an enzyme (HA synthase) in the chondrocyte cell membrane and is immediately transported out of the cell. Next, the hyaluronan is attached (via a linker protein) to aggrecan. Aggregan consists of a core protein (synthesized on the ER) attached to chondroitin sulfate and keratin sulfate sugar +chains (that are added in the Golgi). Aggrecan is then secreted. Thus, the final proteoglycan structure resembles a "centipede" consisting of a hyaluronan molecule ("body") attached to multiple core proteins ("legs"), each of which have multiple sugar groups ("feet"). (Lodish *et al*., 2008) Figure 4 illustrates this structure.
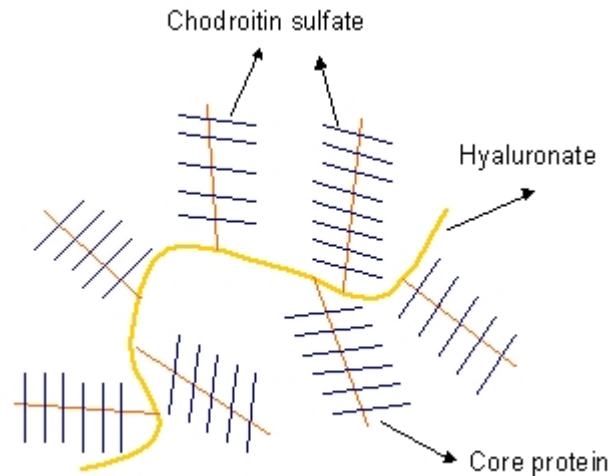
Figure 4: Proteoglycan Structure

Figure 4 shows how proteoglycans are composed of hyaluronan, core protein, and sugar-sulfate groups (chondroitin sulfate and keratin sulfate). The negative charges on these molecules help to draw water to the proteoglycans. (tonga.usip.edu/gmoyna/biochem341/lecture35.html)

Overall, proteoglycan molecules have a number of negative charges on them, attracting water (Lodish *et al.*, 2008, "Articular Cartilage", 2008). As a result, eighty-percent of the cartilage itself is water. The ability to draw water into the cartilage is important for at least two reasons. First, because it is filled with water, cartilage is able withstand compression forces (Erggelet *et al.*, 2008, "Articular Cartilage", 2008). The influx of water is also vital for the diffusion of nutrients to chondrocytes, since there are no blood vessels in cartilage (Erggelet *et al.*, 2008). In the cartilage, the proteoglycans are trapped between the collagen fibers ("Articular Cartilage", 2008). (Figure 2)

Biological Reactions

The biological processes involved with cartilage production can be outlined in six processes. Nutrients (energy and nutrients for synthesis) diffuse into a cell and four general products are being made: collagen, hyaluronan, core protein, and sulfate sugars (keratin and chondroitin sulfate). The core protein combines with the sulfate sugars to

produce the aggrecan complex. Aggrecan then reacts with hyaluronan to form the

complete proteoglycan unit. Figure 5 highlights these biological processes.

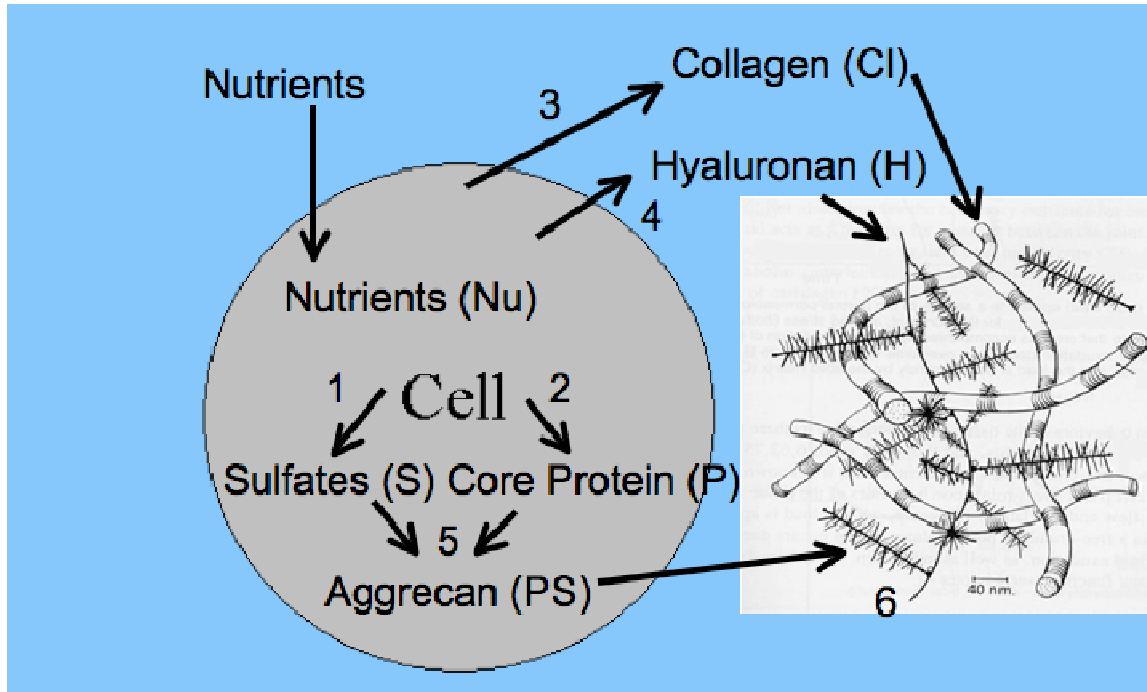Figure 5: Chondrocyte Production of ECM

Figure 5 shows the various compounds produced by a chondrocyte. First, nutrients diffuse into the cell. The cell makes four compounds with the nutrients: sugar sulfates, the core protein, collagen, and hyaluronan (processes 1, 2, 3, and 4). The sugar sulfates and core protein combine to form aggrecan (process 5). Finally, hyaluronan and aggrecan combine to form the completed proteoglycan (process 6).

Challenges in Cartilage Repair:

When cartilage is damaged (whether due to injury or disease), there are several

obstacles to repair. First, there is not a high density of chondrocytes in cartilage; most of

the cartilage is ECM (See Figure 1). Any damage that destroys chondrocytes means

there are even fewer of them to maintain the cartilage. This is compounded by the fact

the chondrocytes loose their ability to mitotically divide (so few new chondrocytes can be

made) and chondrocytes already have a limited natural ability to repair defects. Finally,

cartilage lacks blood vessels, so nutrients must diffuse to the cells. During the initial

formation of cartilage, blood is available to the chondrocytes. In damaged mature

cartilage, however, blood (with the corresponding nutrients, etc) is not as available to assist with repair. (Erggelet *et al.*, 2008) As a result of cartilage's limited natural ability for healing, small injuries (i.e. a bump on the knee) can heal, while larger damage (i.e. sports injury, arthritis) cannot.

Repair via Hydrogel

Because of the limited ability of cartilage to repair itself, medical techniques are being developed to facilitate this healing process. One such technique involves surgically opening the injury site, injecting hydrogel to fill the cavity, and then stitching cartilage/tissue/etc back together (Erggelet *et al.*, 2008). Specifically, hydrogel consists of hyaluronan seeded with chondrocytes (from an external source). Hyaluronan is the backbone of proteoglycans and is also able to bind water, thus explaining the gel consistency of the hydrogel. The main idea is that the chondrocytes, determining (through signaling) that they are not in cartilage, will begin to produce ECM. The hydrogel serves as a biocompatible scaffold, keeping the chondrocytes spaced throughout the injury site and providing some initial structure from which cartilage can be built. The cartilage is better able to heal with this scaffold in place then having to fill a void in the tissue. (NCSU REU, Erggelet *et al.*, 2008) Figure 6 illustrates this repair process.
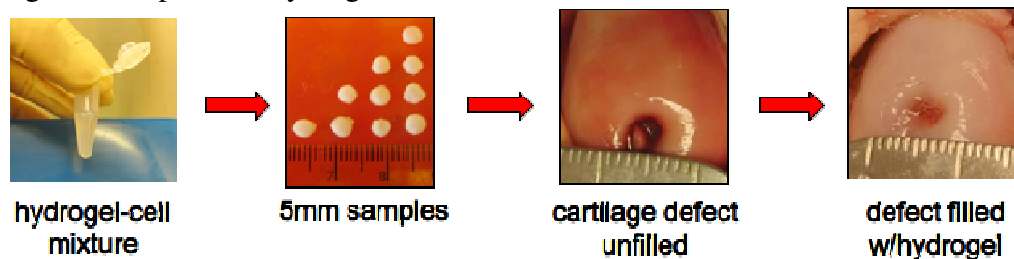
Figure 6: Repair via Hydrogel

Figure 6 shows how a cartilage hole is filled with hydrogel. The chondrocyte/haluronan mixture is made and mixture samples are inserted in an injured site in cartilage. (NCSU REU)

Formation of a Model

As a medical procedure, cartilage regeneration via hydrogel has the potential to help many individuals with damaged cartilage. The focus of this project is to develop a mathematical model for repair via hydrogel. Initially, researchers could collect data (amounts/concentrations, etc) on relevant variables (nutrients, hyaluronan, etc.). The coefficients of the model could then be fit to data obtained in lab. Eventually, a model with fitted coefficients could be used to predict regeneration times for cartilage based on the initial amounts (of various substances) injected into the defect. This would allow for many different starting conditions to be tested, allowing the most effective combinations of substances to be predicted (which then could be confirmed in lab). An accurate mathematical model could greatly expedite the perfection of cartilage regeneration via hydrogel as a medical procedure.

Initial Model

Under the direction of Dr. Mansoor Haider at North Carolina State University (during an REU), an ordinary differential equation model for cartilage regeneration via hydrogel was created. In this model, a distinction is made between "linked" and "unlinked" ECM. These variables do not correspond to particular biological components, just assembled and unassembled cartilage in general. This model, with slight modification, is presented below:

$$1 \quad \frac{dMu}{dt} = a_1 * (Nu - 1) - a_2 * H * Mu$$

$$2 \quad \frac{dMl}{dt} = a_3 * H * Mu$$

$$3 \quad \frac{dNu}{dt} = a_4 * (1 - Ml) - a_5 * Mu$$

$$4 \quad \frac{dH}{dt} = -a_7 * H * Mu$$

The underlying assumption is that the model represents one cell (chondrocyte),

which has an "assigned" volume of cartilage to repair. The injury is considered to be

healed when the cell fills its assigned volume ($Ml = 1$) (Presumably, all of the other cells

in the injury site have filled their volumes as well). This model has four variables

corresponding to mature (linked) ECM (Ml) in the assigned volume, monomeric

(unlinked) EMC (Mu), hydrogel (H), and nutrients inside one average cell (Nu).

Variables are normalized to certain reference amounts. (i.e. the nutrients are normalized

to the minimal survival level, so the baseline nutrient amount is 1; anything above 1 is

used to repair the cartilage. The linked matrix, on the other hand is normalized to the

ideal ECM concentration and so 1 represents the target healthy level.) By convention, the

nutrients are started at 1, the level where no repair is occurring. In healthy cartilage, $Ml =$

1 and $H = Mu = 0$. In damaged cartilage, however, $Ml < 1$ and if hydrogel is injected into

the defect, then $H > 0$. In the model above, the initial kick for the regeneration comes

from the nutrient equation (Equation 3); if $Ml < 1$, then the Nu concentration increases

(the cell sensing it is not in healthy cartilage allows more nutrients into the cell). The

nutrient concentration is lowered as Mu is produced. Mu concentration increases when

Nu is above 1 (as seen from the first term in Equation 1). Next, there is a chemical

reaction between the unlinked matrix, Mu, and the hydrogel, H, thus forming linked

matrix, Ml. This reaction term appears as the last term in three of the equations

(Equations 2, 3, 4). These equations were numerically solved using Mathematica (see

appendix for notebook) for time 0 to 200. The initial conditions were: $H = 0.95$, $Mu =$

Ml = 0, Nu = 1. No data was available to fit the coefficients so they were selected to be:

$a_1 = a_6 = 1$, $a_2 = a_3 = a_5 = 5$, $a_4 = .5$, and $a_7 = 4.75$. A plot of the solution of the DE system

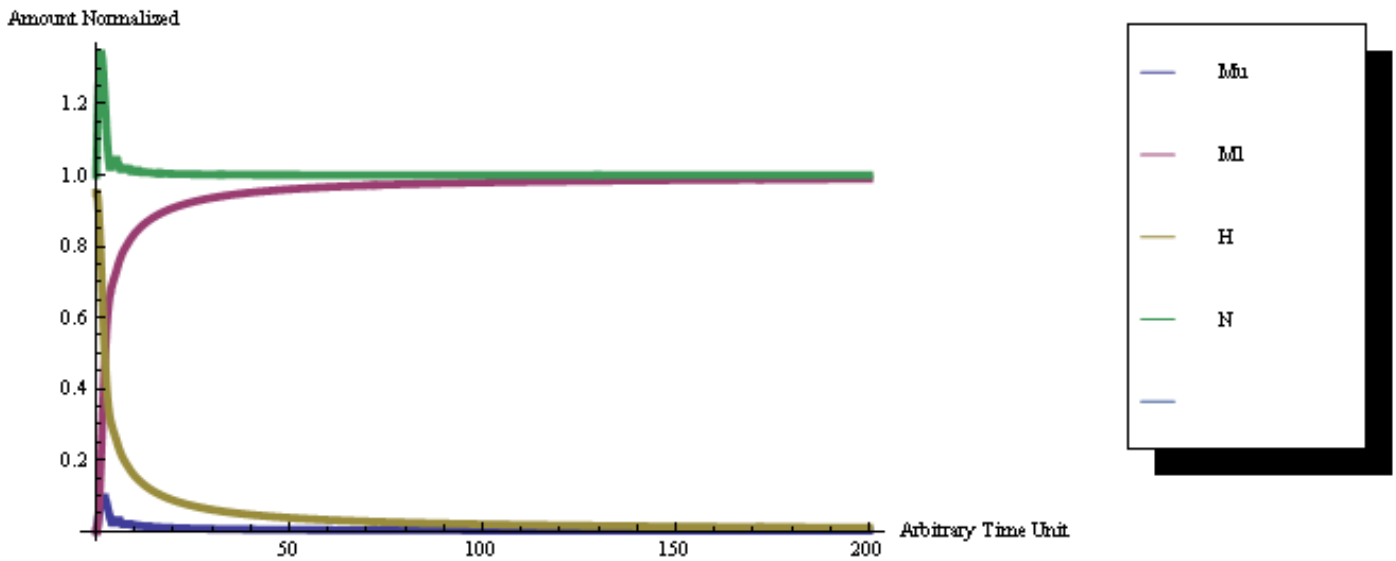appears in Figure 7.

Figure 7: Sample Solution to Initial Model

Figure 7 shows a solution (for Nu, H, Mu, and Ml) under the initial model. The nutrients and unlinked matrix (Mu) peak and level-off, while the linked matrix (Ml) increases to 1 and the hydrogel decreases to 0.

Overall, the DE solution in Figure 7 makes biological sense. The nutrients (green) peak above their base level (one) as they enter cell and then the concentration returns to one as they are used. The hydrogel (yellow) concentration initially starts high and then decreases to zero. Mu (blue) peaks briefly before it is converted to Ml. The Ml concentration begins around zero and increases to one (100%), indicating the regeneration of cartilage.

Problems with Initial Model:

The above model, however, is perhaps too simplistic. One of the main issues is that all of variables do not correspond to specific biological components. The ECM is made of collagen and proteoglycans and these are separate compounds. The Ml and Mu variables group these distinct elements together. Additionally, hydrogel is not really a specific biological compound, although the hyaluronan in the hydrogel is. Perhaps hyaluronan concentration would be a more biologically accurate variable. Overall, the model does not really simulate the actual biology that is occurring. (Though it is a good first approximation since the cartilage, represented by Ml, does heal if hydrogel is injected.)

The New Model: What should it look like?

In order to make the model more biologically relevant, a new system of equations is needed. The new model should have variables corresponding to biological compounds involved with cartilage repair (as described in the Introduction) including: nutrients (Nu) inside the cell (including sugars, amino acids, etc), collagen (Cl), proteoglycans (X), hyaluronan (H), core protein (P), sulfated sugar chains (S), and the complex formed between the core protein and the sulfated sugar chains (PS) (called aggrecan). Each of these compounds plays a unique role in cartilage repair. It is helpful to separate the model into these variables so the effects of altering coefficients and initial conditions for each variable can be assessed. Note that H now represents hyaluronan, not hydrogel as it did in the last model. Hyaluronan is an actual biological molecule and is coincidently the main component in hydrogel. There are other compounds involved with the regeneration process, but these are assumed to be the main ones. Biologically, there are assumed to be three main reactions involving the variables as described under *Biological Reactions*. These reactions are summarized in variables as:

$$Nu \rightarrow Cl, H, S, P$$

$$P + S \rightarrow PS$$

$$PS + H \rightarrow X$$
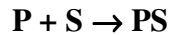
Ideally, the new model would also have some method for modeling healthy cartilage, inflicting damage at time t, and showing how the cartilage responds after the damage. Finally, the model should have a "structural" threshold. The cartilage needs some minimal amount of structure in order to be able to heal (this structure could be fulfilled by the amounts of proteoglycans, collagen, hyaluronan, or some combination thereof). Above this threshold (small injury or larger injury with hydrogel injection), the cartilage should heal, but below the threshold (large injury without hydrogel injection), the cartilage should not heal.

The New Model: System of Equations

Using the above specifications, the following model was created:

1. $\dfrac{dNu}{dt} = a_1 * (1 - X) * thres(H + X + Cl) + a_2 * (1 - Cl) * thres(H + X + Cl) - a_3 * Nu$

2. $\dfrac{dCl}{dt} = a_4 * Nu * (1 - Cl)$

3. $\dfrac{dH}{dt} = a_5 * Nu * (1 - X) * (1 - H) - a_6 * PS * H$

4. $\dfrac{dP}{dt} = a_7 * Nu * (1 - X) * (1 - P) - a_8 * P * S$

5. $\dfrac{dS}{dt} = a_9 * Nu * (1 - X) * (1 - S) - a_{10} * P * S$

6. $\dfrac{dPS}{dt} = a_{11} * P * S * (1 - PS) - a_{12} * PS * H$

7. $\dfrac{dX}{dt} = a_{13} * PS * H$

Note in the above model that there is an equation for every variable that was defined under *The New Model: What should it look like?*. Unlike in the initial model, the baseline nutrient level (for survival, but not cartilage production) is normalized to zero (as opposed to one). The initial "kick" for the system comes when either the proteoglycans or collagen is not at its ideal level (1 or 100%). However, if the total structure (hyaluronan + collagen + proteoglycans) of the system is not high enough, the *thres* function returns a zero (instead of a one) and the nutrients never increase and the cartilage is not healed. For reference, the *thres* function is given below:

$$thres\ (x) = \begin{cases} 0, \text{if } x < 0.8 \\ 1, \text{otherwise} \end{cases}$$

The rate of change of the nutrient concentration decreases as a function of the nutrient concentration itself-the more nutrients that are present, the more the cell uses them to form various compounds. Biologically, the nutrients (Nu) directly create four compounds: hyaluronan (H), core protein (P), sulfated sugars (S), and the collagen, thus a nutrient term appears in the equation for each of these variables. The other two main reactions ($P + S \rightarrow PS$ and $PS + H \rightarrow X$) appear at appropriate locations in the system, depending on which variables they impact. (For instance, the proteoglycan equation has a PS * H term since proteoglycans come from a reaction between aggrecan and hyaluronan). Additionally, the model contains terms such as (1-X). These represent feedback mechanisms whereby an accumulation of a product (direct or eventual) inhibits the creation of more product. For instance, if the aggrecan complex (PS) builds-up, this would inhibit the production of more aggrecan (hence the 1-PS term in the PS equation).

Equation 1 (nutrients) increases when there is a lack of collagen or proteoglycans (either is below one); however, the *thres* function checks to ensure enough "structure" is present, otherwise, the nutrients will not increase. The nutrients are removed by a –Nu

term, the logic of which is described above. Equation 2 (collagen) increases with an

increase in nutrients, but is inhibited as collagen is produced (1-Cl term). Equation 3

(hyaluronan) increases with an increase in nutrients, but is inhibited by the formation of

proteoglycans and hyaluronan. A reaction term between aggrecan (PS) and hyaluronan

(H) removes hyaluronan from the system. Equation 4 (core protein) increases with

nutrients, is inhibited by core protein and proteoglycan formation, and decreases by a

reaction term between the core protein and sugar sulfates. Equation 5 follows a similar

logic to Equation 4, except with sugar sulfates, instead of the core protein. Equation 6

(aggrecan) increases with a reaction term between the core protein and the sugar sulfates,

is inhibited by the formation of aggrecan, and decreases by a reaction term between

aggrecan and hyaluronan. Finally, equation 7 (proteoglycans) increases with a reaction

term between aggrecan and hyaluronan.

This new system was numerically solved on Mathematica (see appendix for

notebooks or contact the author for executable files) for various time intervals. Since

data is not available to fit the coefficients, coefficients were found that yielded physically

reasonable results. These coefficients are: a_1, $a_2 = 0.1$, a_3, a_4, a_6, a_{12}, $a_{13} = 2$, and a_5, a_7, a_8,

a_9, a_{10}, $a_{11} = 1$ (any graphical solutions that follow were run with these coefficients).

First, healthy cartilage was modeled (X and Cl = 1, everything else = 0), with an

inflicted damage at time = 1000. The threshold for cartilage recovery was set to 0.8. A

0.1 damage was inflicted on the proteoglycans and collagen. Since the structure of the

system was still 1.8 (0.9 + 0.9), the cartilage was able to heal (X and Cl went back up to

1). This represents recovering cartilage after a small injury. Next, a damage of 0.7 was

inflicted on the proteoglycans and collagen. This put the total structure of the system

below the threshold and the cartilage was not able to heal (X and Cl stayed at 0.3).   This

represents a significant injury to the cartilage. Graphical solutions of these two runs

appear in Figure 8.
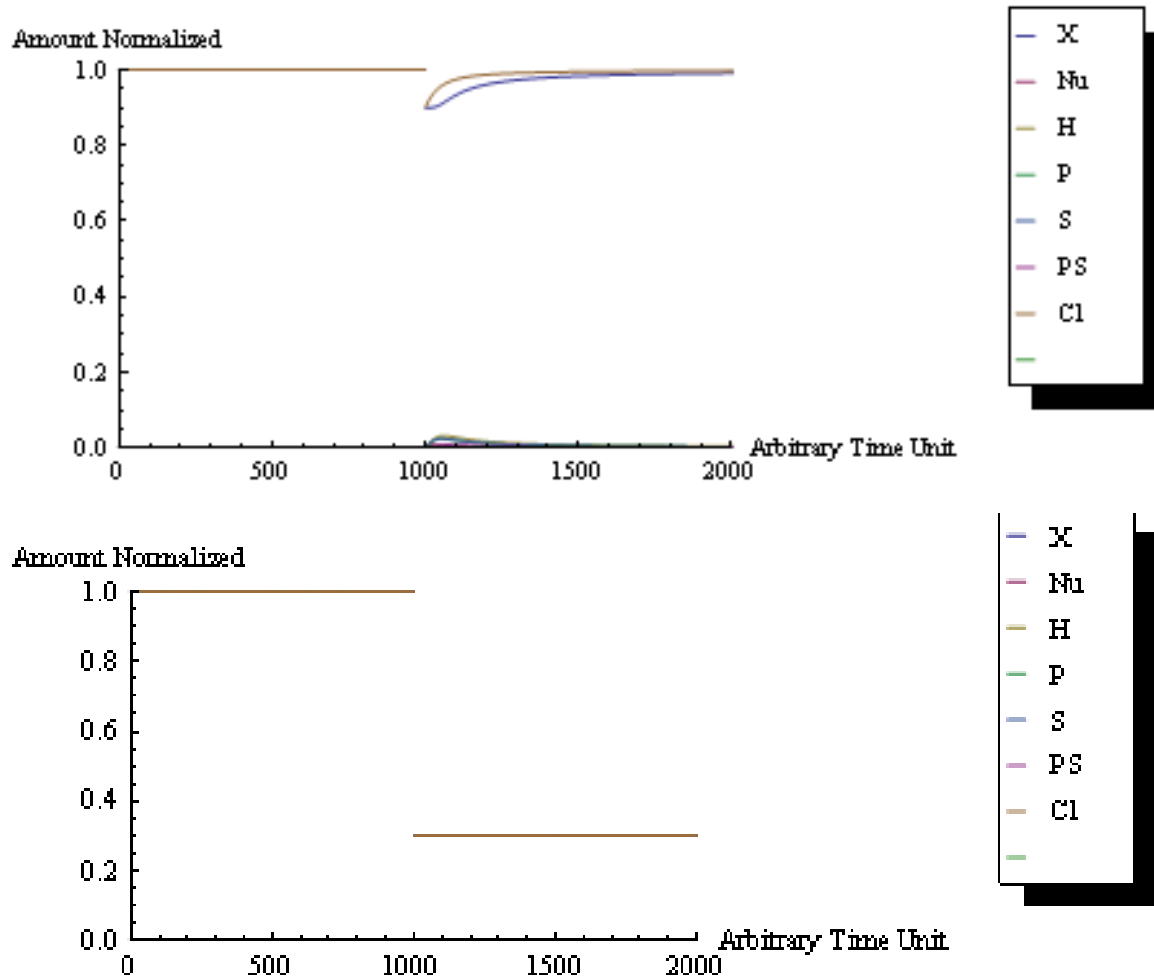
Figure 8: Damage Inflicted to Healthy Cartilage

Figure 8 shows two solutions of the equations where damage (0.1 and 0.7, respectively) was inflicted on the cartilage. The injury occurs at time =1000 and can be seen by the "jump" down in the collagen and proteoglycan levels in both graphs. The cartilage was able to recover in the first instance, as can be seen by the increase in proteoglycan and collagen levels. The proteoglycans seem to lag behind the collagen. This could be the result of the particular coefficients that were used or the fact that more intermediates are required for the production of the proteoglycans in the model. Note in the top graph that the nutrients and other intermediates "kick-on" in response to the damage (seen by the small "bumps" at the bottom of the graph). In the second graph, the cartilage was not able to recover (as can be seen by the flat line at .3 for the collagen and proteoglycans).

Next, using the same coefficients and threshold for recovery, a series of solutions were determined beginning with damaged cartilage (X and Cl below 1) with various amounts of injected hyaluronan (in the form of hydrogel). Figure 9 shows what happens when full damaged cartilage (Cl = X = 0) is injected with 0.4 and 0.8 hyaluronan (no recovery and full recovery, respectively). The third plot is a close-up of time 1-10 of the second plot; note the how the various intermediates are "turned-on" to repair the damage.

Figure 9: Fully Damaged Cartilage with Hydrogel Injections

(0.4 Hyaluronan)
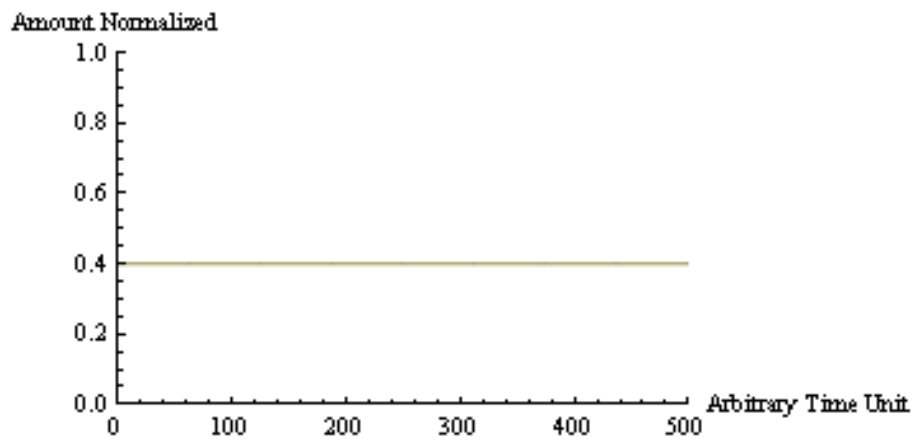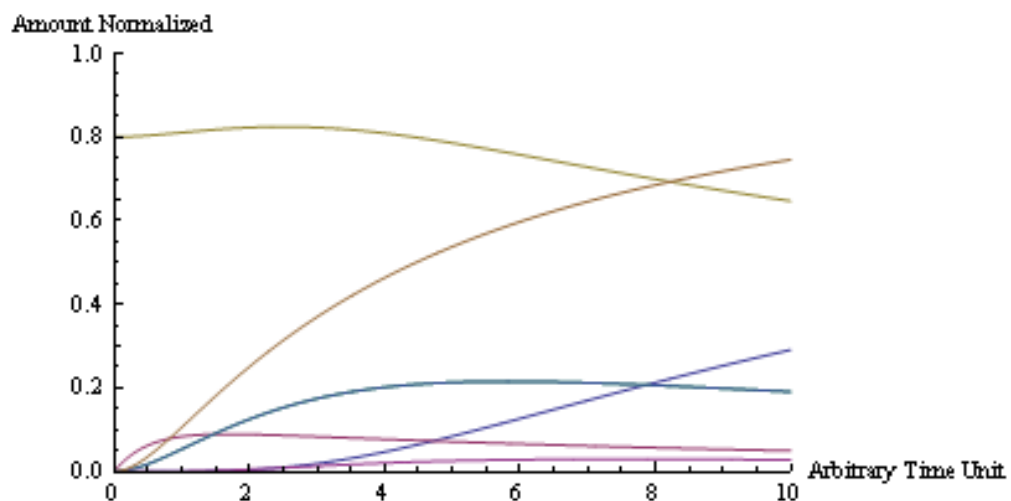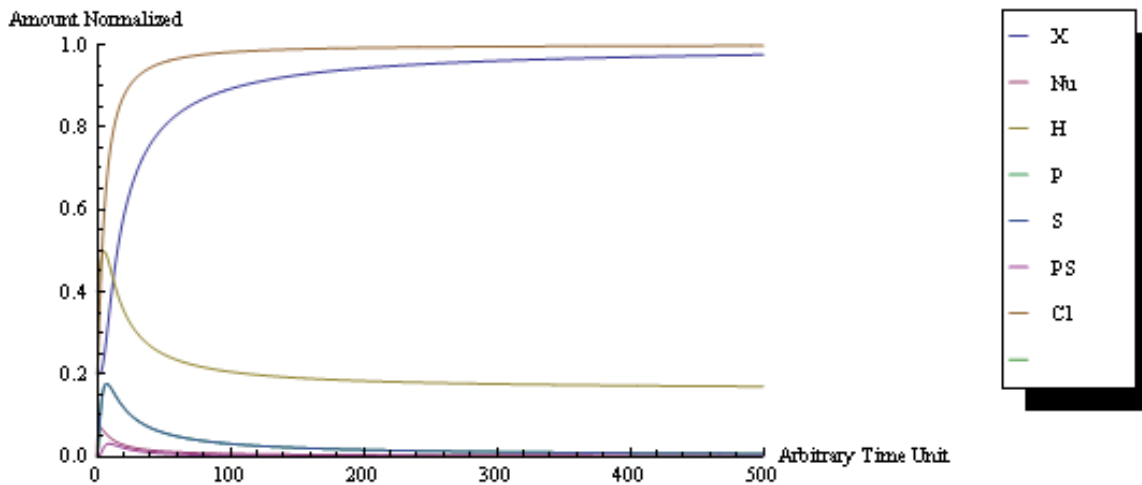

(0.8 Hyaluronan)
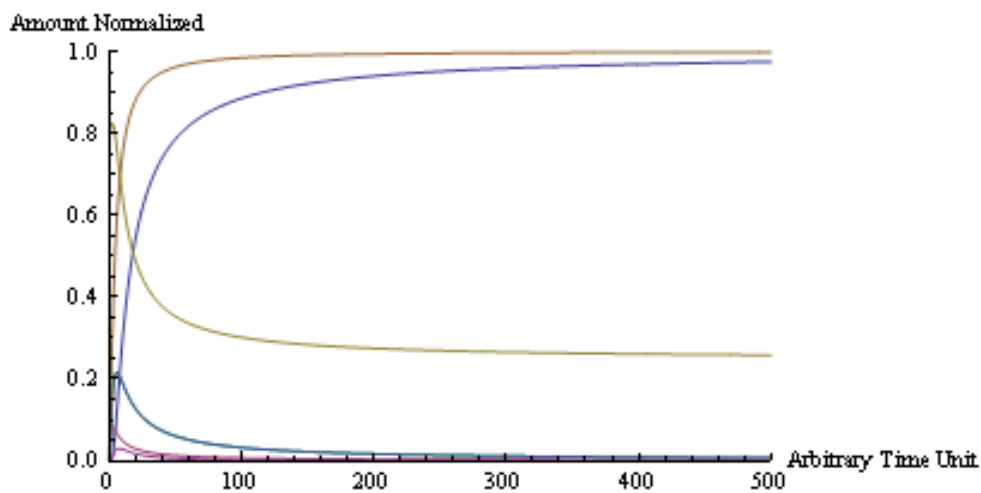
(0.8 Hyaluronan, time 1-10)

Figure 9 shows how damaged cartilage responds with hydrogel injections. In the first plot, 0.4 hyaluronan is injected, which is not enough to heal the cartilage in this instance. Thus, the hyaluronan (H) level stays at 0.4 and the collagen (Cl) and proteoglycan (X) levels stay at 0. In the second plot, 0.8 hyaluronan is injected. This is enough to heal the cartilage as can be seen by the increase of the collagen and proteoglycans towards 1. Various intermediates are also produced and used as can be seen in the second and third plots as the substances that peak and then come back down to zero.

Next, the proteoglycan and collagen levels in the damage cartilage were started above 0 (not completely damaged). Different amounts of hydrogel (hyaluronan) were injected and depending on the total "structure" that was present, the cartilage either was regenerated or stayed damaged. Figure 10 shows some graphical solutions to these runs. In the first plot (in Figure 10), the cartilage is able to recover despite the hyaluronan level only being at 0.4 (where it was not able to recover in Figure 9) because there is some collagen and proteoglycans present. The second plot has low levels of collagen and hyaluronan (0.2 and 0.3, respectively), but is able to recover because of the high level of proteoglycans (0.7). No regeneration is seen in the third plot because the "structure" of the system is not above the threshold level.

Figure 10: Partially Damaged Cartilage with Hydrogel Injections

(H = 0.4, Cl = X = 0.2)

(H = 0.3, Cl = 0.2, X = 0.7)
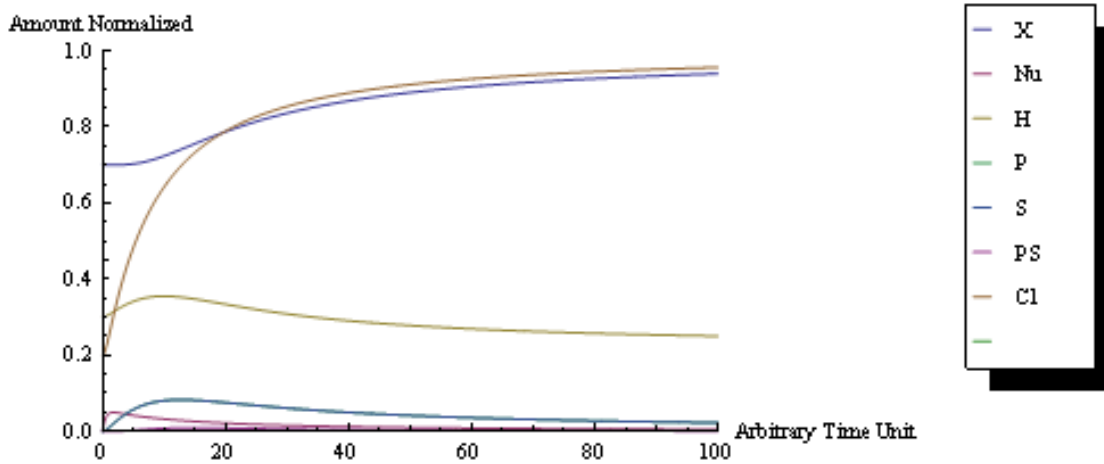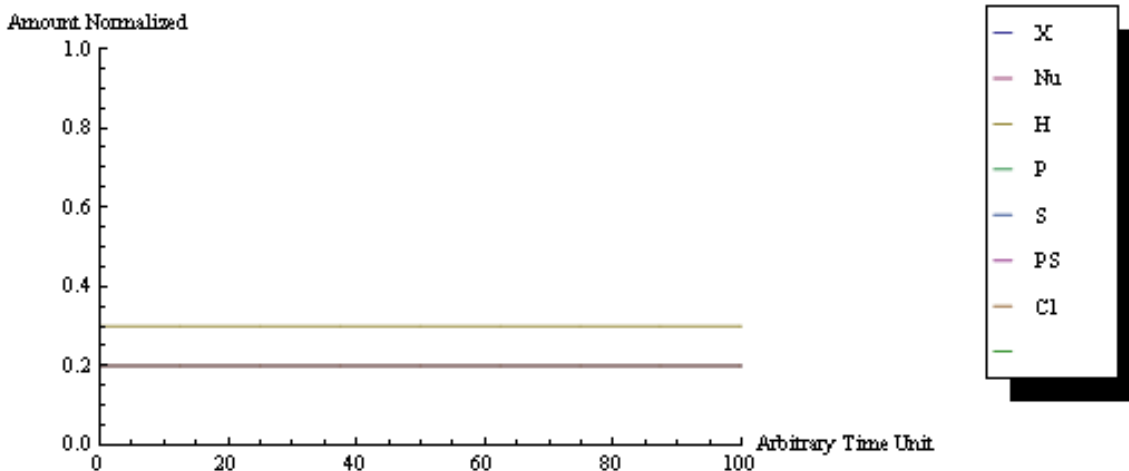

(H = 0.3, Cl = 0.2, X = 0.2)

Figure 10 shows how partially damaged cartilage responds to various hydrogel injections. In the first plot, the collagen and proteoglycans are initially at 0.2, indicating partially damaged cartilage. Hyaluronan is injected (0.4) and the cartilage heals, as can be seen by the increase in proteoglycans and collagen towards 1. In the second plot, the cartilage is still partially damaged (Cl = 0.2, X = 0.7) and 0.3 hyaluronan is injected. Despite the fact that there is less hyaluronan present than in the previous instance, the cartilage still heals because there are more proteoglycans (0.7 vs. 0.2) present initially. In the third plot the cartilage is partially damaged (Cl = 0.2, X = 0.2), but not enough hyaluronan (0.3) is injected to promote healing, as can be seen by the flat line (corresponding to collagen and proteoglycans) at 0.2.

Conclusion

Despite the improvements in the new model, there is still work to be done in modeling cartilage regeneration. First, there are some biological questions that remain to be answered. In the model presented in this paper, there is no reaction term accounting for interaction between the collagen and the proteoglycans. What is the specific interaction between these substances and should this be included in the model? Is the production of these to substances coupled in the cell and how? Additionally, information regarding the relative amounts of compounds (how much core protein is made for each hyaluronan molecule) would be useful for tweaking the model. Furthermore, in the model, the cell obtains some hyaluronan from the hydrogel that is injected. Can the cell use all or some fraction of this hyaluronan? Biologically, there is likely some natural decay occurring in the cartilage. Perhaps the collagen, proteoglycans, and maybe even the hyaluronan should have a decay term in the model. Finally, lab data (such as regeneration times for various starting amounts) from cartilage regeneration experiments would be useful for fitting the coefficients in the model.

Overall, when actually run, the model behaves in a reasonable manner, as seen in Figures 8, 9, and 10. The cartilage can naturally heal itself (Cl and X go back up to 1) from small injuries, but cannot heal itself from large injuries. However, when enough hyaluronan is injected into these larger injuries, the cartilage is able to heal.

Additionally, the new model has several improvements over the initial model. First, the variables are actually biological compounds found in cartilage. The equations themselves are based on processes that occur within cartilage production. Additionally, the new model can simulate both damaged cartilage with injected hydrogel or healthy cartilage that sustains damage at a particular time. Furthermore, the model incorporates the idea of a threshold "structure" level. This is biologically sound since if there is not enough "substance" present, the cartilage will not heal (hence the whole need for developing hydrogel injection as a medical technique).

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Appendix