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DESIGN OF AN IN VITRO SYSTEM FOR THE EVALUATION OF BIODEGRADATION IN COLLAGEN SPONGES USING MRI

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2. degradation
3. MRI

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Abstract

Collagen sponges are widely used in biomedical research. They can be loaded with different drugs, such as growth factors, and they are easy to produce and manufacture, easy to manipulate for desired characteristics, and versatile for use in clinical applications. Current methods for characterizing collagen sponges provide widely accepted data and are, more often than not, destructive to the collagen samples, rendering each sample useful for only one data point in an experiment. The goal of this project was to correlate benchtop degradation measurements with quantitative magnetic resonance imaging (MRI) data. Collagen sponges were degraded in vitro and analyzed using change in dry mass and a ninhydrin assay as well as, MRI. Benchtop methods showed that longer degradation times resulted in increased mass loss. The results of this study suggest that MRI is a viable method to evaluate the degradation extent of collagen sponges.

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1 INTRODUCTION

In excess of 50,000 metric tons of collagen and gelatin are produced annually for a wide variety of medical applications (Olsen, et al, 2003). So much collagen is used each year because the material has many characteristics that make it a multifunctional biomaterial. Collagen is a naturally existing protein in the human body that is found most abundantly in the skin, bones, ligaments, and tendons. Therefore, it is readily harvested from animals and cadavers. Biomaterials derived from natural sources are more advantageous when compared to their synthetic counterparts. Some of the advantages offered by collagen are that it is inexpensive, easily obtained, biocompatible, bioresorbable and biodegradable. Through years of research and development, scientists have learned methods to control the biodegradation rates of collagen through different crosslinking techniques. Controlling the rate of degradation resulted in the use of collagen sponges for a wide variety of biomaterial implant applications including type-I collagen for tissue engineering scaffolds, and the use of types I-III in drug delivery applications (Collagen Application, 2005). That is because degradation rates of collagen are essential for those specific clinical applications. The rate at which the collagen degrades controls the rate at which a drug is released or new cells are allowed to grow into a collagen sponge scaffold.

These applications have proven useful in aiding the regeneration of biological tissues and delivering drugs to the body. As mentioned, those effects are dependant on the degradation of the collagen sponge. However, current techniques of assessing the degradation of a collagen sponges are lacking. This MQP group has proposed a novel and non-invasive technique for assessing the degradation of collagen sponges in vivo by

using MRI. This technique addresses the shortcomings of the current techniques and improves upon them. To prove that this technique can assess the degradation of a collagen sponge, it must be studied in vitro first, and then used for in vivo studies depending on the success of the in vitro experiments. Methods for characterizing collagen sponge degradation have been validated through gold standard benchtop testing and assessment. These methods involve histology assessments, mechanical testing, spectroscopy and observing changes in dry mass. Currently, to use these methods, a sponge is implanted into a large number of animals. Then groups are sacrificed periodically throughout the study to retrieve the sponge, conduct one of the gold standard assays on it, and collect the data. While producing widely accepted data, this takes many man-hours, many animals, and is expensive. This results in a high cost to conduct the experiments. The proposed novel, non-invasive MRI technique for the assessment of collagen sponge degradation would not call for the sacrifice of any animals, would be faster, be non-destructive and would use the same sample for many time points. This results in the consumption of fewer man-hours, fewer animals, the ability to collect multiple data sets from one sponge at each time point, less variability in the data and would therefore cost less money to conduct the assessment while collecting more data of a higher quality.

We hypothesize that the water content of a collagen sponge implanted in the human body will change as it degrades. Therefore, the key is to find and use an MR imaging technique that has parameters that are sensitive to changes in water content. Our plan is to image a sponge before and after degradation, find the change in the parameter, and relate that to amount the collagen sponge has degraded derived from our gold standard benchtop experimentation. This should give us the amount the MRI parameter

changes over time for a specified concentration of degradation-causing enzyme with industrially accepted experimental data to support the MRI results. Assuming this is successful, the studies should move into in vivo experimentation next where an implant is put into rats to see if MRI can still assess degradation of collagen sponges in living creatures.

2 LITERATURE REVIEW

Past research and studies provide a foundation for continued research and development in any field. This section provides an overview of some of the relevant biomedical and MRI topics.

2.1 Collagen

Collagen is a fibrous extra cellular protein found naturally in humans and animals. It is the most abundant protein in the body and with a molecular weight of approximately 300,000g/mol, it provides structural support for blood vessels, ligaments, tendons, skin, and bones (Fujioka, 1998). Figure 1. is an illustration of a collagen molecule.

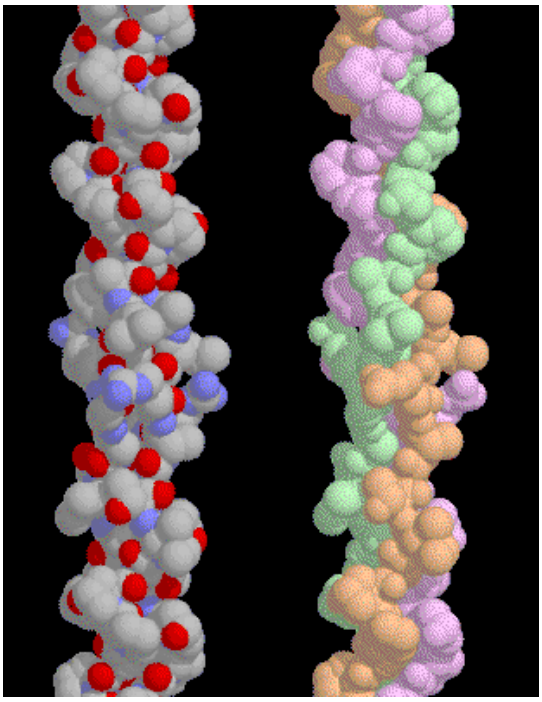


Figure 1: Collagen molecule
(http://www.rcsb.org/pdb/molecules/pdb4_2.html)

2.1.1 Collagen Characteristics

There are at least 27 types of collagens that have been identified in the body as of 2003. (Ylikarppa, 2003). The normal collagen domain is comprised of 1441 amino acids of repeating –Gly-X-Y- sequences that are found folded into their unique triple helix structure (Miller, 1985). The number of identified collagens is constantly being updated by screening cDNA and genomic DNA libraries. Collagens have such varying lengths of domain sequences and various numbers of related proteins that it is difficult to distinguish which proteins are in fact collagens. The large variety of the identified structures is an indicator to the wide range of functions that the collagens form biologically (Prockop and Kivierikko, 1995).

2.1.1.1 Structure

The collagen molecule is a triple helical structure comprised of three polypeptide chains that are each coiled into a left-handed helix. These three left handed-helixes are then wrapped around each other into a right-hand helix that forms the final collagen structure as seen in Figure 2. (a rope-like rod).

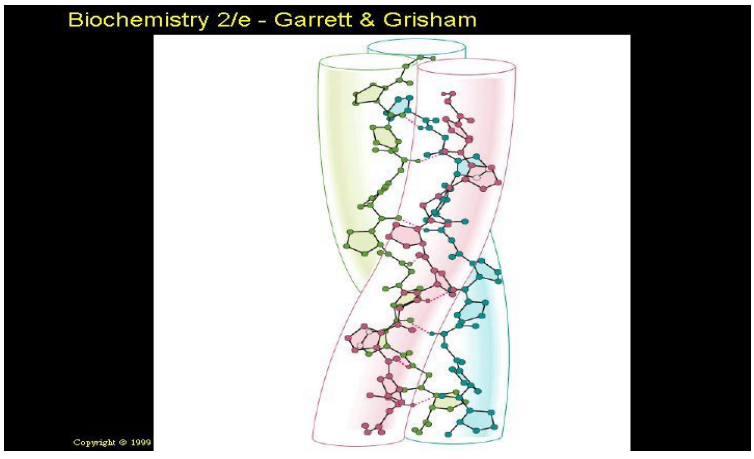


Figure 2: Triple helix of collagen molecule
(http://www.bmb.psu.edu/courses/bmb401/GandGPowerPoints/chapter6/sld040.htm)

The unique sequence of amino acids in the collagen helix (-Gly-X-Y-) provides a tight and stable structure. The strands are typically comprised of the amino acids glycine, proline, and hydroxyproline. Every third amino acid is glycine in each of the strands. Proline typically takes the X position and 4-hydroxyprolene the Y position. This configuration enables the strand to smoothly bend the chain without allowing unnecessary rotation of the polypeptide strands. Hydrogen boding and water bridges, which require 4-hydroxyprolene in the Y position, also act in making the collagen molecule more rigid and stable (Prockop and Kivierikko, 1995). In Figure 3., alanine, which is not normally found in the amino acid sequence, was placed in the position normally taken by glycine. This figure shows how alanine, a much larger compound then glycine, crowds the other neighboring chains. This sequence would make the chain looser and less stable.

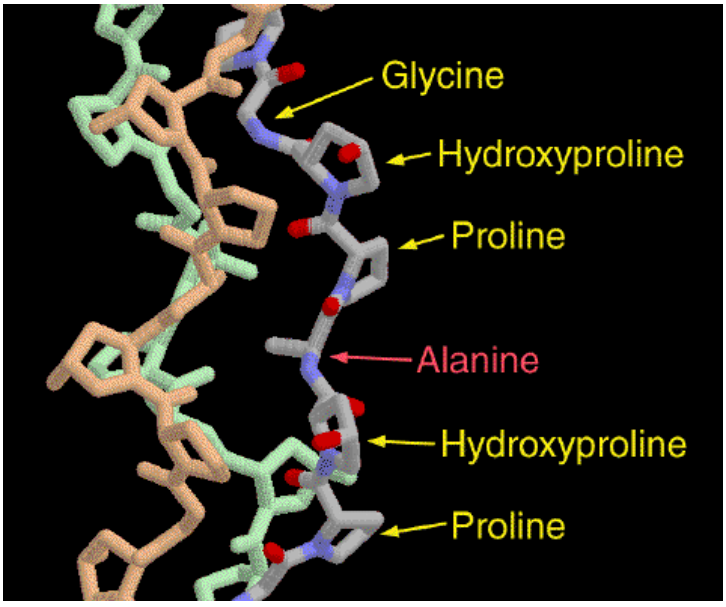


Figure 3: Amino acid placement in collagen triple helix (http://www.rcsb.org/pdb/molecules/pdb4_2.html)

When repeating these three amino acids (glycine, proline, and hydroxyproline) the collagen chain is produced. A normal collagen chain is 1014 amino acids long (Prockop and Kivierikko, 1995).

2.1.1.2 Types

The large family of collagens can be divided into a few classes based on the polymeric structures they form and their related structural features. There are eight major classes of collagens. They are: (1) collagens forming long fibrils, (2) collagens that form network like fibrils, (3) fibril-associated collagens with interrupted triple helices, (4) fibril-associated collagens forming beaded filaments, (5) collagens that form sheets (anchoring fibrils), (6) collagens with a transmembrane domain, (7) partially characterized collagens, (8) proteins that have not yet been identified as collagens but have triple-helix domains (Prockop and Kivierikko, 1995 and Ylikarppa, 2003).

2.1.1.2.1 Collagens forming long fibrils

Collagens types I-III, V, and XI form fibrils and are approximately the same size, with large triple helical domains (about 1000 amino acids per chain). They are also synthesized as large precursors prior to becoming collagens. The large precursors need N- or C-propeptides cleavage to become collagen. These five types of collagen are also similar in how the fibrils are situated. The fibrils are cross-striated and each molecule overlaps its neighbor by about a quarter of its length.

Type I collagen is the major type of collagen that is found in skin, tendon, bone, and dentin. A fetal form of this type of collagen also exists. This is the most abundant type of collagen in the body. Table 1 on the next page lists each of the types of collagen and their location of genes on the human chromosome. The other four types of collagen

in this class are found in various locations throughout the body: type II collagen is specific for cartilage and vitreous humor, type III is often found with type I in skin, muscles, and blood vessels, type V is in fetal tissues, placenta, and interstitial tissues, and type XI is found in cartilage (Prockop and Kivierikko, 1995 and Ylikarppa, 2003).

2.1.1.2.2 Collagens forming network like fibrils

This class of collagens includes type IV, type VIII, and type X collagens. These types consist of approximately 1400 amino acids and are often interrupted by short sequences other than the –Gly-X-Y- sequence. The molecules of this class assemble themselves to form net-like structures. Type IV collagen is found on all basal laminae, type VIII is found in endothelial cells and Descement's membrane of the cornea, and type X collagen is found in the cartilage growth plate (Prockop and Kivierikko, 1995 and Ylikarppa, 2003).

Table 1: Types of Collagen

(Ylikarppa, 2003)

Type	Tissue distribution	Collagen subgroup
I	Most connective tissues	Fibril-forming
II	Cartilage, vitreous humour, intervertebral disc	Fibril-forming
III	Most connective tissues	Fibril-forming
IV	Basement membranes	Nonfibril-forming
V	Tissues containing type I collagen	Fibril-forming
VI	Most connective tissues	Beaded filament-forming
VII	Many tissues, e.g. skin and cornea	Anchoring fibril-forming
VIII	Many tissues, e.g. Descemet's membrane	Hexagonal lattice-forming
IX	Tissues containing type II collagen, e.g. cartilage and vitreous body	FACIT
X	Hypertrophic cartilage	Hexagonal lattice-forming
XI	Tissues containing type II collagen, e.g. cartilage and vitreous body	Fibril-forming
XII	Tissues containing type I collagen	FACIT
XIII	Many tissues, in low amounts	Transmembrane
XIV	Tissues containing type I collagen	FACIT
XV	Basement membrane zones in many tissue	Multiplexin
XVI	Many tissues	FACIT
XVII	Hemidesmosomes	Anchoring filament-forming
XVIII	Basement membrane zones in many tissues	Multiplexin
XIX	Basement membrane zones in many tissues	FACIT
XX	Minor components of several connective tissues	FACIT
XXI	Many tissues	FACIT
XXII	mRNA isolated from cartilage	Not Determined
XXIII	Cornea, lung, cartilage, amnion	Transmembrane
XXIV	Bone, cornea	Fibril-forming
XXV	Brain, neurons	Transmembrane
XXVI	Testis, ovary	Not Determined
XXVII	Cartilage, eye, ear, lung, colon	Fibril-forming

2.1.1.2.3 Fibril associated collagens with interrupted triple helices

Collagen types IX, XII, XIV, XVI, and XIX are found attached to the surfaces of preexisting fibrils of fibril-forming collagens but do not form fibrils themselves. This class of collagens has short triple helix domains and has short segments of non-gly-X-Y- segments interrupting the collagen sequences. Type IX collagen is commonly found attached to the type II fibrils. This type has three collagen triple-helical domains and then four domains that are non-collegenous. Type XII is found in embryonic skin and tendons, type XIV is in fetal skin and tendons, type XVI is found in a number of tissues through out the body, and XIX is found in rhabdomyosarcoma cells (Prockop and Kivierikko, 1995 and Ylikarppa, 2003).

2.1.1.2.4 Fibril associated collagens forming beaded filaments

Type VI collagen is the only type of collagen know to from beaded filaments. The three strands of the collagen chain have short triple helix domains and then a large portion of the strands have N- and C-terminal domains. Type VI collagen is found in most interstitial tissues (Prockop and Kivierikko, 1995 and Ylikarppa, 2003).

2.1.1.2.5 Sheet forming collagens with anchoring fibrils

Type VII collagen is found in the epithelia and anchors skin basal lamin to stroma. This type of collagen links basement membranes to the anchoring plaques of type IV collagen in the extracellular matrix. The triple helix domain of this collagen is very long, with 1530 amino acids in the –Gly-X-Y- repeating sequence (Prockop and Kivierikko, 1995 and Ylikarppa, 2003).

2.1.1.2.6 Collagens with a transmembrane domain

Types XIII, XXIII, and XXV contain a single transmembrane domain that appears to be cytoplasmic and the rest of the molecule is extracellular. Type XIII collagen has been found widely distributed in various tissues and is mostly localized to cell-to-cell and cell-to-matrix contacts. Type XXIII has been found in the cornea, lungs, cartilage, and amnion. Type XXV collagen has been found in the brain and in neurons (Prockop and Kivierikko, 1995 and Ylikarppa, 2003).

2.1.2 Purposes and functions

Collagen has many anatomical and physiological uses in the body and this is seen in the fact that it makes up approximately a quarter of all the protein in the body. It is primarily found in connective tissue. It has great tensile strength that makes it a major component of both ligaments and tendons. It is a major structural protein, forming molecular strands that strengthen the tendons and large tough sheets that support the skin and internal organs. Collagen supports and protects soft tissues of the body and connects them to the skeleton. It is responsible for skin elasticity, and its degradation leads to wrinkles that come with aging. In addition, collagen can combine with mineral crystals to produce bones and teeth. Finally, collagen also fills out the cornea in its crystalline form (Nimni, 1988).

2.2 Sources of Collagen

Collagen can be used for various clinical and research applications. For each of the 27 different types of collagen, there are a variety of different sources in the body where the desired collage type can be harvested. The specific type of collagen to be collected is based on the anatomical or physiological function required for the particular

application. Collagen can be obtained from cadavers or animal donors, and can rebuild connective tissues, providing healthy cell growth.

2.2.1 Types of collagen sources

The most common sources of collagen have been derived from sharks, chickens, and bovine. However, there are numerous other animals from which collagen can be extracted. Shark collagen tends to be very expensive and has some merit in cancer prevention; however, it has a relatively low absorbency rate in humans. Bovine cartilage is the most commonly used collagen supplement due to its higher absorbency rate (Trentham, 1993).

Table 2 is a summary of the common sources of type II collagen.

Table 2: Sources of Type II collagen

(http://www.utmem.edu/ctr/CP_Immunology_CIA/Table_15.5.1.HTML)

Species	Suggested tissue
Chicken	Sternum
Bovine	Knee joint
Porcine	Knee joint
Rat	Knee, hip, shoulder joints Xiphoid process and costal cartilage
Mouse	Sternochondral cartilage

Chicken collagen is a very good supplement for joint pain relief as a supplement for type II collagen. The number of swollen and tender joints was decreased in animal models fed chicken type II collagen I In several animal models, the disease was completely resolved (Trentham, 1993).

Different types of supplements have been proven beneficial for various biological applications. For example, jellyfish type II collagen administered orally at low doses in animal models had a suppressing effect on rheumatoid arthritis. Jellyfish collagen

showed a better effect on the arthritis than bovine or chicken collagen because it is more homogenous than the other options (Hsieh, 2004).

Two of the major sources of type I collagen used for biomedical research are rat tails and bovine tendons. Both are sold commercially and are used for various applications. Type I collagen obtained form rat tails is typically obtained using an acid extraction. The tendons of the rat tails are removed, washed in water, and then dissolved in an acetic acid solution (0.04M). The solution is then prepared, depending on what form of collagen is desired. For example, if films are needed, then the solvent should be evaporated from the solution of collagen poured into glass places and then covered by polyethylene (Sionkowska, 1999).

Bovine tendons are populated sparsely with cells that are surrounded by an extracellular matrix that is primarily composed of type I collagen. The type I collagen in tendons provides it with its strong tensile properties (Samiric, 2004). The collagen is typically extracted from the bovine tendon using acetic acid and then precipitated with 2M NaCl. The product from this extraction is then taken and treated with 10% NaOH to get rid of any contamination of diseases that may have existed in the tendon. It is then purified using ultrafiltration on a molecular sieve and the material that becomes a gel is stratified on suitable tablets and lyophilized for the desired thickness. The collagen is then sterilized using gamma-irradiation (2.5 MRad) and then the collagen content is determined (Trasciatti, 1998).

2.3 Collagen as a Biomaterial

Collagen is known as one of the most useful biomaterials. It has been given this standing due to its ideal biological characteristics, its variety of forms, and its many biomedical applications.

2.3.1 Forms and Applications of collagen

Collagen based biomaterials can be created in many different forms to fulfill the need of a particular application. Some of the common forms of collagen are: films/sheets/discs, shields, sponges, gels/hydrogels, and pellets/tablets.

2.3.1.1 Films, sheets, and discs

One of the major advantages of collagen films in a drug delivery system is their ability to release the drug slowly. Films are used as barrier membranes with a thickness of approximately 0.01-0.5mm. They are made biodegradable and the drugs can be inserted into the film using methods such as hydrogen bonding, entrapment, or covalent bonding. Films have the advantage that they can be sterilized, and pliable while stilling able to maintain enough strength to resist physical damage or change. Films can also be crosslinked with various materials or at various rates to change the release rate of the drug (Lee, 2001 and Li, 2005).

2.3.1.2 Shields

Collagen is used in ophthalmologic uses because of the similarities that exist between the molecules of the eye and certain types of collagen molecules. Collagen shields are primarily used in ophthalmology for drug delivery. The shields are made of porcine sclera tissue whose molecules are very similar to the molecules of the human eye.

The shield protects the healing corneal epithelium from the blinking action of the eyelids (Lee, 2001 and Bourlais, 1998).

Water-insoluble drugs are loaded into the water-soluble collagen matrix prior to insertion into the eye. The collagen entraps the drug molecules and then as the shield dissolves with the tears of the eye, the collagen lubricates the eye, minimizing the eyelid rubbing, increasing the time in which the drug comes in contact with the cornea, and also helps with epithelial healing (Lee, 2001 and Bourlais, 1998).

2.3.1.3 Sponges

Collagen sponges have been useful in many biomedical applications. They have been used widely in the treatment of severe burns and for the treatment of wounds as dressings in pressure sores, donor sites, leg ulcers, and decubitus ulcers (Lee, 2001 and Friess, 1998). Some advantages of using collagen sponges for these applications are that they have smooth adherence to the wet wound bed, they can easily absorb large quantities of tissue exudates, and they can shield against mechanical harm. When using collagen sponges, very often they are loaded with growth factors to enhance wound healing.

The collagen sponges are typically not made of pure collagen but rather a combination of materials to increase their fluid holding capabilities and achieve highly resilient mechanical properties. Collagen from bovine skin combined with elastin, fibronectin, or glycosaminoglycans is one way to generate the ideal properties for the sponge. Collagen sponges can also be crosslinked to change the sponge properties. Crosslinking with chemicals such as glutaraldehyde or polyhydroxyethyl methacrylate (PHEMA) can increase the tensile strength and keep the sponge wet (Lee, 2001 and Friess, 1998).

2.3.1.4 Gels and hydrogels

Hydrogels are excellent in drug delivery systems because they are easy to manufacture and easy to apply at the treatment site. Approximately 50,000 metric tons of gelatins are produced annually for medical use (Olsen, 2003). When produced, they provide a large consistent surface area. Synthetic as well as natural polymers have been combined for use in various hydrogel applications. The different blend of polymers produces a wide range of gel properties such as mechanical strength, drug release, and biocompatibility (Lee, 2001 and Olsen, 2003).

2.3.1.5 Pellets and tablets

Pellets and tablets are useful in drug delivery systems because they are small enough to be injected into the body using a syringe and yet they are large enough to hold higher molecular weight protein drugs. They are an excellent option for localized drug delivery systems. One example of a pellet drug delivery system that fulfills these capabilities is a rod with a diameter of 1 mm and a length of 1 cm. This size pellet is ideal for large molecular weight localized drug delivery (Lee, 2001).

2.3.1.6 Others

Resorbable sutures also have been created using collagen so that physicians no longer have to perform post operational removal of the sutures. Resorbable sutures lose their entire tensile strength within 2-3 months. Collagen is used in both monofilament and multifilament (braided) sutures; however, in recent years, synthetic materials are dominating the sutures market (Tomihata, 1998).

As collagen production decreases with age, wrinkles form because collagen is a large contribution to the elasticity of the skin. Collagen injections are used for cosmetic purposes to keep the younger appearance of the skin.

2.3.2 Suitability

As seen in Table 3, collagen has many advantages that prove it an ideal biomaterial. It is the most widely used biomaterial and its biocompatibility, ease of attainability, and ease of modification to create the optimal characteristics predict that it will remain the most prevalent material for quite some time (Olsen, 2003).

Table 3: Advantages of collagen as a biomaterial

(Lee, 2001)

Advantages
➢ Available in abundance and easily purified from living organisms (constitutes more than 30% of vertebrate tissues) ➢ Non-antigenic ➢ Biodegradable and bioreabsorbable ➢ Synergic with bioactive components ➢ Biological plastic due to high tensile strength and minimal expressibility ➢ Hemostatic – promotes blood coagulation ➢ Formulated in a number of different forms ➢ Biodegradability can be regulated by cross-linking ➢ Easily modifiable to produce materials as desired by utilizing its functional groups ➢ Compatible with synthetic polymers

Table 4 lists the disadvantages of collagen as a biomaterial. The advantages clearly out weigh the disadvantages however, other biomaterials are constantly being researched and used in different applications to overcome the drawbacks of collagen.

Table 4: Disadvantages of collagen as a biomaterial

(Lee, 2001)

Disadvantages:
➢ High cost of pure type I collagen ➢ Variablility of isolated collagen (e.g. corsslink density, fiber size, trace impurities, etc.) ➢ Hydrophilicity which leads to swelling and more rapid release ➢ Variability in enzymatic degradation rate as compared with hydrolytic degradation ➢ Complex handling properties ➢ Side effects, such as bovine spongeform encephalopathy (BSF) and mineralization

2.4 Collagen in Tissue Engineering

Tissue engineering is a field that has been expanding drastically over the past two decades and collagen has played an important role in its progression. Collagen's ideal biomaterial properties make it an excellent tissue engineering tool.

2.4.1 Tissue Engineering

"The aim of tissue engineering is to provide a temporary biodegradable material to substitute for the damaged tissue and to give strength to the tissue until it replaces the biomaterial and starts to function" (Ber, 2005). In the 1980's, interest in tissue engineering emerged and work began towards creating three-dimensional substrates that adequately support cell growth. Since then, the field has spread to working on artificial hips, blood vessels, and mechanical hearts.

2.4.2 Current Devices

There are many different tissue engineering applications of collagen currently in use. A few of the most common applications are Apligraf®, scaffolds, and wound repair.

2.4.2.1 Apligraf®

Apligraf® (See Figure 4) is very similar to human skin. It is composed of two primary layers: the dermal layer and the epidermal layer. The dermal layer is made of human fibroblasts from neonatal foreskin and the epidermal layer made of human keratinocytes. The human cells are in a bovine type I collagen matrix which provides structure and allows for cell growth. The epidermal layer is created by allowing the keratinocytes to multiply and differentiate to create the cellular layers found in natural human skin.

Figure 4: Apligraf®
(http://www.apligraf.com/)

Apligraf® does not contain any normal human cell structures such as blood vessels, melanocytes, lymphocytes, hair follicles, etc. As of 2004, Apligraf® is approved for use in diabetic foot ulcers and venous leg ulcers. Leg ulcers affect 600,000-1 million people in the United States, currently over 80,000 Apligrafs® have been used on patients (Apligraf®, 2005).

2.4.2.2 Scaffolds

Since collagen is the major structural protein in mammalian tissues, it is the most widely used material for tissue engineered scaffolds. Porous collagen scaffolds have been used in cartilage, bone, nerve and skin engineering. They provide support to surrounding tissues, and act as a template for cellular infiltration, proliferation, and differentiation (Ma, 2004).

2.4.2.3 Wound repair

Collagen is an excellent wound repair material. Injectable collagen has been used to speed the closure of wounds. When tested, comparing normal closure of minor soft tissue wounds and collagen/growth factor treated wounds, the rate of wound closure was

increased by 50%. This application is very useful on diabetic ulcers or sores, which heal very slowly in comparison to other minor wounds (Soiderer, 2004).

2.4.3 FDA involvement in Tissue Engineering

The FDA has been closely regulating tissue engineering devices since the enactment of the medical device regulations in 1976. The first medical device approved under this act was approved as a drug (Avitene), and since then, the FDA has considered collagen a "transitional device." This implies that most collagen medical device applications require a pre-market approval with the clinical studies. Collagen topical wound dressings, corneal shields, and catheter cuffs however, have been approved under the 510(k) rule. The 510(k) rule allows the device to be marketed if an adequate amount of similarity is shown to a pre-1976 safe medical device. Table 5 shows some of the collagen based wound dressings that have been approved based on the 510(k) ruling (Pachence, 1998).

Table 5: FDA approved collagen based wound-dressings (Pachence, 1998)

Material Type	Company (Brand Name)
Partially purified skin	LifeCell
Collagen sponges[a]	Integra LifeSciences Corp. (Helistat)
	Johnson & Johnson (Instat)
	MedChem (ActiFoam)
	BioCor (SkinTemp)
Collagen fibers or fleece[a]	Integra LifeSciences Corp. (Helitene)
	Johnson & Johnson (Instat Fibrillar)
	MediChem (Avitene)
Collagen powders	Medifil (BioCore)
Collagen composite dressings	Fibracol (Johnson & Johnson)
	Biobrane (Mylin)
Hydrolyzed collagen	Chronicure (Derma Sciences)

[a] These products approved with a hemostasis claim.

An evaluation method using MRI to determine the exact degradation of a collagen sponge will benefit the FDA because it will allow safer sponges to use when they are involved in clinical applications and will provide more information about the sponges being used.

2.5 Collagen Sponges

One of the best ways to utilize collagen for clinical applications is to fabricate sponges from the protein. Collagen sponges offer many advantages to tissue engineering and drug delivery applications. Their widespread suitability is because their behavior can be controlled for different applications. The degradation kinetics, important in drug delivery and tissue-engineering applications, can be customized by altering the porosity of the sponge, adjusting the degree of cross-linking, and other factors. Sponges are particularly attractive for tissue engineering applications because it is possible to load the sponge with various growth factors and other bioactive molecules that enhance the tissue regeneration.

2.5.1 Fabrication Techniques

Sponges are normally fabricated using freeze-drying techniques (Friess, 1998). An aqueous collagen dispersion, usually acidic, is cooled until frozen. The sponges are then placed under vacuum (less than 100mTorr) to sublimate the frozen ice crystals, creating an interconnected porous structure in the remaining material. The porosity of the sponges can be controlled by altering the collagen content of the aqueous solution, the rate at which the solution is frozen and the final temperature before it is placed under vacuum (Curtil, 1997 and Friess, 1998). The ideal pore size depends on the particular application, however, for cellular infiltration and proliferation, 50-150μm is best (Lee, 2001). It is also necessary to have a large pore volume fraction, 90% or greater within the matrix (O'Brien, 2004).

Temperatures most commonly used fall within the range between -30°C and -80°C, though in some cases -196°C (the boiling point of liquid nitrogen) is used.

Temperature affects pore size by determining the rate of nucleation. The quenching process typically used in the freezing of the collagen solution usually leads to uneven rates of heat transfer and therefore affects pores size (O'Brien, 2004). This leads to variations in pore size within the sponge that are generally undesirable. To address this smaller sponges are fabricated in materials that have improved heat conducting capabilities.

2.5.2 Cross-linking methods

Collagen sponges degrade readily *in vivo*. To increase their lifespan within the body and to control the rate at which they degrade, crosslinking is often employed. Natural collagen has crosslinks that stabilize and strengthen the orderly packing of collagen fibers. These crosslinks are both intra- and inter-molecular (Friess, 1998). Processed collagen does not spontaneously form these crosslinks when it is reconstituted. Consequently, it does not have the same mechanical strength or degradation resilience of natural collagen. This limits the use of many collagen implants to short-term applications. To address this, researchers have developed a variety of methods to create additional bonds in the collagen matrix to stabilize the material. A basic understanding of the various clinically-relevant crosslinking methods will allow a particular method to be selected for investigation of its influence on the degradation measurements performed within this study. In order to understand these crosslinking methods, they can be broadly grouped into two different categories, namely chemical and physical crosslinking methods.

2.5.2.1 Chemical Crosslinking

Chemical crosslinking methods usually involve the use a bifunctional chemical agent that reacts with two collagen molecules, at two different sites, to create a bond between them. The functional groups typically react with the amino acid residues on two different collagen molecules. Ideally the bonds formed should be irreversible and stable. (Khor, 1997). The chemicals that have been used previously to create crosslinks are glutaraldehyde, formaldehyde, polyepoxy compounds, acyl azide, carbodiimides, hexamethylene diisocyanate and chromium tanning (Khor, 1997 and Friess, 1998).

2.5.2.1.1 Glutaraldehyde

Glutaraldehyde has been used extensively in many applications and has gained widespread acceptance. Its success is based on the fact that it is easily obtained, inexpensive and forms the crosslinks in collagen in a relatively short period of time (Khor, 1997). This method has proven its effectiveness in the manufacture of bioprosthetic heart valves. Glutaraldehyde reduces the rate of biodegradation while maintaining other material properties, such as viscoelasticity and anatomic integrity (Jayakrishnan, 1996).

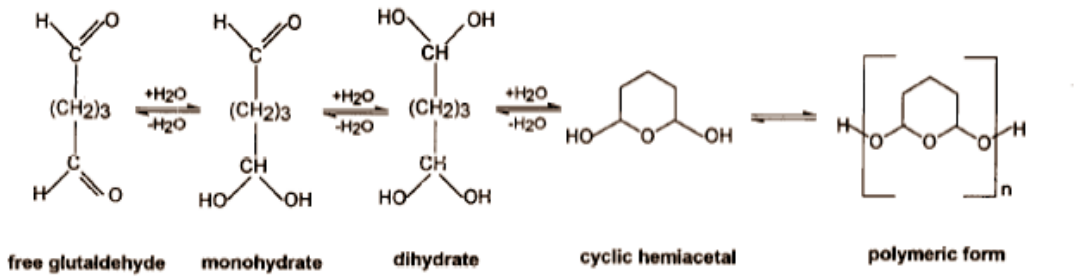


Figure 5: Forms of glutaraldehyde in solution
(Khor, 1997)

Glutaraldehyde is a five-carbon aliphatic molecule whose structure in solution is shown in **Error! Reference source not found.**. The molecule creates the crosslinks by interacting with different collagen chains and creating a chemical bridge between them. The reaction depends on the solvent used, the pH, concentration of glutaraldehyde and the purity of glutaraldehyde. It has been reported that increasing concentration of glutaraldehyde, while it leads to shorter crosslinking times, results in less efficient crosslinking (Friess, 1998). The reaction mechanism is shown below in Figure 6. The details of this reaction are beyond the scope of this project.

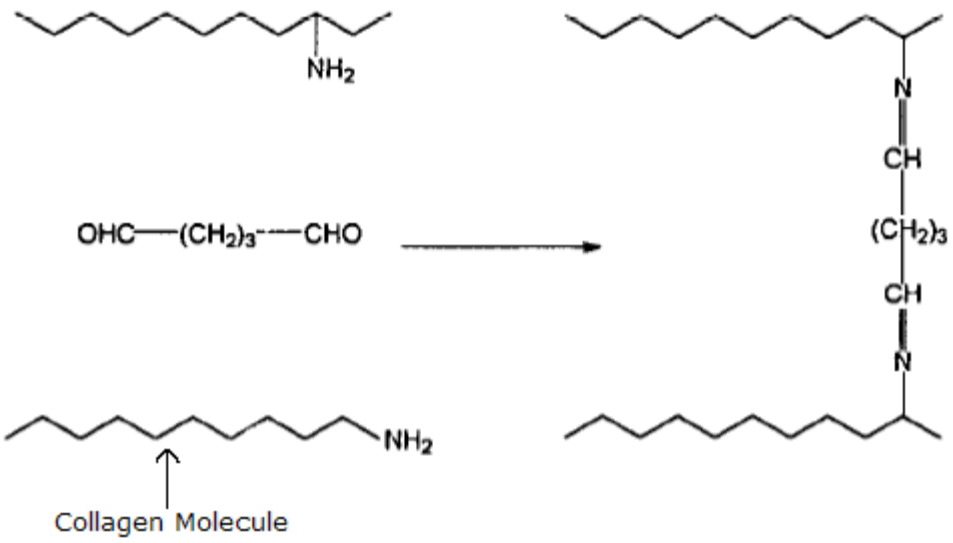


Figure 6: Glutaraldehyde crosslinking of collagen

(Khor, 1997)

Glutaraldehyde is commonly used for crosslinking, however, it does have some drawbacks. One of the disadvantages of using glutaraldehyde is that it has a tendency for causing treated implants to become calcified. Additionally, it has cytotoxic effect on cells caused from residual, unreacted glutaraldehyde. Finally, it forms unstable glutaraldehyde polymers within the collagen matrix. To address the problem with

residual glutaraldehyde, L-glutamic acid and glycine have been shown to quench the remaining particles and reduce the toxic effects (Jayakrishnan, 1996).

2.5.2.1.2 Hexamethylene diisocyanate

Hexamethylene diisocyanate (HMDI), whose structure is shown below in Figure 7, is a straight chain molecule where the two functional ends of the molecule are separated by ten atoms.

Figure 7: Structure of hexamethylene diisocyanate

(Khor, 1997)

This molecule differs from other chemical crosslinking agents in that the crosslinking must be carried out in anhydrous conditions. This is necessary because the isocyanate functional group is highly reactive with water and would not react with collagen molecules in an aqueous environment. Consequently, crosslinking by HMDI is performed in 2-propanol. When HMDI degrades, it is presumed to produce hexamethlylene diamine, which is less cytotoxic when compared to other crosslinking agents (Khor, 1997). This makes residual amounts of the crosslinking agent less of a problem.

2.5.2.1.3 Carbodiimides and Acyl Azide

The principle advantage of using carbodiimides and acyl azide is that these molecules are not required to be bifunctional in order to create the stabilizing bonds (Friess, 1998). The most common chemical used in the class of carbodiimides is 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). The molecule works by activating the carboxylic acid groups of aspartic and glutamic acid residues in one amino acid chain

which then reacts with the amine group of another polypeptide chain to create amide bonds (Damink, 1996). The mechanism for this reaction is shown in Figure 8.

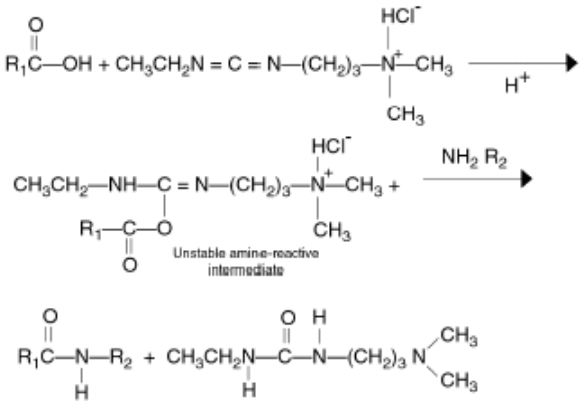


Figure 8: Carbodiimide reaction with carboxylic groups and then with amines (http://chem.ch.huji.ac.il/~eugeniik/edc.htm)

Acyl azide is similar in that it also activates the carboxylic acid group. However, part of the molecule is incorporated into the crosslink, unlike the carbodiimide. The carboxylic group undergoes a series of reactions that produces an acyl azide functionality. This functionality then reacts with an amine group on another chain to produce the amide bond. Diphenylghosphoryl azide (DPPA) has been used to improve on acyl azide with faster crosslinking performance being reported (Khor, 1997).

2.5.2.1.4 Polyepoxy compounds

Polyepoxy compounds are based on the structure for glycerol where the hydroxyl groups have been replaced with carbon polymers terminating in an epoxy functional group. Typical structures for compounds in this class are shown in Figure 9.

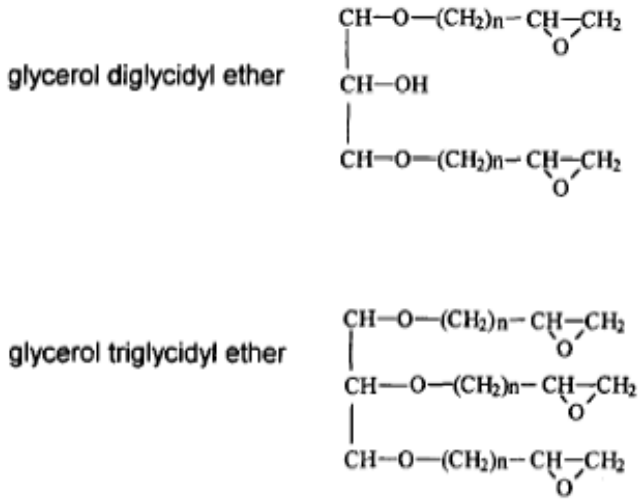


Figure 9: Structure of polyepoxy compounds

(Khor, 1997)

The epoxy functional group reacts primarily with lysine residues. This crosslinking method gives tissues acceptable cytotoxicity levels (Friess, 1998). The resulting material is more hydrophilic than glutaraldehyde treated tissue and is less susceptible to calcification (Khor, 1997).

2.5.2.1.5 Chromium Tanning

Chromium tanning, originally developed as an industrial process to treat leather, has been used with some success to improve collagen implant resistance to collagenase activity (Raghuraman et al, 2000). The trivalent metal ion forms "stable basic oligomeric complexes with acidic residues on the collagen molecule at a pH of 3-4" (Friess, 1998). However, this method has not been widely used in medical device applications.

2.5.2.2 Physical Crosslinking

Alternative methods to chemical crosslinking have been proposed to address the shortcomings of chemical crosslinking, namely high cytotoxicity and poor

biocompatibility. The two most commonly used methods are dehydrothermal treatment (DHT) and ultraviolet irradiation.

2.5.2.2.1 Dehydrothermal Treatment

DHT crosslinking is performed by placing the sample under vacuum, less than 0.2 mbar, and heating at 105°C for one to five days (Ma et al, 2004). The method works by removing all the water within the collagen matrix. This causes condensation reactions between the carboxylic groups and amino groups on adjacent polypeptide chains. The principle advantage of this method is that does not introduce any toxic chemicals while still increasing the materials resilience to degradation. However, it does result in increased hydrophobicity and partially degrades the collagen matrix. If this method is used in the project, then the MRI measurements may have to be adjusted for the partial degradation before the sponge is placed in the collagenase solution (Weadock et al, 1995).

2.5.2.2.2 Ultraviolet Irradiation

Exposing the collagen material to radiation at 254 nm is thought to result in the formation of free radicals on the aromatic ring of certain amino acid residues. The reaction between these radicals then creates the crosslinks. This method is limited by the number of aromatic amino acid residues present in the polypeptide chain, namely phenylalanine, tyrosine and typtophan (Weadock et al, 1995). The thickness of the sample may also be a problem because the UV irradiation may not penetrate the entire sample due to attenuation. This will be a particular problem for thicker collagen sponges. It is simply not possible to radiate the material longer as this will cause molecular fragmentation and unwinding of the collagen triple helix. Molecular damage and partial

denaturation in the form of chain scissions is unavoidable. However, complete denaturation does not occur after limited exposure due to local constraints that prevent the helix from unwinding (Ohan et al, 2002).

2.5.2.3 Evaluating degree of crosslinking

Thermal stability and the material's resilience to degradation are used to determine the extent to which collagen has been crosslinked and the effectiveness of the crosslinking method, both *in vivo* and *in vitro*. These methods have become well established and are extensively used. It is also possible to determine if the collagen has been denatured using the same methods.

The thermal stability measures the temperature at which the collagen becomes denatured as the triple helix unwinds and becomes a random coil. This temperature will be increased by bonds between the helices and between molecules within the same helix. To measure this temperature various methods have been developed. Hydrothermal isometric tests are performed by holding the sample with clamps at its unloaded length while the temperature of the surrounding solution is then increased. At the denaturation temperature, the sample will produce a force as it tries to coil up. The temperature at which this occurs is recorded. The parameter measured is usually termed the "shrinkage temperature" even if it is measured by some other means, such as holding the sample at constant thickness and measuring the pressure as the temperature is increased (Lee, 1995).

Differential scanning calorimetry is another possible method to measure the thermal stability. This is done by placing a sample in a bathing solution in one pan and only the bathing solution in another pan. The temperature of both pans is increased while

the heat flow to each pan is monitored. A difference in the heat flows indicates an enthalpy-dependant transition. This occurs at the denaturation temperature (Lee, 1995).

The materials resilience to degradation in proteolytic solutions is another possible method to measure the extent of crosslinking in the collagen. Crosslinks increase resistance to digestion via steric hindrance that prevents enzymes from binding to the activation sites on the collagen molecules. The crosslinks also keep enzyme-cleaved chains together (Ohan, 2002). Collagen materials are incubated in solutions containing enzymes for a specified amount of time. The amount of material remaining or the amount that was released by the enzymes is recorded and used as a measure of crosslinking.

2.5.3 Sponge use in Tissue Engineering

Sponges have become well established for use as hemostatic agents and wound coverings. Sponges have been used as dressings for severe burn wounds, pressure sores, leg ulcers and decubitus ulcers. The properties of collagen, such as biocompatibility and active cellular ingrowth, make it very suitable for these types of applications (Friess, 1998). It is also possible to incorporate drugs into the sponge matrix that further promote tissue healing. This has been used to enhance the functionality of collagen sponges as a bone scaffold by introducing bone morphogenetic protein (BMP). Collagen sponges loaded with recombinant human-BMP-2 has gain FDA approval for use in combination with a titanium spine fusion cage for anterior lumbar spinal fusion. In Europe, the scaffold is approved for use as an adjuvant in acute tibia fractures (Geiger 2003).

2.6 Degradation methods

The principle method for inducing the degradation of collagen sponges is to treat the sponge with an enzyme that will cleave the collagen molecule and cause the matrix to disintegrate. It is also possible to compromise the collagen matrix by applying mechanical forces; however, the equipment and greatly complicated experimental procedure do not make a feasible method to use in this project. Collagenases have been isolated from a variety of sources and are the only enzymes known to cleave the intact collagen super coil (Peterkofsky, 1982). Collagenases derived from mammalian sources belong to a group of enzymes known as matrix metalloproteinases (MMPs). These enzymes are all structurally related and are zinc (Zn^{2+}) dependant. They are responsible for tissue remodeling in the body and are very specific to the different types of collagen. Collagenases from other sources, such as bacteria, may also have the same Zn^{2+} dependency, but do not share the same structural characteristics to be grouped with the other MMPs. In general, they are less specific and will degrade multiple types of collagen (Watanabe, 2004). After the initial cleavage, the collagen molecule is susceptible to other non-specific enzymes such as trypsin and pepsin present within the *in vivo* environment.

2.6.1 Bacterial Collagenases

A commonly used type of enzyme that is well documented within the literature is bacterial collagenase from *Clostridium histolyticum*. The enzymes isolated from this source usually represent a mixture of several different enzymes that each has slightly different binding to collagen; however, they are all closely related (Watanabe, 2004). The minimum sequence required by these enzymes to cleave the intact collagen molecule

is R-Pro-X-Gly-Pro, where the proline residues can be replaced by hydroxyproline. The cleavage occurs between the X and glycine molecules (Peterkofsky, 1982). The ColH and ColG are specific enzymes present in the collagenolytic mixture isolated from *C. histolyticum*. The structure for the ColH enzyme is shown in Figure 10.

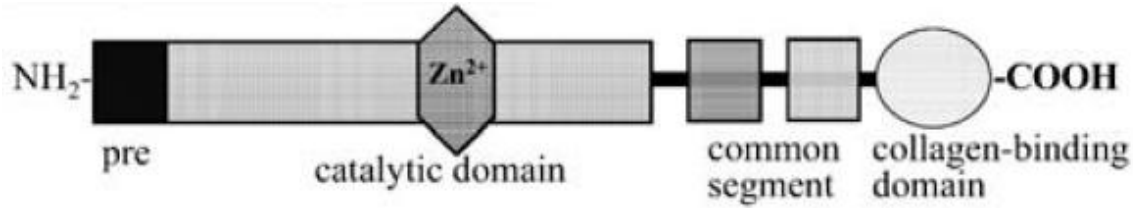


Figure 10: ColH from *C. histolyticum*

(Wantanabe, 2004)

2.7 Evaluating degradation

Various methods *in vitro* have been developed to measure the extent of degradation that has occurred during in vitro experimentation. The most rudimentary of these is visual inspection of the sponge. Another simple technique is to measure the change in mass of the sponge because of molecules being released from the sponge. The bulk mechanical properties of the sponge can also be used to determine the integrity of the sponge and the hence the degradation extent. There are also chemical methods where the amount of released collagen is measured. The principle means for this type of evaluation is to use a dye that binds to the released particles, where the amount of binding can be measured by an absorbance measurement.

2.7.1 Dry Mass

The simplest method to determine the degradation extent of a sponge would be to measure the change in mass of the sponge before and after degradation. The release of

amino acids during the degradation reduces the mass of the sponge. The extent of degradation can thereby be quantified as a percent reduction in mass (Damink, 1996).

The most accurate mass measurement is obtained by using a dry sponge. Changes in water content, due to different crosslinking and/or degradation extents, will cause unwanted variations in the results. After freeze-drying, the final step in fabrication, and the dry sponges can easily be weighed before they are degraded. After the degradation, the sponges will need to be lyophilized to return the sponge to a dry state prior to the second weighing.

The change in dry mass is a straightforward technique that will provide a reasonable quantification of the degradation. This method does not require specialized equipment beyond what is available for this project, or any hazardous chemicals. To perform this technique does not require any advanced lab skills.

However, the accuracy of this method is limited. Changes are detected only through the physical result of collagen fragments being separated from the bulk sponge. The accuracy of the standard laboratory scales may not be sufficient to quantify the degradation reliably. During the digestion, larger fragments may be released from the sponge that may be impossible to recover prior to massing. Furthermore, the extra handling of the sponge to remove the water content could result in additional fragment loss and adversely affect the reliability of the results. Despite these disadvantages, the method's simplicity and ability to provide a good approximation still make it worthwhile to use.

2.7.2 Spectroscopy

Spectrophotometric methods provide a means to quantify the amount of a particular molecule within a solution. In general, a dye that binds to the molecule of

interest is added to the solution to create a new compound with an absorbance at a specific wavelength. The absorbance of the solution at this wavelength is proportional to the amount of binding that has taken place and hence proportional to the concentration of the substance of interest in the solution. Dimethylmethylene blue, the Bradford assay, and ninhydrin have all been used previously in collagen-related studies.

2.7.2.1 Dimethylmethylene Blue

Dimethylmethylene blue dye selectively binds to sulfated glycosaminoglycans (GAG), a component of many collagen sponges, and has gained wide acceptance in quantifying its concentration (Templeton, 1988). The dye is also used in histology applications because of its high affinity for nuclei acids. The accuracy and ease of this technique has contributed to its popularity. Drawbacks include the fact that the assay cannot be used in the presence of pyridine and pyridine nucleotides. Solutions containing high salt concentrations are also excluded because the salts result in false positive readings (Templeton, 1988).

The GAG-chains are commonly incorporated into collagen sponges tailored for skin regeneration applications (Pek, 2004) and for anterior cruciate ligament repair (Murray, 2003). The GAG portion of these sponges is approximately 8wt% (Pek, 2004). This means that relatively small amounts of the degraded GAG component will be released by using enzymes specific for it, limiting the total degradation range. The results obtained from this technique will cover a range narrower than what would be ideal for developing a corresponding MRI technique.

2.7.2.2 Bradford Assay

The Bradford assay uses the Coomassie brilliant blue G-250 dye that binds to a variety of free amino acids (Compton, 1985). The largest response is seen with arginine, while binding with other proteins, such as histidine, lysine, tryptophan, tyrosine, or phenylalanine causes variations in color (Lopez, 1993; Compton, 1985). The non-specificity of this dye, its significant susceptibility to interference and the variations caused by changes in pH are a major limitation to this particular method.

However, the low cost, speed and simplicity of the method has lead to the development of modifications to improve the accuracy. The addition of sodium dodecyl sulphate (SDS) at a very low concentration was shown to increase the response to collagen proteins fourfold (Lopez, 1993). This modification does not address the non-specificity of the dye or reduce its susceptibility to interferences from other molecules that might be present in the solution. This assay has become useful as a fairly sensitive, general, semi-quantitative assay. Accurate quantitative results can be obtained in certain solutions with the judicious selection of a standard (Sapan, 1999).

This project will require accurate quantification of protein concentration to determine the extent of sponge degradation. This objective outweighs benefits of the Bradford assay, namely speed and cost. Furthermore, the amino acids to which the response of this method is greatest are not going to be released in significant amounts by bacterial collagenase degradation.

2.7.2.3 Ninhydrin

This assay is widely used in many disciplines to detect amino acids (Friedman, 2004). It is another method that could be used to quantify any amino acids released

because of degradation. Ninhydrin and a reducing agent, usually hydrindantin (Cahn, 2002) or $SnCl_2$ (Yin, 2002), are added to the solution containing the amino acid of interest. A reaction between the amino acid and ninhydrin then occurs to produce the chromophore. The most common chromophore produced by the reaction with primary amines has a maximal absorbance at $\lambda = 570$ nm and has been called Ruhemann's purple (Friedman, 2004). The chemical reaction cascade that leads to the formation of Ruhemann's purple is shown in Figure 11.

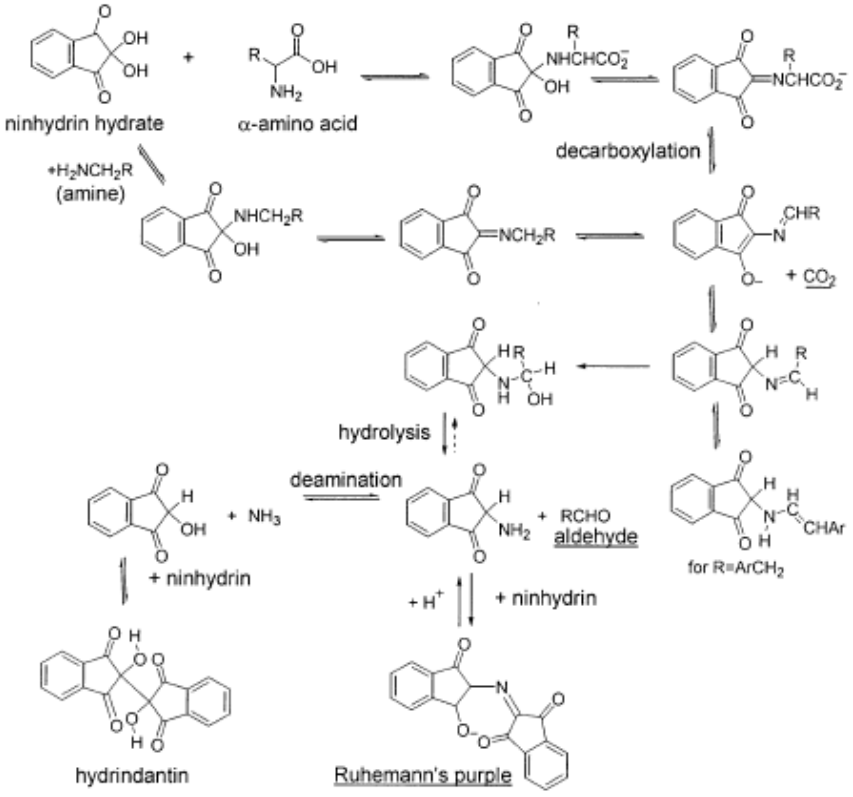


Figure 11: Ninhydrin reaction cascade

(Friedman, 2004)

The collagen sequence is dominated by the repeating tripeptide glycine-proline-hydroxyproline and would release mostly these proteins when undergoing complete degradation. The ninhydrin complex formed by the reaction with secondary amines

proline and hydroxyproline would create a chromophore that has optimal absorbance at λ = 440nm. This falls within the yellow visible range.

Degradation by bacterial collagenase will cleave the bonds at the location R-Pro-X-Gly-Pro, as mentioned previous. This will expose one amino group, leucine, for the ninhydrin to react with per cleavage site. As mentioned previously, type-I collagen is comprised of two α1(I) chains and one α(2) chain. The α1(I) chain consists of 19 leucine and 6 isoleucine residues and the α(2) chain contains 30 leucine and 14 isoleucine residues, each per 1000 total residues. Each chain is 1014 amino acids long. Therefore, from the digestion of one complete collagen molecule, approximately 95 residues are expected to be released.

The overwhelming advantage of this particular assay is that it is extremely reliable and accurate. It has been used as a standard to measure other colorimetric methods (Sapan, 1999). No method is infallible; interference from high sugar content has been found but this will not be a problem in this project (Magne, 1992). However, modifications to the standard technique are possible to eliminate this interference. The method, while very dependable, requires the solution be boiled for approximately twenty minutes (Cahn, 2002). This heat treatment is not always ideal when dealing with thermally sensitive solutions. The time required to obtain the results is also greater than other methods, such as the Bradford assay.

Ninhydrin would be the optimal assay for the detection of released amino acids. When performed correctly, the results can be compared with the corresponding MRI measurements with greater confidence than would be afforded by the other methods. Moreover, there is nothing, such as interfering molecules, which would preclude the use of this assay.

2.7.3 Mechanical testing

In addition to the spectrophotometric methods and change in dry mass to quantify the degradation of collagen sponges, it is possible to measure reductions in the mechanical properties of sponges and relate those to the degradation extent. The erosion of the collagen fibers within the sponge will affect the bulk mechanical properties of the material. Studies have measured changes in tensile strength (Damink, 1995), unconfined compressive measurements (Pek, 2004) and stress relaxation (Cahn, 2004). These methods are very sensitive to degradation. However, the need for additional testing equipment and complications that arise in controlling variables, such as sample geometry and water content, when no new data will be obtained does not justify their use.

2.7.4 Implant Evaluation

The methods described up to this point all deal with *in vitro* degradation and degradation measurements. The performance of a collagen device must ultimately be proved in an *in vivo* environment where enzymes responsible for tissue remodeling would degrade the sponge. The methods used to measure the degradation need to be modified accordingly. The evaluation of collagen implants is usually performed by excising the implant and dividing it for processing and testing using different methods. This is necessary because the methods used to obtain different types of information from the implant are usually destructive, such as the mechanical testing. Parameters that are generally important when evaluating collagen implants are cellular infiltration, extent of degradation, deposition of new tissue, vascularization, calcification, and immune response. The most common method to obtain this data is to perform some type of histological staining.

2.7.4.1 Histology

Histological staining is used to determine the extent of cellular infiltration, sponge degradation and vascularization (Daamen, 2005). This is a well-established method performed by using a variety of staining methods. Toluidine blue (Van Wachem, 1999), hematoxylin/eosin, and safranin O/fast green have been used previously. These staining methods allow the researchers to visualize the cells, the remains of the implant, and connective tissue. The immune response is characterized by determining the types of cells present in the implant and their quantity. The extent of degradation can be determined using histomorphometry. Histomorphometry is performed by measuring the area of each of the different regions on a slide, such as regions occupied by the implant, new tissue, blood vessels or empty space. This is usually carried out with the aid of a computer. However, this technique is limited because the tissue section obtained is a sample of the implant and may not be representative of the entire implant. It would be necessary to take multiple sections throughout the implant to get a more reliable measurement of the degradation extent. This is very resource intensive. The development of a MRI procedure to evaluate the degradation would provide the degradation extent of the entire implant without needing to take multiple measurements from the same sample.

2.7.4.2 Immunostaining

The stains mentioned previously are used to identify general types of tissues or particular cellular regions. Greater specificity can be obtained using immunostaining. This technique uses antibodies that have extremely specific binding domains. The general procedure is to allow these antibodies to attach. Once the initial binding has

taken place, a fluorescent protein can then be attached via a secondary antibody to the first antibodies. This allows the antibodies to be visualized and together they can be used to determine if a particular molecule is present or not. Immunostaining has been used to identify new collagen production in rats by using antibodies that are specific to rat collagen type I after implantation with a scaffold produced with bovine Achilles tendon collagen (Daamen, 2005).

2.8 Magnetic Resonance Imaging

Nuclear Magnetic Resonance (NMR) is a spectroscopic technique used to obtain physical and chemical data about molecules. Magnetic resonance imaging (MRI) is an imaging method primarily used for clinical diagnostic applications. This data is then used to produce high quality images of the inside of the human body for medical analysis. MRI is actually based on the principles of nuclear magnetic resonance imaging (NMRI); however, the nuclear part of the name was dropped in the 1970's due to the negative connotations it brought during the Cold War (Hornak, 2002). Both names are synonymous with the imaging technique used within this study.

MR imaging came about through a complex series of discoveries and inventions starting with the discovery of X-rays in 1895 by Wilhelm Roentgen. In the years to follow, scientists worked to prove and use this phenomenon for different applications. In 1942, I.I. Rabi invented the molecular beam magnetic resonance method. Despite this, the two men to whom credit is given for the discovery of NMR are Purcell and Bloch, who found NMR to be present in condensed matter in 1950. In the early 1970's Peter Mansfield and Paul C. Lauterbur made significant contributions to the field of NMR that later led to its use as a medical imaging device ("The 2003 Nobel Prize", 2003).

MR is an imaging technique based on spectroscopy. "Spectroscopy is the study of the interaction between electromagnetic radiation and matter". Signals are received from protons by putting them in a high magnetic field to cause them to spin. Then, RF pulses are emitted around the sample of increasing frequency. When the frequency of the RF pulse is the same as the frequency of the spinning proton, the proton receives the pulse and it temporarily has an increased energy. Once the field is off, the proton releases its energy and returns to its ground state. A receiving RF coil detects this and records that information. After an entire scan, all the information is processed to produce an image of the presence of protons in the sample. This produces a proton density image.

NMR's data acquisition is derived from the angular momentum of the nucleus of an atom. Many atoms exist in the samples that are imaged and the total angular momentum is composed of angular moments from individual nuclei. Atoms are induced by the strong magnetic field to spin. The entire quantity is referred to as "spin." As mentioned previously, we focused on changes in water content of our sponges, so MRI is a great technique to analyze it. Water, lipids and metabolites have large quantities of hydrogen, the proton spin clinical MRI uses to produce images (Lahti, 1998). For example, the hydrogen protons in water molecules have both a charge and a spin, which produces a nuclear magnetic moment vector (M). When these protons are placed within a magnetic field (B_0), their protons begin to rotate. The frequency of the protons will be equal to the frequency of the field, causing a transition between the two energy levels of the spin (Hornak, 2002). When an RF pulse (B_1) is applied at the same frequency as the magnetic field, the energy of the proton is raised to the higher of the two energy levels. The magnetic field created by the RF pulse rotates the nuclear moment vectors around the

horizontal axis. This pulse will eventually rotate M 90^{o} from the directional axis of B_0 onto the XY plane, shown Figure 12.

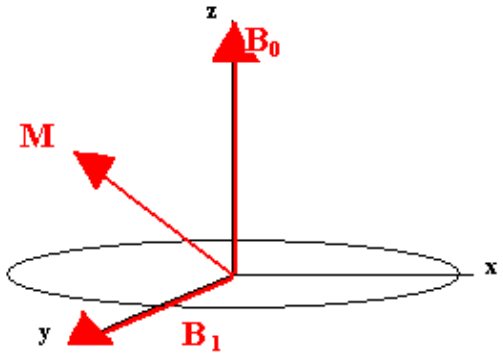


Figure 12: The nuclear moment vector with a magnetic field and an RF pulse

(Hornak, 2002)

When the RF pulse is removed, M will precess, or rotate about perpendicular to B_0 and return to its original energy level and spin, which can also be seen in Figure 12. This precession will have an angular frequency (ω) based on Equation 1:

Equation 1: Angular Frequency

Hornak, 2002

$$\omega_0 = \gamma B_0$$

The proportionality constant relating frequency and field is the ratio magnetogyric (gyromagnetic) ratios (γ), which depends on the charge to mass ratio of the nucleus.. This rotation perpendicular to the B_0 field, when occurring within the MR imager system, is what causes the magnetic resonance signal to be produced.

The RF coil is an integral part in producing the MR signal. This coil excites the protons into higher energy levels and causes the shift in orientation of the nuclear moment vector. The RF coil also plays an integral part in the resolution of images in smaller samples, such as the samples being used within this study. The smaller the sample, the smaller the field of view is going to be and the more likely it will be that

noise will play a significant role in distortion of the image. The useable volume within the coil should be maximized by the sample regardless of its size, but it particularly important for smaller samples. The extent to which the coil is filled by the sample is referred to as the filling factor. The greater the internal volume of the coil that the sample occupies, the better the signal-to-noise ratio will be and the better the image will be.

The radiofrequency coil produces a pulse at the same frequency, or resonance, of the magnet. This is done by tuning the coil when it is placed in the desired location within the magnet, just before imaging begins. There are different types of coils that can be used to maximize the resolution of the image depending on size and shape of the desired sample. The two main types are volume coils, which includes solenoid coils, and surface coils. A volume coil completely contains the sample, while a surface coil is rested on top of or underneath the sample during imaging.

"NMR imaging is based on a superposition of a linear magnetic field gradient on Bo to spatially encode the position of nuclei in the sample," (Lahti, 1998). Images can be created by applying linear magnetic field gradients to a sample. The signal that is then acquired from the RFC contains amplitude, frequency, and phase. That is all that is needed to create an image. Amplitude controls the image intensity, frequency controls the position in one dimension, and phase controls the position in a perpendicular dimension (Lahti, 1998). There are different types of pulse sequences that cater to the different material compositions of the sample, as well as the desired resolution and imaging time.

One pulse technique uses a multi-echo spin-echo sequence where multiple parts of the sample are recorded by different signals within the same repetition time (TR) period. This technique is useful for shortening the overall imaging time, producing

images in one fourth of the previously required time (Hornak, 2002). This fast spin-echo technique uses the k-space trajectory to illustrate the signal acquisition and image reconstruction strategies. K-Space is filled in the region of the field of view where MR signal is actively being sampled; however, it is still traversed even when data sampling is dormant (Bernstein et al, 2004). This k-space is divided into equal parts depending on the number of echoes in the sequence. Multi echoes will take a slice from each uniform section of the k-space and then repeat the process. This speeds up the imaging process a great deal. However, the sample needs to be large enough and divided into the proper amount of sections in order to ensure that RF pulses and acquisition do not interfere with one another.

Another pulse sequence that can be introduced to provide additional contrast in T1-weighted images is *inversion recovery* (IR). This pulse sequence employs an inversion pulse followed by a time delay and then an RF excitation pulse. The time delay is referred to the inversion time, or TI (Bernstein et al, 2004). An inversion on pulse followed by a time delay is used as the first half of an imaging sequence, and then a self-contained pulse sequence is employed as the second half. This second sequence can be any type of typical pulse sequences, such as RF spin-echo or gradient echo pulses, and is referred to as the host sequence (Bernstein et al, 2004).

2.9 Measurable parameters

The key to this project is being able to relate an MR image to the amount of degradation of a collagen sponge. Through the experiments in the project, enzymatic collagen degradation is going to be assessed with gold standard assays to determine how much collagen had degraded. The collagen will then be imaged with an MRI machine

and the relationship between the bench-top degradation assessment model and the MR image will be established. Once the relationship has developed between a known amount of degradation and the image produced, MRI can be used to assess unknown amounts of degradation non-destructively and non-invasively. In other words, the goal of the project is to find the amount of collagen degradation by means of only an MR image.

Many different techniques can possibly be employed to produce an MR image. Each technique has different applications, depending on its benefits and limitations. Some techniques are very specialized and can image very specific events. The different imaging techniques have to do with the order and method by which the software collects the data of the measurable parameters of MRI (the data the MRI machines measures). Some of the measurable parameters of an MRI are the T_1 relaxation time, the T_2 relaxation time, and the spin density.

2.9.1 T_1 relaxation time

The spin-lattice relaxation time (T_1) is the time to reduce the difference between the longitudinal magnetization (M_Z) and its equilibrium value by a factor of *e* (Hornak, 2002). In other words, the relaxation time T_1 is the time required for the *z* component of **M** to return to 63% of its original value following an RF excitation pulse. The term spin-lattice refers to the fact that the excited proton ("spin") transfers its energy to its surroundings ("lattice") rather than to another spin (Brown and Semelka, 1999). At equilibrium, the magnetization vector (M_o) lies only along in the direction (+Z direction) of the applied magnetic field of the MRI magnet as shown in Figure 13.

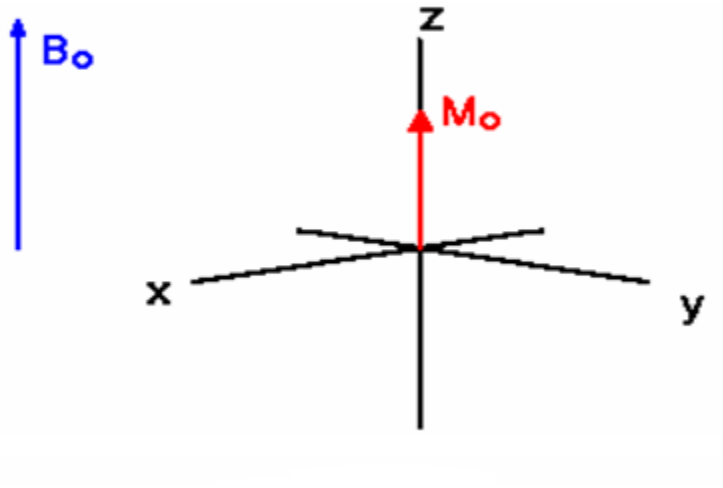


Figure 13: Magnetization vector of the MRI magnet

Hornak, 2002

By applying an RF pulse at a certain frequency from the radio frequency coil, enough energy can be applied to the protons to make the magnetization vector rotate from entirely aligned in the same direction of the applied magnetic field (B_o) to completely perpendicular to it (i.e., A 90° RF pulse). When the magnetization vector is perpendicular to the B_o field, no part of the vector is directed in the same direction as the field; therefore, the magnetization vector in the B_o- field direction is equal to zero (M_Z=0). Once the RF pulse field is turned off, the magnetization will return to equilibrium withr a certain time constant called spin-lattice relaxation time or T_1. Typical values for this time constant, T_1, in human tissue are hundreds of milliseconds. The equation below describes the relationship of T_1 to the magnetic field vector.

Equation 2: T_1 relationship to magnetic field vector

Hornak, 2002

$$M_z = M_o (1 - e^{-t/T_1})$$

2.9.2 T_2 Relaxation time

While T_1 relaxation time dealt with magnetization of protons in the same direction of the applied magnetic field, T_2 relaxation time is a measurable parameter dealing with the magnetization of a proton in a direction perpendicular to the applied magnetic field. The relaxation time T_2 is the time required for the transverse component of M to decay to 37% of its initial value via irreversible processes (Brown and Semelka, 1999). It is also known as spin-spin relaxation time because a proton ("spin") transfers its energy to another proton ("spin") rather than to the surrounding lattice.

If the net magnetization is placed in the XY plane, following a 90° RF pulse, it will rotate about the axis of the applied B_o field at a frequency equal to the resonance frequency of the proton. This frequency is called the Larmor frequency (Hornak, 2002). Each spinning proton rotates at its own Larmor frequency due to experiencing a slightly different magnetic field depending on its location within the bore of the MRI and its applied magnetic field. The net magnetization starts to become forced out of phase due to the difference in each proton's Larmor frequency; the more time that elapses, the more out of phase the net magnetization becomes.

The time constant associated with the decay of the transverse magnetization is called the spin-spin relaxation time or T_2. T_2 is always less than or equal to T_1. The equation that relates the T_2 relaxation time to the magnitude of the transverse magnetization is as follows:

Equation 3: T_2 relationship to magnetic field vector

Hornak, 2002

$$M_{XY} = M_{XY_0}\, e^{-t/T_2}$$

In summary, the spin-spin relaxation time, T_2, is the time to reduce the transverse magnetization by a factor of e. In the previous sequence, T_2 and T_1 processes are shown separately for clarity. That is, the magnetization vectors are shown filling the XY plane completely before growing back up along the Z-axis. Actually, both processes occur simultaneously with the only restriction being that T_2 is less than or equal to T_1 (Hornak, 2002).

Equation 4: T_1 and T_2 relation
Wehrli et al, 1988

$$T_1 = T_2 \text{ for pure liquids, while } T_2 < T_1 \text{ for biological samples}$$

2.9.3 Spin Density

Spin density is a very important parameter of MRI. Signal density is related to the concentration of the spinning protons in the sample being imaged. The more protons that there are in a given volume, the higher the spin density. Since MR images are generated from spinning protons, the higher the number of spinning protons within a volume, the more intense the image will be. Spin density is vital for image production, especially for pathological assessment and others that require a high contrast image. Tissue that is normal and healthy will have different properties than that of diseased tissue. For example, the density of hydrogen may be much different (usually higher) in the diseased tissue. Contrast in the imaging is needed to differentiate between the healthy and diseased tissue. When the healthy tissue has a different spin density than the diseased tissue, it will have a different image intensity and a pathological condition will be more apparent in the MR image.

2.10 MRI Techniques

2.10.1 Diffusion Measurements

Diffusion measurements assess the translation of molecules in the sample. It requires the use of a bipolar magnetic field gradient. A bipolar gradient pulse is one in which the gradient is turned on in one direction for a period of time then turned on in the opposite direction for an equivalent amount of time (Hornak, 2002). A bipolar gradient pulse has no net effect on a stationary proton. In this technique, any signal that is generated by a positive field will be cancelled out by a negative field and vice versa. If a proton is stationary at a specific location, its signal will be unaffected. However, if a molecule moves during the first half of the imaging process, then when the bipolar magnetic gradient is reversed, the spatial encoding imparted by the first gradient will not be removed. This will result in a slight cancellation of signal intensity when summed with the signal from other protons in the sample. The amount of signal attenuation is proportional to the diffusion coefficient of the molecules.

The diffusion measurement imaging technique is applicable to the project because as the collagen sponge degrades the water diffusivity though the sponges it will be affected. The thought is that as the sponge degrades, there is more room for water to pass through the sponge and therefore diffusion through it changes. It looks hopeful that an MRI diffusion measurement will produce images that show the difference in water diffusion for the sponges degraded different amounts. It is also hopeful that a correlation between the amount of diffusion and the amount of degradation of the sponge can be determined. Then an image of a sponge can be taken, the diffusion determined, and then the unknown amount of degradation can be derived from that image.

2.10.2 Signal Averaging

Signal averaging is a simple procedure that is applied to most MR images. The signal-to-noise ratio (SNR) of a tissue in an image is the ratio of the average signal for the tissue to the standard deviation of the noise in the background of the image. The signal-to-noise ratio may be improved by performing signal averaging (Hornak, 2002). Signal averaging is in essence a collection of many images of the same sample that are then averaged together. It improves the signal-to-noise ratio because signal received from the sample add with each image taken while the noise is partially cancelled out. Noise is averaged out because it is random and does not appear in the same location in each image while the sample does produce signal in the same location in each image. Averaging, therefore, reduces the noise without degrading the intended image. The improvement of the signal-to-noise ratio is proportional to the square root of the number of images averaged (also called N_{ex} as in the number of excitations). The equation below shows the relationship.

Equation 5: Signal-to-Noise relation to N_{ex}

Hornak, 2002

$$SNR \propto Nex^{1/2}$$

2.10.3 Magnetization Transfer Contrast

Magnetization transfer contrast is a new method of increasing the contrast between tissues by physical rather than chemical means. For this technique to be effective there must be at least two spin systems in the imaged anatomy that are capable of exchanging energy between themselves and one of the systems must have a much shorter T_2 than the other system. A saturation pulse is applied with a frequency

approximately 1 kHz from the center frequency. The saturation pulse is followed by a gradient-echo or spin-echo sequence (Hornak, 2002). A good way to understand the process is to think of the two separate spin systems as energy reservoirs. There is some spin system (referred to as X in Figure 14) whose reservoir is connected to a water energy reservoir and another independent water energy reservoir as shown in Figure 14.

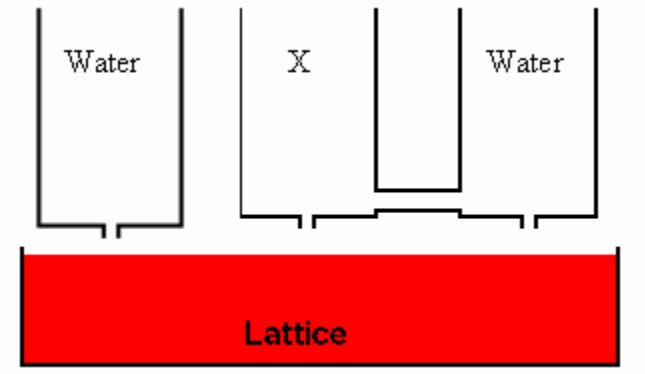


Figure 14: Spin System Reservoir

Hornak, 2002

Energy can be applied to any of the reservoirs and it will return to the surrounding molecules by means of spin-lattice relaxation. Spin system X can be energized with a frequency selective saturation pulse without directly energizing either water reservoir. However, the water reservoir connected to spin system X and is influenced by the energy in X. A pulse sequence is then used to measure the magnetization of the two water reservoirs while there is still energy in spin system X. The water connected to spin system X will produce an image as if a short TR was used and the water not connected to the spin system X will produce an image as if a long TR was used. This produces a contrast between the two types of water and therefore makes them differentiable in an MR image even thought their T_1 values are the same.

This technique is applicable for the project because there is water in the collagen sponge and there is water in the surrounding solution. If it is possible to generate an

image that will differentiate between the free water and the bound water (the water that is actually in the sponge material) then it may be possible to generate an image where degradation can be assess by analyzing the change in the amount of bound water.

2.10.4 Multislice imaging

Multislice imaging is a technique that speeds up the imaging process. One of the downfalls of MR imaging is the length of time it takes to create enough images for diagnostics purposes. When searching for pathological problems, the length of time for imaging is unacceptable if only one slice is acquired at a time. Multislicing helps remedy that problem. Figure 15 shows the timing of the imaging sequence and it is apparent that most of the imaging time during the TR interval is unused.

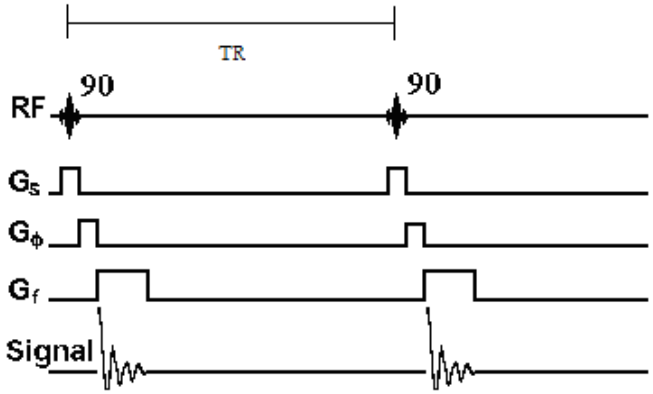


Figure 15: Timing of Imaging Sequence

Hornak, 2002

The multislicing technique employs that unused time by exciting other slices of the subject being imaged. The only constraint with the technique is that the excitation of one slice cannot affect the excitations of any other slice. This can be accomplished by applying one magnitude of the slice-selection gradient and then changing the frequency of the 90° RF pulses (Hornak, 2002). Each RF pulse has a different center frequency and does not overlap. This results in pulses that affect different slices of the subject. It is

anticipated that many images will need to be taken throughout the project due to a number of reasons. Additionally, time is a rather large factor in the project. The faster images can be produced, the more images that can be acquired. The more images acquired, the better the collagen degradation can be assessed. This all adds to the quality and accuracy of the data collected in this project.

2.11 Clinical Applications

MRI has become one of the most effective and important diagnostic devices in clinical medicine. Clinical MRI generally uses a 1.5 Tesla magnet to create a black and white three-dimensional view of the tissue of the body with higher contrast than CT scans are capable. Sometimes, tracers are injected into the blood to enhance the image and create greater contrast. MRI's are most often used for analyzing soft tissues, such as, tumors in the body, the brain, spinal cord, and internal organs in addition to injuries of the bones and joints. The primary quality that makes MRI the tool it is today is minimally invasive. It gives a very accurate view of the soft tissue inside the body without having to cut into it and look around or put a camera inside.

Tumors are commonly investigated using MRI. 3D MR imaging is well-suited for analyzing a tumor's size, shape, location, and depth into the tissue. Patients greatly appreciate the fact that a non-invasive MRI can be done to assess the extent of most tumors in order to avoid multiple painful biopsies. Besides that sometimes it is important to know what blood supply is being affected by the tumor and the MRI does all of this with minimal invasiveness. Additionally, once removed after surgery, MRI can again be used to see if any of the tumor is left and if blood supply has returned to normal. MRI has become an essential tool for diagnosing and treating tumors in recent years.

As mentioned before, the brain is commonly imaged using MRI. Abnormalities such as multiple sclerosis and countless other diseases are diagnosed with the assistance of MRI. MRI is used following stroke to analyze brain tissue that may have been damaged due to a lack of oxygen. A tracer can be injected into the blood stream and imaged in the brain. The tracer will show up bright white in an image as shown in Figure 16. Any changes in blood flow, such as clots or other threats of additionally strokes, will appear in the MRI and can be properly dealt with by the doctor.

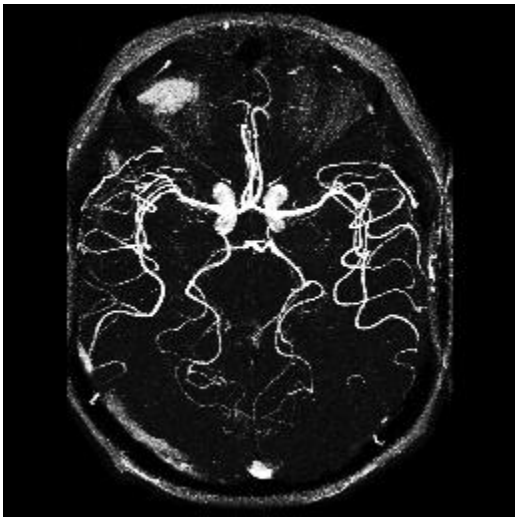


Figure 16: MR image with a tracer

Hornak, 2002

MRI's are also very helpful in curing epilepsy in some patients. In some cases of epilepsy, a surgical procedure can be done on the affected temporal lobe of the brain. However, whenever surgery is performed on the brain, there is a risk of permanent damage to surrounding tissue resulting in verbal memory defects. Therefore, it is necessary to identify and lateralize the seizure focus with a high degree of accuracy. These may be accomplished with magnetic resonance imaging. MRI demonstrates atrophy and/or high signal intensity in the affected hippocampus (Mukherji, 1998).

Another use of MRI is to non-invasively view and investigate soft tissue such as organs. The liver, kidneys, and spleen are some of the most common organs imaged. Damage to and functioning of each organ can be assessed with a great deal of accuracy. The heart requires imaging very often as well. The purpose is to look at the heart and the surrounding major blood vessels. MRI is very useful for recognizing and diagnosing heart defects. It also is used for recognizing changes in muscles thickness of the heart following a heart attack.

MRI's have become common in sports medicine as well. Injuries affecting the knee, shoulder, or any other joint often have to be imaged to assess the extent and type of injury. For example, torn ligaments in the knee are very common in football and basketball and frequently imaged by MR. The reason that they are so effective for diagnostic purposes in sports medicine is that when there is an injury to the soft tissue, there is acute hemorrhaging, which fills the interrupted portion of lower-intensity cartilage or ligament, at the site of the tear (Wehrli, 396). Tears to muscles, tendons, ligaments or other soft tissues show up bright white while the tissue itself is a dull gray.

Radio frequency coils are essential hardware for MRI. They vary greatly depending on what is being imaged. When the desired sampled to be imaged is close to the surface of the skin, a surface coil, shown in Figure 17 can be used to create the image.

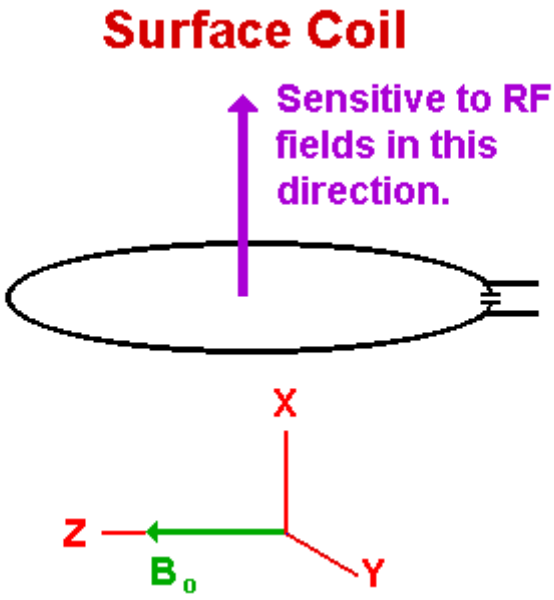


Figure 17: Surface coil

Hornak, 2002

However, it is not effective for imaging tissue deep inside the body. The magnetic field near the surface coil drops off rapidly as distance increases as shown in Figure 18.

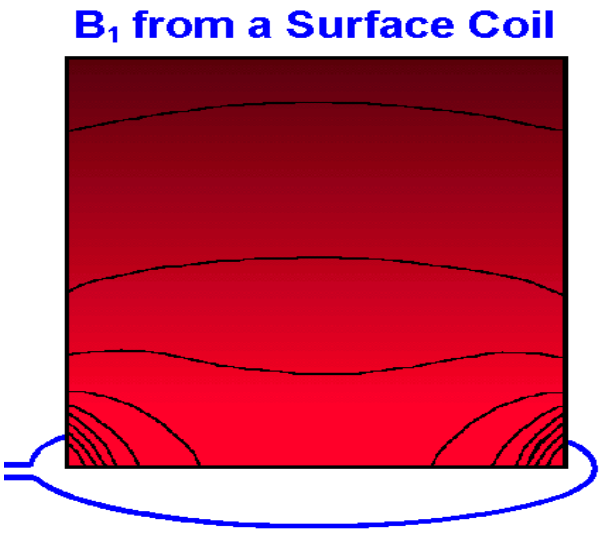


Figure 18: Magnetic field from surface coil

Hornak, 2002

Another type of radio frequency coil that is very common is a birdcage coil shown in Figure 19. A birdcage coil is a volume coil and actually contains whatever is being

imaged within its volume. It is commonly used for imaging the brain, but it is possible to image anything that will fit inside it. It is essential to maximize the filling factor, or to fill as much of the birdcage coil volume as possible. If that is not done, the signal-to-noise ratio of the image will be low.

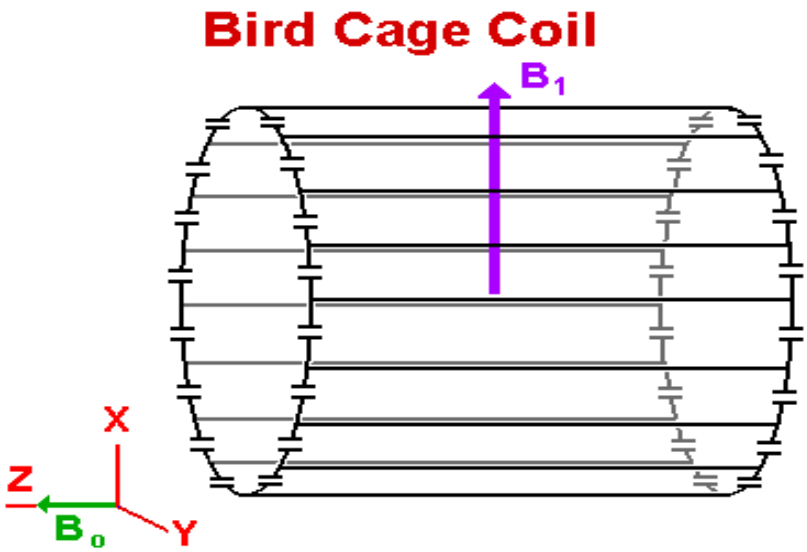


Figure 19: Birdcage coil

Hornak, 2002

One final common type of radio frequency coil is the solenoid coil. A solenoid is a coil of wire that is wrapped around the sample by multiple turns as shown below in Figure 20. It is similar to a birdcage coil in that it is a volume coil, but they both have different qualities. Volume coils have to be used with discretion, because since they encompass the sample, they also encompass the unfilled space around the sample. This reduces the filling factor, which also results in a lower quality image.

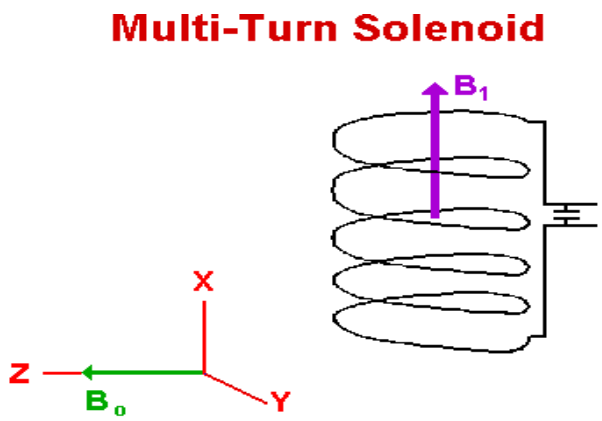


Figure 20: Multi-turn solenoid coil

Hornak, 2002

2.12 Current Research Applications

MRI has been used in research for a long time. There are many different types of research currently underway in addition to the countess number of experiments using MRI that have been in the past. To give a quick overview of some examples of MRI used in research, the Bioimaging Center at Yale School of medicine is currently researching on several different topics. They are using a 4.0 Tesla magnet to do research on diabetes, epilepsy, depressive disorders, and the metabolic control of brain energy and neurotransmitter metabolism. They are also using at 7.0 Tesla MRI to image and research the metabolic control of brain energy and neurotransmitter metabolism (Magnetic Resonance Resource Center, 2003).

All of that research is on very important topics in the medical field. However, some current research has been particularly interesting. Nieminen et al wrote a journal article in 2004 about their research using MR microscopy to analyze quantitatively the enzymatic-degraded cartilage. This analogous project provides much helpful information about many subjects that go hand-in-hand with our project. The difference between the two is that the Yale group used MR microscopy while we will be looking at lower-

resolution images. Additionally, they enzymatically degraded the collagen as a part of cartilage; we will be degrading pure collagen and results will differ. Their research team feels that MRI may provide a noninvasive quantitative technique for the evaluation of cartilage degeneration at the early stages of disease process. It would therefore be advantageous to identify MR parameters that could sensitively detect changes in cartilage structure (Nieminen et al, 2000). Part of our project is identifying the MR parameters that sensitively detect changes in collagen degradation. This is very similar to their project because collagen makes up much of cartilage. In their project, they found a statistical significant increase in the cartilage T_2 after a 44-hour collagenase treatment (Nieminen et al, 2000). These results suggest that MRI also has the ability to assess the extent of degradation of a collagen sponge. Additionally, a collagen sponge will have greater diffusion properties and possibly less crosslinkage and therefore would need a much shorter period of degradation than the 44-hour period needed for the cartilage.

In summary, due to recent research using MRI to assess enzymatic cartilage degradation, the group feels that it is likely that we will have success in our project. We feel that at the least, if not a better imaging technique, we can obtain and image using T_2 weighting that will allow us to assess the degradation of the collage sponge.

3 PROJECT APPROACH

Collagen biodegradation rates are an important consideration for the design of many medical devices and implants. The methods available to biomaterial researchers for measuring the degradation rates are resource intensive, creating a potential rate-limiting factor in the development of collagen devices. The majority of these methods require destructive testing, preventing repetitive long-term studies on the same sample. MRI presents a possible means to measure the extent of degradation of a collagen sample nondestructively.

3.1 Hypotheses

Enzymatic degradation of collagen sponges will alter the matrix to affect the behavior of water molecules within the matrix. It will do this because as the collagen degrades through bulk degradation, pore size will increase. In the experiments, the pores will be filled with water. There will be unbound water or free water in the pores as well as bound water that is molecularly bounded to the collagen. This change in water behavior will be detected by a change in MRI parameters that are sensitive to bound and unbound water. It will be possible to determine the extent of degradation of the collagen sponge by customizing and optimizing the MR imaging technique to be as sensitive as possible to the particular changes caused by enzymatic degradation. Multiple degradation-extent measurements of the same sponge, taken during the lifespan of the sponge in a proteolytic solution, will lead to possible kinetic modeling of the degradation process.

3.2 Assumptions

The first assumption made is that current, destructive methods for measuring the extent of degradation in collagen sponges can be replaced with an MRI technique. MRI has undergone significant development and has proven its usefulness in numerous applications due to its noninvasive nature and sensitivity to small changes in behavior of water molecules.

The second assumption is that bovine tendon collagen is a suitable collagen source for this project. It provides an appropriate substrate for collagenase enzyme activity and is representative of collagen derived from different sources that are used in various types of implants and devices. This is because it is comprised of type-I collagen which is the most common type and undergoes similar processing.

The third assumption is that the sponges manufactured for this project are homogenous in their composition and pore structure and contain insignificant amounts of impurities. The pore structure will allow for complete hydration of the sponge and for dissolved particles to move freely throughout the sponge. This will allow the enzyme to penetrate the sponge completely and cause uniform bulk degradation throughout sponge, rather than surface degradation.

The fourth assumption is that the various crosslinking techniques that the sponges may undergo will only decrease the rate of degradation in experiments with constant collagenase activity. Any other changes in the sponge caused by crosslinking will not be relevant to this project nor will they affect the results in any way.

The fifth assumption is that the bacterial collagenase enzymes will alter the collagen matrix in a manner that changes the water binding and diffusion throughout the matrix. It is believed that as collagenase degrades the collagen sponge, the hydrated

sponge will have larger pore size and therefore more room for water to reside. Additionally, as collagenase cleaves collagen molecules, water that used to be bound to the molecules become unbound or free water within the sponge.

The sixth assumption is that MRI can detect the change in water behavior through a particular imaging technique, such as T_1-weigthed imaging, T_2-weighted imaging or diffusion measurement. As we image sponges with different amounts of degradation, they should have different amounts of bound and unbound water. T_1 or T_2 imaging is sensitive to bound and unbound water. The prediction is that images using those techniques will show the change in bound water, and therefore the change in the collagen structure due to degradation. Diffusion imaging shows water diffusing through a sample over time. It is assumed that the rate of diffusion will increase as the amount of degradation increases. Therefore, we assume diffusion weighted imaging could be used to assess degradation.

The last assumption made for this project is that the particular imaging techniques used can be performed within a sufficiently small time frame such that the degradation that occurs during imaging is insignificant. It will not be possible to halt the degradation to imaging the sponge and then resume enzyme activity. This will allow discrete data points to be collected with which to model the degradation.

3.3 Specific Aims

The result of this project will be a MRI and benchtop protocol that can be used to determine the extent of degradation in a collagen sponge and a collagen holder in which multiple sponges can be imaged simultaneously. The protocol will be suitable for collagen sponges at all stages of degradation and manufactured from all types and

sources of collagen. It will also be able to determine the degradation extent of crosslinked sponges. The data collected using this imaging protocol will be appropriate for the modeling of the degradation kinetics of various implant and devices.

The collagen holder will allow sponges to be imaged using a single radiofrequency coil. The holder will keep the sponges hydrated during the imaging to prevent water loss from affecting the results and to maintain the sponge state.

4 DESIGN

The following is the process the team used to create our collagen holder and radiofrequency coil prototypes. The justifications for the final design choice reflect the team's knowledge of the background literature and input from the two advising research scientists.

4.1 Stakeholders

A stakeholder is any individual or group that can affect or is affected by an organization's activities. For example, a stakeholder could be a local community who want to know that a factory will not be releasing harmful pollutants, consumers who want product information, or investors who wish to see a company prosper. In this project the primary stakeholders identified are the biomaterials research and development scientists, the Food and Drug Administration (FDA) and the designers. Also, other less obvious stakeholders need to be taken into account prior to designing the collagen sponge holder and radio frequency coil. Health insurance companies, physicians and patients will also be affected by this design.

4.1.1 Biomaterial Research and Development Scientists

Research and development (R+D) scientists are concerned with a number of factors of the design of the collagen sponge holder and radio frequency coil. These scientists want a safe product that produces accurate degradation measurements. Safety is important for their researchers using the data or design as well as future stakeholders like manufacturing companies because they want to produce a safe product that performs very well. This will create better sales profits without the risk of selling unsafe products.

The accuracy of the degradation is crucial; if there is no correlation between bench top and MRI measurements then the design cannot be used in the future. The biomaterial scientists also want it to be easily marketable, is easy to use, and compatible with the current methods and equipment they are using. The design should also be inexpensive and promote efficient use of time by analyzing several sponges at the same time, as time is money in the business world.

Biomaterials research and development scientists are ranked the most important stakeholders in this design. As seen in Table 6, later at the conclusion of the stakeholder's section, they hold 45% of the weight in the *in vitro* section of this project and 35% in the *in vivo* section of this project.

4.1.2 Food and Drug Administration (FDA)

The FDA's primary concern with any medically related design is safety. The biomaterials scientists that may be using this method to characterize collagen sponge degradation will have to prove to the FDA that the data obtained is accurate and that the collagen sponge is still safe to use following degradation readings using the MRI. The FDA will need evidence proving that this technique for measuring sponge degradation is accurate, in that it is compatible with data from gold standard techniques such as histology. The FDA is also concerned with the animals used in the Phase II testing of this project, the in vivo experiments using rats.

The FDA was ranked high in comparison to many of the other stakeholders that were taken into consideration for this project. As seen in Table 6, the FDA holds 20% of the weight in the design for the *in vitro* system and 30% in the design of the *in vivo* system.

4.1.3 Designers

The designer's goal is to design a device that will encompass all of the stakeholders' objectives in a realistic fashion. The designer's concerns are cost, accuracy, and safety. The stakeholders' objectives are important in the creation of the device. However, if funding is not available, or the safety of the designers is put at risk, then the device may not be created. For this reason, the designer holds 25% of the weight of the stakeholders in both the *in vitro* and *in vivo* phases. These weights are show in comparison to the other stakeholders in Table 6 at the conclusion of the stakeholders' section.

4.1.4 Health Insurance Companies

Insurance companies are concerned with the products safety as well as the cost of the collagen sponge implant and the consumer's confidence in the sponge implant. If the collagen sponge was implanted into a patient, assuming a certain rate of degradation in the body, and the sponge did not degrade as predicted, then further expenses would result. The insurance company is most successful when the device performs as it should, and the physician does not have to re-implant the sponge or need unnecessary follow-up visits due to sponge degradation problems.

Health insurance companies received 6% of the total stakeholders' weight (100%) in both the *in vitro* and *in vivo* phases (Table 6). The reason this percentage is small in comparison to the biomaterials scientists, the FDA, or the designer is that the device has a long way to go before this method is used in determining sponge degradation for sponges used in a clinical setting. The weight of the health insurance companies will increase the

when this method reaches later phases, such as use by biomaterials research and development scientists.

4.1.5 Physicians

Physicians are minor stakeholders at the current stage of the creation of this method for characterizing sponge degradation. However, their importance will only grow as other stakeholders such as the FDA and biomaterial R+D scientists adapt the method. Physicians are concerned with the accuracy of the collagen sponge (knowing exactly how it degrades based on the MRI image), the quality of the implant (to limit future patient visits due to sponge malfunctions), and the cost of the sponge.

When applying a sponge to a patient, the physician needs to be sure that he is not harming the patient and that the sponge will do exactly what the manufacturer says it will. For this reason, physicians are stakeholders in the development of the collagen sponge holder and radio frequency coil in this project. Physicians receive 2% of the total weight of the stakeholders in both of the phases (Table 6). Similar to the health insurance companies, physicians' weight is not largely significant in this design process due to the early stages of the design and testing.

4.1.6 Patients

Patients are concerned with how the collagen sponge implant will function. Patients, or families of patients, will most likely desire to know the quality of the device and the evidence to back up the product being used. Patients want to be safe and will want a sponge that degrades precisely as it is meant to. Patients also received 2% of the total weight of the stakeholders (Table 6) for both the *in vitro* and *in vivo* tests.

4.1.7 Stakeholders Weights

As this method to characterize sponge degradation proceeds in different phases, the weights of the stakeholders will continuously change. In this project, Phase I (*in vitro* degradation of the sponges) and Phase II (*in vivo* degradation) are of relevance. Table 6 weights the stakeholders in comparison to one another. We arrived at these weights after thorough discussion with our advisors and after many drafts and changes. These weights will later be used when determining the design of the collagen sponge holder and radio frequency coil.

Table 6: Stakeholders Weights in 2-phase Design Process

	In vitro	In vivo
	Phase I	Phase II
Health Insurance Company	6%	6%
Food and Drug Administration	20%	30%
Biomaterial Research and Development Scientist	45%	35%
Designer	25%	25%
Physician	2%	2%
Patient	2%	2%

4.2 Defining the Problem

The objectives, constraints, and functions of the design of the collagen holder and radio frequency coil need to be taken into account to fulfill the needs of each of our stakeholders. We used these lists to then define our problem and propose a purpose statement for this project.

4.2.1 Objectives, Constraints, and Functions

Since the stakeholders have been defined, it is necessary to think of their concerns and create a common list of objectives, constraints, and functions the device must accommodate for the device to be successfully designed.

4.2.1.1 Objectives

Objectives are the goals or desired attributes of the design. The collagen sponge holder and radio frequency coil should be:

- Safe to use
- Inexpensive
    - Durable
    - Able to accommodate multiple samples
- Easy to use
    - Easy to clean
    - Able to house the radio frequency coil
    - Portable
    - Able to connect to current setup
    - Easy to calibrate
- Able to produce high quality data
    - Reliable
    - Able to maintain experimental conditions
    - Able to maintain collagen state
        - Able to maintain temperature
        - Able to maintain physical state
        - Able to maintain chemical state
    - Stable
    - Accurate
        - Able to provide adequate resolution
        - Able to provide a high filling factor
    - Reproducible
    - Able to visualize the samples

4.2.1.2 Constraints

Constraints of a design are restrictions or limitations that the design must meet to be acceptable. The constraints for this project are that the material used in the design must be non-magnetic in order to not interfere with the imaging the collagen sponges using the MRI. Additionally, the design must not be larger then the 14.5cm diameter of the MRI bore, the samples should be placed at a height of 7.25cm, the size of the wells of the collagen sponge holder must not be smaller then the collagen sponges and the design must cost less then $600.

4.2.1.3 Functions

Functions are what a successful design must do when created. The collagen holder and radio frequency coil must keep the sponge hydrated during imaging. The holder needs to provide separation between the sponges and hold the collagen samples at the center of the MRI bore (approximately 7cm above the bottom). The radio frequency coil must resonate the sample(s) at the magnet's frequency, it should excite the entire sample homogenously, should attach the radio frequency coil to the collagen holder, and the design should achieve the best resolution image possible.

4.2.2 Purpose Statement

After further defining the problem, the following revised client statement was created:

> *The goal of this project is to design and develop a noninvasive imaging protocol to correlate implant biodegradation with quantitative gold standard experimental data. To do this, we will need to design a suitable collagen holder and radio frequency coil. This design should be safe to use, inexpensive, easy to use, and able to produce high quality data. Generating a design that is inexpensive should be accomplished by making it durable and able to accommodate multiple samples. The design should be easy to clean, able to house the radio frequency coil, portable, able to connect to current setup, and easy to be calibrated. It should also be reliable, able to maintain experimental conditions, able to maintain collagen state, stable, accurate, reproducible, and able to visualize the samples. The collagen holder and radio frequency coil should keep the sponge hydrated, provide separation between samples, hold the collagen samples 7.25cm above the base of the MRI bore, resonate at the Larmor Frequency of the MRI magnet, excite the entire sample homogenously, attach the radio frequency coil to the collagen coil, and achieve the best resolution image possible. In order to perform these functions, the design should: not be magnetic, not be larger then the 14.5cm diameter of the MRI bore, be placed at a height of 7.25cm, have wells that have a larger diameter then the width of the sponges they hold, and cost less then $600.*

4.3 Needs Analysis

The interests of each stakeholder were used to create the list of objectives we, the designers, felt represented the desired outcomes the device should embody. These stakeholders have considerable influence over whether the device ultimately meets the needs of the clients. Their interests in the device were carefully considered and evaluated based on the amount of possible use each group would see in regards to the final design. The final groups of stakeholders considered for this collagen holder and radio frequency coil includes research and development (R & D) scientists, the Insurance Company, the Food and Drug Administration (FDA), the designers, the patients and the physicians.

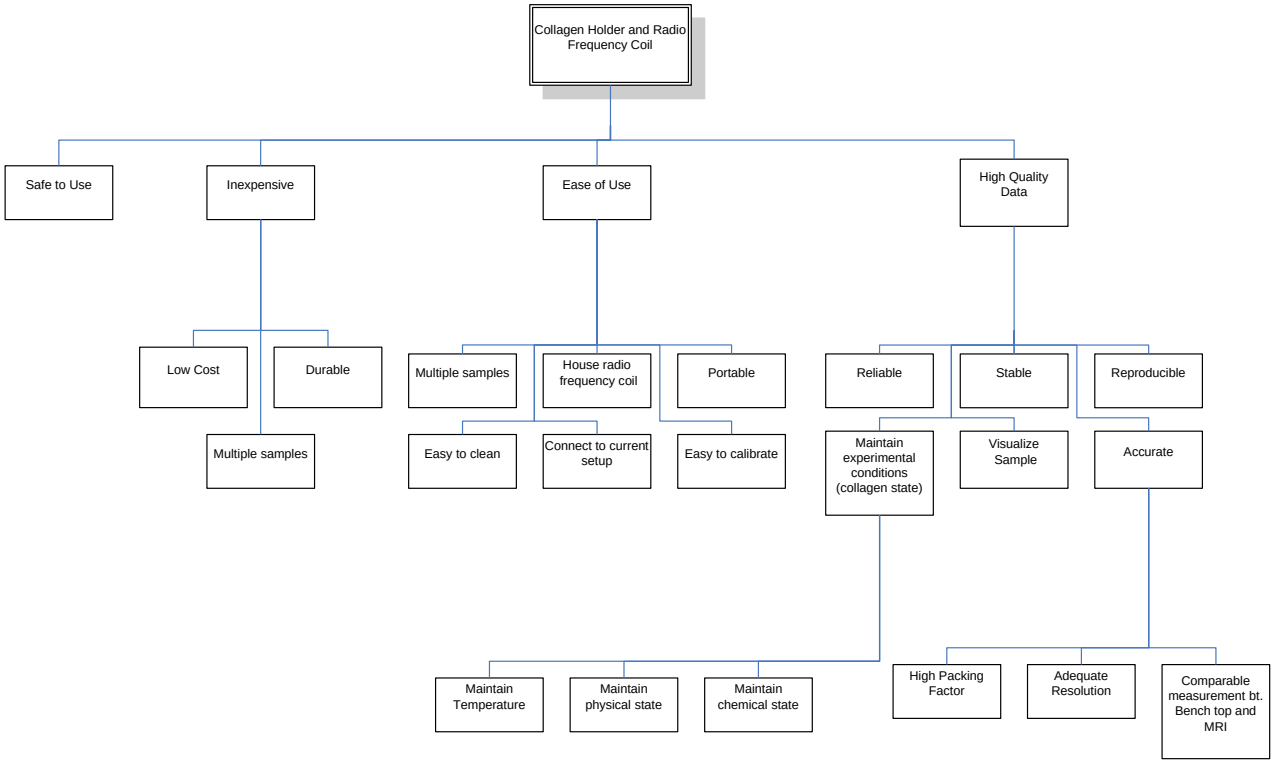


Figure 21: Unweighted objectives tree

To determine the concerns of each stakeholder, a list of possible objectives, constraints and functions was compiled. The objectives were arranged based on their common characteristics in the unweighted objectives tree in Figure 21.

The level 1 objectives include ease of use of the device, the cost (Inexpensive) associated with the device, its safety while in use and the quality of data (High Quality Data) produced by the entire system. The safety of the device relates to the device being handled by researchers, as well as the safety of any living specimens being contained within the device. This means no sharp edges or corners, no spring-loaded mechanisms that can catch hands and no materials that are toxic to animals and humans.

Inexpensive is broken down into three level 2 objectives: low cost, durable and multiple samples. Low cost is defined by this design team as the cost of building and testing our prototype, as well as the final estimated cost to R & D companies to purchase the device when on the market. The durability of the device relates to the number of times samples can be imaged successfully within the setup, how well the device can survive the vibrations from within the MRI magnetic, or a fall from the height of a typical bench-top in a lab.

The aim of the Multiple Samples objective is to reduce the cost of man-hours spent preparing and imaging the samples. It would also result in collecting much more data to model the sponge degradation. Multiple Data points also reduces cost of operating the MRI by getting the desired model using less imaging.

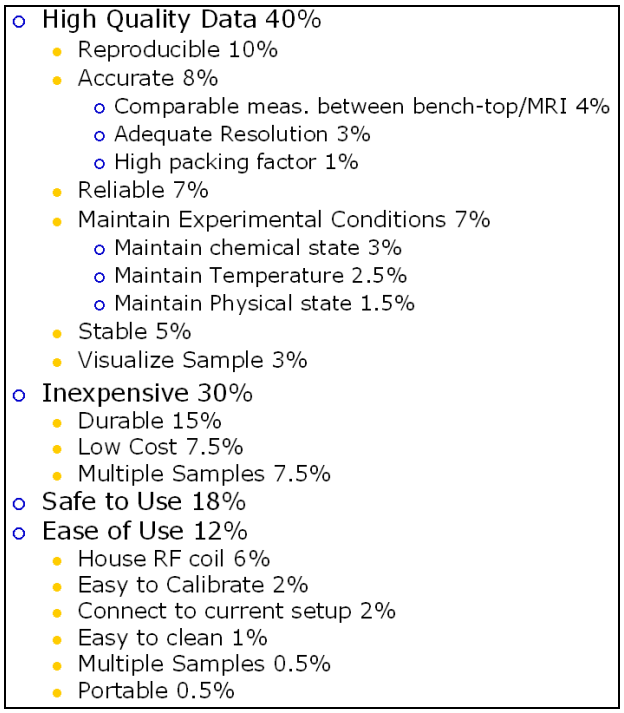


Figure 22: Objectives, sub-objectives with weights

Ease of Use is broken down into six, level-2 objectives including multiple samples, portable, easy to clean, connect to current set-up, easy to calibrate and house radio frequency coil. The multiple samples sub-objective named here is the same as stated above. The portability of the device translates to the size and weight of the entire system. Connect to current set-up addresses the number of adaptors needed to interface our device with different MRI systems. Easy to calibrate refers to the difficulty of tuning the coil's frequency to that of the magnet.

High quality data is broken down into six, level-2 objectives as well and they are as follows: reliability of the data, stability of the device in the magnet bore, reproducibility of the data, ability to visualize the sample within the device, accuracy of the data and the device's ability to maintain the experimental conditions (see Figure 22). Accuracy has three, level-3 objectives including high filling factor of the sample and coil, adequate resolution of the images and comparable measurements between the bench-top

experiments and MRI. Maintaining experimental conditions is also broken down into three, level-3 objectives involving maintaining the physical, chemical and thermal states of the sample.

4.3.1 Pairwise Comparison Charts

In order to organize, rank and weight these objectives, the design team created pairwise comparison charts (PCCs) for each stakeholder and each level of objectives. All of the level-1 objectives, or primary objectives, were ranked against each other in the following manor. Each objective was compared against all the others across a row. If that objective was considered more important than another was, it received a score of 1. If it was not considered more important than the objective it was being compared to, it received a score of 0 (see Table 7). This process was repeated for each stakeholder for all three levels of objectives. The weight of each stakeholder was taken into consideration when rating the objectives. Then the six stakeholder PCC's were combined to create the master PCC for each level. This was done by adding the scores given to an objective by each stakeholder within each cell of the chart taking into account the weight of the specific stakeholders, as seen in Table 8 for level 2 and Table 9 for level 3.

Table 7: Level 1 Pairwise Comparison Chart

	Safe to use	Ease of use	Inexpensive	High quality data	Total	Weighted score
Safe to use	X	0+1+1+1+0+1	0+0+1+1+0+0	0+0+1+1+0+0	8	1.05
Ease of use	1+0+0+0+1+0	X	0+0+1+0+1+0	0+0+0+0+0+0	4	0.69
Inexpensive	1+1+0+0+1+1	1+1+0+1+0+1	X	0+1+0+1+0+0	10	1.83
High quality data	1+1+0+0+1+1	1+1+1+1+1+1	1+0+1+0+1+1	X	14	2.43

Table 8: Level 2 pairwise comparison

Goal	Low Cost	Multiple Samples	Durable	Reliable	Experi-mental Conditions	Stable	Accurate	Reproducible	Visualize Sample	Easy to Clean	House Radio Frequency Coil	Portable	Connect to Current Set Up	Easy to Calibrate	Totals	Weighted Score	Normalized
Low Cost	X	1+1+0+1+0+0	0+0+0+1+0+0	0+0+0+0+0+0	0+0+0+0+0+0	0.5+0.5+0+0+0+0	0+0+0+0+0+0	0+0+0+0+0+0	0+1+1+1+0.5+0.5	1+1+1+1+1+1	0+0+0+0+0+0	1+1+1+1+1+1	0+0+0+0+0+0	1+0+0+0+0+0	**22**	4	5
Multiple Samples	0+0+1+0+1+1	X	0+1+0+0+1+1	0+0+0+0+0+0	0+0+0+0+0+0	0+0+0+0+1+1	0+0+0+0+0+0	0+0+0+0+0+0	0+1+1+0+1+1	0+0+0+0+1+1	0+0+0+0+0+0	1+0+1+0+1+1	0+0+1+0+0+0	0+0+1+0+0+0	**20**	2	2
Durable	1+1+1+0+1+1	1+0+1+1+0+0	X	0+0+0+0+0+0	0+0+0+0+0+0	0+0+1+1+1+1	0+0+0+0+0+0	0+0+0+0+0+0	1+1+1+1+1+1	0+0+1+0+0+0	0+0+0+0+0+0	1+0+1+1+1+1	0+0+0+0+0+0	0+0+1+0+0+0	**25**	4	5
Reliable	1+1+1+1+1+1	1+1+1+1+1+1	1+1+1+1+1+1	X	1+0+1+1+1+1	1+1+1+1+1+1	1+0+0.5+0.5+0.5+0.5	0+0+0.5+0.5+0.5+0.54	1+1+1+1+1+1	1+1+1+1+1+1	0+0+0+0+0+0	1+1+1+1+1+1	1+0+1+0.5+1+1	1+0+1+0.5+1+1	**61**	10	11
Experi-mental Conditions	1+1+1+1+1+1	1+1+1+1+1+1	1+1+1+1+1+1	0+1+0+0+0+0	X	1+0+1+1+1+1	0+1+0+0+1+1	0+0+0+0+0+0	1+1+1+1+1+1	1+1+1+1+1+1	0+0+0+0+0+0	1+1+1+1+1+1	0+0+1+1+1+1	1+0+1+0+1+1	**53**	8	9
Stable	0.5+0.5+1+1+1+1	1+1+1+1+0+0	1+1+0+0+0+0	0+0+0+0+0+0	0+1+0+0+0+0	X	0+0+0+0+0+0	0+0+0+0+0+0	1+1+1+1+1+1	0+1+1+0+0+0	0+0+0+0+0+0	0+1+1+0+1+1	0+0+1+0+0+0	0+0+0+0+0+0	**25**	5	5
Accurate	1+1+1+1+1+1	1+1+1+1+1+1	1+1+1+1+1+1	0+1+0.5+0.5+0.5+0.5	1+0+1+1+0+0	1+1+1+1+1+1	X	0+0+0.5+0.5+0.5+0.5	1+1+1+1+1+1	1+1+1+1+1+1	0+0+0+0+0+0	1+1+1+1+1+1	1+1+1+1+1+1	1+1+1+1+1+1	**62**	10	11
Reproducible	1+1+1+1+1+1	1+1+1+1+1+1	1+1+1+1+1+1	1+1+0.5+0.5+0.5+0.5	1+1+1+1+1+1	1+1+1+1+1+1	1+1+0.5+0.5+0.5+0.5	X	1+1+1+1+1+1	1+1+1+1+1+1	0+0+0+0+0+0	1+1+1+1+1+1	1+1+1+1+1+1	1+1+1+1+1+1	**68**	12	13
Visualize Sample	1+0+0+0+0.5+0.5	1+0+0+1+0+0	0+0+0+0+0+0	0+0+0+0+0+0	0+0+0+0+0+0	0+0+0+0+0+0	0+0+0+0+0+0	0+0+0+0+0+0	X	0+0+0+0+0+0	0+0+0+0+0+0	0+0+0+0+0+0	0+0+0+0+0+0	0+0+0+0+0+0	**4**	1	1
Easy to Clean	0+0+0+0+0+0	1+1+1+1+0+0	1+1+0+1+1+1	0+0+0+0+0+0	0+0+0+0+0+0	1+0+0+1+1+1	0+0+0+0+0+0	0+0+0+0+0+0	1+1+1+1+1+1	X	0+0+0+0+0+0	1+1+1+1+1+1	0+0+1+0+0+0	0+0+0+0+0+0	**26**	5	5
House Radio Frequency Coil	1+1+1+1+1+1	1+1+1+1+1+1	1+1+1+1+1+1	1+1+1+1+1+1	1+1+1+1+1+1	1+1+1+1+1+1	1+1+1+1+1+1	1+1+1+1+1+1	1+1+1+1+1+1	1+1+1+1+1+1	X	1+1+1+1+1+1	1+1+1+1+1+1	1+1+1+1+1+1	**78**	13	14
Portable	0+0+0+0+0+0	0+1+0+1+0+0	0+1+0+0+0+0	0+0+0+0+0+0	0+0+0+0+0+0	1+0+0+1+0+0	0+0+0+0+0+0	0+0+0+0+0+0	1+1+1+1+1+1	0+0+0+0+0+0	0+0+0+0+0+0	X	0+0+0+0+0+0	0+0+0+0+0+0	**11**	2	2
Connect to Current Set Up	1+1+1+1+1+1	1+1+0+1+1+1	1+1+1+1+1+1	0+1+0+0.5+0+0	1+1+0+0+0+0	1+1+0+1+1+1	0+0+0+0+0+0	0+0+0+0+0+0	1+1+1+1+1+1	1+1+0+1+1+1	0+0+0+0+0+0	1+1+1+1+1+1	X	1+0+0+1+0+0	**44.5**	8	9
Easy to Calibrate	0+1+1+1+1+1	1+1+0+1+1+1	1+1+0+1+1+1	0+1+0+0.5+0+0	0+1+0+1+0+0	1+1+1+1+1+1	0+0+0+0+0+0	0+0+0+0+0+0	1+1+1+1+1+1	1+1+1+1+1+1	0+0+0+0+0+0	1+1+1+1+1+1	0+1+1+0+1+1	X	**46.5**	7	8

Table 9: Level 3 pairwise comparison chart

	Maintain Temp	Maintain physical state	Maintain chemical state	High Packing Factor	Adequate Resolution	Comparable measurement between MRI/ bench top	Total	Weighted Score	Normal-ized
Maintain Temp	X	1+1+0+1+1+0	0+0+0+0+0+0	1+0+0+1+1+1	0+0+0+0+0+0	0+0+0+0+0+0	8	1.33	8.866667
Maintain physical state	0+0+1+0+0+1	X	0+0+0+0+0+0	1+1+0+1+1+1	0+0+0+0+0+0	0+0+0+0+0+0	7	1.02	6.8
Maintain chemical state	1+1+1+1+1+1	1+1+1+1+1+1	X	1+1+0+1+1+1	0+1+0+0+0+0	0+0+0+0+0+0	18	3.05	20.33333
High Packing Factor	0+1+1+0+0+0	0+0+1+0+0+0	0+0+1+0+0+0	X	0+0+0+0+0+0	0+0+0+0+0+0	4	0.85	5.666667
Adequate Resolution	1+1+1+1+1+1	1+1+1+1+1+1	1+0+1+1+1+1	1+1+1+1+1+1	X	0+0+0+0+0+0	23	3.75	25
Comparable measurement between MRI/ bench top	1+1+1+1+1+1	1+1+1+1+1+1	1+1+1+1+1+1	1+1+1+1+1+1	1+1+1+1+1+1	X	30	5	33.33333

The scores were then added up across a row to give the objective its total score given by all the stakeholders. The total score was then multiplied by the stakeholder's weight to obtain a weighted score. The weighted scores from each stakeholder were then added to give a total score for that objective. Finally, the weighted scores were normalized. The normalized score represents the percentage of 100% for each level that an objective receives. This score is then used in the Weighted Objectives Tree in Figure 23.

The weighted objectives tree shows a set of numbers for each objective. The number on the right represents the score out of the initial 100% for each level an objective received based on the pairwise comparison charts. The left hand number represents what percentage of the higher-level objective's score that sub-objective received.

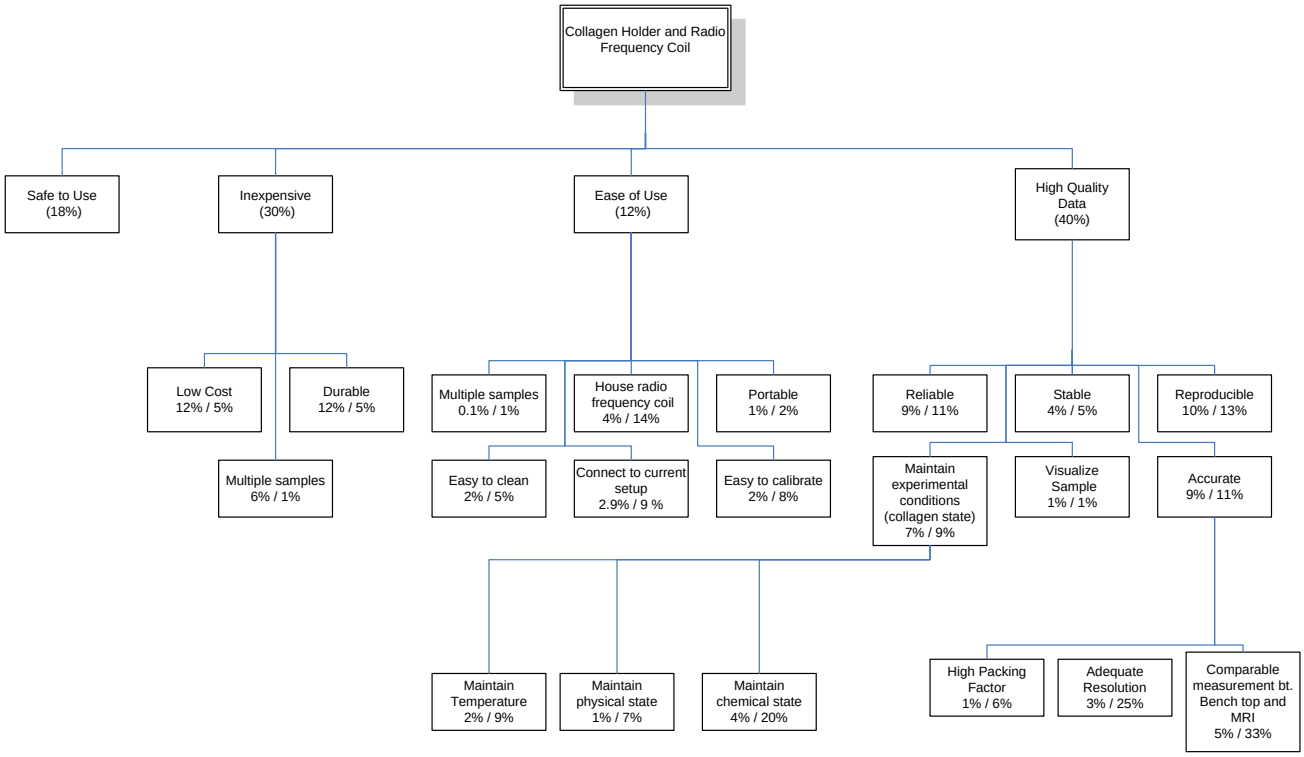


Figure 23: Weighted objectives tree

In order to determine what percentage of a level-1 objective's weighted score a sub-objective received, the PCCs were consulted. For example, when dividing the weight of a primary objective such as inexpensive, the normalized scores of low cost, durable and multiple samples were added together to created a total. Then to find what percentage of inexpensive's score each one received their normalized scores were each divided by this total, which gave us the percentage of inexpensive's weight each would receive. Low cost and durable both scored equally, so they both received 12% each adding up to 24 of a possible 30%. Multiple samples received a lower percentage than the other two, so it received the remaining 6%, making all three sub-objectives equal Inexpesive's 30% (see Figure 24). When necessary, adjustments were made to the final scores in order to add up to the proper level one objective's weight.

Normalized Score Total:

$5 + 2 + 5 = 12$

Finding percentages for each sub-objective:

Low Cost - $5 \div 12 = 0.41667$

Multiple Samples - $2 \div 12 = 0.1667$

Durable - $5 \div 12 = 0.41667$

Finding percentage of Inexpensive's weight:

Low Cost - $30 \times 0.41 = 12.3 = 12$

Multiple Samples - $30 \times 0.1667 = 5.0 = 6$

Durable - $30 \times 0.41 = 12.3 = 12$

Goal	...	Totals	Weighted Score	Normalized
Low Cost	...	22	4	5
Multiple Samples	...	20	2	2
Durable	...	25	4	5

Figure 24: Calculations for obtaining left-hand weights

4.3.2 Functions-Means Tree

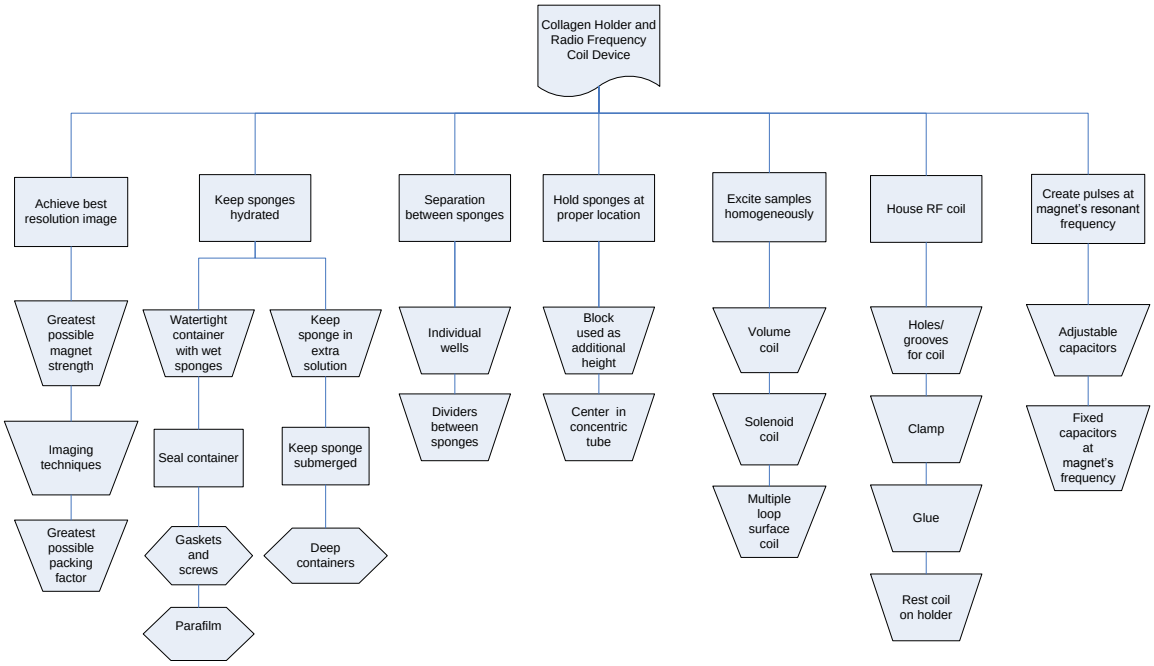


Figure 25: Functions-means tree

When the objectives for the device have been clearly outlined and weighted, the team moved on to determining what functions the designs would need to perform. There

are seven different objectives relevant to the device functioning in a manner suitable for this study. They are as follows: achieve best image resolution, keep sponges hydrated, maintain separation between sponges, hold sponges in the proper location, excite samples homogeneously, house RF coil and create pulses at the magnet's resonant frequency.

The tree outlines not only the desired functions of this device, but the feasible means of obtaining these functions in practice. The text in rectangles represents different functions and their sub-functions. The text located within the trapezoids outline the means the team decided were feasible for obtaining these functions. Finally, the text in the 3 hexagons shows the means for the sub-functions above them. The means were decided upon by including every possible method of obtaining a desired function. This was then used to generate the design alternatives.

4.3.3 Constraints

Once the objectives and functions are outlined, the next step is to determine what constraints limit certain means of obtaining the desired design. These constraints are based mainly on the restrictions and limitations set forth by the team's design budget, the dimensions of the existing Oxford® MRI unit, the physics behind MR imaging techniques and stakeholder concerns. The following constraints were used in eliminating alternative design choices: the device must fit MRI bore, it has to be made of non-magnetic materials, there must be sub-divisions sufficiently large to hold collagen sponges, the device must be rigid to support its own weight within the MRI bore, the RF coil must have low electrical resistance, the RF coil must be compatible with collagen holder and the RF coil must include adjustable capacitors.

Any device designs that do not meet these constraints cannot be considered for use in this study. If the design does not allow the device to fit within the bore diameter of 14.5 cm, or the device is magnetic in any capacity, imaging cannot take place. Likewise, is the collagen compartments are not large enough to hold the sponges completely flat and level, they cannot be imaged properly. The device must also support its own weight, as well as that of the samples, because there are no provisions within the MRI bore to compensate for this. The RF coil must include the appropriate physical properties as described above, as well as fit onto the collagen holder within the MRI bore.

Once a design has proven it conforms to these constraints, it can then be considered for its ability to meet the functions and objectives previously outlined by the design team.

4.4 Generating Design Alternatives

Once the design problem had been fully specified and the needs of the design explicitly stated along with the design constraints, it was necessary to produce the maximum number of possible design alternatives that may successfully solve the design problem. While many of the possible designs may not initially seem feasible, an unlikely idea may be developed to fulfill the needs better than a design that seems like the obvious choice with only a cursory consideration of the alternatives. In this project, two methods were used to generate the design alternatives, namely the C-Sketch method and the Morphological Chart.

4.4.1 C-Sketch

The C-sketch method was carried out by each team member sketching a possible design on paper, along with annotations and labels to explain further the design. No

talking about the designs was permitted during the entire process. Once team members had sketched to their satisfaction, the paper with the design was passed to the team member on the left. Modifications, suggestions and remarks were made directly on the paper. This was repeated until each team member had seen every possible design. The results of this exercise follow.

4.4.1.1 Concentric Tube

The concentric tube, shown in Figure 26, works by loading the samples into a single tube with spacers between each sponge sample. The tube, MRI and circular sponge samples all share a common axis line along which their centers are placed, hence the name. The spacers prevent the sponges from coming into contact with each other and provide the necessary spacing to identify each sponge in the MR image. The end plates would be sized to the MRI bore to ensure the sponges would be in the center. A volume coil of some description would need to be used with this design, as a surface coil would be too cumbersome and most likely not work effectively to excite the sponge as necessary to produce an image.

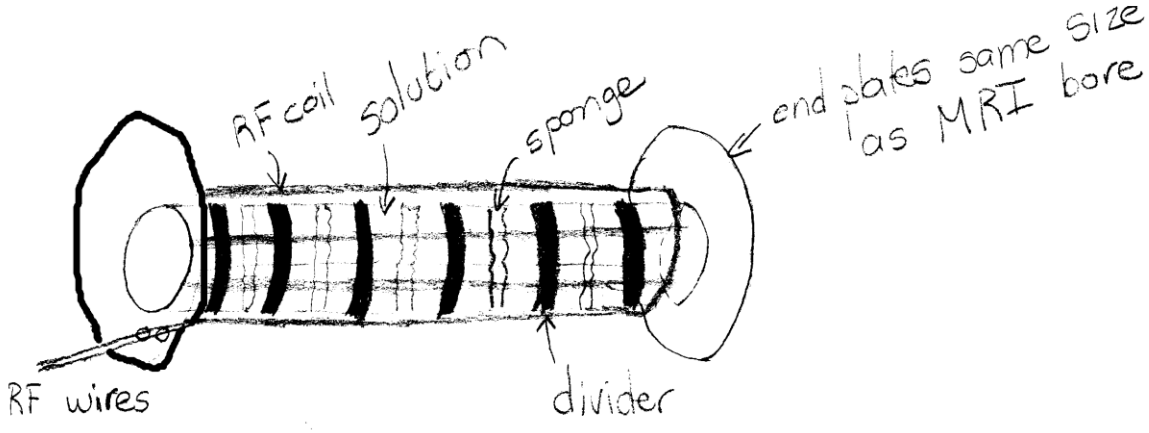


Figure 26: Concentric Tube

4.4.1.2 Contact Lens Cases

The contact lens case design that came out of the C-sketch method, shown below in Figure 27 uses ordinary contact lens cases to hold the sponges. These cases are watertight and prevent the sponges from dehydrating. It is compatible with a wide range of radiofrequency coils, unlike the concentric tube. A surface coil could be placed on top of the case or a solenoid could be wrapped around the case. It is also possible to create a birdcage coil that encloses all the cases. The cases are supported by a block that can be fabricated from a variety of materials, such as plastic or wood. Each sponge would be loaded into a separate lens case.

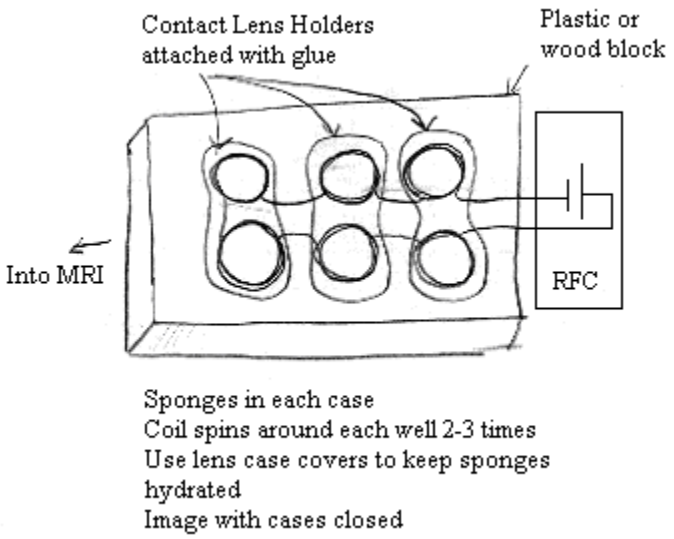


Figure 27: Contact Lens Cases

4.4.1.3 12-Well Plate

The 12-well plate design uses a standard 12-well plate to hold the sponges. The sponges are fabricated to have the exact dimensions of the wells in a 12-well plate. There would be little to manufacture for this design and the loading the sponges would be

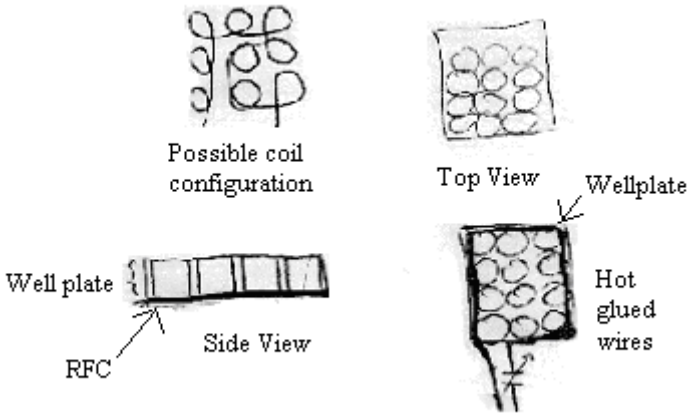


Figure 28: 12-well Plate

greatly simplified. The 12-well plates also come with a lid that could be wrapped in parafilm to prevent any solution loss through dehydration or spillage. The plate could be placed on a block in the same manner as the contact lens cases to ensure the samples were held at the correct height within the MRI bore. The sketch for this design is shown above in Figure 28.

These three possible designs, obtained from the C-Sketch method, do not exhaust the entire set of design alternatives. There are many other possible designs that should be considered. A morphological chart was used to enumerate more of the possible designs.

4.4.2 Morphological Chart

The morphological chart is constructed by listing all the functions that the device must perform along the left edge of the table. The possible means, parts or methods by which these functions could be fulfilled are then listed horizontally across in line with each function. This creates a table with all possible means specifically enumerated. The morphological chart constructed by the team is shown below in Table 8. The chart is

used by selecting one means from each row to create the design alternative. The chart below lists seven functions with a varying number of means to fulfill each function. The chart suggests that there are 3 x 2 x 3 x 6 x 2 x 4 x 2 = 1,728 possible designs.

Table 8: Morphological Chart

Means **Function**	**1**	**2**	**3**	**4**	**5**	**6**
Excite entire sample homogeneously	solenoids	multiple loop surface coils	birdcage coils	X	X	X
Resonate sample at magnet's frequency	adjustable capacitor	fixed capacitor at desired frequency	X	X	X	X
Achieve best resolution image	greatest possible frequency	imaging techniques	greatest possible filling factor	X	X	X
Attach RFC to collagen holder	glue	String	rest on holder	holes/grooves for coil	electrical tape	soldering
Keep sponges hydrated	submerge in solution	watertight container	X	X	X	X
Separate sponges	multi-well plate	dividers between sponges	pill containers	contact lens cases	X	X
Hold sponges at proper height	block	concentric tube	X	X	X	X

This chart allows numerous combinations of designs. The design combinations are obtained by taking one mean listed after each of the seven functions. For example, one design could use:

- a birdcage coil that excites the entire sample homogenously
- an adjustable capacitor that resonates the sample at the magnet's frequency
- the greatest possible frequency to achieve best resolution image
- electrical tape to secure the RFC to the collagen holder
- water-tight containers to keep the sponges hydrated

- a multi-well plate to separate the sponges
- a concentric tube to hold the sponges at the proper height

From this morph chart, hundreds of design ideas are proposed. However, not all combinations are possible and it is not feasible or efficient to evaluate every possible design. For example, a fixed capacitor would most likely not be used because it would be very challenging to build the radiofrequency coil to have a specific frequency. Also, this capacitor could only be used with that specific circuit, making any later modifications more expensive without any other benefits. The concentric tube is also limited by some of the means of performing specific functions.

Twelve top designs were found realistic after using the morph chart method to generate design alternatives. They are listed below:

- Well plate with multiple loop surface coil
- Well plate with birdcage coil
- Concentric tube with solenoid coil
- Concentric tube with birdcage coil
- Contact lens case with solenoids
- Contact lens case with birdcage
- Contact lens case with multiple loop surface coil
- Pill container with solenoids
- Pill container case with birdcage
- Pill container case with multiple loop surface coil
- Tray with dividers with multiple loop surface coil
- Tray with dividers with birdcage coil

The C-Sketch method and the morphological chart helped to generate as many ideas as possible in an effort to ensure no possible designs were overlooked. After this, it was possible to proceed with confidence, knowing that nothing significant was being overlooked, to narrowing down the many different design alternatives. There is no way

we could be sure that nothing was excluded from the design space, however, we felt that we could continue with reasonable confidence.

4.4.3 Modeling of Top Design Choices

The designers then sketched the top twelve design alternatives that were generated from the C-sketch method and the morphological chart. This allowed the designer to see what the actual device would look like, what materials would be need to create these prototypes, and what/if any problems may be encountered when manufacturing the designs. Appendix A contains the sketches of the top 12 designs.

4.4.4 Feasibility Considerations

The feasibility of designing a proper collagen holder and radio frequency coil for this project has to be considered. Is it possible and worth the time and effort? The method of using Magnetic Resonance Imaging was also considered for its feasibility.

4.4.4.1 Collagen Holder and Radio Frequency Coil

The top twelve designs listed above in the section title “Design Alternatives” are all made of inexpensive and easily assessable materials. Contact lens cases, concentric tubes, trays with dividers, and pill containers all cost less than $10 and can easily purchased through a catalog or at a local pharmacy or convenient store. The bases for the designs would be made of plastic or wood, which can be supplied by the Worcester Polytechnic Institute machine shop or purchased at a hardware store. The radio frequency coils will be created at the UMASS Imaging Center and the supplies will be provided.

4.4.4.2 Magnetic Resonance Imaging

MRI has been used in biomedical research for over 30 years, imaging all organs of the body. In medicine, it has been primarily used to look at damage to soft tissues of the body such as the muscle, tendons, ligaments, and the brain. One disadvantage of MRI is that whatever is being imaged must remain completely still for high quality images.

Using MRI to image collagen sponges should provide high quality images that will show the change in degradation between various sponges. MRI is an excellent modality to image the sponges because it is non-invasive, provides detailed images, and the samples will be stationary.

4.4.5 Device Metrics

The top 12 design alternatives were measured to determine which design best meets the established device objectives. Device metrics were created, each with a scale from 0-4, and then each design was given the appropriate score for each of the objectives. These scores were then added together and the designs were ranked accordingly from the design that best meets the objectives (highest score) to the design that does not meet the device metrics on a very high standard (lowest score).

Each of the established objectives has one or more parameters it should meet. Below all of the objectives are listed with the parameter(s) desired. Each metric has a few sentences listed below their score/parameter table providing further explanations or clarification.

- Radio frequency coil (RFC) wire should be 40 degrees C or cooler

Score	Parameter
0	Wire exceeds 40 degrees C
1	Wire reaches 40 degrees C
2	Wire reaches 35 degrees C
3	Wire reaches 30 degrees C
4	Wire stays below 30 degrees C

If the coil exceeds 40 degrees Celsius then the wire would be unable to be touched to move or adjust. If a hot wire were accidentally touched, it would not be safe to the device user. The wire could also be considered a fire hazard, or damage the collagen holder and samples, if it exceeds a certain temperature.

- Device should have smooth edges that so that the skin is not punctured when a force is applied by leaning on the device

Score	Parameter
0	Skin is punctured when pressure is below 20 lbs
1	Skin is punctured at a pressure of 20 lbs
2	Skin is punctured at a pressure of 30 lbs
3	Skin is punctured at a pressure of 40 lbs
4	Skin is not punctured when the pressure applied exceeds 40 lbs

The device designed should have smooth edges so that it safe to use in the laboratory and when imaging. The collagen holder should be made of materials that are not sharp. The wires of the radio frequency coil should be secured so if bumped into, the wire does not puncture the skin.

- Contains compounds with hazardous material classification

Score	Parameter
0	Contains compounds of a hazardous material classification of 4
1	Contains compounds of a hazardous material classification of 3
2	Contains compounds of a hazardous material classification of 2

3	Contains compounds of a hazardous material classification of 1
4	Contains no hazardous materials (no classification)

Hazardous materials are very harmful and require a lot of additional precautions. Ideally, each of the top designs will receive a 4 in this category, and not be considered a hazardous material.

- The Radio Frequency Coil (RFC) and collagen holder combined cost should fall within the budget

Score	Parameter
0	Costs $600 or more
1	Costs $400 or more
2	Costs $200 or more
3	Costs $50 or more
4	Costs less than $50

Worcester Polytechnic Institute refunds each student completing their Major Qualifying Project is provided with a $156 reimbursement for project costs. With four students completing this project, there is a $624 budget. The supplies and the manufacturing costs should fall within this budget.

- Hold samples

Score	Parameter
0	Holds 1 or no sponges
1	Holds 2-4 sponges
2	Holds 4-6 sponges
3	Holds 6-8 sponges
4	Holds more then 8 sponges

The more samples that can be imaged at a time, the smaller number of images will need to be produced to see samples. In addition, the variability will decrease, increasing the accuracy of the results. If an error occurs with the image, it will be constant among all the samples, rather than just a single sample.

o Radio Frequency Coil must encompass as many sponges as possible

Score	Parameter
0	RFC encompasses 1 or no sponges
1	RFC encompasses 2-4 sponges
2	RFC encompasses 4-6 sponges
3	RFC encompasses 6-8 sponges
4	RFC encompasses more then 8 sponges

The RFC must be able to image as many samples that are placed in the MRI bore, to the best resolution and image quality as possible. The more samples the RFC can encompass at a time, the smaller the number of images will be produce, saving time and money.

o Should maximize filling factor by proximity to the sample

Score	Parameter
0	Coil is more then 8mm away from the sample
1	Coil is 6-8mm away from the sample
2	Coil is 4-6mm away from the sample
3	Coil is 2-4mm away from the sample
4	Coil is less then 2mm away from sample

The further the RFC is away from the sample, the worse the image will be. Measuring how close the RFC can get to the sample is an important parameter in the design of our device.

o Should withstand shaking of machine without circuit failing or wires and other pieces coming loose

Score	Parameter
0	Collagen holder and RFC become detached at a shaker setting of 1 after 10 minutes
1	Collagen holder and RFC become detached at a shaker setting of 2 after 10 minutes
2	Collagen holder and RFC become detached at a shaker setting of 3 after 10 minutes
3	Collagen holder and RFC become detached at a

	shaker setting of 4 after 10 minutes
4	Collagen holder and RFC withstand maximum shacking (setting 5) without being effected after 10 minutes

In a laboratory or in clinical application, all devices have been dropped, shaken and damaged due to human use. Making this device durable through being able to withstand shaking will make it a more durable device.

o Should be able to handle a 6-foot drop when assembled and still function normally

Score	Parameter
0	Pieces of collagen holder and RFC break and completely separate
1	Spacing and movement of parts, however still one apparatus
2	3 or more dents and scrapes in apparatus
3	1-2 scrapes or dents on apparatus
4	There are no signs of physical damage

Human error is something that will always occur in some form or another. When using this device, it is possible for it to fall from a lab bench or storage location therefore making it withstand a drop from a reasonable height will make the device more durable.

o Easy to Clean

Score	Parameter
0	Apparatus can no longer be used after exposure to cleaning agent
1	Apparatus is physically altered after exposure to cleaning agent
2	It takes over 10 minutes to clean entire apparatus effectively
3	It takes 5-10 minutes to clean entire apparatus effectively
4	It takes less then 5 minutes to clean entire apparatus effectively

The device should be able to be used multiple times and an important factor of this multiple usage is to be sure that it is clean, and does not have any liquids or materials contained from a previous image that will alter the samples degradation.

- House Radio Frequency Coil (RFC)

Score	Parameter
0	Collagen holder does not have an adequate way to attach the RFC so current is not flowing
2	Collagen holder has room for RFC coil however either current flow is not the maximum amount possible, or holder shakes or rocks (is not completely stable)
4	Collagen holder has proper room to house the RFC coil, allowing maximum current flow, keeping holder stable

The collagen holder will have to be compatible with the RFC for an adequate image to be produced. When combining these two components of the design, the stability and current should not be affected.

- Portable

Score	Parameter
0	Weights more then 50 lbs or larger then 10 sq feet
1	Weighs 40-50 lbs or has 8-10 sq feet
2	Weighs 30-40 lbs or has 6-8 sq feet
3	Weighs 20-30 lbs or has 4-6 sq feet
4	Weighs less then 20 lbs or less than 4 sq feet

The devices should be easy to move from the lab, where collagen sponges are produced, to the MRI machine. An average person can lift around 30 pounds so the device will hopefully weigh less than this amount. The dimensions should also be considered, a very large and bulky device will be awkward and a hassle to transport.

- Connect to current setup

Score	Parameter
0	Is unable to connect to current setup
2	Connects to current setup allowing partial current flow
4	Connects to current setup allowing maximum current flow

Current is necessary to produce an image so the current flow should not be distorted in any form. Maximum current flow produces a detailed image.

- Easy to calibrate

Score	Parameter
0	Can not be calibrated
1	Can only be calibrated before placed in the MRI bore
2	Can be calibrated by using multiple people and adjusting the entire apparatus setup
3	Can be calibrated while in the MRI bore using two people, adjusting just a part of the apparatus
4	Can be performed while in the MRI bore using only one person, adjusting just part of the apparatus

The MRI radio frequency coil must be calibrated to the software used for imaging each time an image is taken. If the device has to be removed from the MRI bore for each calibration it will be very time intensive. If the device cannot be calibrated at all, an image may not be able to identify the changes in degradation of the sponges.

- Reliable

Score	Parameter
0	Performs as expected less than 60% of the time
1	Performs as expected between 60-70% of the time
2	Performs as expected between 70-80% of the time
3	Performs as expected between 80-90% of the time
4	Performs as expected between 90-100% of the time

Ideally, the device will perform as it is expected to each time the sponges are imaged. If data results were skewed or inaccurate on some images taken, the results would not be valid.

- Maintain Temperature

Score	Parameter
0	Is not able to be used with animal heater

1	Variation in sample temperature is greater than 15°C
2	Variation in sample temperature is greater than 10°C
3	Variation in sample temperature is greater than 5°C
4	Variation in sample temperature is less than 15°C

The temperature conditions need to remain unaltered because various temperature changes can change how quickly the sponges degrade. The sponge temperature should be maintained from when the sponge is initially degraded to the time it is imaged.

o Maintain Physical State

Score	Parameter
0	Sponge is permanently deformed or torn
2	Sponge is deformed from hydrated/normal shape
4	No deformation is observed

The sponge should not be altered when it is in the collagen holder and radio frequency coil being imaged. It can be determined if the physical state is maintained seeing if the sponge is still hydrated after imaging or if the shape is changed.

o Maintain Chemical State

Score	Parameter
0	Sample appearance is significantly altered (color/translucency change)
2	Sample appearance is slightly altered (color/translucency change)
4	No change in sample appearance

The device created should not alter the sample in any way. The image needs to resemble accurately the sponge degradation and so the collagen holder should not change the color or translucence. The sponges should be able to be used after imaging.

o Stable

Score	Parameter
0	Difficult to place level in bore and rocks from bumping
2	Easy placement in bore and rocks from bumping **OR** Difficult to place in bore and does not rock from bumping
4	Easy to place level within bore and does not rock from bumping

A stable device is necessary for an accurate MR image. The design cannot rock or move to image the samples.

- Accurate

Score	Parameter
0	Measurement is more than 40% from bench top value
1	Measurement is more than 30% from bench top value
2	Measurement is more than 20% from bench top value
3	Measurement is more than 10% from bench top value
4	Measurement is less than 10% from bench top value

This parameter cannot be scored until after the prototypes are tested. The greater the accuracy from the bench top value, the better the design.

- High Filling factor

Score	Parameter
0	Filling factor of less than 65%
1	Filling factor between 65-75%
2	Filling factor between 75-85%
3	Filling factor between 85-95%
4	Filling factor of greater than 95%

The greater the filling factor, the better the MR image. The filling factor is how much empty space is left between the sample being imaged and the radio frequency coil. The image will provide a greater extent of information on the degradation of the collagen sponge, the greater the filling factor is.

- Reproducible

Score	Parameter
0	Measured values for same sample exceed 40% variability
1	Measured values for same sample exceed 30% variability
2	Measured values for same sample exceed 20% variability
3	Measured values for same sample exceed 10% variability

4	Measured value for same sample is less than 10% variability

This parameter cannot be assessed until after the prototypes are tested. The design that produces the highest variability should be given a higher score because the accuracy of the measurements is considered to have greater validity.

- Visualize Sample(s)

Score	Parameter
0	Cannot see samples
1	Can locate samples but not assess state
2	Can locate and partially assess sample state
3	Can locate and assess sample state with minor visual obstructions
4	Can locate and assess sample state without visual obstructions

Visualizing the samples will enable the designers and researchers to visualize the degradation in the collagen holders. This will allow for more accurate data because the degradation can occur in the same holder the samples will be imaged.

4.5 Numerical Evaluation Charts

The purpose of a numerical evaluation chart is to arrive at a final design choice using a logical and scientific approach. A numerical evaluation chart is the means to narrowing down all of the possible designs derived from the Morphological Charts into what will become the final design. This not only gives the best design possible, but it also shows how the design team arrived at its decision for the final design and supports it. This is important when other people study or review the design process; it gives a systematic illustration of how the team's final design was achieved. It does so by eliminating designs that violate constraints and by ranking the remaining designs by how well they achieve each objective. It also takes into account that not every objective is as important as another; therefore, each weight from the weighted objectives tree is factored

in for each objective. The charts are set up (such as in Table 9) with all the alternate designs listed across the top. The constraints are listed down the left hand column followed by the objectives. The weights of each objective is taken from the weighted objective tree and put in the column next to its objective. The left hand weight from the weighted objective tree was used; it is the weight that relates the objective's importance to the entire design (rather than the right hand weight from the weighted objectives tree, which relates that objectives importance only on its level). Since the weights relate to the entire design, their sum in the Numerical Evaluation adds up to 100%. The following table shows a small generic numerical evaluation chart to get and idea of how it is setup.

Table 9: Example Numerical Evaluation Chart

	Weight %	Alternative design 1	Alternative design 2	Alternative design 3	Alternative design 4
CONSTRAINTS					
Constraint 1					
Constraint 2					
Constraint 3					
OBJECTIVES					
Objective 1	V%				
Objective 2	W%				
Objective 3	X%				
Objective 4	Y%				
Objective 5	Z%				

As mentioned before, the numerical evaluation chart arrives at the final design by grading each alternative design's ability to complete each objective. From there, the weighted scores are added up and the device that has the most points should be the final design. For our design process, the grading system was on a zero to four-point scale. The better the design completed each objective, the higher the score it received. A few objectives, due to their nature, were scorred on a 0, 2, 4 point scale, but the majority were

on the traditional zero-four point scale. An example of one objective's grading system follows in Table 10.

Table 10: Objective Grading System

Accurate

Score	Parameter
0	Measurement is more than 40% from bench top value
1	Measurement is more than 30% from bench top value
2	Measurement is more than 20% from bench top value
3	Measurement is more than 10% from bench top value
4	Measurement is less than 10% from bench top value

Table 10 is an example of how the objective of accuracy was scored. If the imaging technique gave results that varied less than 10% from the gold standard bench top model, it would receive 4 points. If it was more than 10% and less that 20% of the bench top model, it would score 3 points and so on. The complete grading system for each objective is described and defined in the design metrics section.

Some objectives are complicated, generally the level-1 objectives. For example, in our design, two of the objectives we established were "safe to use," and "durable." In each of these objectives, the design team felt there were several qualities the device had to have in order to fulfill each one. Therefore, "safe to use" was broken down into 3 separate scores and "durable" into 2 separate scores. For example, "safe to use," was reached by meeting adequate measures of temperature, sharp edges, and hazardous materials while durability took into account shaking from the MRI machine and accidental dropping. For both objectives, the average score was taken in order to normalize the score before it was multiplied by the weight percent to get the weighted score. Figure 29 is a section of our final numerical evaluation. It shows the two objectives just described and each of their weight percentage (18 % and 12 %

respectively). Notice that in the next column, "safe to use," has received three scores (4+3+4) for that specific design. The score is normalized by dividing it by 3 to get an average of the three scores. Then, that average is multiplied by the weight to get the final weighted score for that objective under that design. The same is done for "durable"; except it receives two scores and they are normalized by dividing by two before multiplying by the weight (12 %) to get the weighted score.

Objectives			
Safe to Use	18	(4+3+4)/3*0.18 = 0.66	(4+1+4)/3*0.18 = .54
Durable	12	(4+2)/2*0.12 = 0.36	(2+0)/2*0.12 = .12
Multiple samples	6.1	4* 061 = 0.244	4*.061 = 0.244

Figure 29: Section of Final Numerical Evaluation Matrix

For the rest of the objectives, only one score is given. The score is multiplied by the weight percentage to get a final weighted score for each objective for each design.

4.5.1 Arriving at our numerical evaluation chart

This section explains how the team arrived at each score for all of the different objectives for each alternate design. Once again, all of the scores and definition of scores throughout this entire section can be viewed back in the device metrics section. The following tables (Table 11 and Table 12) are the design team's final numerical evaluation charts.

Table 11: Numerical Evaluation Matrix A

Design	Weight (%)	Well plate with multiple loop surface coil	Concentric tube with birdcage coil	Contact lens case with solenoids	Contact lens case with birdcage	Well plate with birdcage coil	Contact lens case with multiple loop surface coil
Constraints							
Must not be larger than MRI bore							
Must not effect collagen sponge							
Must not be magnetic							
Objectives							
Safe to Use	18	(4+3+4)/3*0.18 = 0.66	(4+1+4)/3*0.18 = .54	(4+4+4)/3*0.18 = 0.72	(4+4+4)/3*0.18 = 0.72	(4+2+4)/3*0.18 = 0.6	(4+4+4)/3*0.18 = 0.72
Durable	12	(4+2)/2*0.12 = 0.36	(2+0)/2*0.12 = .12	(4+4)/2*0.12 = 0.48	(4+0)/2*0.12 = 0.24	(4+0)/2*0.12 =0.24	(4+3)/2*0.12 = 0.42
Multiple samples	6.1	4*.061 = 0.244	4*.061 = 0.244	4*.061 = 0.244	4*.061 = 0.244	4*.061 = 0.244	4*.061 = 0.244
Low Cost	12	4*.12 = 0.48	3*.05 = 0.15	4*.12 = 0.48	4*.12 = 0.48	4*.12 = 0.48	4*.12 = 0.48
Easy to clean	2	3*0.02 = 0..06	2*0.02= 0.04	4*0.02 = 0.08	4*0.02 = 0.08	3*0.02 = 0.06	4*0.02 = 0.08
House RFC	4	4*0.04 = 0.16	4*0.04 = 0.16	4*0.04 = 0.16	4*0.04 = 0.16	4*0.04 = 0.16	4*0.04 = 0.16
Portable	1	4*0.01 = 0.04	4*0.01 = 0.04	4*0.01 = 0.04	4*0.01 = 0.04	4*0.01 = 0.04	4*0.01 = 0.04
Connect to current setup	2.9	4*0.029 = 0.116	4*0.029 = 0.116	4*0.029 = 0.116	4*0.029 = 0.116	4*0.029 = 0.116	4*0.029 = 0.116
Easy to calibrate	2	3*0.02 = 0.06	3*0.02 = 0.06	3*0.02 = 0.06	3*0.02 = 0.06	3*0.02 = 0.06	3*0.02 = 0.06
Reliable	9						
Maintain temperature	2	4*0.02 = 0.08	4*0.02 = 0.08	4*0.02 = 0.08	4*0.02 = 0.08	4*0.02 = 0.08	4*0.02 = 0.08
Maintain physical state	1	4*0.01= 0.04	0 = 0	2*0.01= 0.02	2*0.01 = 0.02	4*0.01 = 0.04	2*0.01 = 0.02
Maintain chemical state	4	4*0.04 = 0.16	4*0.04 = 0.16	4*0.04 = 0.16	4*0.04 = 0.16	4*0.04 = 0.16	4*0.04 = 0.16
Stable	4						
Comparable measurement bt bench top and MRI	5						
Adequate resolution	3						
High packing factor	1	3*0.01 = 0.03	0 = 0	4*0.01 = 0.04	0 = 0	0 = 0	3*0.01 = 0.03
Reproducible	10						
Visualize sample	1	4*0.01 = 0.04	3*0.01 = 0.03	1*0.01 = 0.01	1*0.01 = 0.01	4*0.01 = 0.04	1*0.01 = 0.01
Total Scores	**100**	2.53	1.74	2.69	2.41	2.32	2.62

Table 12: Numerical Evaluation Matrix B

Design	Weight (%)	Tray with dividers with multiple loop surface coil	Tray with dividers with birdcage coil	Pill container with solenoid	Pill container with birdcage	Pill container case with multiple loop surface coil	Concentric tube with solenoid coil
Constraints							
Must not be larger than MRI bore							
Must not effect collagen sponge							
Must not be magnetic							
Objectives							
Safe to Use	18	(4+4+4)/3 x 0.18 = 0.72	(4+4+4)/3 x .18=0.72	(4+4+4)/3 x .18=0.72	(4+4+4)/3 x .18=0.72	(4+4+4)/3 x .18=0.72	(4+1+4)/3 x .18=0.54
Durable	12	(2+1)/2 x .12=0.18	(2+1)/2 x .12=0.18	(4+3)/2 x .12 =0.42	(4+1)/2 x .12 =0.3	(4+2)/2 x .12 = .36	(2+0)/2 x .12= .12
Multiple samples	6.1	4 x .061 =.244	4 x .061 =.244	4 x .061 =.244	4 x .061 =.244	4 x .061 =.244	4 x .061 =.244
Low Cost	12	4 x .12 = .48	4 x .12 = .48	4 x .12 = .48	4 x .12 = .48	4 x .12 = .48	4 x .12 = .48
Easy to clean	2	2 x .02 = .04	2 x .02 = .04	3 x .02 = .06	3 x .02 = .06	3 x .02 = .06	2 x .02 = .04
House RFC	4	4 x .04 =.16	4 x .04 =.16	4 x .04 =.16	4 x .04 =.16	4 x .04 =.16	4 x .04 =.16
Portable	1	4 x .01 = .04	4 x .01 = .04	4 x .01 = .04	4 x .01 = .04	4 x .01 = .04	4 x .01 = .04
Connect to current setup	2.9	4 x .029 = .116	4 x .029 = .116	4 x .029 = .116	4 x .029 = .116	4 x .029 = .116	4 x .029 = .116
Easy to calibrate	2	3 x .02 = .06	3 x .02 = .06	3 x .02 = .06	3 x .02 = .06	3 x .02 = .06	3 x .02 = .06
Reliable	9						
Maintain temperature	2	4 x .02 = .08	4 x .02 = .08	4 x .02 = .08	4 x .02 = .08	4 x .02 = .08	4 x .02 = .08
Maintain physical state	1	4 x .01 = .04	4 x .01 = .04	4 x .01 = .04	4 x .01 = .04	4 x .01 = .04	0 x .01 = 0
Maintain chemical state	4	4 x .04 = .16	4 x .04 = .16	4 x .04 = .16	4 x .04 = .16	4 x .04 = .16	4 x .04 = .16
Stable	4						
Comparable measurement between bench top and MRI	5						
Adequate resolution	3						
High packing factor	1	4 x .01 = .04	0 x .01 = 0	2 x .01 = .02	0 x .01 = 0	4 x .01 = .04	3 x .01 = .03
Reproducible	10						
Visualize sample	1	4 x .01 = .04	3 x .01 = .03	3 x .01 = .03	4 x .01 = .04	4 x .01 = .04	1 x .01 = .01
TOTAL	**100**	2.4	2.35	2.63	2.5	2.6	2.08

4.5.2 Well plate with multiple loop surface coil

The well plate with multiple loop surface coils scored very well for safe to use. It received the highest score possible for two of the three components of the objective, and a 3 on the remaining component. The design scored this alternate design fours for "safe to use" because it was felt that the device would have an RFC that would stay below 30 degrees C, and contained no hazardous materials. For the remaining component, have safe edges so that the skin is not damaged when pressure is applied, the design scored a 3. Since the objective fulfilled the first two listed components completely, as defined in the device metrics, it was scored 4 points in each for this objective. For the pressure component of the durable objective, the team felt that with a pressure near 40 lbs, the well plate might crack. Given the relatively high pressure, so the team awarded that component 3 points. Since there were three components of this objective, the three scores were added together and then divided by three to get an average score of normalized results. That resulted in (4+3+4)/3=3.67. That score was then multiplied by the weight percentage of "safe to use," which was 18%. This gave a weighted score of 0.66 for safe to use.

After that, durability for the design was scored. Durable has two components and the design team felt that it did not achieve each one of them very well. The first component of durable is that the device should be able to withstand the shaking and vibrations of the MRI without pieces coming lose. For this component, this design received a 4. It received a 4 because the team felt that the design was as sturdy as the others were and a surface coil would remain in its proper placement when the machine reached its highest intensity. For the other component, the ability of the device to

withstand a 6-foot drop when assembled and still function as intended, the design received a score of 2. The team scored it as a 2 because a fall of that magnitude could damage part of parts of the device, but it would still function normally. By definition, that results in a score of 2. The two components were averaged to normalize the score and then multiplied by the weight percentage of the objective (12%). The final weighted score for durability of the well plate with multiple loop surface coils was 0.36.

Next, the ability of the design to hold and image multiple samples was scored. It scored as high as possible for this objective. It received a 4 because it holds 12 sponges, and by the definition in the device metrics, it warrants that score as long as it holds 8 or more. The 4 was multiplied by the weight percentage, which was 6.1%, and its final weighted score was 0.244.

The following objective was low cost for building the device. This particular design scored very well in this objective. Not much money would need to be spent for the design. We already have several well plates and the material for building surface coils is available to the team in the lab. Therefore, the total cost for putting it together would be less than $30. In the device metrics, that means it receives a score of 4. When that is multiplied by the weight percentage, 12%, it has a final weighted scored of .48.

Subsequently, the design was scored on how well it completed the objective of being easy to clean. The design team felt that well plates would not be very hard to clean, but also felt that it was not the best design to make cleaning as easy as possible, so it received a score of 3 for the objective. Since the device has wells, the team felt that it would take a little extra time to clean, at least more than the 5 minutes required to score a 4. It would take more than 5 minutes but less than 10 minutes in order to wipe out each

well and clean the device effectively. Its score of 3 was multiplied by its weight percentage, 2%, and its final weighted scored was calculated to be 0.06.

Next, the design's ability to house the radio frequency coil was scored. The radio frequency coil would not have much trouble being incorporated into the well plate with multiple loop surface coils. It could simply rest on what every the well plate was placed on during imaging. It received a score of 4 and when multiplied by the weight % of 4 %, it received a final weighted score of 0.16.

The design's portability was the next objective that was scored. The team decided that this device should receive a score of 4 for this objective. In the device metrics, a design that gets a 4 must weigh less than 20 lbs and take up less than 4 square feet. This design would be a little bigger than a well plate and not much heavier than the wire of the RF coil and the well plate itself, so nothing even close to 20 lbs. Its score of 4 was multiplied by its weight percentage of 1 %. Its final weighted score for this objective was 0.04.

Connecting to the current setup in the MRI lab was the following objective that was scored by the design team for the well plate with multiple surface coils. The metric for receiving a grade of 4 was that the device had to carry to appropriate current through the radio frequency coil and set up correctly in the MRI. The design team felt that this design completed this objective as well as any other and scored the objective a 4. The weight percentage for the objective is 2.9%. The final weighted score for the objective of this alternate design is 0.116.

Calibration is another important part of starting an MR imaging process and is the next objective that was scored. The team felt that this design could not be calibrated

easily by one person and that two people would be required to calibrate the device easily. Therefore, according to our metrics, the design team assigned a grade of 3 to this objective. When the score was multiplied by the weight percentage of 2%, it calculated the final weighted score to be 0.06.

Next, maintaining the temperature of the sample was the objective that was scored. Its weight percentage was 2% of the entire design. The team felt that this design would maintain the temperature as well as any other design and scored it as a 4 for the objective. That was based upon the fact that the well plate had a cover that protected the sponges from exposure to the open air and therefore maintain its starting temperature as well as have a proper circuit in the RF coil that does not generate heat. This design received a 4 and when multiplied by the weight %, the final weighted score was calculated. It was 0.08.

Maintaining the physical state of the sponges was the next objective the team scored. It also received a 4 for this objective because according to the device metrics, the design had to maintain the hydration and hold the sponge so that it was not deformed. The design received a 4 because the well plate is where the sponges are made, so they fit extremely well and are not deformed when placed in the well and also because the cover prevents any dehydration of the sponges. The score of 4 was multiplied by the weight % of 1% to reach the final weighted score of 0.04.

After that, the ability of the design to maintain the chemical state of the sample was scored by the design team. The team decided that this objective would also receive a 4 for this particular design. This score was reached by considering the fact that the only part of the device to touch the sponge is the well plate. They are plastic and are, in fact,

the very tool in which the sponges were created. They will not chemically alter the sponges and therefore received a score of 4 for that reason. Its weight percentage was 4 % of the entire design. The final weighted score for the objective was 0.16.

The next objective scored by the design team was the high filling factor of the radio frequency coil. High filling factor is important for getting quality MR images. The design received a score of 3 for this objective. The reasoning behind the score was that a surface coil that loops up and down a 12 well plate would include much area that is not the sponges. Although in theory it would work well, it just did not have the 95% sample coverage of the area inside the radio frequency coil in order to score the objective higher. The score of 3, multiplied by the weight % of 1 resulted in a final weighted score of 0.03.

Finally, the ability to visualize the sample while it degrades in the device is the last objective scored for the well plate with multiple loop surface coils. This design for the device completes this objectively to the highest degree possible. Some of the other designs had collagen holders that are solid or translucent, but this design has clear holders to give the researcher the ability to see the sample inside the case to tell when it has completely degraded. Therefore, the design team gave it a score of 4. When multiplied by the weight percentage, 1%, the final weighted score was reached and it was 0.04.

With all of the weighted objectives scored to see how well the design completed each of them, a total weighted scored was reached for each objective. It took into effect how well each objective was performed and how important it was in the grand scheme of things. Each weighted score was added up and the total weighted score for the well plate with multiple loop surface coils was 2.53.

4.5.3 Concentric Tube with Birdcage Coil

The concentric tube design did not score very well when the design team scored it on the objective of safe to use. It received a score of 4, 1, and 4 on the three components of safe to use. The design scored a 4 on the component that dealt with having a RFC that did not overheat. The device metrics defined that a design that has a coil that stays under 30 degrees Celsius receives a 4, and that is what this design does. The design, however, did not fair so well when a force was applied to it. The design team felt that the glass tube would break and no longer be safe when a force of 20 lbs was put on it. Therefore, as defined in the metrics, it received a 1 for that component. Finally, the design received a 4 on the final component because it does not contain any hazardous materials and its score is defined that way in the metrics. The scores for the three components were added and divided by three to normalize the score. The normalized score was 3. This score was multiplied by the weight percentage, which is 18%, and the final weighted score was reached. The weighted score for safe to use for this design is 0.54.

The design also did not have much success with grading for durability either. The design team gave the device a 2 for the "ability to withstand shaking" component of the durable objective. The team arrived at this score because the tubes containing the sponges inside the large glass tube are not fixed by any means. Therefore, they can become dislodged if the intensity of the vibrations reached a moderate level and the device would fail during imagining. The team also scored the ability to withstand a fall, a component of durability, a 0. This is because the device would be destroyed if dropped from ten feet. The glass would shatter and nothing would hold the collagen anymore.

The two scores were added and divided by 2 to normalize the score. That value, 1, was multiplied by the weight percentage, 12%, to get the final weighted score of 0.12.

The design team next scored the concentric tube design a 4 at holding multiple samples. The design team arrived upon this score because the device could hold more than eight samples in a line of small tubes inside a large glass tube. The score was multiplied by the weight percentage, 6.1 %, to find the final weighted score. It turned out to be 0.244.

After that, the low cost of the design was evaluated by the design team. The team felt that the design fulfilled this objective relatively well, but not the best. The team gave the design a 3 for this objective because it would cost more than $30. A glass tube would be expensive and a birdcage coil may have to be custom made to fit the design and therefore would not be the most cost effective of all the designs. The score of 3 was multiplied by the weight percentage of 12 to get the final weighted score of 0.15.

Following that, easy to clean was the next objective scored by the team. The team decided that the concentric tube design deserved a 2 for this objective. The team felt that have tubes inside a large glass tube would be reasonably complicated when easy cleaning is intended. The entire design would have to be dismantled to clean and then reassembled to prepare for another image and the team felt that this would take more than 10 minutes. The score of 2 was multiplied by the weight percentage of 2 to get the final weighted score of 0.04.

Housing the radio frequency coil was the next objective to be scored by the team for this alternative design. The team scored it a 4. There were examples of tubes that the rats were imaged in at the MRI lab with birdcage coils around them. They all contained a

working RFC and housed it during imaging without much problem. The score of 4 was multiplied by the weight percentage of 4. The final weighted score of 0.16 was the result.

Portability was the following objective that was analyzed for the concentric tube design. The design team gave this particular design a 4 because it weighed less than 20 lbs and took up less than 4 square feet. When the score of 4 was multiplied by the 1 weight percentage, it arrived at the weighted score of 0.04.

After that, the ability for the design to connect to the current setup in the MRI lab was evaluated. The team felt that this design would completely fulfill that objective and gave it a 4. There would be no need to vary from the conventional RFC and its connection to the MRI machine. Therefore, a 4 was awarded to the objective with the weight % of 2.9. The final weighted score was 0.116.

Then, easy to calibrate was evaluated by the design team. The team awarded 3 points for the score of the objective. The reason that the objective received 3 points is because it would not be easy for one person to calibrate this design. However, it would be easy for two people to do it and that is why it was awarded 3 points. That multiplied by the weight percentage of 2 gives a weighted score of 0.06.

Next, the concentric tube's ability to maintain the temperature of the sample was evaluated. The design team awarded 4 points to this device because it has several tubes inside a large tube, which eliminates exposure of the sample to air. It keeps it insulated to a certain extent. The score of 4 was multiplied by the weight percentage of 2% to get the weighted score of 0.08.

After that, the ability to maintain the physical state of the sample was evaluated. The team gave this a score zero because when the tubes were placed inside a large tube,

they would have to be put into the MRI bore sideways. This would cause all of the sponges to be on their sides and deform. Since its score was zero, when multiplied, it still had a weighted score of 0.

Next, the design team gave the concentric tube a 4 for its ability to maintain the chemical state of the sponge. Only glass or plastic would be touching the sample and they would not affect the chemistry of the sponges. That is how the team arrived at the score of 4 and it is defined in the device metrics.

The next objective that was scored was the high filling factor of the RFC. The design team gave this alternate design a 0 for this objective as well. The reason is that the smaller tubes containing the sponges would have to go inside a larger tube. This makes the large distance from the tubes to the sample even larger. Then it was put inside a birdcage coil. There is just too small of a filling factor to be awarded any points in this objective. The weighted score for this objective is 0.

Finally, the ability of view the sample during degradation and imaging was considered. The design team felt that the concentric tube deserved to be awarded a score of 3 for this objective. The tubes containing the sponges are clear and easily visualized, but the birdcage coil will make viewing the sponges a little difficult. That is why the design received a 3. The score was multiplied by the weight percentage of 1 and the final weighted score was the result. The weighted score was 0.03.

Each objective for this design was scored to see how well the design fulfilled the necessary objectives. In addition, the importance of the objectives was taken into account. When all of the weighted scores were added up, the total weighted score for the concentric tube was 1.74.

4.5.4 Contact Lens Cases with Solenoid Coils

The contact lens cases with solenoid coils scored very well for safe to use. It scored 4s in each of the three components of the safe to use objectives. The reason the design team gave this alternate design 4 points for each component is that the RFC wires will stay below 30 degrees Celsius, will not injure skin when pressure exceeding 40 lbs is applied, and contains no hazardous materials. In each component, this design was as good as it gets. The three scores of 4 were added and divided by 3 to normalize the score. The average was 4 and was multiplied by the weight % of the objective, 18%. This gives the weighted score for the objective; which was 0.72, the highest possible.

In addition, for the objective of durable, the design scored the highest possible. It scored 4s for each of the two components of the durability objective. The reason the design team scored the components 4 points apiece is because the contact lens cases would hold the sponges tightly in place because they are roughly the same size. The cases could then be wrapped with RFC wire for solenoids and firmly attached to a base. This would cause the design to withstand shaking and failing and withstand damage when dropped from 10 feet, the two components of durability. The two scores of 4 were added and divided by two to average them and normalize the score. Then that value, 4, was multiplied by the weight of the objective, 12%, to get the weighted score, 0.48. That is the highest score possible for that objective.

Next, the ability to hold multiple samples was evaluated by the design team. The design team scored this objective a 4 for the contact lens cases and solenoid coils. The lens cases are small and cheap and many were arranged on a plastic board. More than 8 samples can be imaged at a time with this design and according to the device metrics; the

design gets a score of 4. That value is multiplied by the weight of the objective, 6.1%. That gives the weighted score for the design and the objective and it is 0.244.

Low cost is the next objective that was evaluated. The design team awarded the design 4 points for this objective. The contact lens cases were very inexpensive, and the plastic board was scrap from the Mechanical Engineering Department at WPI. The team did not spend more than $30 building this prototype and under the definitions in the device metrics, which means this objective receives a score of 4. That score is multiplied by the weight, 12%, to give the weighted score of 0.48.

Easy to clean was the next objective the design team scored. The score the team came up with was a 4. The entire device could go under a faucet to be washed. It would take less than 5 minutes to clean and scrub. According to the device metrics, that warrants a score of 4. That score of 4 multiplied by the weight, 2%, gives the weighted score of 0.08.

Following that, housing the RFC needed evaluation. The design team gave this objective 4 points as well. This design would not cause any deviation from the normal imaging devices and they all house RFC's with ease. The metrics award the objective 4 points. That multiplied by its weight, 4%, gives the weighted score of 0.16.

Next, the portability of the device was scored. The team once again decided to award 4 points to the contact lens cases with solenoid coils because it was very light (less than 20 lbs), very small (less than 4 square feet), and very easy to carry around. The size and weight are what the score is based on, and that is why the device was awarded 4 points. That score multiplied by the weight, 1%, to give the weighted score of the objective, 0.04.

Next, the connection to the current setup in the MRI lab was evaluated by the design team. The score was determined by the fact that the RFC is setup like ever other one in the lab. It did not cause any problems with the imaging; it carries the right amount of signal and current. Since it connects to the current setup as well as it could, it received the score of 4. That score was multiplied by the weight of the objective, 2.9%, and it arrived at the weighted scored for the objective of the design, 0.116.

After that, the calibration had to be easy. The design team evaluated the score by assessing how easy it was for one person to calibrate the device. The team learned that the device was not easy to calibrate with one person, but was with two. According to the device metrics, the objective was awarded 3 points for the extent it was completed. Those 3 points were multiplied by the weight of the objective, 2%, and the weighted scored was arrived at, 0.06.

The next objective the design team evaluated was how well the temperature of the sample was maintained. The design team scored this objective similar to the previous designs. Since this design included tops to each lens case, protecting it from being exposed to the air, the design team felt that the samples temperatures would be maintained less than 15 degrees Celsius from the starting temperature. According to the device metrics, the design team scored the objective to receive 4 points. Those points were multiplied by the weight of the objective, 2%, and that value is the weighted score, 0.08.

Subsequently, the physical maintenance of the samples in the device was evaluated. The contact lens cases have a slight concavity to them but also some ridges inside to support the sponge off the curved bottom somewhat, but not completely. The

covers to the lens cases prevent dehydration. Considering both of these facts, the design team still had to give a score of 2 for the objective. That score was multiplied by the weight of the objective, 1%, and that value is the weighted score, 0.02.

After that, the maintenance of the chemical state of the samples was evaluated. The team felt that since only the soft plastic of the contact lens cases would be touching the sample, and that they would not affect the sample chemically, the device should receive the score of 4 for this objective of the design. The weight of 4% was multiplied by the score of 4 to get the weighted score of 0.16.

Next, the filling factor of the RFC was evaluated to make sure it was high. The lens cases are about the same diameter of the sponges, therefore, the filling factor is maximized because there is very little wasted space inside each of the solenoids. In other words, there is very little space inside those coils that is not sponge. This gives a filling factor greater than 95% and the team scored it 4 points. This is defined in the metrics. The score of 4 was multiplied by the weight, 1%, and the result, 0.04, is the weighted score.

Finally, the last objective for this alternate design is the ability to visualize the samples as they degrade during imaging. The team awarded the design 1 point because the cases are not clear. They are translucent and the cases are covered and wrapped in a solenoid coil. When the sample was large, they could be visualized from the top of the case, but after any degradation, it was hard to see them. Therefore, the team awarded it 1 point. That was multiplied by the weight, 1%, and that result is the weighted scored, 0.01.

After the weighted score was established for each one of the objectives, they were all added together to get a total weighted score for that particular alternate design. Each objective was weighted to take into account how important that objective was to the entire device and the design was scored on how well it completed each objective. That is how the team got the results it did. The total weighted score for contact lens cases with solenoid coil was 2.69.

4.5.5 Contact Lens Case with Birdcage Coil

The contact lens cases with birdcage coil scored very well for safe to use. It scored 4s in each of the three components of the safe to use objectives. The reason the design team gave this alternate design 4 points for each component is that the RFC wires will stay below 30 degrees Celsius, will not injure skin when pressure exceeding 40 lbs is applied, and contains no hazardous materials. In each component, this design was a good as it gets. The three scores of 4 were added and divided by 3 to normalize the score. The average was 4 and was multiplied by the weight % of the objective, 18%. This gives the weighted score for the objective; which was 0.72, the highest possible.

After that, the durable objective scored a little lower for this specific alternate design. It scored a 4 for one of the two components of the durability objective and a 0 for the other. The reason the design team scored the components at 4 and a 0 because the contact lens cases would hold the sponges tight in place because they are roughly the same size. Then the lens cases are firmly attached to a base. The shaking would not be a problem, this design would handle vibrations at the highest level for a long time and that is why the design team scored it a 4. However, the other component of durability scored a 0. The birdcage coil is the problem for the second component of durability. The design

team felt that the birdcage coil would be extensively damaged if dropped from 10-feet and that it would not work properly after that. That is what warranted the score of 0 on the second component of durable. The two scores of 4 and 0 were added and divided by two to average them and normalize the score. Then that value, 2, was multiplied by the weight of the objective, 12%, to get the weighted score of 0.24.

Next, the ability to hold multiple samples was evaluated by the design team. The design team scored this objective a 4 for the contact lens cases and birdcage coils. The lens cases are small and cheap and many were arranged on a plastic board. More than 8 samples can be imaged at a time with this design and according to the device metrics; the design gets a score of 4. That value is multiplied by the weight of the objective, 6.1%. That gives the weighted score for the design and the objective and it is 0.244.

Low cost is the next objective that was evaluated. The design team awarded the design 4 points for this objective also. The contact lens cases were very inexpensive, and the plastic board was scrap from the Mechanical Engineering Department at WPI. The team did not spend more than $30 building this prototype and under the definitions in the device metrics, which means this objective receives a score of 4. That score is multiplied by the weight, 12%, to give the weighted score of 0.48.

Easy to clean was the next objective the design team scored. The score the team came up with was a 4. The entire device could go under a faucet to be washed. It would take less than 5 minutes to clean and scrub. According to the device metrics, that warrants a score of 4. That score of 4 multiplied by the weight, 2%, gives the weighted score of 0.08.

Following that, housing the RFC needed evaluation. The design team gave this objective 4 points as well. The RFC for this design is a birdcage and it would be custom built to fit over the base and lens cases. This design would not cause any deviation from the normal birdcage coils or normal imaging devices and they all house RFC's with ease. The metrics award the objective 4 points. That multiplied by its weight, 4%, gives the weighted score of 0.16.

Next, the portability of the device was scored. The team once again decided to award 4 points to the contact lens cases with birdcage coil because it was very light (less than 20lbs), very small (less than 4 square feet), and very easy to carry around. The size and weight are what the score is based on, and that is why the device was awarded 4 points. That score multiplied by the weight, 1%; to give the weighted score of the objective, 0.04.

Next, the connection to the current setup in the MRI lab was evaluated by the design team. The score was determined by the fact that the RFC is setup like every other one in the lab. It did not cause any problems with the imaging; it carries the right amount of signal and current. Since it connects to the current setup as well as it could, it received the score of 4. That score was multiplied by the weight of the objective, 2.9%, and it arrived at the weighted scored for the objective of the design, 0.116.

After that, the calibration had to be easy. The design team evaluated the score by assessing how easy it was for one person to calibrate the device. The team learned that the device was not easy to calibrate with one person, but was with two. According to the device metrics, the objective was awarded 3 points for the extent it was completed.

Those 3 points were multiplied by the weight of the objective, 2%, and the weighted scored was arrived at, 0.06.

The next objective the design team evaluated was how well the temperature of the sample was maintained. The design team scored this objective similar to the previous designs. Since this design included tops to each lens case, protecting it from being exposed to the air, the design team felt that the samples temperatures would be maintained less than 15 degrees Celsius from the starting temperature. According to the device metrics, the design team scored the objective to receive 4 points. Those points were multiplied by the weight of the objective, 2%, and that value is the weighted score, 0.08.

Subsequently, the physical maintenance of the samples in the device was evaluated. The contact lenses have a slight concavity to them but also some ridges inside to support the sponge off the curved bottom somewhat, but not completely. The covers to the lens cases prevent dehydration. Considering both of these facts, the design team still had to give a score of 2 for the objective. That score was multiplied by the weight of the objective, 1%, and that value is the weighted score, 0.02.

After that, the maintenance of the chemical state of the samples was evaluated. The team felt that since only the soft plastic of the contact lens cases would be touching the sample, and that they would not affect the sample chemically, the device should receive the score of 4 for this objective of the design. The weight of 4% was multiplied by the score of 4 to get the weighted score of 0.16.

Next, the filling factor of the RFC was evaluated to make sure it was high enough. The lens cases have about the same diameter as the sponges, therefore, the filling factor

for the solenoid coils (which wrap right around each lens case) was high and this objective received a high score in the previous design. However, the major difference between the last design and this one is that now a birdcage coil is being used. It can be custom built to maximize its filling factor, however, the nature of birdcage coils is that there is a lot of space within its volume that contains no sample and that is the definition of a low filling factor. The team estimated that less than 65% of the volume in the birdcage coil would be samples. That is the reason the design team scored this design a 0 for this objective. The definition is in the device metrics. The weighted score for this objective of the design is 0.

Finally, the last objective for this alternate design is the ability to visualize the samples as they degrade during imaging. The team awarded the design 1 point because the cases are not clear. They are translucent and the cases are blocked somewhat by the birdcage coil surrounding all of the samples. It was possible to view the sample from time to time, but after degradation it gets harder and with more obstacles in the way, it makes it even more difficult. That is why the team scored this objective 1 point for the lens cases with birdcage coil. That score was multiplied by the weight, 1%, and that result is the weighted scored, 0.01.

After the weighted score was established for each one of the objectives, they were all added together to get a total weighted score for that particular alternate design. Each objective was weighted to take into account how important that objective was to the entire device and the design was scored on how well it completed each objective. That is how the team got the results it did. The total weighted score for contact lens cases with birdcage coil was 2.41.

4.5.6 Well Plate with Birdcage Coil

The well plate with birdcage coil scored fairly well for safe use. There were three components of the objective. This design scored 4s on two of them, the RFC wire does not heat up higher than 30 degrees Celsius and it does not contain hazardous materials. Therefore, by the definitions listed in the metrics, and by the design team's opinions, these two components each received 4s. The remaining component was the pressure that the device could withstand without causing damage to human skin. The design team felt that because there was a plastic well plate and a birdcage coil, that moderate pressure around 30 lbs. could possibly break one or both parts of the device. The team scored it a 2 for the design under this objective for that reason. That number was added to the first two 4s and averaged in order to obtain a normalized score of 3.33. That number was then multiplied by the weight of the objective, 18%, to get the weighted score of 0.6.

After that, durability for the design was scored it. Durable has two components and the design team felt that it did not achieve each one of them very well. The first component of durable is that the device should be able to withstand the shaking and vibrations of the MRI without pieces coming lose. For this component, this design received a 4. It received a 4 because the team felt that the design was as sturdy as the others were and a surface coil would remain in its proper placement when the machine reached its highest intensity. For the other component, the ability of the device to withstand a 6-foot drop when assembled and still function as intended, the design received a score of 0. The team scored it a 0 because a fall of that magnitude would damage the birdcage coil to the point where it would not function anymore. By the definition in the metrics, that results in a score of 0. The two components were added

together and divided by 2 to average them and normalize the score. Then they were multiplied by the weight percentage of the objective (12%). The final weighted score for durability of the well plate with multiple loop surface coils was 0.24.

Next, the ability of the design to hold and image multiple samples was scored. It scored as high as possible for this objective. It received a 4 because it holds 12 sponges, and by the definition in the device metrics, it warrants that score as long as it holds 8 or more. The 4 was multiplied by the weight percentage, which was 6.1%, and its final weighted score was 0.244.

The following objective was low cost for building the device. This particular design scored very well in this objective. Not much money would need to be spent for the design. Well plates are inexpensive if more are needed and the material for building birdcage coils is available the lab. Therefore, the total cost for putting it together would be less than $30. Based on the device metrics, it receives a score of 4. When that is multiplied by the weight percentage, 12%, it has a final weighted scored of .48.

Subsequently, the design was scored on how well it completed the objective of being easy to clean. The design team felt that well plates would not be very hard to clean, but also felt that it was not the best design to make cleaning as easy as possible, so it received a score of 3 for the objective. Since the device has wells, the team felt that it would take a little extra time to clean, at least more than the 5 minutes required to score a 4. It would take between 5 minutes and 10 minutes to wipe out each well and clean the device effectively. Its score of 3 was multiplied by its weight percentage, 2%, and its final weighted scored was calculated to be 0.06.

Next, the design's ability to house the radio frequency coil was scored. The radio frequency coil would not have much trouble being incorporated into the well plate. There is already existing birdcage RFC's at the MRI lab that fit well plates. It received a score of 4 and when multiplied by the weight percentage of 4 %, it received a final weighted score of 0.16.

The design's portability was the next objective that was scored. The team decided that this device should receive a score of 4 for this objective. In the device metrics, a design that gets a 4 must weigh less than 20 lbs and take up less than 4 square feet. This design would be a little bigger than a well plate and not much heavier than the wire of the RF coil and the well plate itself, so nothing even close to 20 lbs. Its score of 4 was multiplied by its weight percentage of 1 %. Its final weighted score for this objective was 0.04.

Connecting to the current setup in the MRI lab was the following objective that was scored by the design team for the well plate with birdcage coil. The metric for receiving a score of 4 was that the device had to carry the appropriate current through the radio frequency coil to produce an MR image. The design team felt that this design completed this objective as well as any other and scored the objective a 4. The weight percentage for the objective is 2.9%. The final weighted score for the objective of this alternate design is 0.116.

Calibration is another important part of starting an MR imaging process and is the next objective that was scored. The team felt that this design could not be calibrated easily by one person and that two people would be required to calibrate the device easily. Therefore, according to our metrics, the design team scored this objective a 3. When the

score was multiplied by the weight percentage of 2%, it calculated the final weighted score to be 0.06.

Next, maintaining the temperature of the sample was the objective that was scored. Its weight percentage was 2% of the entire design. The design team felt that this design would maintain the temperature as well as any other design and scored it a 4 for the objective. That was based upon the fact that the well plate had a cover that protected the sponges from exposure to the open air and therefore maintain its starting temperature as well as have a proper circuit in the RF coil that does not generate heat. This design received a 4 and when multiplied by the weight %, the final weighted score was calculated. It was 0.08.

Maintaining the physical state of the sponges was the next objective the team scored. It also received a 4 for this objective because according to the device metrics, the design had to maintain the hydration and hold the sponge so that it was not deformed. The design received a 4 because the well plate is where the sponges are made, so they fit extremely well and are not deformed when placed in the well and also because the cover prevents any dehydration of sponges. The score of 4 was multiplied by the weight percentage of 1% to reach the final weighted score of 0.04.

After that, the ability of the design to maintain the chemical state of the sample was scored by the design team. The team decided that this objective would also receive a 4 for this particular design. This score was reached by considering the fact that the only part of the device to touch the sponge is the well plate. They are plastic and are, in fact, the very device in which the sponges are created. They will in no way chemically alter

the sponges and therefore received the 4 for that reason. Its weight percentage was 4 % of the entire design. The final weighted score for the objective was 0.16.

The next objective scored by the design team was the high filling factor of the radio frequency coil. This design received a score of 0 for this objective. The reasoning was that a birdcage coil would have less than 65% filling factor. In other words, the volume of the sample coil would be less than 65% of the birdcage coil. The weighted score for the design for this objective is 0.

Finally, the ability to visualize the sample while it degrades in the device is the last objective scored for the well plate with birdcage coil. This design for the device completes this objectively to the highest degree possible. This design has clear well plates as a holder for the collagen. This allows the design team to view into the wells and be able to tell when the sponge has degraded completely. Based on the definition in the device metrics, the objective was given a 4 as a score. When multiplied by the weight percentage, 1%, the final weighted score was reached and it was 0.04.

With all of the weighted objectives scored to see how well the design completed each of them, a total weighted scored was reached for each objective. The numerical evaluation took into account how well each objective was met by the design and how important it was when looking at the big picture. Each weighted score was added up and the total weighted score for the well plate with a birdcage coil was 2.32.

4.5.7 Contact Lens Case with Multiple Loops of a Surface Coil

The contact lens cases with a surface coil that loops around the well plate a few times scored very well for safe to use. It scored 4s in each of the three components of the safe to use objectives. The design team gave this design 4 points for each component

because the RFC wires stayed below 30 degrees Celsius, did not injure/damage skin when pressure exceeded 40 lbs, and contained no hazardous materials. In each component, this design was as good as it gets. The three scores of 4 points each were added together and divided by 3 to get an average. The average score was the normalized score. The 4 average was multiplied by the weight % of the objective, 18%. This gave the weighted score for the objective; which was 0.72, the highest possible.

Additionally, the design scored very high for durable as well. It scored a 4 and a 3 for the two components of the durability objective. The first component was about the device's ability to resist the vibrations and shaking of the MRI while remaining in working order and not falling apart. The team decided that the contact lens cases held the sponges tightly in place and were securely fastened to the supporting plastic plate. The team therefore concluded that the design could withstand the highest amount of shaking and vibrations the MRI machine could create and was awarded 4 points for the first component of the objective. The second component was surviving a 6-foot drop. The team felt that the surface coil or the well plate would receive some cosmetic damage, but not anything too serious. Therefore, according to the device metrics, that second component was awarded 3 points. The two numbers were added together and divided by 2 to get the average. The average was a normalized score for the objective. The normalized score, 3.5, was multiplied by the weight for the objective, 12%. That resulted in the weighted score for the objective of the device being 0.24.

Next, the ability to hold multiple samples was evaluated by the design team. The design team scored this objective a 4 for the contact lens cases and surface coil. The lens cases are small and inexpensive and many were arranged on a plastic board. More than 8

samples can be imaged at a time with this design and, according to the device metrics, the design gets a score of 4. That value is multiplied by the weight of the objective, 6.1%. That gives the weighted score of 0.244 for the design and the objective.

Low cost is the next objective that was evaluated. The design team awarded the design 4 points for this objective also. The contact lens cases were very inexpensive and the plastic board was scrap from the Mechanical Engineering Department at WPI. The team did not spend more than $30 building this prototype and, under the definitions in the device metrics, this objective receives a score of 4. That score is multiplied by the weight, 12%, to give the weighted score of 0.48.

Easy to clean was the next objective the design team scored. The score the team came up with was a 4. The entire device could go under a faucet to be washed. It would take less than 5 minutes to clean and scrub. According to the device metrics, that warrants a score of 4. That score of 4 multiplied by the weight, 2%, gives the weighted score of 0.08.

Following that, housing the RFC needed evaluation. The design team gave this objective 4 points as well. This design would not cause any deviation from the normal imaging devices and they all house RFC's with ease. Based ont he metrics, the objective was awarded a score of 4 points. That multiplied by its weight, 4%, gives the weighted score of 0.16.

Next, the portability of the device was scored. The team once again decided to award 4 points to the contact lens cases with surface coils because it was very light (less than 20lbs), very small (less than 4 square feet), and very easy to carry around. The size and weight are what the score is based on, and that is why the device was awarded 4

points. That score multiplied by the weight, 1%; give the weighted score of the objective, 0.04.

Next, the connection to the current setup in the MRI lab was evaluated by the design team. The score was determined by the fact that the RFC is setup like every other one in the lab. It did not cause any problems with the imaging; it carries to right amount of signal and current. Since it connects to the current setup as well as it could, it received the score of 4. That score was multiplied by the weight of the objective, 2.9%, and the weighted scored for the objective of the design was 0.116.

After that, the calibration had to be easy. The design team evaluated the score by assessing how easy it was for one person to calibrate the device. The team learned that the device was not easy to calibrate with one person, but was with two. According to the device metrics, the objective was awarded 3 points for the extent it was completed. Those 3 points were multiplied by the weight of the objective, 2%, and the weighted scored was arrived at, 0.06.

The next objective the design team evaluated was how well the temperature of the sample was maintained. The design team scored this objective similar to the previous designs. Since this design included caps for each lens case, protecting it from being exposed to the air, the design team felt that the samples temperatures would be maintained less than 15 degrees Celsius from the starting temperature. According to the device metrics, the design team scored the objective 4 points. Those points were multiplied by the weight of the objective, 2%, and that value is the weighted score, 0.08.

Subsequently, the physical maintenance of the samples in the device was evaluated. The contact lens cases have a slight concavity to them but also some ridges

inside to support the sponge off the curved bottom somewhat, but not completely. The covers to the lens cases prevent dehydration. Considering both of these facts, the design team still had to give a score of 2 for the objective. That score was multiplied by the weight of the objective, 1%, and that value is the weighted score, 0.02.

After that, the maintenance of the chemical state of the samples was evaluated. The team felt that since only the soft plastic of the contact lens cases would be touching the sample, and that they would not affect the sample chemically, the device should receive the score of 4 for this objective of the design. The weight of 4% was multiplied by the score of 4 to get the weighted score of 0.16.

Next, the filling factor of the RFC was evaluated to make sure it was high. The lens cases are about the same diameter of the sponges, therefore, the filling factor is maximized because there is very little wasted space inside each of the surface coils. In other words, there is very little space inside the cross section of those coils that is not sponge. However, the team still did not feel that it was the best filling factor and that more like 90%, and not 95% of the cross sectional area was sponge. Due to the device metrics, the team scored it 3 points. The score of 3 was multiplied by the weight, 1%, and the result, 0.03, is the weighted score.

Finally, the last objective for this alternate design is the ability to visualize the samples as they degrade during imaging. The team awarded the design 1 point because the cases are not clear. Seeing a nearly degraded white collage sponge is impossible through the translucent cases. When the sample was large, the sponge could be seen from the top of the case. However, after any degradation began, it was virtually

impossible to see. Therefore, the team awarded that aspect of the design 1 point. That was multiplied by the weight, 1%, and that result is the weighted scored, 0.01.

After the weighted score was established for each one of the objectives, they were all added together to get a total weighted score for that particular alternate design. Each objective was weighted to take into account how important that objective was to the entire device and the design was scored on how well it completed each objective. The total weighted score for contact lens cases with surface coil was 2.62.

4.5.8 Tray with Dividers with Multiple Loop Surface Coil

The tray with dividers with multiple looped surface coils was first evaluated on how well it completed the objective of being safe to use. As mentioned previously, safe to use is broken down into three components. This design scored a 4 in each of the three components: keeping the RFC wire under 30 degrees Celsius, not puncturing skin when 40 lbs of force is applied, and contains no hazardous materials. All three scors were averaged to yield a normalize value. That normalized score was then multiplied by the weight of the objective, 18% of the total design, to get the weighted score, 0.72.

The next objective that the design team scored was the durability of the design. This objective had two components and the design scored low for each one. The design scored a 2 for the first component because the team felt that it would not be able to withstand much more than moderate shaking because the dividers in the tray would be dislodged and the design would then fail. The same thought was behind the score of 1 for its ability to survive a 6-foot fall. The fall would destroy the arrangement of the spacers in the tray and cause the design to fail. The two scores were averaged to get a normalized

score. The normalized score was multiplied by the weight of the objective, 12%, to get the weighted score, 0.18.

The next objective that was evaluated was the design's ability to hold and image multiple samples at the same time. The design team scored this device a 4 for this objective because a tray could easily be divided into 8 or more sections to hold samples. As the definition in the device metrics says, the design would deserve a score of 4. The score was then taken, multiplied by the weight of the objective, 6.1%, to get the weighted score, 0.244.

After that, the cost of the design was scored; the lower the cost, the better the score. The design team scored this device a 4 for the low cost. The entire device could be fabricated for $30 or less and, by the definition of low cost in the device metrics, that warrants the score of 4. That score is multiplied by the weight of the objective, 12%, to get the weighted score, 0.48.

Next, the easy to clean objective was assessed. The design team felt that this device could not be completely cleaned and reassembled in less than ten minutes and that metric was assigned a grade of 2 for easy to clean. The score of 2 was multiplied by the weight of the objective, 2%, to get the weighted score of 0.04.

After that, the device was evaluated to see how well it accommodated the RFC. The design team gave this device a 4 because they felt that it should have no problem housing a fully functioning RF coil in and around the collagen. The collagen was supported by a plastic block where the circuit board of an RF coil could rest. Other than that, all that was needed is the surface coils around the samples. This design fully houses

an operating RFC and therefore received a 4 from the design team. That score was multiplied by the weight of the objective, 4%, to get the weighted score of 0.16.

The portability of the design was scored next. The design team felt that this device was worthy of a 4 because it was less that 20 lbs and it took up less than 4 square feet, the definition of a 4 score from the device metrics. The score was multiplied by the weight of the objective, 1%, to get the weighted score, 0.04.

Following that, how well the device connected to the current setup in the MRI lab was scored. The device received a score of a 4 because the device has no unusual design or parts that would interfere with a normal connection to the MRI equipment. The score was multiplied by the weight of the objective, 2.9%, to get the weighted score, 0.116.

The difficulty of calibrating the RFC was the next objective evaluated. The design team gave this device a score of 3 because the device could not easily be calibrated by one person. However, two people working together could calibrate it easily and, according to the device metrics, that warrants a score of 3. That was multiplied by the weight of the objective, 2%, to get the weighted score of 0.06.

Next, the ability to maintain the temperature of the sample was evaluated. The design team felt that the sample was not heated up due to the RF coil and remained within 15 degrees Celsius of its starting temperature during imaging. That is the definition of a 4 in the device metrics. The score was multiplied by the weight of the objective, 2%, to get the weighted score of 0.08.

After that, the ability to maintain the physical state of the samples was evaluated. The team felt that the design would do nothing to physically deform or damage the

sample and awarded the design 4 points for this objective. The score was multiplied by the weight, 1%, to get the weighted score for the objective, 0.04.

Next, the objective of maintaining the chemical state was scored. The design team felt that the objective deserved a score of a 4 because the only materials that would be exposed to the samples would be plastics and they would not cause any kind of chemical reaction with the samples. Therefore, the score of 4 was multiplied by the weight of the objective, 4%, to get the weighted score or 0.16.

High filling factor was the next objective that the design team assessed. The design team felt that multiple looped surface coils could be made to have a high filling factor. If the coils were well-constructed, the sample would comprise 95% of the cross-sectional area of the coils. By the definition in the device metrics, the design warrants a score of 4. That, multiplied by the weight of the objective, 1%, gave the weighted score, 0.04.

The final objective to be assessed was the ability to visualize the sample during degradation. The design team felt that there would be no visual obstructions with this design and viewing the samples would easy. Therefore, the team awarded the design 4 points. The score was multiplied by the weight of the objective, 1%, to get the weighted score of 0.04.

All of the objectives were weighted to take into account that some objectives are more important than others are and each one was scored on how well it was completed by the tray with dividers and multiple loops of surface coils. All of the weighted scores were added together to get the total weighted score of 2.4 for the design.

4.5.9 Tray with Dividers with Birdcage Coil

The tray with dividers and birdcage coil design was first evaluated on how well if completed the objective of being safe to use. Safe to use is broken down into three components. This design scored a 4 in each of the three components: keeping the RFC wire under 30 degrees Celsius, not puncturing skin when 40 lbs of force is applied, and contains no hazardous materials. The design team scored 4 points for each component per the definitions in the device metrics. All three scores were averaged together to normalized them. The normalized score was then multiplied by the weight of the objective, 18% of the total design, to get the weighted score, 0.72.

The next objective evaluated was the durability of the design. This objective had two components and the design scored low for each one. The design scored a 2 for the first component because the team felt that it would not be able to withstand much more than moderate shaking because the dividers in the tray would be dislodged and the design would then fail. The same thought was behind the score of 1 for its ability to survive a 6-foot fall. The fall would destroy the arrangement of the spacers in the tray and cause the design to fail. The two scores were averaged to get a normalized score. The normalized score was multiplied by the weight of the objective, 12%, to get the weighted score, 0.18.

The next objective that was evaluated was the design's ability to hold and image multiple samples at the same time. The design team scored this device a 4 for this objective because a tray could easily be divided into 8 or more sections to hold samples. As the definition in the device metrics says, the design would deserve a score of 4. The score was then taken, multiplied by the weight of the objective, 6.1%, to get the weighted score, 0.244.

After that, the cost of the design was scored; the lower, the better. The design team scored this device a 4 for the low cost. The entire device could be fabricated for $30 or less and, by the definition of low cost in the device metrics, that warrants the score of 4. That score is multiplied by the weight of the objective, 12%, to get the weighted score, 0.48.

Next, the easy to clean objective was assessed. The design team felt that this device could not be completely cleaned and reassembled in less than ten minutes and that is the metric to score the device a 2 for easy to clean. The score of 2 was multiplied by the weight of the objective, 2%, to get the weighted score of 0.04.

After that, the device was evaluated to see how well it accommodated for the RFC. The design team gave this device a 4 because they felt that it should have no problem housing a fully functioning RF coil in and around the collagen. The collagen was supported by a plastic block where the circuit board of an RF coil could rest. Other than that, all that was needed is the surface coils around the samples. This design fully houses an operating RFC and therefore received a 4 from the design team. That score was multiplied by the weight of the objective, 4%, to get the weighted score of 0.16.

The portability of the design was scored next. The design team felt that this device was worthy of a 4 because it was less that 20 lbs and it took up less than 4 square feet. The score was multiplied by the weight of the objective, 1%, to get the weighted score, 0.04.

Following that, how well the device connected to the current setup in the MRI lab was scored. The design team gave the device a score of a 4 because the device has no unusual design or parts that would interfere with a normal connection to the MRI

equipment. The score was multiplied by the weight of the objective, 2.9%, to get the weighted score, 0.116.

The difficulty of calibrating the RFC was the next objective evaluated. The design team gave this device a score of 3 because the device could not easily be calibrated by one person. However, two people working together could calibrate it easily and according to the device metrics, that warrants a score of 3. That was multiplied by the weight of the objective, 2%, to get the weighted score of 0.06.

Next, the ability to maintain the temperature of the sample was evaluated. The design team felt that the sample was not heated up due to the RF coil and remained within 15 degrees Celsius of its starting temperature during imaging. That is the definition of a 4 in the device metrics. The score was multiplied by the weight of the objective, 2%, to get the weighted score of 0.08.

After that, the ability to maintain the physical state of the samples was evaluated. The team felt that the design would do nothing to physically deform or damage the sample and awarded the design 4 points for this objective. The score was multiplied by the weight, 1%, to get the weighted score for the objective, 0.04.

Next, the objective of maintaining the chemical state was scored. The design team felt that the objective deserved a score of a 4 because the only materials that would be exposed to the samples would be plastics and they would not cause any kind of chemical reaction with the samples. Therefore, the score of 4 was multiplied by the weight of the objective, 4%, to get the weighted score or 0.16.

High filling factor was the next objective that the design team assessed. The design team felt that birdcage coil would not have a high filling factor. Even if the

birdcage coil were constructed perfectly, it would be hard to get the sample to comprise more than 65% of the volume of the birdcage coil. Therefore, the team gave this objective a 0 by the definition in the device metrics. The weighted score for this objective was 0.

The final objective to be assessed was the ability to visualize the sample during degradation. The design team felt that there would a minor obstruction because the RFC was a birdcage coil and it would make viewing the samples easy, but not as easy as other designs. Therefore, the team awarded the design 3 points. The score was multiplied by the weight of the objective, 1%, to get the weighted score of 0.03.

Each of the objectives was scored on how well it was completed by the design. Additionally, the importance of each objective was taken into account when the scores were multiplied by the weight % of each objective. The weighted scores were all added up and the tray with dividers and birdcage coil had a final total weighted score of 2.35.

4.5.10 Pill Container with Solenoid

The pill containers with the solenoid coil scored very well for safe to use. There are three components of the objective and they are the following: to keep the RFC wire less than 30 degrees Celsius, to not puncture skin when 40 lbs of pressure is applied, and contain no hazardous materials. Those are the definitions of a device that would receive a 4 in each component according to the device metrics. The three scores were averaged to normalize the scores to 4. The score was multiplied by the weight of the objective, 18%, to get the weighted score of 0.72.

Next, the durability was assessed. The design scored very well for this objective. There are two component of durability and this design scored a 4 and a 3 on them. The

first component is the ability to withstand the shaking and vibrating of the MRI machine. The team felt that this device could withstand the highest degree of shaking and vibration without affecting the functionality of the design, which is what warranted the score of 4. The other component of durability is the ability to survive a 6-foot drop and still function. The team felt that a 6-foot drop could affect the spacing of a solenoid coil slightly and that would affect its ability to produce an image. Therefore, the team decided to score it at a 3 for that component. The two scores were averaged to normalize the score, which was then multiplied by the weight of the objective to get the weighted score of 0.42.

After that, the ability of the design to hold and image multiple samples was assessed. The design scored a 4 because many pill containers could be lined up and placed on a block or plastic platform to hold and image collagen sponges. It would hold more than 8 samples and that is why it received a 4. The score was multiplied by the weight of the objective, 6.1%, to get the weighted score of 0.244.

Next, low cost was evaluated. The only expenses that would need to be made for this design are to buy pill containers, a supporting block and a means to fasten the pill containers to the block. This would cost less than $30 and, according to the device metrics, that warrants a score of 4 for the design. The score was multiplied by the weight of the objective, 12%, to get the weighted score, 0.48.

Subsequently, the objective of easy to clean was scored. The design team felt that this design accomplished this objective very well, but not perfectly. Because there are individual containers that need to be scrubbed out, the team scored it a 3 because they felt it would not be able to be cleaned effectively in less than 5 minutes. The score was then multiplied by the weight of the objective, 2%, to get the weighted score of 0.06.

After that, housing the RFC was scored. The design received a 4 because the support block that the pill containers sit on can be used to put a circuit board required by the RFC. Besides that, there are not any unusual designs of the device that would prevent it from housing a fully functional RFC. That is why it received the score of 4. That score was multiplied by the weight of the objective, 4%, to get the weighted score, 0.16.

Next, the portability of the device was scored. The design would not be any heavier than 20 lbs and would not be large enough to take up more than 4 square feet of area. According to the device metrics, that warrants the score of 4. That score was multiplied by the weight of the objective, 1%, to get the weighted score of 0.04.

After that, the objective of how well it connects to the current setup was assessed. The design team found that this design connects to the current setup at the MRI lab as efficiently as possible. It connects to the setup perfectly; there is nothing in the design that would obstruct or interfere with the connection. Therefore, it received a score of 4. That score was multiplied by the weight of the objective, 2.9%, to get the weighted score, 0.116.

Following that, the objective of easy to calibrate was assessed. The team scored this objective a 3 because it could not easily be calibrated with one person but it could be easily calibrated with two people. The score was multiplied by the weight of the objective, 2%, to get the weighted score of 0.06.

The objective of maintaining the temperature of the sample was next to be assessed. The design team felt that the covers of the pill container would help insulate the sample from any external temperature changes. In addition, the team felt that the RFC coil would not cause heat. In either case, the team felt the sample would not change

by more than 15 degrees Celsius and according to the device metrics, which warrants a score of 4. The score was multiplied by the weight of the objective, 2%, to get the weighted score of 0.08.

After that, maintaining the physical state of the samples was evaluated. The design team felt that the pill containers would prevent dehydration and would not deform or compress the samples in any way. Therefore, the team awarded the objective 4 points. That score was multiplied by the weight of the objective, 1%, to get the weighted score of 0.04.

Next, the maintenance of the chemical state of the sample was scored. The team scored it a 4 because they felt that the only thing that would be in contact with the sample was the pill containers and they would not affect the sponge chemically in any way. That is why the design received a 4. The score was multiplied by the weight of the objective, 4%, to get the weighted score of 0.16.

After that, the filling factor was evaluated. The pill containers are a little larger than the sponges, so there is a fair amount of wasted filling space within the wells that the sponge does not take up and therefore reduces the filling factor because the RFC is limited by the walls of the wells. The team estimated that about 75-80% of the volume coil would be filled with the sample and therefore, according to the metrics, it was scored a 2. The score was multiplied by the weight of the objective, 1%, to get the weighted score, 0.02.

Finally, the ability to visualize the sample was assessed. The team awarded the design 3 points for this objective because the pill containers were clear and easy to see through, but it became more difficult when the solenoid coils wrapped around the wells.

That made the sponge harder to see, especially when degradation started. The score was multiplied by weight of the objective, 1%, to get the weighted scored of 0.03.

Each of the objectives of the design was assessed to see how well each was completed by the design and scored. Additionally, the importance of each objective was taken into account. All the weighted scores were added and the total was 2.63.

4.5.11 Pill Container with Birdcage Coil

The pill containers with the solenoid coil scored very well for safe to use. There are three components of the objective and they are the following: to keep the RFC wire less than 30 degrees Celsius, to not puncture skin when 40 lbs of pressure is applied, and contain no hazardous materials. Those are the definitions of a device that would receive a 4 in each component according to the device metrics. This design does all of those and therefore, it received a score of 4 for each component of the safe to use objective. The three scored were averaged to normalize the score and the result was 4. That was multiplied by the weight of the objective, 18%, to get the weighted score of 0.72.

Next, the durability was assessed. The design scored average for this objective. There are two component of durability and this design scored a 4 and a 1 on them. The first component is the ability to withstand the shaking and vibrating of the MRI machine. The team felt that this device could withstand the highest degree of shaking and vibrating without affecting the functionality of the design, which warranted the score of 4. The other component of durability is the ability to survive a 6-foot drop and still function. The team felt that a 6-foot drop would damage the birdcage coil to such an extent that pieces would still be connected, but may need to be fixed before it worked correctly again. Therefore, the team decided to score it at a 1 for that component. The two scores

were averaged to normalize them. The normalized score was multiplied by the weight of the objective to get the weighted score of 0.3.

After that, the ability of the design to hold and image multiple samples was assessed. The design scored a 4 because many pill containers could be lined up and placed on a block or plastic platform to hold and image collagen sponges. It would hold more than 8 samples and that is why it received a 4. The score was multiplied by the weight of the objective, 6.1%, to get the weighted score of 0.244.

Next, low cost was evaluated. The only expenses that would need to be made for this design are to buy pill containers, a supporting block and a means to fasten the pill containers to the block. This would cost less than $30 and according to the device metrics, that warrants a score of 4 for the design. The score was multiplied by the weight of the objective, 12% to get the weighted score, 0.48.

Subsequently, the objective of easy to clean was scored. The design team felt that this design accomplished this objective very well, but not perfectly. Because there are individual containers that need to be scrubbed out, the team scored it a 3 because they felt it would not be able to be cleaned effectively in less than 5 minutes. The score was then multiplied by the weight of the objective, 2%, to get the weighted score of 0.06.

After that, housing the RFC was scored. The design received a 4 because the support block that the pill containers sit on can be used to put a circuit board required by the RFC. Besides that, the design the device is normal and does not have anything else that would prevent if from housing a fully functioning RFC. That is why it received the score of 4. That score was multiplied by the weight of the objective, 4%, to get the weighted score, 0.16.

Next, the portability of the device was scored. The design would not be any heavier than 20 lbs and would not be large enough to take up more than 4 square feet of area. According to the device metrics, that warrants the score of 4. That score was multiplied by the weight of the objective, 1%, to get the weighted score of 0.04.

After that, the objective of how well it connects to the current setup was assessed. The design team found that this design connects to the current setup at the MRI lab as efficiently as possible. It connects to the setup perfectly, there is noting in the design that would obstruct or interfere with the connection. Therefore, it received a score of 4. That score was multiplied by the weight of the objective, 2.9%, to get the weighted score, 0.116.

Following that, the objective of easy to calibrate was assessed. The team scored this objective a 3 because it could not easily be calibrated with one person but it could be easily calibrated with two people. The score was multiplied by the weight of the objective, 2%, to get the weighted score of 0.06.

The objective of maintain the temperature of the sample was next to be assessed. The design team felt that the covers of the pill container would help insulate the sample from any external temperature changes. In addition, the team felt that RFC would not cause heat. In either case, the team felt the sample would not change by more than 15 degrees Celsius and according to the device metrics, which warrants a score of 4. The score was multiplied by the weight of the objective, 2%, to get the weighted score of 0.08.

After that, maintaining the physical state of the samples was evaluated. The design team felt that the pill containers would prevent dehydration and would not deform

or compress the samples in any way. Therefore, the team awarded the objective 4 points. That score was multiplied by the weight of the objective, 1%, to get the weighted score of 0.04.

Next, the maintenance of the chemical state of the sample was scored. The team scored it a 4 because they felt that the only thing that would be in contact with the sample was the pill containers and they would not affect the sponge chemically in any way. That is why the design received a 4. The score was multiplied by the weight of the objective, 4%, to get the weighted score of 0.16.

After that, the filling factor was evaluated. The pill containers are a little larger than the sponges, so there is a fair amount of wasted filling space just within the walls of the pill containers. However, a birdcage coil further extends the wasted space and continues to reduce the filling factor to below 65% of the volume coil. Therefore, according to the metrics, it earned a score of 0. The weighted score for this objective was 0.

Finally, the ability to visualize the sample was assessed. The team awarded the design 4 points for this objective because the pill containers are already transparent, and easy to see through, but now it does not have the solenoid coils obstructing the view as in the last design. The birdcage coil makes the sample completely visible during degradation. That is why the score of 4 was decided on. The score was multiplied by weight of the objective, 1%, to get the weighted scored of 0.04.

Each of the objectives of the design was assessed to see how well each was completed by the design and scored. Additionally, the importance of each objective was

taken into account. All the weighted scores were added and pill containers within the birdcage coil received 2.5.

4.5.12 Pill Container with Multiple Loop Surface Coil

The pill containers with the surface coil scored very well for safe to use. There are three components of the objective and they are the following: to keep the RFC wire less than 30 degrees Celsius, to not puncture skin when 40 lbs of pressure is applied, and contain no hazardous materials. Those are the definitions of a device that would receive a 4 in each component according to the device metrics. The three scored were averaged to normalize the score and the result was 4. That was multiplied by the weight of the objective, 18%, to get the weighted score of 0.72.

Next, the durability was assessed. The design scored very well for this objective. There are two component of durability and this design scored a 4 and a 2 on them. The first component is the ability to withstand the shaking and vibration of the MRI machine. The team felt that this device could withstand the highest degree of shaking and vibration without affecting the functionality of the design, which warranted the score of 4. The other component of durability is the ability to survive a 6-foot drop and still function. The team felt that a 6-foot drop would affect the placement of the surface coils, but after some minor adjustments and fine-tuning, should be able to image without a problem. Therefore, the team decided to score it at a 2 for that component. The two scores were averaged to normalize them. The normalized score was multiplied by the weight of the objective, 12%, to get the weighted score of 0.36.

After that, the ability of the design to hold and image multiple samples was assessed. The design scored a 4 because many pill containers could be lined up and

placed on a block or plastic platform to hold and image collagen sponges. It would hold more than 8 samples and that is why it received a 4. The score was multiplied by the weight of the objective, 6.1%, to get the weighted score of 0.244.

Next, low cost was evaluated. The only expenses that would be needed for this design are to buy pill containers, a supporting block and a means to fasten the pill containers to the block. This would cost less than $30 and, according to the device metrics, that warrants a score of 4 for the design. The score was multiplied by the weight of the objective, 12%, to get the weighted score, 0.48.

Subsequently, the objective of easy to clean was scored. The design team felt that this design accomplished this objective very well, but not perfectly. Because there are individual containers that need to be scrubbed out, the team scored it a 3 because they felt it would not be able to be cleaned effectively in less than 5 minutes. The score was then multiplied by the weight of the objective, 2%, to get the weighted score of 0.06.

After that, housing the RFC was scored. The design received a 4 because the support block that the pill containers sit on can be used to put a circuit board required by the RFC. Then the surface coil could simply sit on top of or under the pill containers. Besides that, this is a very normal design for a device that is being MR imaged. Therefore, there was nothing to prevent if from using and housing a fully functionally RFC. That is why it received the score of 4. That score was multiplied by the weight of the objective, 4%, to get the weighted score, 0.16.

Next, the portability of the device was scored. The design would not be any heavier than 20 lbs and would not be large enough to take up more than 4 square feet of

area. According to the device metrics, that warrants the score of 4. That score was multiplied by the weight of the objective, 1%, to get the weighted score of 0.04.

After that, the objective of how well it connects to the current setup was assessed. The design team found that this design connects to the current setup at the MRI lab as efficiently as possible. It connects to the setup perfectly, there is nothing in the design that would obstruct or interfere with the connection. Therefore, it received a score of 4. That score was multiplied by the weight of the objective, 2.9%, to get the weighted score, 0.116.

Following that, the objective of easy to calibrate was assessed. The team scored this objective a 3 because it could not easily be calibrated with one person but it could be easily calibrated with two people. The score was multiplied by the weight of the objective, 2%, to get the weighted score of 0.06.

The objective of maintaining the temperature of the sample was next to be assessed. The design team felt that the covers of the pill container would help insulate the sample from any external temperature changes and there would be no heat from the RFC. Therefore, nothing would change the sample temperature by 15 degrees Celsius and, according to the device metrics, that warrants a score of 4. The score was multiplied by the weight of the objective, 2%, to get the weighted score of 0.08.

After that, maintaining the physical state of the samples was evaluated. The design team felt that the pill containers would prevent dehydration and would not deform or compress the samples in any way. Therefore, the team awarded the objective 4 points. That score was multiplied by the weight of the objective, 1%, to get the weighted score of 0.04.

Next, the maintenance of the chemical state of the sample was scored. The team assigned a scored of 4 because they felt that the only thing that would be in contact with the sample was the pill containers and they would not affect the sponge chemically in any way. The score was multiplied by the weight of the objective, 4%, to get the weighted score of 0.16.

After that, the filling factor was evaluated. The pill containers are a little larger than the sponges, so the surface coils need to be well constructed to encompass as little of the area beside that of the sponge. The team estimated that they could be constructed so 95% of it contained the entire sample. According to the metrics, it was scored a 4. The score was multiplied by the weight of the objective, 1%, to get the weighted score, 0.04.

Finally, the ability to visualize the sample was assessed. The team awarded the design 4 points for this objective because the pill containers are already transparent and, by the nature of surface coils, there will be nothing in the way of seeing the sample during degradation. The surface coils will be out of the way and viewing samples in the containers will be easy. That is why the score of 4 was assigned. The score was multiplied by weight of the objective, 1%, to get the weighted scored of 0.04.

Each of the objectives of the design was assessed to see how well each was completed by the design and scored. Additionally, the importance of each objective was taken into account. All the weighted scores were added together and the pill container with surface coil design received 2.6.

4.5.13 Concentric Tube with Solenoid Coils

The concentric tube design did not score very well when the design team scored it on the objective of safe to use. It received scored of 4, 1, and 4 on the three components

of safe to use. The design scored a 4 on the component that dealt with having a RFC that did not overheat. The device metrics defined that a design that has a coil that stays under 30 degrees Celsius receives a 4, and that is what this design does. The design, however, did not fair so well when a force was applied to it. The design team felt that the glass tube would break and no longer be safe when a force of 20 lbs was put on it. Therefore, as defined in the metrics, it received a 1 for that component. Finally, the design received a 4 on the final component because it does not contain any hazardous materials and its score is defined that way in the metrics. The scores for the three components were added and divided by three to normalize the score; which was 3. This score was multiplied by the weight percentage, 18%, and the final weighted score for safe use for this design was 0.54.

The design also did not have much success with grading for durability. The design team gave the device a 2 for the "ability to withstand shaking" component of the durable objective. The team arrived at this score because the tubes containing the sponges inside the large glass tube are not fixed by any means. Therefore, they can become dislodged if the intensity of the vibrations reached a moderate level and the device would fail during imagine. The team also scored the ability to withstand a fall as 0. This is because the device would be destroyed if dropped from ten feet. The glass would shatter and nothing would hold the collagen anymore. The two scores were averaged to normalize the score. That value, 1, was multiplied by the weight percentage, 12%, to get the final weighted score of 0.12.

The design team next scored the concentric tube design a 4 at holding multiple samples. The design team arrived upon this score because the device could hold more

than eight samples in a line of small tubes inside a large glass tube. The score was multiplied by the weight percentage, 6.1 %, to find the final weighted score, 0.244.

After that, the low cost of the design was evaluated by the design team. The team felt that the design fulfilled this objective relatively well, but not the best. The team gave the design a 4 for this objective because it would not cost more than $30. A glass tube would be expensive but a solenoid coil would not cost anything more. Therefore, the total cost will not be more than $30. The score of 4 was multiplied by the weight % of 12 to get the final weighted score of 0.48.

Following that, easy to clean was the next objective scored by the team. The team decided that the concentric tube design deserved a 2 for this objective. The team felt that have tubes inside a large glass tube would be reasonably complicated to clean. The entire design would have to be dismantled to clean and then reassembled to prepare for another image and the team felt that this would take more than 10 minutes. The score of 2 was multiplied by the weight % of 2 to get the final weighted score of 0.04.

Housing the radio frequency coil was the next objective to be scored by the design team for this alternative design. The team scored it a 4. There were examples of tubes that the rats were imaged in at the MRI lab with birdcage coils around them. They all contained a working RFC and housed it during imaging without much problem. Solenoid coils are smaller that the birdcage coil and will be used in this design. Therefore, it should be easier to fit than a birdcage. The score of 4 was multiplied by the weight % of 4. The final weighted score was 0.16.

Portability was the following objective that was analyzed for the concentric tube design. The design team gave this particular design a 4 because it weighed less than 20

lbs and took up less than 4 square feet. When the score of 4 was multiplied by the 1 weight %, the weighted score of 0.04 was derived.

After that, the ability for the design to connect to the current setup in the MRI lab was evaluated. The team felt that this design would completely fulfill that objective and gave it a 4. There would be no need to vary from the conventional RFC and its connection to the MRI machine. Therefore, a 4 was awarded to the objective with the weight % of 2.9. The final weighted score was 0.116.

Then, easy to calibrate was evaluated by the design team. The team awarded 3 points for the score of the objective. The reason that the objective received 3 points is because it would not be easy for one person to calibrate this design. However, it would be easy for two people to do it and that is why it was awarded 3 points. That multiplied by the weight % of 2 gives a weighted score of 0.06.

Next, the concentric tube's ability to maintain the temperature of the sample was evaluated. The design team awarded the device 4 points because it has several tubes inside a large tube that eliminates exposure of the sample to air. It keeps it insulated to a certain extent. The score of 4 was multiplied by the weight % of 2% to get the weighted score of 0.08.

After that, the ability to maintain the physical state of the sample was evaluated. The team gave this a zero score because when the tubes were placed inside a large tube, they would have to be put into the MRI bore sideways. This would cause all of the sponges to be on their sides and deform. This resulted in a weighted score of 0.

Next, the design team gave the concentric tube a 4 for its ability to maintain the chemical state of the sponge. Only glass or plastic would be touching the sample and

they would not affect the chemistry of the sponges. The score was multiplied by the weight, 4%, to get the weighted score of 0.16.

The next objective that was scored was the high filling factor of the RFC. The design team gave this alternate design a 3 for this objective as well. The reason is that the smaller tubes containing the sponges would have to go inside a larger tube. This makes the large distance from the tubes to the sample even larger. Then it was put inside the solenoid coil. There is no space between the large tube and the solenoid coil, but since the large tube creates a space between the coil and the samples, the team scored the device a 3 for this objective. The score was multiplied by the weight of the objective, 1%, to get the weighted score, 0.03.

Finally, the ability of view the sample during degradation and imaging was considered. The design team felt that the concentric tube deserved to be awarded a score of 1 for this objective. The tubes that hold the sponges are clear but the solenoid coil will make viewing the sponges a little difficult. The coil would interfere with viewing the sponges greatly. That is why the design received a 1. The score was multiplied by the weight percentage of 1 and the final weighted score was the result. The weighted score was 0.01.

Each objective for this design was scored to see how well the design fulfilled the necessary objectives. In addition, the importance of the objectives was taken into account. When all of the weighted scores were added up, the total weighted score for the concentric tube with a solenoid coil was 2.08.

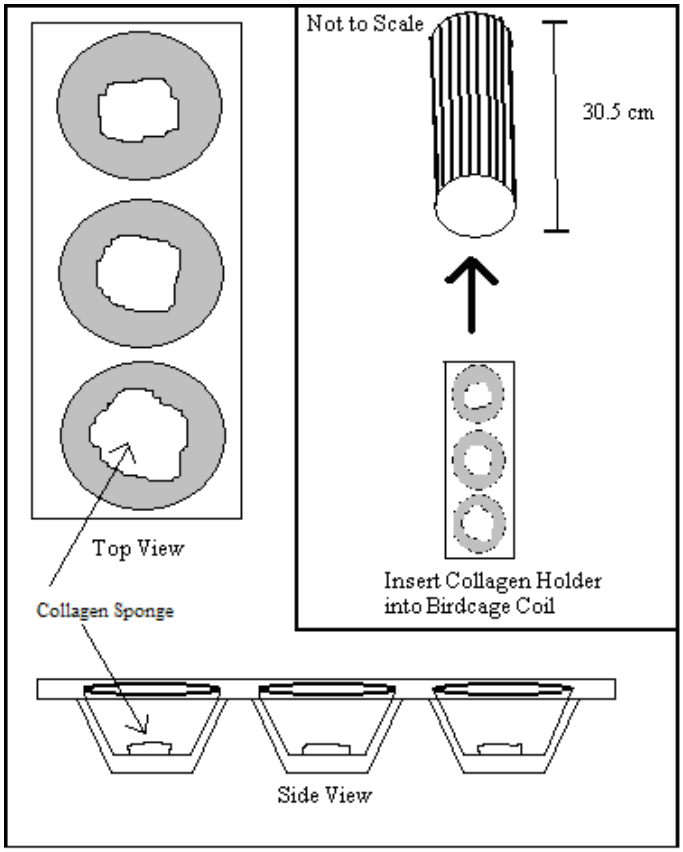
4.6 Final Design

The contact lens case with a birdcage radio frequency coil was chosen as our final design based on the numerical evaluation matrix. A plastic base was cut to proper size and contact lens cases were secured with Velcro®.

The design worked very well in creating MR images. It also imaged four samples at a time, which we learned is the best that could be done. The problem that was encountered could not be foreseen during the design process. During experimentation, it was learned that after the initial imaging, the sponges needed to be taken out, degraded and replaced in the holder. The transporting of degraded sponges was too complicated and delicate. During transporting or rinsing attempts, often times pieces of sponge would be physically removed during our efforts. This would then change the change in dry mass results that we planned to use to quantify our MRI results. If we altered the change in dry mass results so significantly there would be no way to prove that the MRI results were showing degradation. After numerous experimental attempts that included different means of transporting the sponges, the group learned that it was necessary to modify our design. It was necessary to keep the sponges in the same well over the course of the entire experiment. That meant that we had to come up with a way to get solutions off the sponge and conduct rinses without physically altering the delicate degraded sponges. The group decided that Transwells® (Corning, Corning, NY, product #3428) were the best means of accomplishing this feat. Transwells® are portable wells with a fine membrane on the bottom. The pore size of the membrane was 8 micrometers. Each Transwell® sat in its own individual well in a 6 well plate. There was a distance of 1 mm between the bottom of the well and the bottom of the Transwell®. This allowed us to

keep the sponges hydrated when desired by leaving them in the well. Additionally, it gave the design team a way to remove solutions by lifting the Transwells® out of the wells and letting the solutions drip through. This was much easier when conducting rinses because it gave us solutions that were already filtered of particles that could interfere with our ninhydrin assay. In our previous methods, we had to centrifuge the solution numerous times and was very time consuming. It also did not disturb the sponges, which was the main objective in changing the design. The main downfall was that the Transwells® were larger than the contact lens case so only three could be imaged at a time. In addition, we had to redesign the holder and RFC to make them larger. Since we had to increases the size of the collagen holder, it could not fit in the birdcage coil anymore. Then we had to find another birdcage coil to accommodate the larger holder. Another problem with the new design, it was difficult to drip dry the Transwells® and get the sponges to a constant state of hydration between wells and between different experiments. Overall, however, this gave the design team much better results, and made experimentation easier. This was our final design shown in figure 28.

Figure 30: Final Design-Transwell®



4.6.1 Dimensions

The dimensions of our final design were modified to fit within the width of the bore of the magnet, which is 14.5 cm. A platform for the initial birdcage coil was constructed by the design team to orient the coil in the middle of the bore. This ensures that the samples are receiving homogenous magnetic fields and that the data is processed correctly. The final birdcage coil that was used had an outside diameter equal to that of the bore, therefore, it fit inside of the magnet without the need for a platform for proper orientation. The coil was also about 30.48 centimeters long. It was a commercial birdcage coil as opposed to the original coil that was created at the MRI lab by graduate students.

5 MATERIALS and METHODS

The following chapter describes the materials and methods used to complete this project. The experiments for this project are divided by the laboratory in which they are performed. In Professor Pin's laboratory, sponges were fabricated, treated with collagenase and the degradation measured using ninhydrin and change in dry mass. All MRI experiments were performed at University of Massachusetts Medical Imaging Center (UMMIC) in Prof Sotak's laboratory.

5.1 Collagen Sponge Protocols

5.1.1 Sponge fabrication

Sponges were fabricated using bovine Achilles tendon type-I collagen (Sigma, St. Louis, Mo, Cat# C9879). This is an insoluble collagen extracted using Na_2HPO_4 according to the protocol developed by Einbinder and Schubert (1951). The collagen dispersion is prepared by placing collagen flakes in 10mM HCl (pH 2.0) to create a solution that is 1%w/v. This solution is then blended at 4°C for four minutes, allowed to sit for ten minutes and then blended for another four minutes. The solution is then centrifuged for five minutes at 5,000rpm/4000g and stored at 4°C.

Sponges are made in 12-well multi-well plates by placing 2.3mL of the dispersion in each well. The well plates were centrifuged at 800rpm for 15 minutes to remove any air bubbles that may have appeared while measuring out the collagen. The plate is then placed in liquid nitrogen (-196°C) or in the -80°C freezer to freeze the dispersion. The

sponges were then lyophilized using *Recipe 2*. The slower freezing rate that occurs when the collagen is placed in the -80°C freezer results in larger ice crystals. When these crystals are lyophilized in the freeze-dryer they produce larger pores than those obtained by freezing in liquid nitrogen. For this reasoning, the -80°C freezer was only used for the first batch of sponges fabricated. All sponges used for results and analyses were frozen in liquid nitrogen.

Some sponges were crosslinked using dehydrothermal (DHT), UV, or carbodiimide. To perform the DHT crosslinking the sponges were wrapped in tinfoil and placed in the oven with a vacuum of less than 200mTorr and a temperature of 105°C for 24-hours. UV crosslinking was performed by exposing each side of the sponge to UV irradiation. The total time the sponge was under UV irradiation classified the sponge as to its crosslinking extent. For example, if a sponge was exposed to 5 minute of UV irradiation on each side, then the sponge had been UV crosslinked for 10 minutes. Carbodiimide crosslinking was performed by using N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide HCl (Sigma, Cat# E6383) dissolved in DI water at a concentration of 10mg/ml. The sponges were hydrated in this solution for 24 hours and then rinsed thoroughly with PBS. For a detailed procedure, please see Appendix B: Sponge Fabrication.

5.1.2 Collagenase treatment

The sponges were treated with collagenase according to the protocol found in Appendix C: Degradation Protocol. Briefly, sponges were incubated in 0.1M Tris-HCl buffer containing calcium ions at a concentration of 0.025M and with bacterial collagenase (Calbiochem, San Diego, CA, and Cat # 234153) at varying concentrations

for a specific amount of time. The bacterial collagenase, derived from *Clostridium histolyticum*, is a mixture of several different types of enzymes that uses Ca^{2+} ions as a cofactor. The enzyme activity is inhibited at a specified time point by the addition of ethylenediaminetetra-acetic acid (0.25M), a chelate, which removes all the Ca^{2+} ions from the solution thus preventing the enzyme from any further activity.

5.1.3 Change in Dry Mass

The change in dry mass of the sponge was selected as a method to determine the degradation extent of the sponges, as detailed in Appendix D: Change in Dry Mass Protocol. The initial dry mass was compared to the final mass of the sponge after it had been lyophilized to remove any liquid mass. This method provides a good approximation of the degradation extent. The simplicity of the measurement makes it very worthwhile to perform. This test along side the ninhydrin results were intended to provide the determination of the degradation extent.

5.1.4 Ninhydrin

This spectroscopic method measures the amount of free amino acids in the solution. The cleaved collagen molecule will expose a free amino group, on the leucine residue, that will react with the ninhydrin to produce a purple dye that absorbs light at 570nm. The concentration can then be determined from the absorbance at this wavelength. If the degradation was not performed in Transwells®, the solution in which the sponges were degraded is centrifuged to remove insoluble particulates and the supernatant is drawn off. If sponges were degraded in Transwells® the thin film should keep the solution free from insoluble particles. The ninhydrin reagent solution (Sigma, St

Louis, MO, Cat# N7285) is then added to the degraded solution and boiled for 10 minutes using a hot water bath to help the reaction proceed to completion. After this, 95% ethanol is added and the absorbance read. The absorbance value is directly proportional to the amount of free amino acids in the solution. From the absorbance measurement the amount of collagen that was enzymatically cleaved can be determined. If the absorbance of the samples is beyond that range covered by the standard leucine curve, the sample can be diluted with PBS before the ninhydrin reagent is added. For detailed procedures please refer to Appendix E: Ninhydrin Protocol.

5.2 Imaging Protocol

The goal of this project is to develop an imaging technique that best measures the degradation extent of collagen sponges. Standard imaging techniques will be employed to characterize the sponges first. Then the sponges will be degraded a specified amount and imaged again to see if any MR parameters have changed.

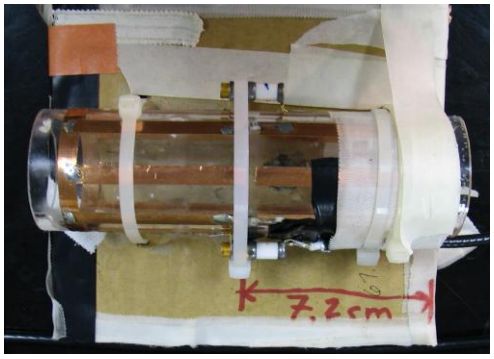
5.2.1 Magnet Set-up

Experiments were conducted at 85 MHz in a 147-cm long, horizontal bore 2 Tesla magnet (Oxford Instruments, Oxford, UK). The collagen samples and RF coil were inserted into the bore of the magnet, and the coil was tuned in that position prior to imaging. ParaVision imaging software (Bruker version 2.1.1, Bruker Instruments, Billerica, MA) was used to acquire and reconstruct the image data.

5.2.2 Radio Frequency Coil

A birdcage coil tuned to 85 MHz, the resonant frequency of the magnet, was used to produce the time varying magnetic fields during acquisition. The original RF coil was

built for the group by Dave Bennett, a graduate student conducting research in the NMR lab. This birdcage coil had an internal diameter of 3.2 cm and a total length of 12.4 cm, including base assembly. This coil featured a polymer cylinder with copper coil exterior and 2 adjustable capacitors. When the holder set-up was switched to the Corning® 6-well plate set-up, a larger coil had to be used. This larger coil was a commercial radio frequency coil, and was chosen because it was able to accommodate the final holder used for this study. This coil was 30.5 cm in length. Please refer to Figure 31 for images of the RF coils used.



3.2 cm diameter birdcage coil

Final birdcage coil

Figure 31 – RF coils

5.2.3 MRI Acquisition

For each imaging technique, images were obtained by taking a single coronal slice encompassing the entirety of the samples. Each image had a matrix size of 256 frequency encodes and 128 phase encoding steps. The field of view (F.O.V.) was a square 12 cm with a slice thickness varying between 5 and 8 mm, depending on the

geometries of the samples at the time. Signal averaging was also employed to reduce random noise in the final images.

Two different imaging sequences were used in order to acquire the MR data and measure the 3 different parameters. T_1-weighted rapid acquisition with relaxation enhancement (RARE) sequence was chosen for its ability to considerably reduce imaging time. With this sequence, 11 TRs ranging from 180 – 7650 msec were used along with a constant TE of 40 msec. T_2-weighted multi-slice multi-echo bio (MSME Bio) scanning was also chosen for its relatively short acquisition times. This sequence maintained a TR of 2230 msec and sampled echoes every 20 msec during that period, or at 12 different TEs.

5.2.4 Data Analysis

Once the raw data was reconstructed by the Bruker system, a number of different programs had to be used to analyze the data. First, two programs were written in interactive data language (IDL) in order to process the data into maps relating the signal intensity of each pixel into proton density (M0′), T_1 relaxation time and T_2 relaxation times. These IDL programs were run using Research Systems® IDL versions 5.5 and 5.6, depending on which lab the data was being processed in at the time. These programs also allowed for different scaling to be applied to the data in order to remove noise or unwanted artifacts still remaining after scaling. Once these maps were created, the program ImageJ was used to create histograms of the parameter values of each map on a pixel by pixel basis.

Each image contained 3 samples in order to reduce acquisition time. When processing the data using ImageJ, each sponge was isolated and analyzed for its MR

parameter values. ImageJ created histograms based on the frequency of specific pixel values within the chosen area of the image. Then, these lists were transferred to Microsoft Excel, and the pixel values generated by ImageJ were converted back to the desired MR parameters by using the following equation:

Equation 6 - Conversion from ImageJ value to MR Parameter value

$$Value_{MRParameter} = PixelValue_{ImageJ} \frac{\left(UpperLimit_{MRScaling} - LowerLimit_{MRScaling}\right)}{256} + LowerLimit_{MRScaling}$$

ImageJ output pixel values on a scale of 0-255 when creating the histograms. When IDL processed the data, the user defined upper and lower limits on the scaling of the maps in order to display the data within the dynamic range of colors. The above equation converts the 0-255 scale of values in ImageJ back to the scaling values the user defined in IDL for processing using Excel. Histograms displaying the pre- and post-imaging data sets were then created in Excel.

6 RESULTS

Following is a compilation of the results obtained, including all preliminary and unsuccessful experiments. Preliminary experiments were performed to refine laboratory skills and to ensure the methods selected from the literature review would actually work in this specific application. The initial results were used to refine the experimental procedure. The preliminary degradation experiments as well as all the ninhydrin assays were performed in Professor Pin's laboratory. The degradation experiments that were used for the final results were performed at UMMIC to decrease the amount of time between degradation and imaging, hence eliminating additional variance. Imaging of the undegraded and degraded sponges was performed at UMMIC in Professor Sotak's laboratory.

6.1 Ninhydrin Response to Leucine

The purpose of these experiments was to verify the ninhydrin response to free amino acids using leucine as a standard and to identify the useful working range of this assay. The first experiment used leucine concentrations varying over several decades. This was necessary to generate the s-shaped response curve and to identify the linear region in which all the concentrations of subsequent experiments should fall to ensure the highest accuracy possible. The concentrations selected were (all in μmol/ml): 100; 50; 10; 5; 1; 0.5; 0.1; 0.05; 0.01; 0.005; 0.001 and 0.0005.

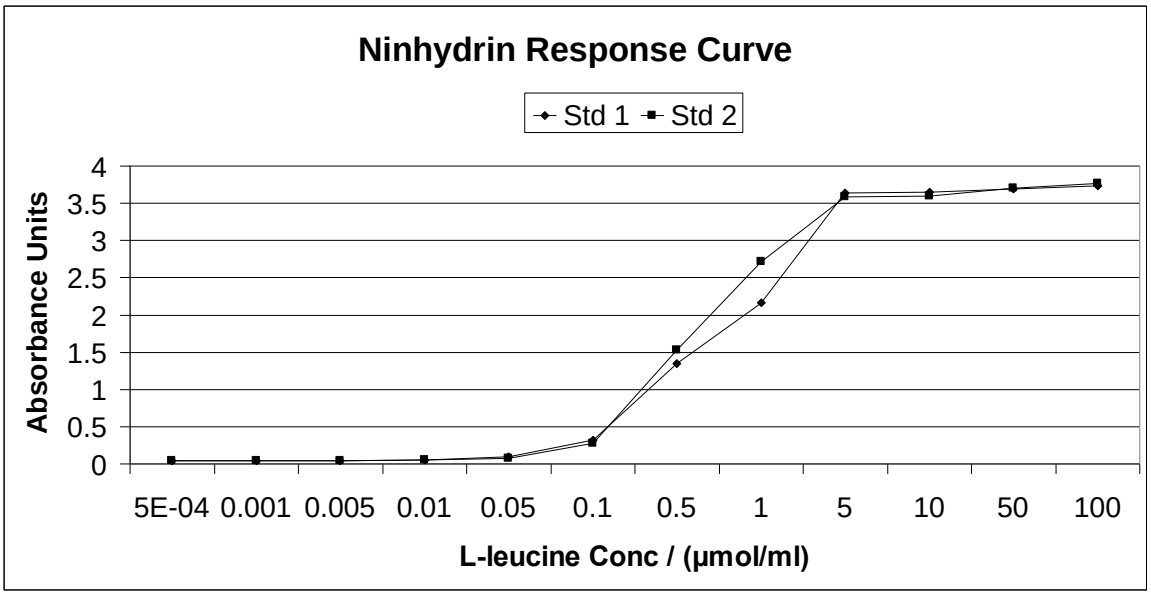


Figure 32: Ninhydrin Total Response Curve

The results of this experiment are shown in Figure 32. The graph clearly shows the desired s-shaped curve. The two standards are fairly close to each other, given the large range over which the concentrations span.

Based on these results, concentrations within the linear region were selected to verify the working range. The expected concentration of solutions from the enzyme degradation was also taken into consideration (see Appendix E: Ninhydrin Protocol). The concentrations selected were (all in µmol/ml): 1; 0.9; 0.8; 0.7; 0.6; 0.5; 0.4; 0.3; 0.2; 0.1; 0.05; 0.01. The results of this experiment are shown in Figure 33. This figure shows the desired linear result. Concentrations within this range can be accurately quantified using this assay. If the digestion solutions have concentrations higher than those shown, then it will be possible to dilute them. However, if the concentrations are lower, the measurements could become more difficult.

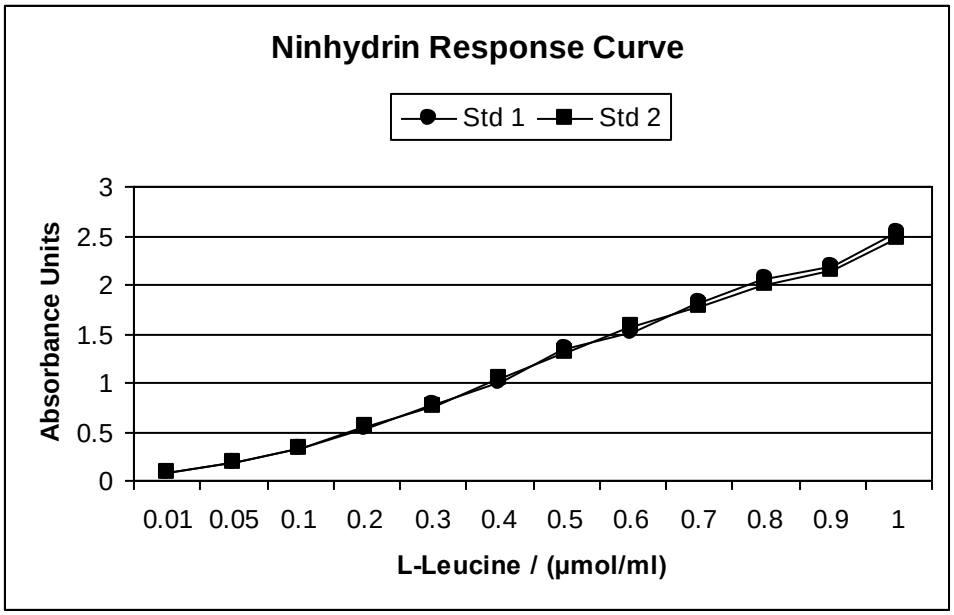


Figure 33: Ninhydrin linear response

After using this standard curve for a few experiments, it was realized that there was no zero on the curve. Having a zero will allow more accurate analysis of the results. To accommodate this, the 0.01 dilution was removed from the curve and a zero was added. Fresh standards were made each time the ninhydrin assay was performed and fitted. This ensured that our sample values are correctly correlated to the standard leucine curve.

6.2 Preliminary Degradation Experiments

The first degradation experiments were performed to investigate the end points obtained with different collagenase and calcium concentrations. Furthermore, sponges that were uncrosslinked and UV-crosslinked were used to see if this made a difference in the degradation times. For these experiments, pieces of sponge weighing approximately 2mg were used to conserve collagen. The results from the first experiment are shown in Figure 34 and Figure 35.

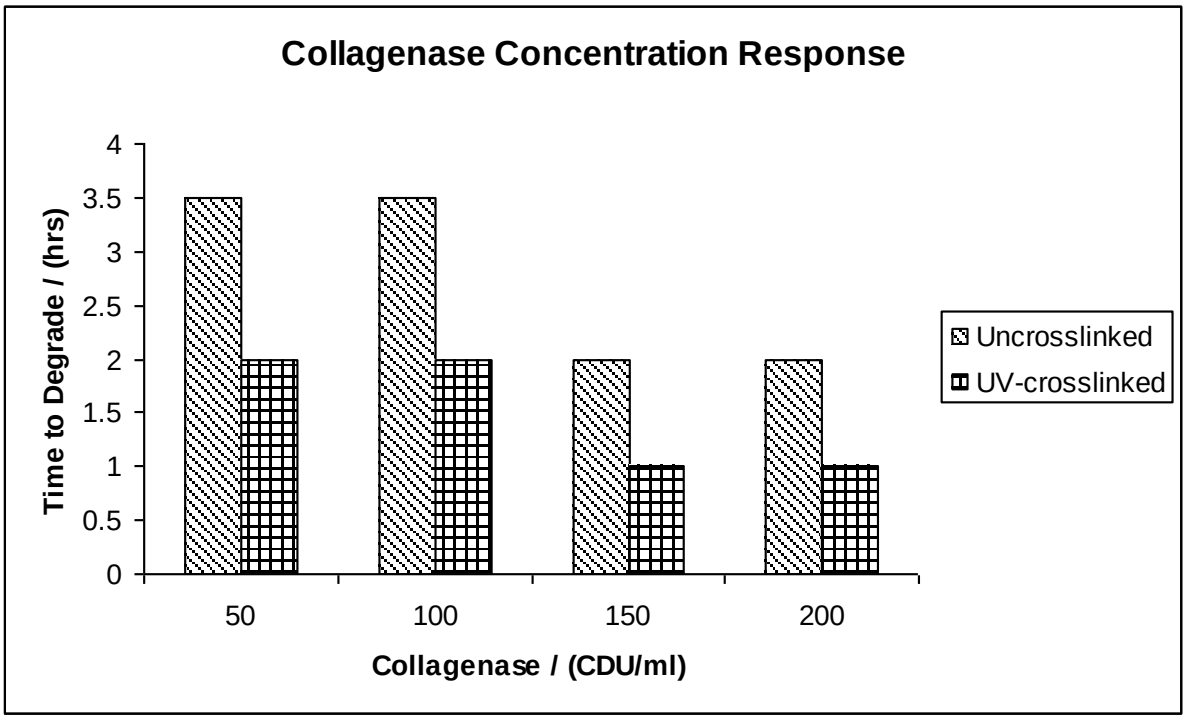


Figure 34: Collagenase concentration response

Figure 34 shows the surprising result that the crosslinked sponges degraded faster than the uncrosslinked ones. It was expected that the crosslinking would increase the sponges' resistance to degradation. A decrease in resilience would occur if the crosslinking technique caused the sponge to partially denature or damaged the sponge through some other mechanism. The experiment would need to be repeated to ensure the validity of these results.

The degradation times are also much shorter than would be ideal. The MR imaging technique may require as much as 45 minutes to complete a single measurement. If there is significant degradation within this timeframe, then it would be averaged into the image and it would no longer represent a snapshot of the degradation at a single time point. An increase in the degradation times can be achieved by decreasing the collagenase concentration or the calcium ion concentration.

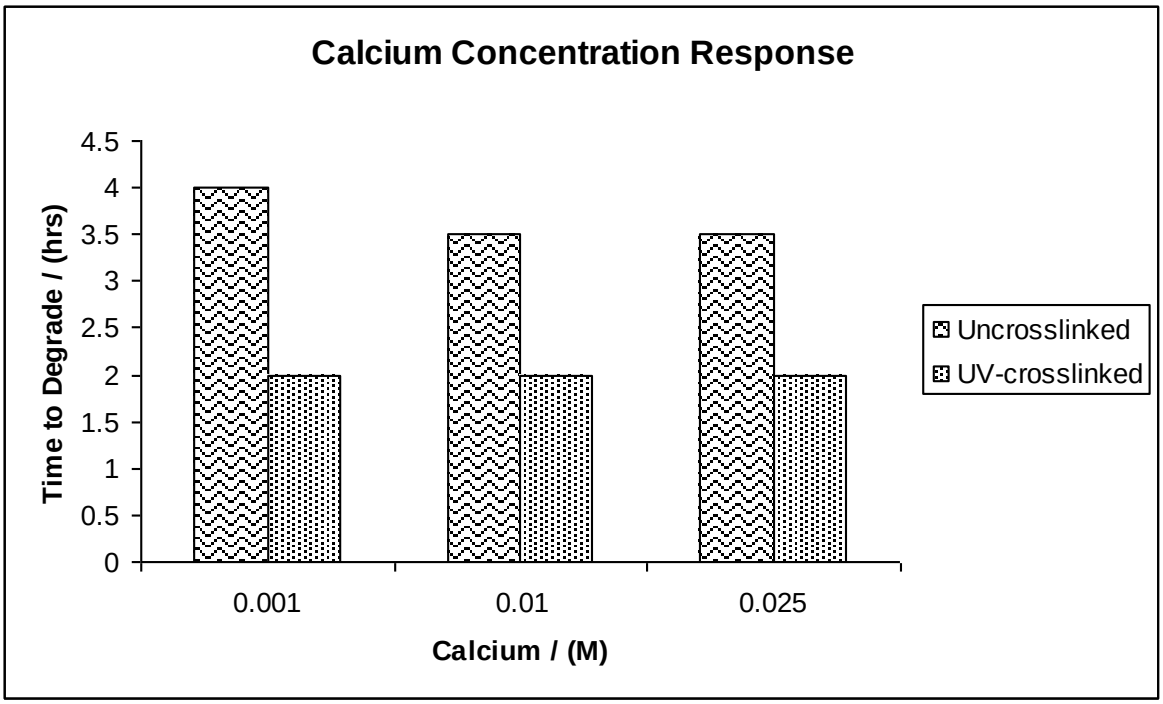


Figure 35: Calcium concentration response

The effect of Ca^{2+} concentration is shown in Figure 35. There is no change in degradation times with varying calcium ion concentrations over the range selected for this experiment, except for a slight increase for the uncrosslinked sponge at a concentration of 0.001M. However, this is not significant. Unless there are problems inhibiting the degradation at a concentration of 0.025M, it will be used for all following experiments. The lack of response to varying calcium concentrations means decreasing the collagenase concentration would be a more suitable method for increasing the degradation times.

The degradation experiment was repeated using collagenase concentrations much lower than for the preliminary experiments. The concentrations selected were (all in CDU/ml): 1; 5; 10 and 20. The degradation times for these concentrations were all greater than 12 hours, when observation was stopped, with none of the sponges having

degraded completely. This timeframe is too long to be feasible for the project. A complete sponge, weighing approximately 10mg, rather than 2mg would increase this degradation time even more. Experiments need to be completed quicker than this if the project is to finish within the allowed time.

The concentrations of 100CDU/ml and 200CDU/ml were also repeated to verify the results obtained in the first experiment. These results are shown below in Figure 36.

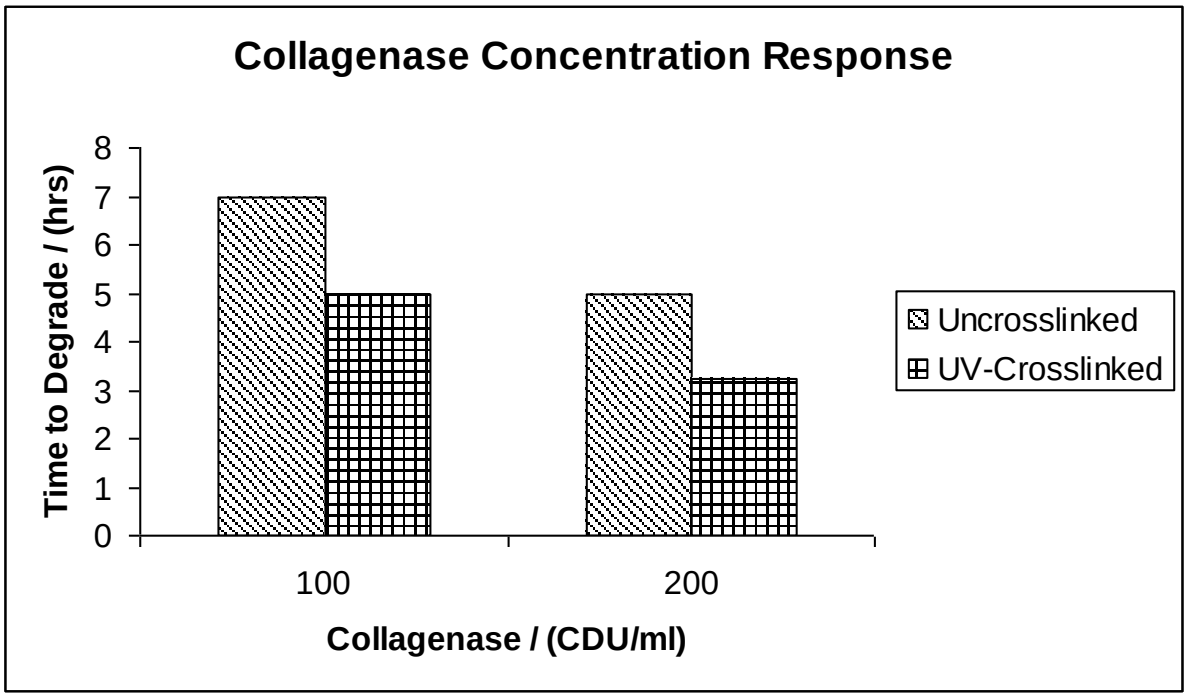


Figure 36: Second collagenase concentration response

Again the crosslinked sponges degraded faster than the uncrosslinked sponges. This confirms that the UV-crosslinking is not having the desired affect, in fact, quite the opposite. The crosslinking procedure will either have to be modified, perhaps by decreasing the crosslinking time, or another clinically relevant technique should be selected to fabricate the sponges.

The degradation times for the second experiment were greater than the times obtained in the first experiment. This may be due to aging of the stock collagenase

solution. It was not stored in the refrigerator between experiments. A new stock solution will be made and stored in the refrigerator for subsequent experiments. The sponges used in the second experiment were also from a second fabrication batch. There may have been differences between the batches. The fabrication technique is still fairly new and has not yet been repeated often enough to ensure reproducibility.

6.3 Degradation Experiments

6.3.1 Experiment 1

Based on the preliminary experiments, 200CDU/ml was used for the degradation. The first experiment performed produced uncrosslinked sponges, frozen in liquid nitrogen that had degraded for 4, 8, 12, and 16 hours. Two sponges were degraded at each of the time points for a total of 8 sponges. The ninhydrin assay was performed on these sponges using dilutions in an attempt to get the absorbencies for digestion solutions within the working range of the ninhydrin curve. We used dilutions ranging from 1:100 to 1:100000. The results from the ninhydrin assay are seen in Figure 37.

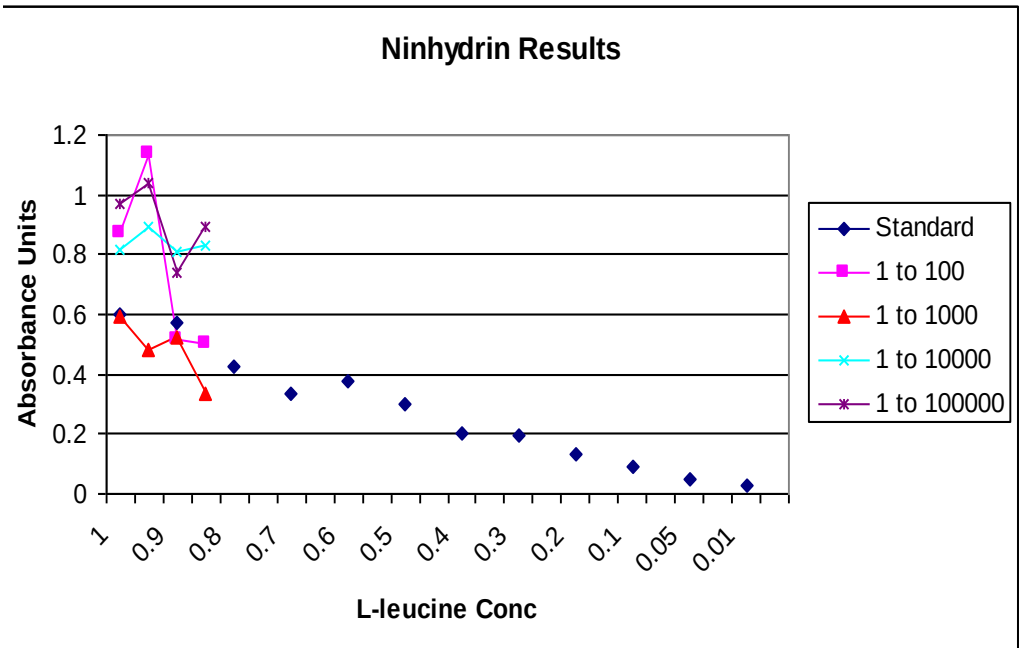


Figure 37: Experiment 1 - Ninhydrin Results

These results do not show an increasing ninhydrin response for increasing degradation. The data points do not map well onto the standard curve and there is almost no effect from the dilutions. The 1 to 1000 dilutions were closest to the standard curve.

6.3.2 Experiment 2

Thirty-six sponges were prepared in two batches. Eighteen sponges from each batch were placed in degrading solution at a collagenase concentration of 200CDU/ml. It was anticipated that 3 sponges from each batch (6 total) would be halted every 2 hours for six time points up to 12 hours of degradation. However, when the experiment was set-up and run, it was noticed that the sponges were degrading much faster than anticipated. After 2 hours, it was observed that the sponges had been degraded too far to be imaged. At the time, it was decided to halt the degradation every 1 hour thereafter. The adjusted time points were 2, 3, 4, 5 and 6 hours. The remaining 6 sponges were left to degrade to determine the new end point.

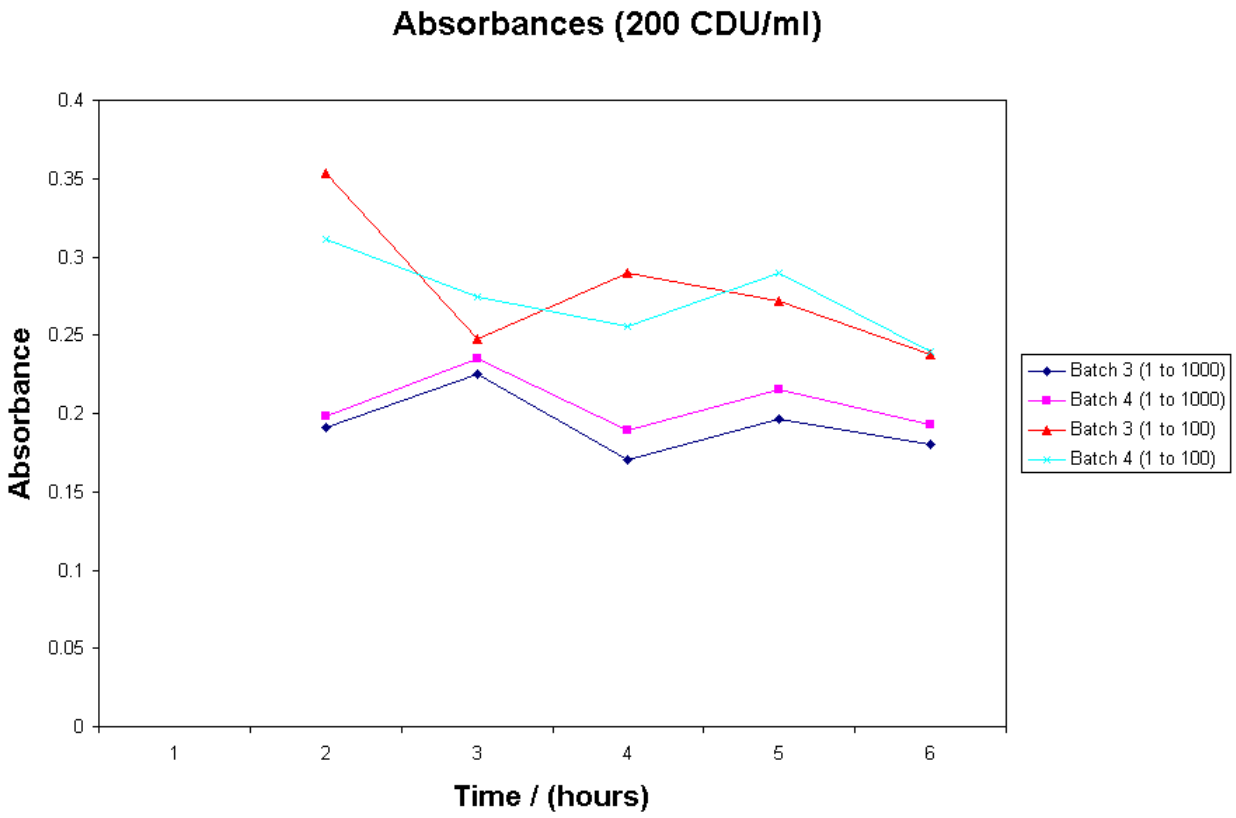


Figure 38: Experiment 2 – Ninhydrin Results

The ninhydrin results are shown in Figure 38. The samples were diluted by 1:1000 according to the results from experiment 1. This dilution still did not map well on to the standard curve so a 1:100 was also performed.

6.3.3 Experiment 3

A new set of sponges was degraded using a collagenase concentration of 50CDU/ml due to the rapid degradation observed in experiment 2, which was performed at 200CDU/ml. Five sponges from each batch were prepared for a total of 10 sponges. The degradation was halted on 2 sponges every hour for a total of five time points. We

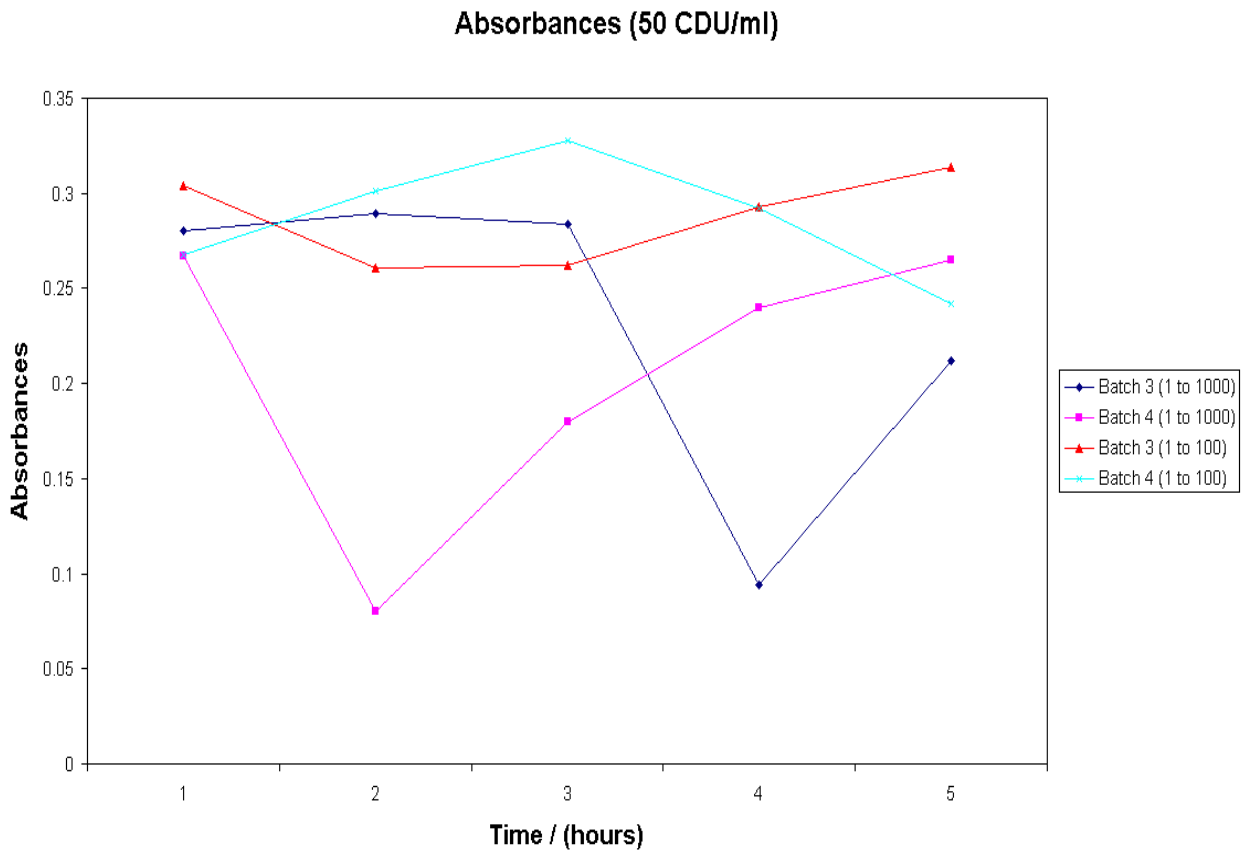


Figure 39: Experiment 3 – Ninhydrin Results

found that this concentration was more successful because it produced partially degraded sponges that were still intact and could be moved to the MRI holder. The ninhydrin assay was performed using the same dilutions as experiment 2. The results are shown in Figure 39.

After performing this experiment, it was decided that the definition for the degradation end point needed to be changed. Rather than being the time at which there are no longer pieces of sponge visible, it was changed to be the time at which the sponge can no longer be imaged. This occurs when the sponge is no longer intact as a single piece.

6.3.4 Experiment 4

In experiment 3, 50 CDU/ml proved successful so additional sponges were degraded at this concentration for experiment 4. Eighteen sponges, 9 that were UV crosslinked for 30 minutes on each side, 1 hour total, and 9 that were uncrosslinked, were degraded for 1, 2 and 3 hours. Three sponges were used for each time point. Dilutions of 1:50 and 1:25 of our samples were performed and 1:25 was found to work best. In Figure 40, are the results from the 1:25 dilution. The 1:50 dilution was discarded.

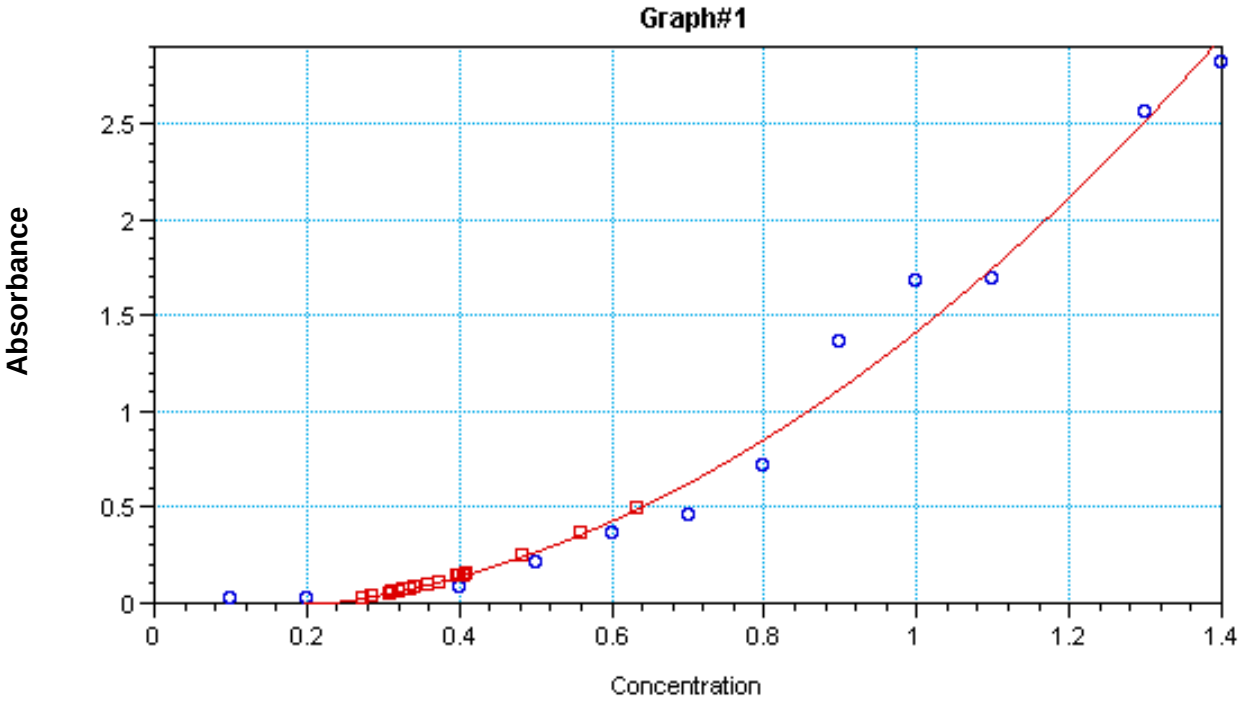


Figure 40: Experiment 4 - Ninhydrin Results

6.3.5 Experiment 5

Eighteen sponges were used in this experiment, 9 UV crosslinked for 60 minutes total and 9 uncrosslinked. Two sponges of each type were degraded for each time interval. There were four time intervals: 1, 2, 3 and 4 hours. One sponge of each type was degraded completely. The end point of the UV crosslinked sponge was found to be 6

hours and the uncrosslinked sponge was 5 hours. Figure 41 shows are the results from experiment 4 using a 1:25 dilution.

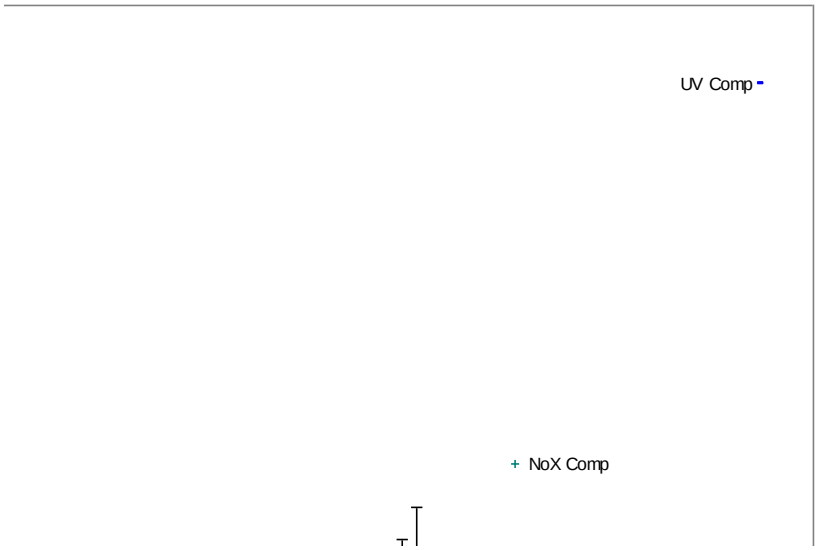


Figure 41: Experiment 5 - Ninhydrin Results

6.3.6 Experiment 6

Sponges were screened for homogeneity using gradient echo fast images (GEFI). See Appendix A: MRI Data for these images. From these images, sponges with an excessive amount of air bubbles or varying intensities across the sponge were discarded. The remaining sponges were grouped according to when their degradation would be halted so that one holder could be removed from the incubator without affecting the other sponges. All sponges were degraded directly in the MRI collagen holders so that the sponges did not have to be moved after they had been degraded. Previously, this extra moving step had resulted in unnecessary damage to the sponges. All the solution

volumes were halved to fit in the collagen holders, while keeping the concentration values the same.

Six uncrosslinked sponges and 9 UV crosslinked (60 minutes total) were then degraded with a concentration of 50 CDU/mL. The uncrosslinked sponges were degraded for 1 and 2 hours and the UV crosslinked sponges were degraded at 1, 2 and 3 hours. There were 3 sponges at each time point. Figure 42 are the ninhydrin results obtained using a 1:10 dilution. The 1:25 dilution was attempted, but all samples were too light and did not fall within the working range of the standard curve.

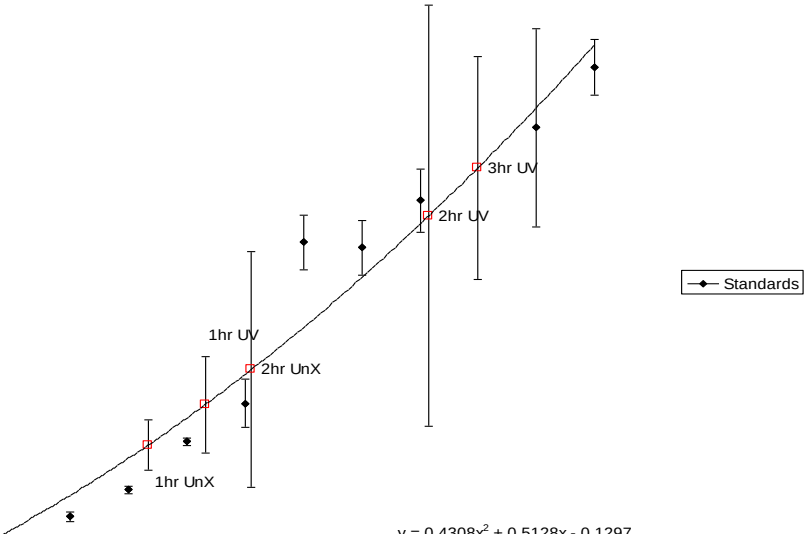


Figure 42: Experiment 6 - Ninhydrin Results

6.3.7 Compiled Results for Preliminary 50 CDU/mL

Figure 43 shows are the complied ninhydrin results for all the degradation experiments performed at 50 CDU/mL. It shows the expected trend of increasing

concentrations of free amino acids with increased degradation times. It also shows that the 60-minute UV crosslinked sponges release more amino acids than the uncrosslinked sponges. This could be due to partial denaturing.

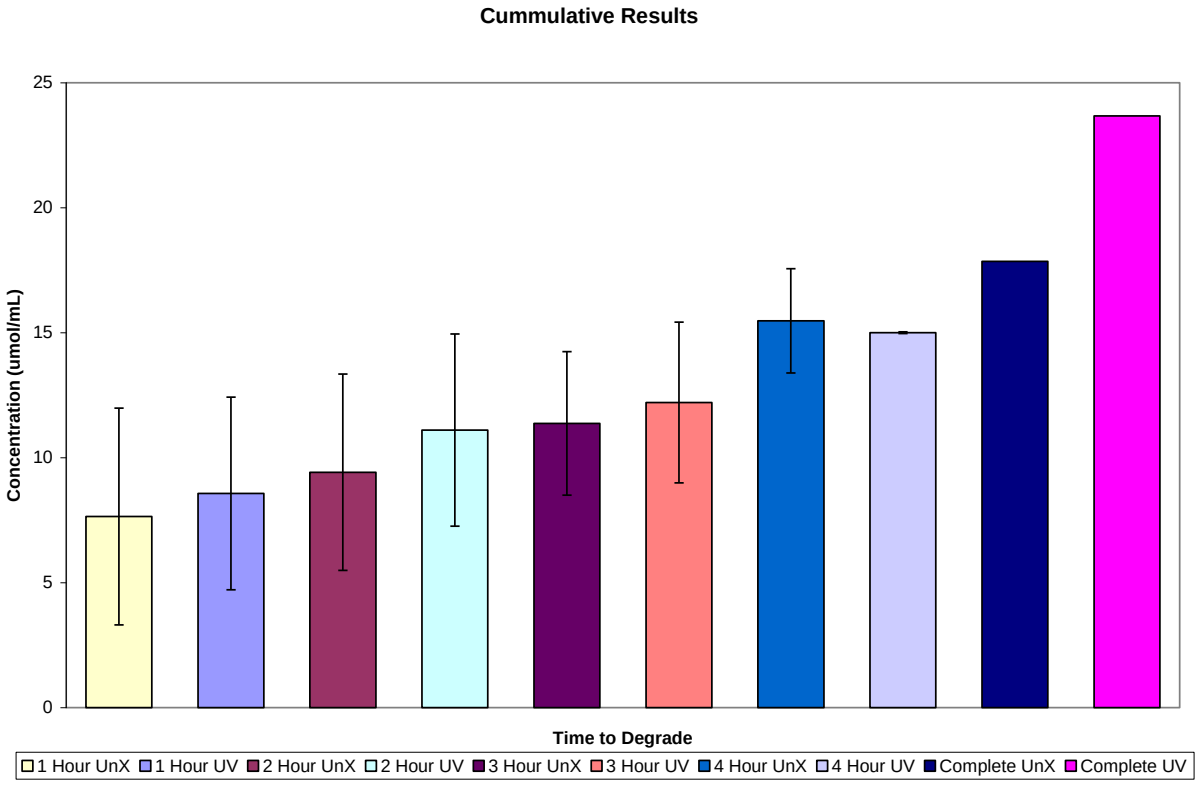


Figure 43: Compiled Preliminary Ninhydrin Results - 50 CDU/mL

6.3.8 Experiment 7

In the next experiment four holders were degraded, 4 sponges in each holder. Sponges had been crosslinked by either carbodiimide, 10 minutes UV, 20 minutes UV, or 40 minutes UV. Two sponges of each crosslinking type were degraded for either 1 or 2 hours. In this experiment, the 60-minute UV sponges were eliminated because Experiment 6 suggested denaturing of the extended crosslinking. Figure 44 shows the data we found from the comparison of the different crosslinking types. The UV-crosslinking times in Figure 44 through Figure 48 refer to irradiation time for each side.

All other times are total irradiation times. This graph shows that the carbodiimide keeps the sponges most intact. The graph also shows that 20 minute UV crosslinked sponges generally have a higher absorbance then 10-minute UV crosslinked sponges. The 40-minute UV sponges also have greater absorbance of the 10-minute UV sponges and also the 20-minute UV sponges.

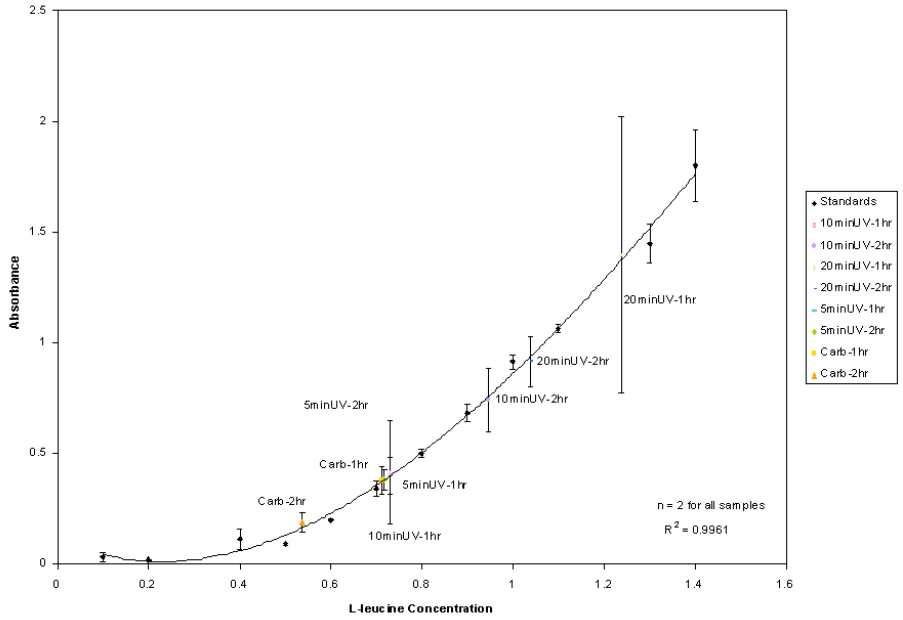


Figure 44: Experiment 7 – Ninhydrin Results

After creating this graph we realized the absorbance's had not yet been normalized and was therefore inaccurate. The sponges' absorbance's were averaged and normalized as shown in Figure 45.

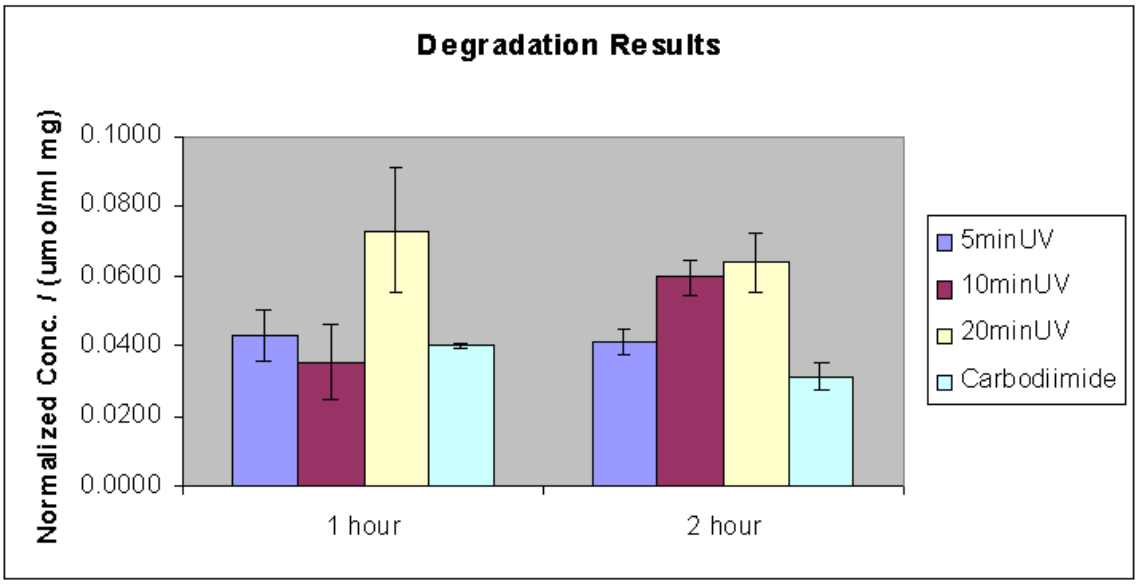


Figure 45: Experiment 7 - Bar graph of ninhydrin results

The change in dry mass was also calculated from the same set of sponges. The Y-axis represents the change in dry mass. Figure 46 displays this information. As you can see, the carbodiimide change in mass came up negative; this may be because the sponges were still wet. There also may have been an error or inaccuracy when weighing them.

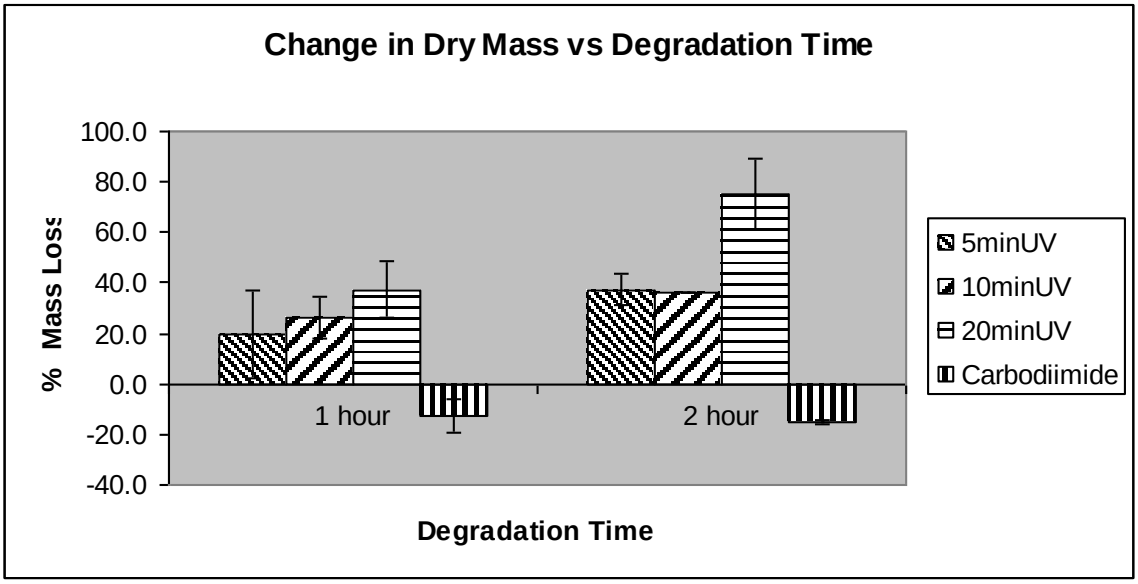


Figure 46: Experiment 7 - Change in dry mass

Figure 46 was then altered slightly after determining that change in dry mass was not the optimal way to represent the data obtained. Change in dry mass was adjusted to percent mass loss and so that the starting weight of the sponges did not affect the results. Figure 47 below shows this data. As you can see, the carbodiimide results have also been removed from the graph because after re-lyophilizing the sponges, the masses did not change. It was decided not to use carbodiimide crosslinking any longer because a greater concentration of collagenase would have to be used with these sponges due to the strength of the crosslinking.

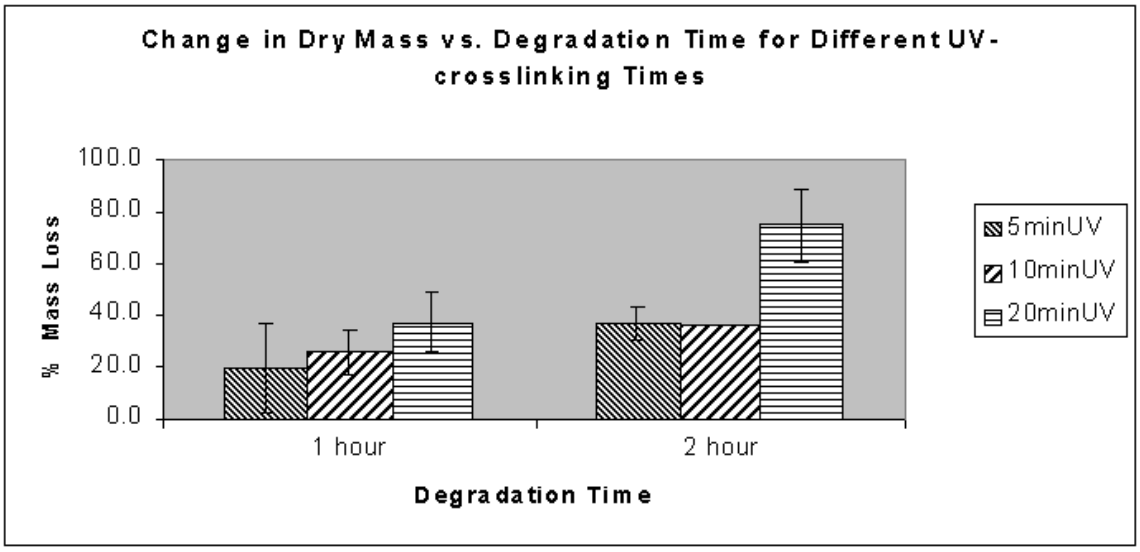


Figure 47: Experiment 7 - % Mass Loss

Carbodiimide was also removed from the ninhydrin results from this experiment producing the graph seen in Figure 48.

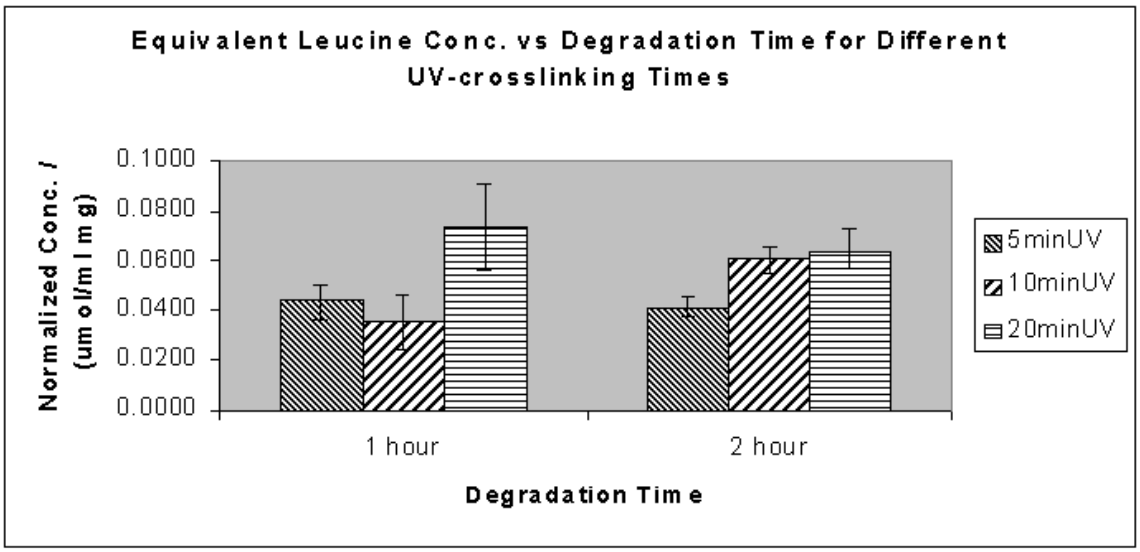


Figure 48: Experiment 7 - Ninhydrin results without carbodiimide

6.3.9 Experiment 8

A final experiment was run in four steps based on all of the previous data obtained. 10-minute UV sponges and 20-minute UV sponges were chosen for this experiment. The 40-minute sponges showed possible degradation and inconsistent results in Experiment 7 and the carbodiimide sponges were not used because of reasons stated in the previous section. Sponge degradation for this experiment occurred directly in the Transwells® and sponges were all imaged within 2 hours of the necessary rinses following degradation. In most cases, the sponges were placed in the MRI bore for post-imaging approximately 10 minutes following the degradation process. The Transwell® plates proved excellent in the rinsing steps. After each rinse, the Transwell® was removed from the well and placed on a paper towel. At this time, the solution in the well was dumped and the new rinse was added. The Transwell® was then returned to the proper well.

Sponges were degraded in four groups of various specifications. At the end of the experiment data had been obtained from the following samples: (6) 10-minute UV

sponges degraded for 1 hour, (6) 10-minute UV sponges degraded for 2 hours, (6) 20-minute UV sponges degraded for 1 hour, (6) 20-minute UV sponges degraded for 2 hours. The results of this experiment can be seen in Figure 49 and Figure 50.

6.3.10 Compiled Final Results

Experiment 8 was the accumulation of 7 experiments that each pushed forward with the research. Figure 49 and Figure 50 express the degradation data found most valuable in this study. From these results, one can see that the ninhydrin assay was not as successful as anticipated. The results between the 1 hour versus 2 hours of degradation were insignificant and the results comparing the 10 minute UV sponges with the 20 minute UV sponges were also insignificant.

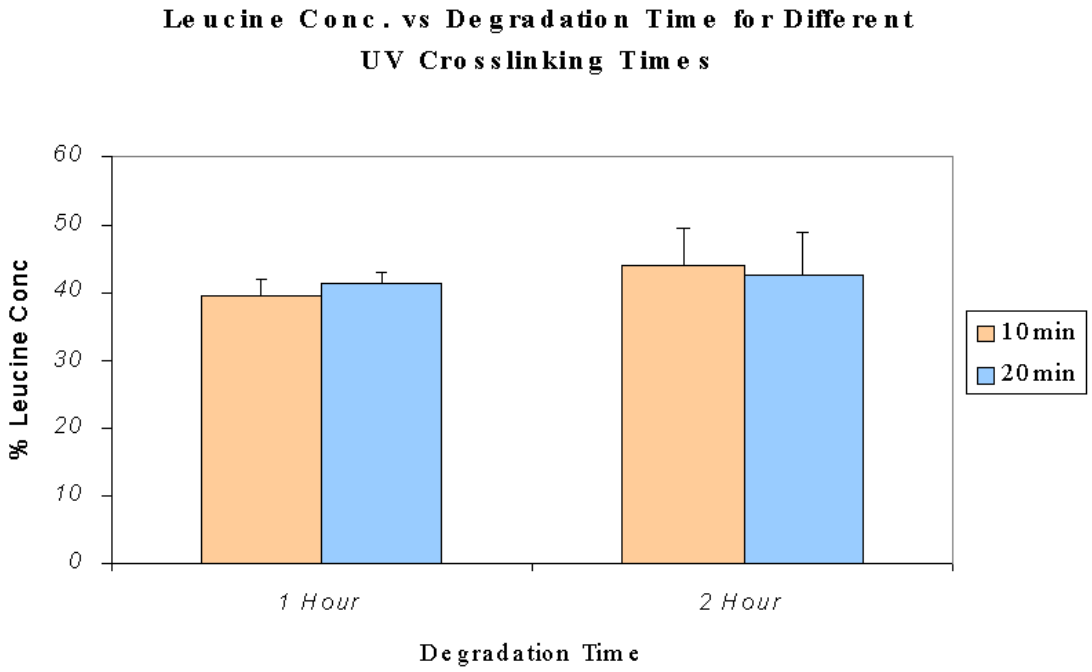


Figure 49: Experiment 8 - Final Ninhydrin Results

The percent mass loss data, however, produced the results that had been anticipated. Figure 50 shows that percent mass loss increases when the sponges are

degraded for longer periods of time. The percent mass loss is also greater for the 20-minute UV sponges than the 10-minute UV sponges. This could be because the sponges are denaturing after 20-minutes under the UV irradiation. It would follow in line with the hypothesis we previously had with regards to the 60-minute UV sponges.

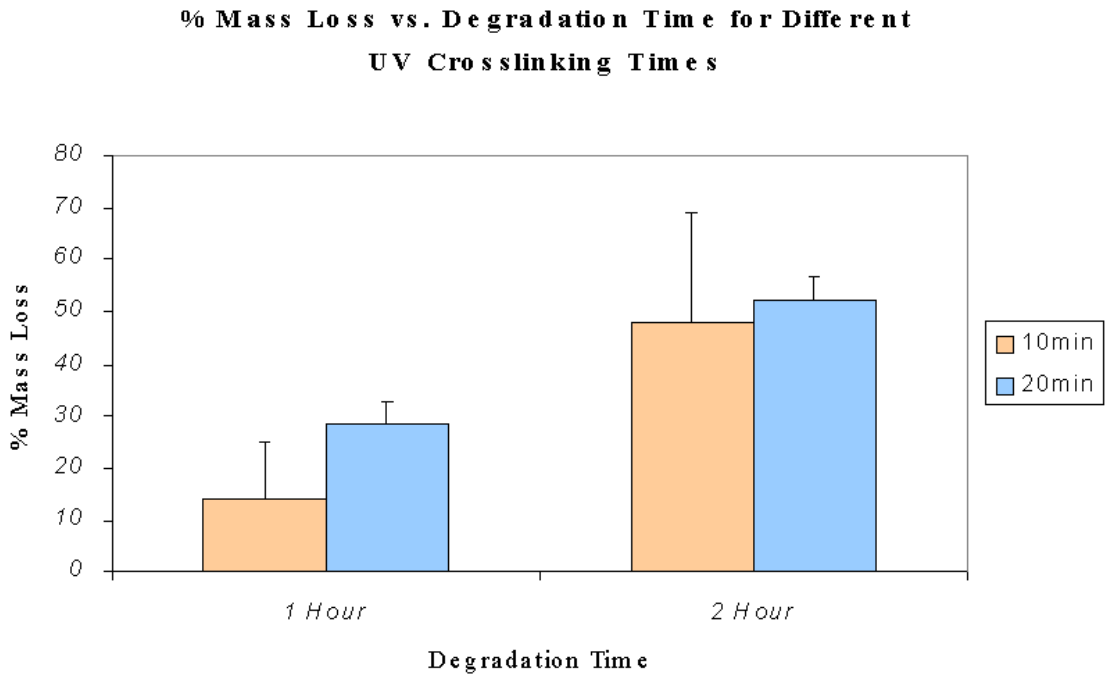


Figure 50: Experiment 8 - Final Percent Mass Loss

6.4 MR Imaging

6.4.1 Collagen Holder Tested

The initial testing of some imaging techniques produced mixed results. For the preliminary MR imaging, the entire model established through the design process was not used. This was because there was no radio frequency coil made for the design yet. Instead, an individual contact less case was used with a single solenoid coil around it. This would at least let the team know whether contact lens cases could possibly be used.

If so, then the team could create a radio frequency coil to accommodate the original design of multiple lens cases for multiple samples. Additionally, an alternate design with multiple lens cases equally spaced in a single line was used for preliminary imaging because there were some things that the alternate design accomplished that were very desirable, namely simultaneous imaging of multiple samples. It also tested how well a volume coil around an entire well plate would work to produce an image.

The next steps of testing the design was to image control sponges using the collagen holder and examine the images obtained using the design. It was found that due to the curvature of the bottom of the contact lens cases, the collagen sponges did not lie flat as required to obtain a good image. It was determined that the curvature was too severe to overlook and had to be addressed.

Epoxy was injected into the lens cases to create a flat bottom once it cured. That worked for the day and the holder was successfully used to obtain images. However, there were no means to inject a controllable volume of epoxy. Once it had cured, the sponges were held at different heights. Additionally, either the epoxy shrank upon curing or, more likely, simply would not bond to the interior of the lens cases because they became loose and prone to falling out. A more permanent insert was needed, but the overall design of the collagen holder was a success. Two additional holders were then produced that were similar to the prototype, but with a few minor improvements. For example, the space between the lens cases was reduced to make sure the samples fit into the RFC. Additionally, the holder's base was adjusted to a precise width so that it fit in the plastic tube of the RFC such that it oriented the collagen sponges at the exact center of the RFC and hence bore.

Poly(dimethly siloxane) (PDMS) was later injected into the bottom of each contact lens case. According to manufacturer's instructions, 10 parts of the base was mixed with 1 part of the curing agent. The mixture was then de-gassed under vacuum, 1 ml was injected by syringe into each case, and the mixture was de-gassed again to remove all air bubbles. The holders were then placed in the drying oven at 55° C to cure the PDMS. The protocol called for at least 6 hours in the oven. The holders were left in the oven for more than 7 hours but the PDMS was still tacky and not fully cured.

The original holders were scraped clean of any uncured PDMS and more contact lens cases were attached to more bases to create nine holders, labeled A through I. A new batch of PDMS was mixed and the application was repeated. Again, 1 ml of PDMS mixture was added by a syringe to each lens case in each of the nine holders and de-gassed twice just as before. This time, the mixture was cured at 60° C for over 30 hours before it was totally complete. Although the process took longer than expected, they all came out level, uniform, and maintained a watertight seal around the edges. This prevents water from getting under the polymer to the bottom of the lens case and producing distorted images. The epoxy used in the first attempt could not accomplish this.

Dave Bennett, a graduate student that has been assisting the MQP group at the MRI lab, suggested that a surface coil be used to create a high-resolution image of one of the sponges. Table 13 and Table 14 show the MRI parameters for the image and the sponge type, respectively.

Table 13: High-Resolution surface coil parameters

Coil Type	TE	TR
Surface (4 loop)	7 msec	300 msec

Table 14: Sponge description

Sponge	Batch #	Freeze-Drying Type	Cross-linking
Control 1	Batch 1	Liquid Nitrogen	None

This technique gave a very detailed picture of what the sponges looked like in an MR image. The image can be seen in Appendix A: MRI Data. This allowed some MRI data to be collected before the holders were completely ready.

These holders, as mentioned above, were labeled A through I. Each holder was also given a dot on the left side in order to orient each holder the same way. For example, each holder is always put into the magnet "dot first." Additionally, each of the four lens cases on each holder is numbered starting from the dot. The illustration is shown below in Figure 51, summarizing how each holder is organized.

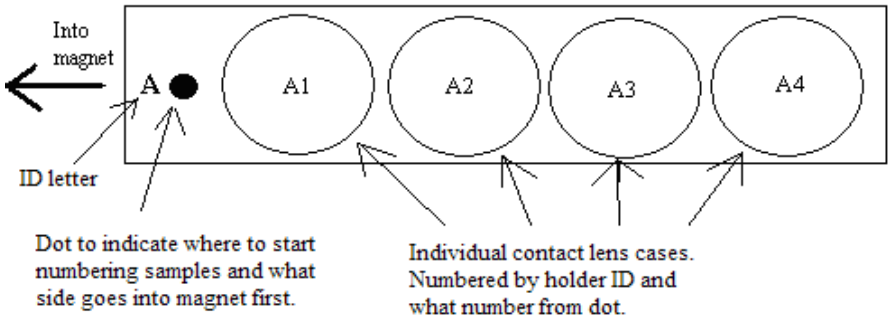


Figure 51: Labeling and organization of each holder

6.4.2 Sponges Tested

There were different sponges tested during the preliminary imaging. This was to decide which type of sponge would be best to produce the best images. The first four control sponges that were imaged were all non-degraded. There were four sponges total. Two were freeze-dried in a liquid nitrogen bath and the other two were freeze-dried at -80°C.

Additionally, one of the liquid nitrogen sponges was UV crosslinked for 30 minutes each side and one of the -80°C sponges was cross-linked by 24 hours DHT. A summary of the sponges appears in Table 15 below.

Table 15: Initial Control Sponges

Sponge Number	Freeze Drying Method	Cross-linking Method
1	Liquid Nitrogen	Ultraviolet Radiation
2	Liquid Nitrogen	None
3	- 80° C	None
4	- 80° C	DHT

The images obtained from these sponges may be found in Appendix A: MRI Data. The holder held the sponges at the correct height and alignment.

Holders A and B were first used to start analyzing sponges degraded different amounts. Table 16 shows what sponges were located in what holder:

Table 16: Sponges from preliminary degraded imaging

Location	Sponge Type
A1	UV X-linking, 1 hour degradation
A2	No X-linking, 1 hour degradation
A3	UV X-linking, 2 hour degradation
A4	No X-linking, 2 hour degradation
B1	UV X-linking, 3 hour degradation
B2	No X-linking, 3 hour degradation
B3	Control, no degradation
B4	*Empty*

These sponges were then imaged using proton density, T1 and T2 weighted imaging techniques. The images were done in a coronal-head foot orientation. Table 17, Table 18 and Table 19 below show the MRI parameters for each. The images produced by this imaging technique are available in Appendix A: MRI Data.

Table 17: Parameters for Proton Density Scan

Field of View	Slice Thickness	TR (msec)	TE (msec)	# of averages	# of slices	Isodist A	T_x Attenuator
12 cm	5 mm	3000	20	1	1	-.5	38.0

Table 18: Parameters for T1 weighted Imaging

Scan #	TE (msec)	TR (msec)
1	20	30
2	20	50
3	20	80
4	20	150
5	20	400
6	20	800
7	20	1500
8	20	3000

Table 19: Parameters for T2 weighted Imaging

Scan #	TE (msec)	TR (msec)
9	50	3000
10	100	3000
11	200	3000
12	250	3000

There were some problems with the preliminary imaging of the degraded sponges. It took some time for the MQP team to get the concentration of the enzymes correct in order to degrade sponges in a consistent manner. Initially, the sponges were degraded too fast and there was nothing to image. Following that, the sponges degraded too slowly and all the sponges were the same as each other. After some time working it out, the team was able to get consistent degradation results and moved ahead with imaging the degraded sponges. One holder was prepared with UV cross-linked sponges that were degraded for 1 hour and 2 hours as shown in Table 20.

Table 20: Sponge location of UV cross-linked sponges

Sponge Location	Cross-linking	Degradation Time
A1	UV	1 Hour
A2	UV	1 Hour
A3	UV	2 Hours
A4	UV	2 Hours

Images showed inconsistent degradation again based on intensity and visual inspection. It was expected that sponge A1 and A2 would degrade similarly and A3 and A4 would be the same as each other. This did not happen. The images can be seen in Appendix A: MRI Data. It was decided at this point that before any degradation was performed on a sponge, it had to undergo a scout scan to ensure that it was homogeneous. This was done before and after degradation from then on.

A new batch of collagen and sponges, both UV crosslinked and uncrosslinked, were made to start with a clean slate. These were hydrated and put into five different holders to undergo scout imaging to see what sponges were homogeneous and which were not. The inhomogeneous sponges were discarded and the uniform sponges would be used for further experimentation. Table 21 shows the location and cross-linking method for each sponge:

Table 21: Sponges imaged with some cross-linked and some uncross-linked

Sponge Holder/Location	Cross-linking
D1	None
D2	None
D3	UV
D4	UV
C1	None
C2	None
C3	UV
C4	UV
F1	None
F2	None
F3	UV
F4	UV
E1	None
E2	None
E3	UV
E4	UV
G1	*empty*
G2	None
G3	UV
G4	*Empty*

The sponges were imaged quickly to see where there was water and where there was not. This ensured that the collagen was homogeneous throughout the sponge, but also that it was properly hydrated. The fast scans resulted in the discarding of sponges F1, F2, and D2 due to their heterogeneous appearance. The images that resulted from these fast scans can be seen in Appendix A: MRI Data.

Once the "bad" sponges were removed, the remaining sponges were reorganized. This was necessary because the degradation needs to take place in an incubator and when a holder is removed after a certain amount of time, the degradation of all the sponges in that holder would have to be stopped because it would no longer be incubated. Therefore, the sponges were arranged in each holder such that all the sponges in a single holder had the degradation halted at the same time. Notice that three uncrosslinked

sponges and three crosslinked sponges were degraded 1 hour and 2 hour each. Additionally, notice that only crosslinked sponges were degraded for 3 hours. This is because uncrosslinked sponges cannot be imaged after 3 hours of degradation. The reorganization and degradation times of the sponges can be seen in Table 22.

Table 22: Fast scanned sponges

Location	Cross-linking	Degradation time
C1	None	1 hour
C2	None	1 hour
C3	None	1 hour
C4	*Empty*	*Empty*
F1	*Empty*	*Empty*
F2	UV	1 hour
F3	UV	1 hour
F4	UV	1 hour
E1	None	2 hour
E2	None	2 hour
E3	None	2 hour
E4	*Empty*	*Empty*
D1	*Empty*	*Empty*
D2	UV	2 hour
D3	UV	2 hour
D4	UV	2 hour
G1	UV	3 hour
G2	UV	3 hour
G3	UV	3 hour
G4	*Empty*	*Empty*

Not much information can be derived from these images, but they do ensure the uniformity and homogeneity of the sponges. The images from this imaging session can be seen in Appendix A: MRI Data.

The next imaging session was spent imaging 16 total sponges that were crosslinked by four alternative methods (four sponges for each method). This was performed because there was some evidence from the scout scans and the ninhydrin assay that suggests the UV radiation may be denaturing the collagen. This could give rise to

heterogeneities within the sponge and could cause degradation to take place at a higher rate. To test this theory, the team changed the crosslinking method from the normal 30 minutes of UV irradiation to 5, 10, and 20 minutes. Additionally, a set of four sponges was cross-linked chemically using carbodiimide. The sponges were placed into holders according to Table 23. These sponges were imaged using the same T1 (Table 18) and T2 (Table 19) parameters as mentioned earlier.

Table 23: New crosslinking methods imaged

Location in holder	Cross-linking type	Amount
A1	UV	5 min
A2	UV	5 min
A3	UV	5 min
A4	UV	5 min
B1	UV	10 min
B2	UV	10 min
B3	UV	10 min
B4	UV	10 min
C1	UV	20 min
C2	UV	20 min
C3	UV	20 min
C4	UV	20 min
D1	Carbodiimide	N/A
D2	Carbodiimide	N/A
D3	Carbodiimide	N/A
D4	Carbodiimide	N/A

IDL was used to create T1 and T2 maps from all the data that was collected. The team assessed the different crosslinking methods for the appropriate technique to degrade consistently and image. Please see the MRI appendices for all the data collected for this study.

7 CONCLUSIONS

The original hypothesis of this project is the MRI is a viable way to assess the extent of degradation in collagen sponges. Based on the experimentation and analysis of the results obtained at this point, this hypothesis has not been proven. As see in Figure 52 the experimental data from the gold standard benchtop analyses of the change in dry mass due to degradation shows a significant increase in the percent loss in dry mass due to degradation of the sponges.

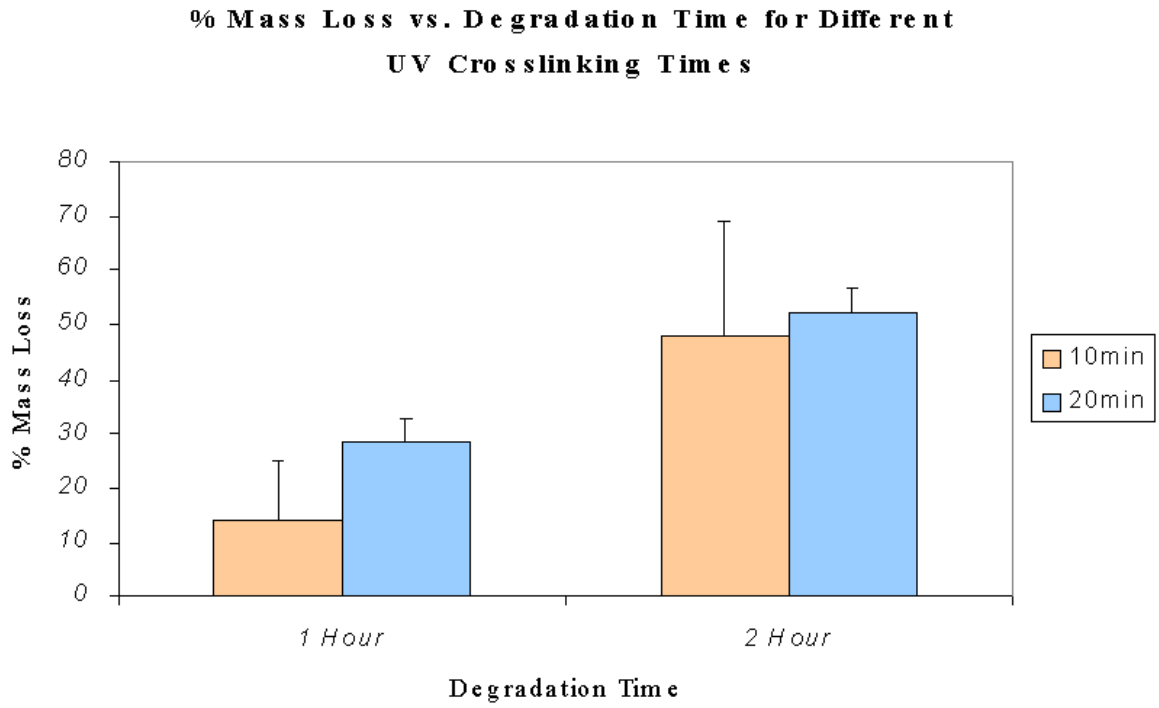


Figure 52: Percent Change in Dry Mass

Overall, 3 separate MR parameters were measured both pre- and post-degradation. Based on the data collected for the proton density (M0′), there was no

statistically significant change after degradation. The same can be said for both the T_1 and T_2 relaxation times. There was no significant change in any of the MR parameters when comparing pre degradation data to post-degradation data. The following figures are representative of the typical results for each MR parameter. Please see Appendix A: MRI Data for all of the MRI data.

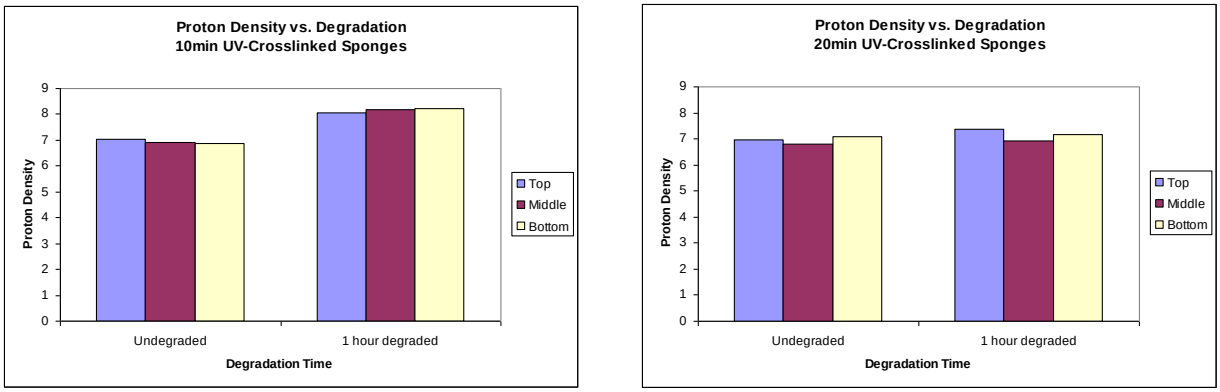


Figure 53 - Proton density relationship to degradation

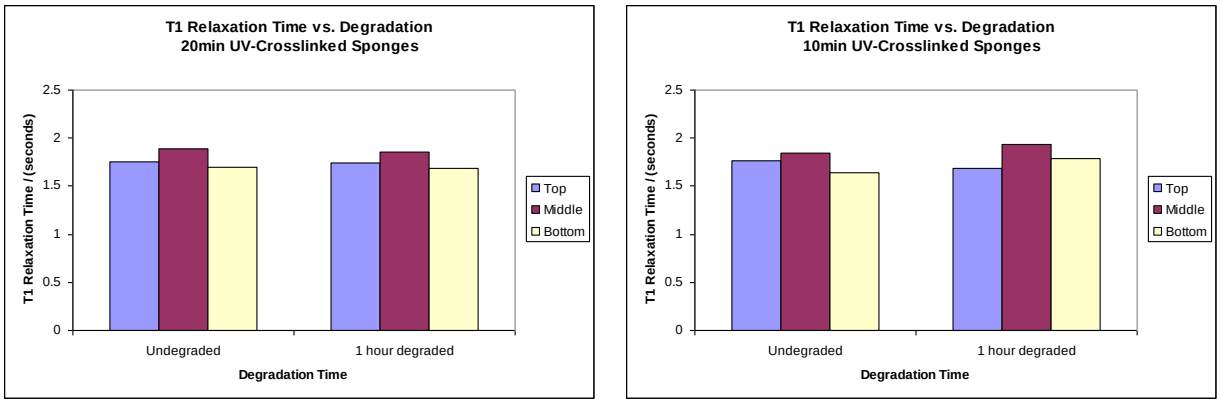
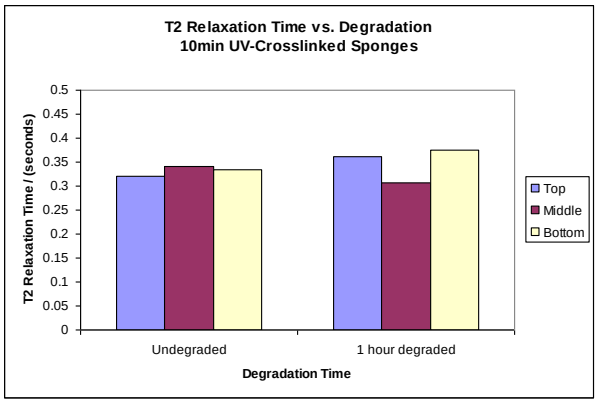


Figure 54 - T_1 relaxation time relationship to degradation



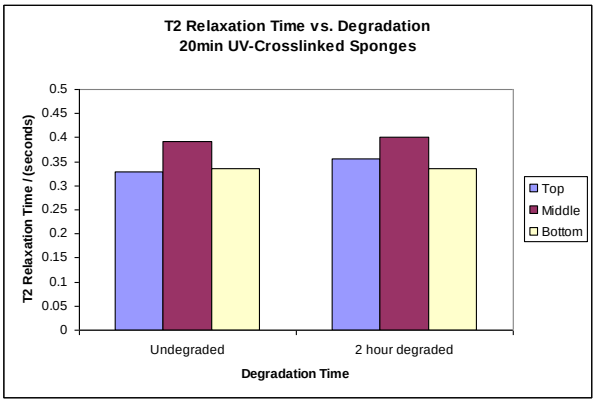


Figure 55 - T_2 relaxation time relationship to degradation

The MRI data showed changes in the three parameters; however they were inconsistent and not statistically powerful enough to prove that our results were anything beyond random sampling. We could not say with any confidence that there was a trend in the change in MR parameters due to degradation. Based on the data collected and the analysis up to this point, MRI may still prove to be a viable method for characterizing the extent of degradation in collagen sponges, just not using these specific imaging techniques and methods. This project team would like to offer some recommendations in the following chapter which may provide data showing a significant change to prove the original hypothesis.

8 RECOMMENDATIONS

Although this project did not prove the hypothesis that MRI could accurately assess the degradation of collagen sponges, the project team still believes it is possible by other means. These experiments were limited by time constraints and the collective MRI experience of this project team. This project has provided a solid foundation from which other projects may stem from. Many areas of this project can be improved upon in order to test different MR parameters, as well as to replicate degradation in the human body more accurately. This would give a higher overall clinical significance and experimental quality to any continuance of this research.

The project team's first recommendation for the expansion of the project is to conduct experiments using different crosslinking techniques. All of this study's experiments were conducted using ultraviolet (UV) radiation crosslinked sponges. The problem with UV radiation is that overexposure actually denatures the collagen proteins instead of crosslinking them. This makes the overall structure of the sample weaker instead of fortifying the fibers against degradation. The use of aqueous crosslinking methods, such as carbodiimide and dehydrothermal (DHT) produced poor and unexpected results when assessing the degradation on the bench top, as well as distorting the overall structure of the sponges and causing problems with hydration during the imaging process. Further experimentation with differently crosslinked sponges may offer more consistent degradation results. Consistent, linear bulk degradation should provide a clearer correlation between the benchtop analyses and the MRI data. Additionally, it may

be of value to attempt the experiments with no crosslinking at all. At this time, it is unclear what role, if any, crosslinking plays in affecting MR parameters.

In order to overcome the complications associated with the different holder set-ups used during the course of this project, the use of a well plate system with built in ports would be highly desirable. The built in ports would overcome the problems associated with sponge transference between holders during degradation. Enzymes and wash solutions could be added and removed through these ports without disturbing the sponge and stirring up any large pieces of the samples which may have come loose during degradation. The ports would remove the damage caused to the sponge by repeated insertion of pipette tips into the wells. By keeping the sponges in the well plates, this would also overcome the hydration issues experienced with the transwell system. By allowing the sponge to rest on a solid, flat surface, it would ensure the sponge was as hydrated as possible without sitting in excess liquid; or allowing liquid to continuously drip out, dehydrating the sponge during long imaging periods, as the transwell membranes did.

There are many different MR imaging techniques and pulse sequences available, and it is necessary to attempt different imaging techniques. This project focused on the changes in bound and unbound water content in each sponge. Therefore, T_1 and T_2 weighted imaging were used in an effort to characterize this. We learned that these parameters were not sensitive enough to the extent to which we degraded the collagen sponges *in vitro*. However, this project team feels that different techniques may be used to better relate the extent of degradation in collagen sponges.

The imaging technique that may show the most promise is diffusion weighted imaging (DWI). DWI is based on the amount of water that diffuses through the sponge over time. Since degradation will increase the pore size in the sponge, the diffusion rates should change with degradation. Therefore, a quantitative relationship between the diffusion weighted images and the amount of degradation of the sponge may be determined. If not, other imaging techniques should be tested for their sensitivity, such as gradient spin echo imaging, etc.

Another recommendation would be to mimic collagen digestion in the human body more closely. This can be done by replacing the collagenase enzyme, used for this project, with matrix metalloproteases (MMP). MMP is the enzyme the human body uses to degrade collagen, providing more relevant results in respect to biomaterial degradation in clinical applications. This would be useful after having previous success in relating changes in MR parameters to degradation using collagenase, *in vitro*. Matrix metalloproteases are much more expensive than the bacterial collagenase used in this project, which may be a concern if there are budget constraints on a future project. Should any future *in vitro* experiments prove successful, the next step should involve *in vivo* animal studies; prior to seeking FDA approval for human clinical trials. This is necessary because they will need to show how the experiment will work and how their study parallels the human body before the FDA will even consider it. Using better digestive enzymes like metalloproteases will be necessary eventually.

It is the project team's hope that this study will be the foundation for a device and/or technique that is approved by the FDA for use in industry or clinical applications. In order to receive the FDA's approval, the clinical relevance and significance needs to

be shown through *in vivo* animal testing. One of the original goals of this project was to implant collagen sponges into the backs of lab rats and image them with MRI at various time points to characterize degradation *in vivo*. It is the recommendation of the project team that if a relationship between MR parameters and collagen sponge degradation can be determined, these experiments should move on to animal testing. To do this, collagen sponges should be implanted in the backs of rats subcutaneously, and the biodegradation rate assessed by the same MR imaging technique proven effective in the *in vitro* experiments. If success is shown with the *in vivo* studies as well, seeking FDA approval for human clinical trials would be the final iteration for this project.

9 APPENDICES

9.1 Appendix A: MRI Data

The following pages document all the MRI data collected and processed for this project. The first set of data is organized to allow comparison of undegraded and degraded measurements for each of the three parameters measured. The data is organized chronologically and each set maintains the order proton density (M0), T_1 relaxation times, and T_2 relaxation times.

Appendix in separate file

IQP/MQP SCANNING PROJECT

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9.2 Appendix B: Sponge Fabrication

Materials
Bovine Achilles Tendon Collagen (Sigma, Cat# C9879)
10mM HCl
Water-cooled blender
Conical Tubes
12 well plates
Freeze-dryer

Procedure

1. Start cooling the water for the blender and freezing the shelf in the freeze dryer
2. Combine collagen and 10mM HCl to make a 1%w/v mixture
3. Place mixture in blender and blend for 4 min. Allow to sit for 10 min and then blend for another 4 minutes.
4. Place dispersion into conical tubes and centrifuge to remove all the bubbles. The solution should be homogenous and white.
5. Pour collagen dispersion into wells of 12 well plate, adjusting volume to achieve desired thickness (*Assume wet volume is the same as dry volume. Area of well is 3.8cm^2).*
6. Freeze well plate by placing in liquid nitrogen or in -80°C freezer.
7. Place well plate in freeze-dryer (make sure shelf temperature is at -45°C or lower) and perform the following steps:
    a. Bring vacuum to below 200mTorr.
    b. Raise shelf temperature to 0°C
    c. Dry overnight at 0°C
    d. Increase shelf temperature to 20°C
8. When shelf reaches 20°C, release vacuum and remove sponges
9. Crosslink sponges if required
    - To UV-crosslink the sponges, place sponge in open Petri dish and place in the UV-crosslinker for the desired time. Turn the sponge over and repeat for the other side.
    - To DHT crosslink the sponges, wrap in each sponge in tin foil, making sure to leave on edge open so air is free to move in and out of the sponge. Place in vacuum oven and turn on the vacuum pump. Once pressure is below 150mTorr, turn heater on. As the temperature increases, check the oven frequently to ensure pressure does not exceed 200mTorr. If it approaches this pressure, turn off heater until pressure has recovered. Once temperature has reached 105°C, start timing 24 hours.
    - Carbodiimide crosslinking is performed by dissolving N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide HCl (Sigma, Cat# E6383) at a concentration of 10mg/ml in DI water. The sponges are then hydrated in this solution and stored at 4°C for 24 hours to complete the crosslinking. Sponges are then rinsed 3 times for 10 minutes in PBS.

Note:
The freeze-dryer has been programmed to perform the above steps, with the correct timing. To have the freeze-dryer perform these steps, use *Recipe 2*.

9.3 Appendix C: Degradation Protocol

Materials:

1x PBS (Fisher, BW17517Q, $24.12)

Dilute stock solution (10x PBS) at 1:10 ratio.

To prepare 150ml dilute 15ml 10x stock with 135ml distilled water.

Tris-HCl buffer

To prepare 100ml of 0.1M Tris-HCl buffer add 1.144g Tris-HCl and 0.332g Tris-Base to 100ml distilled water.

Tris-HCl Buffer with $CaCl_2$

To prepare 100ml of 0.1M Tris-HCl buffer with $CaCl_2$ add 0.2775g $CaCl_2$ to 100ml 0.1M Tris-HCl buffer.

Collagenase (Calbiochem, Cat# 234153)

To prepare 10ml collagenase solution at $100CDUml^{-1}$, add 4.075 mg to 0.1M Tris-HCl w/ Ca^{2+}. Dilute as necessary to achieve desired concentration

Ethylenediaminetetra-acetate (0.25M EDTA, MW = 292.24)

To prepare 2ml of 0.25M EDTA (Sigma, 100g) add 0.1461g to 2ml distilled water

2 x12-well plates

Procedure for a sponge weighing approximately 15mg:

1. Prepare collagen sponges in 12-well plates, see Appendix A: Sponge Fabrication
2. Prepare fresh stock solutions as described above
3. Weigh dry sponge and record weight. Ensure that sponge is properly labeled throughout degradation and imaging so that final weight may be compared to initial weight.
4. Hydrate sponge in 2ml 0.1M Tris-HCl w/ Ca^{2+} in 12-well plate (or 6-well plate with transwells) by placing it under vacuum.
5. Incubate at 37°C for 15 minutes.
6. Add 2 ml collagenase solution (5 ml if using transwells) to well at concentration double the desired final concentration and incubate at 37°C for required time.
7. Remove from incubator and place immediately on ice at desired time point. Add 0.2ml of 0.25M EDTA (0.5 ml of 0.25M EDTA if using transwells) to halt the degradation.
8. Remove sponges and rinse three times with 10mM EDTA (10 min each).
9. Pipette remaining solution into another container to analyze later using ninhydrin protocol (Appendix C).
10. Rinse three times with 1x PBS water (30 minutes total)
11. Transfer sponge to another 12-well plate (or 6-well with transwells) containing 2ml (5ml if using transwells) 1x PBS to keep sponge hydrated.
12. Seal with lid and parafilm until ready to image.

Note: If performing the degradation in the collagen holder, all the volumes of sponges in 12-well plate should be halved.

Collagen Standard:

To create collagen standard, grind 5mg of collagen sponge and treat with collagenase according to above procedure. Allow sponge to incubate with collagen for 24 hours to achieve complete degradation.

9.4 Appendix D: Change in Dry Mass Protocol

Change in Mass Protocol:

Materials:

Collagen sponge
12-well plate
Mass balance
Freeze-dryer

Procedure: (to be performed before and after collagenase treatment)

1. Place degraded sponge in a 12-well plate and remove any excess solution
2. Freeze well plate at -80°C until ready for lyophilization
3. Place well plate in freeze-dryer and lyophilize using *Recipe 2*.
4. Re-lyophilize as often as necessary to ensure all water is removed
5. Determine final mass of sponge and compare to original mass.

9.5 Appendix E: Ninhydrin Protocol

Materials:

2% Ninhydrin Reagent solution (Sigma, Cat# N7285)

Note: Liquid should be a burgundy color. If liquid is yellow it has been exposed to air for too long and should not be used.

0.05% glacial acetic acid

To prepare 1000ml of 0.05% glacial acetic acid, combine 0.5ml 100% acetic acid with 999.5ml distilled water

96 well plate (1)

0.015 g L-leucine (Sigma, Cat# 61819, 25g, MW = $131.17^{g}/_{mol}$)

15ml conical tubes (15)

DI Water

95% ethanol

Procedure:

1. Make stock solution of l-leucine by dissolving 0.0184g L-leucine in 100ml 0.05% glacial acetic acid.

$$100 \times 10^{-6}\, moles \times \frac{131.17 g}{mole} = 0.0131 g$$

2. Dilute stock solution to achieve the following concentrations in 0.375ml: (all in μmol/ml)

   1.4; 1.3; 1.1; 1.0; 0.9; 0.8; 0.7; 0.6; 0.5; 0.4; 0.02 and 0

3. Place 0.375ml solution recovered from degradation and standard solutions in eppendorf tubes.
   a. ***Note:*** *It may be necessary to dilute the solutions recovered from degradation to ensure the absorbance values fall into the range of the standard curve. A 1:5 dilution using PBS works best.*
4. Add 0.125ml of the 2% ninhydrin reagent solution
5. Mix contents of all tubes gently
6. Place in boiling water bath for 10 minutes exactly
7. Cool to room temperature
8. Add 0.625ml 95% ethanol to each tube and mix contents together
9. Load solutions into a 96 well plate using a clean pipette tip for each sample, taking note of where the standards and samples are placed.
10. Load blank wells with 0.1M Tris-HCl w/ Ca^{2+} and collagenase.
11. Read the absorbance at 570nm
12. Calculate the leucine concentration in the samples by computing the absorption ratio with respect to the standard solutions
13. To calculate the extent of collagen degradation use the following equations, based on the assumption that for every collagen molecule digested, 95 leucine molecule is exposed to react with ninhydrin:

Concentration x *5ml = # moles released.* (1.0)

Initial sponge weight/$MW_{collagen}$ = Total initial moles (2.0)

$MW_{collagen}$ = 300,000g/mol

$$\frac{\#\,Moles\,\mathrm{Re}\,leased}{95} = \#\,CollagenMolesDegraded \quad (3.0)$$

 # collagen moles degraded/Total initial moles x 100 = *amount degraded* (3.0)

Note: The complete degradation of a 10mg sponge should produce a solution with a leucine concentration of ***0.792 µmol/ml****, based on the following calculations:*

$$\frac{0.010g}{300{,}000\frac{g}{mol}} = 3.33\times10^{-8}mol$$

$$3.33\times10^{-8}mol\times\frac{6.0221\times10^{23}molecules}{mol} = 2.007\times10^{16}molecules$$

$$2.007\times10^{16}molecules\times95\frac{cleaves}{molecule} = 1.907\times10^{18}\,\mathrm{exp}\,osed$$

$$1.907\times10^{18}\times\frac{mol}{6.0221\times10^{23}molecules} = 3.167\times10^{-6}mol$$

$$1.907\times10^{18}\times\frac{mol}{6.0221\times10^{23}molecules} = 3.167\times10^{-6}mol$$

9.6 Appendix F: Top 12 Design Alternatives

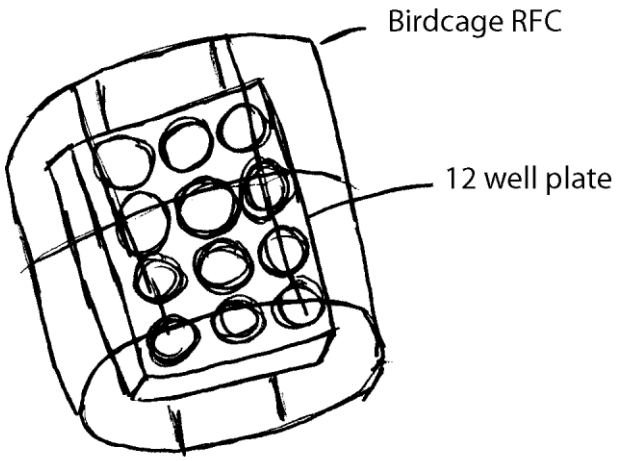

Figure 56: Well plate with birdcage coil

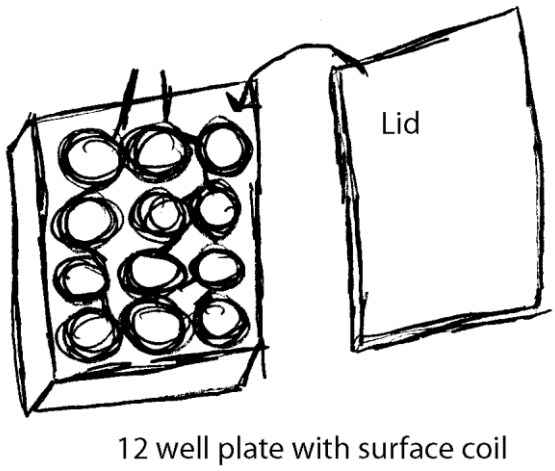

Figure 57: Well plate with surface coil

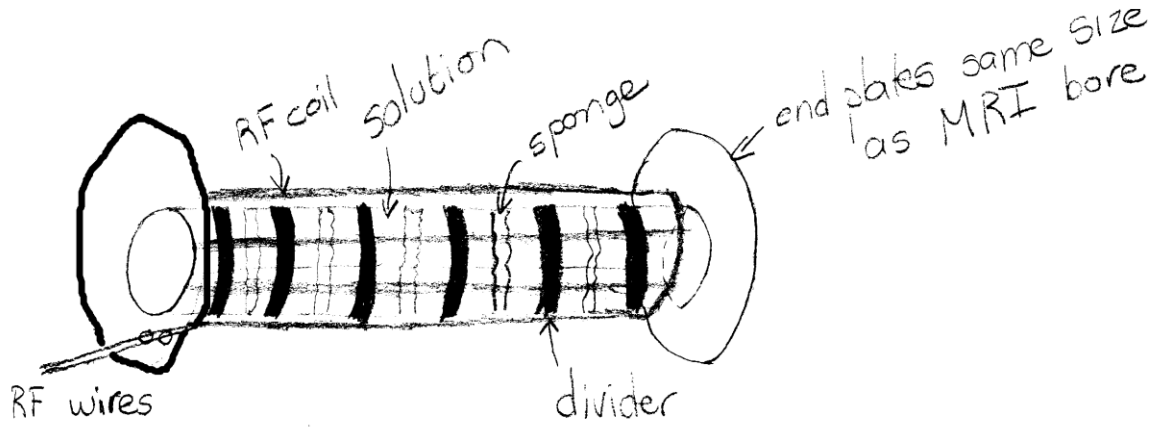

Figure 58: Concentric tube

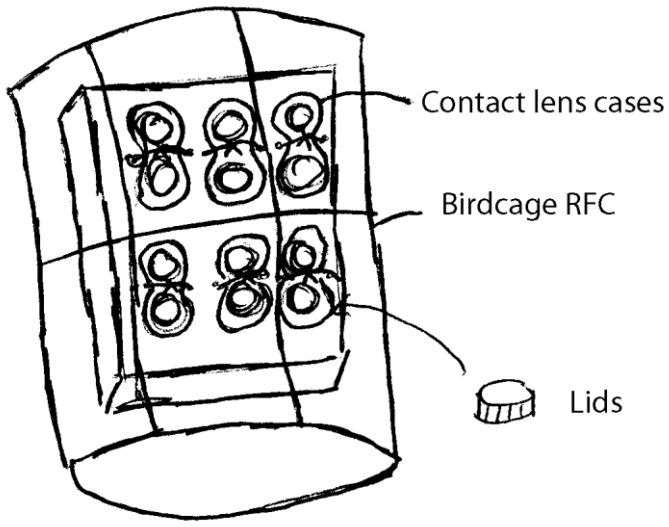

Figure 59: Contact lens cases with birdcage coil

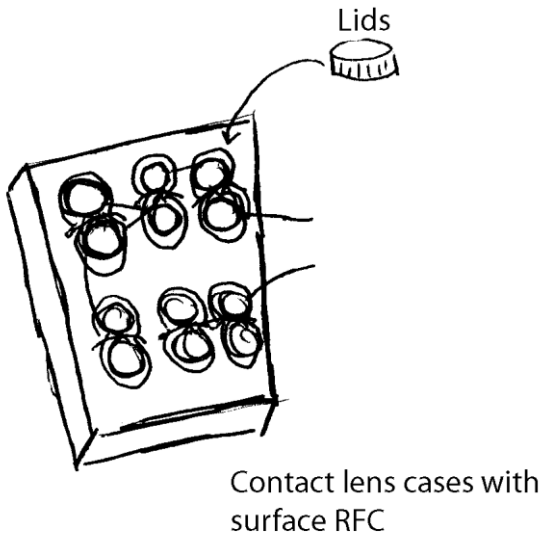

Figure 60: Contact lens cases with surface coil

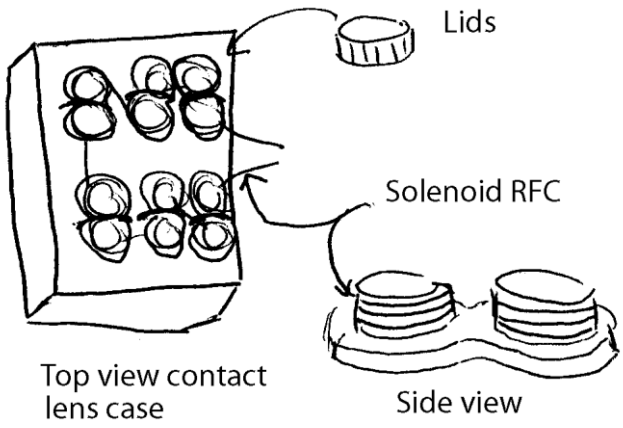

Figure 61: Contact lens cases with solenoid coil

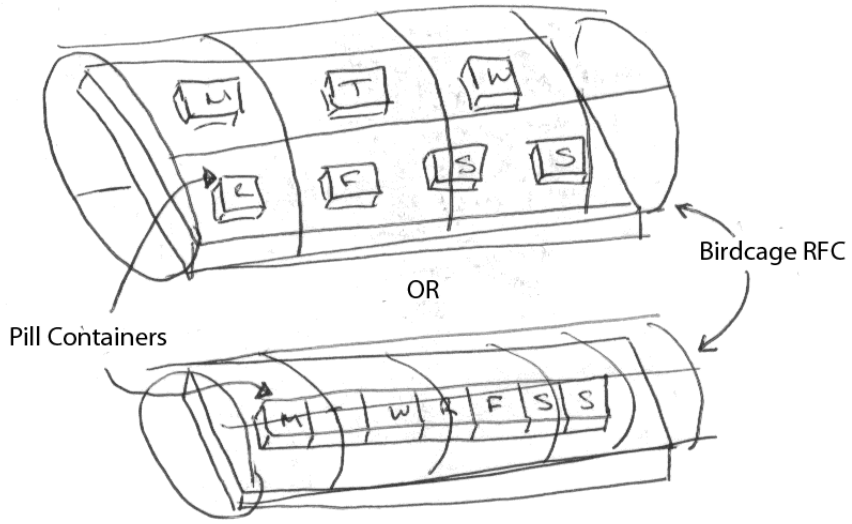

Figure 62: Pill container with birdcage coil

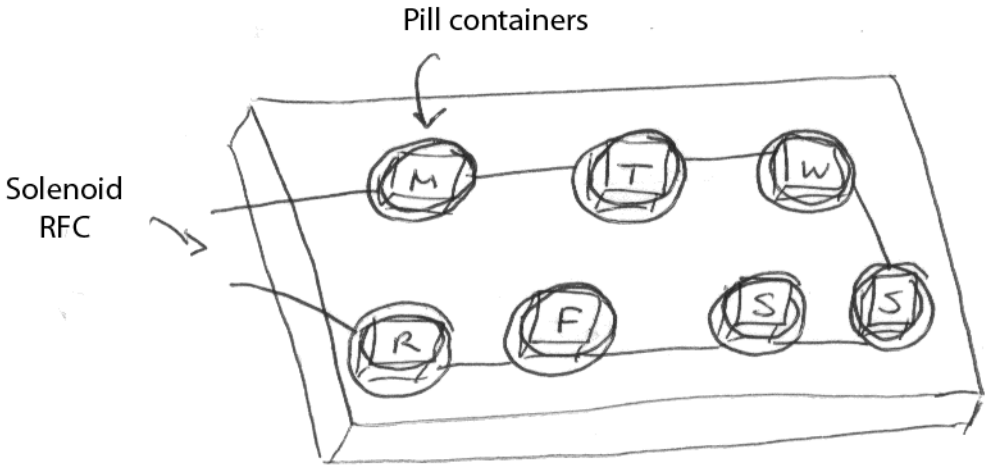

Figure 63: Pill container with solenoid coil

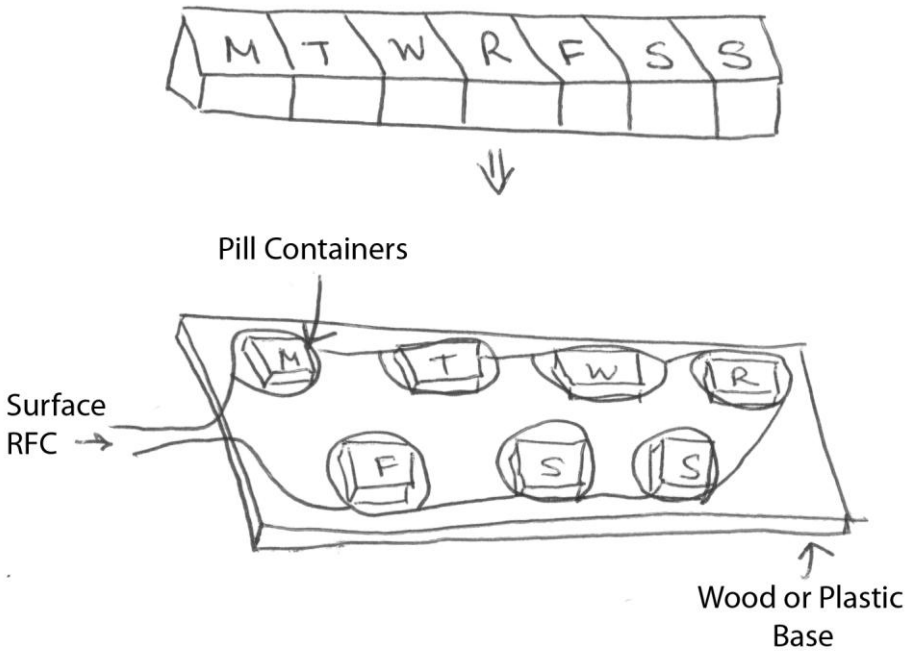

Figure 64: Pill container with surface coil

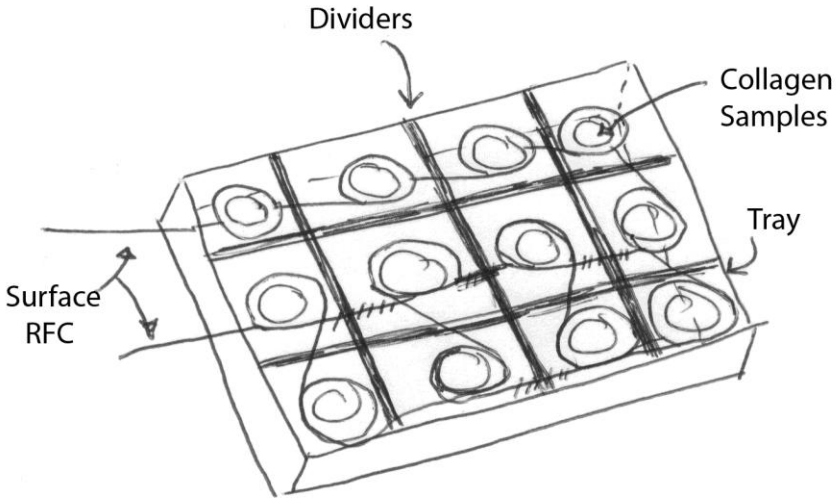

Figure 65: Tray with surface coil

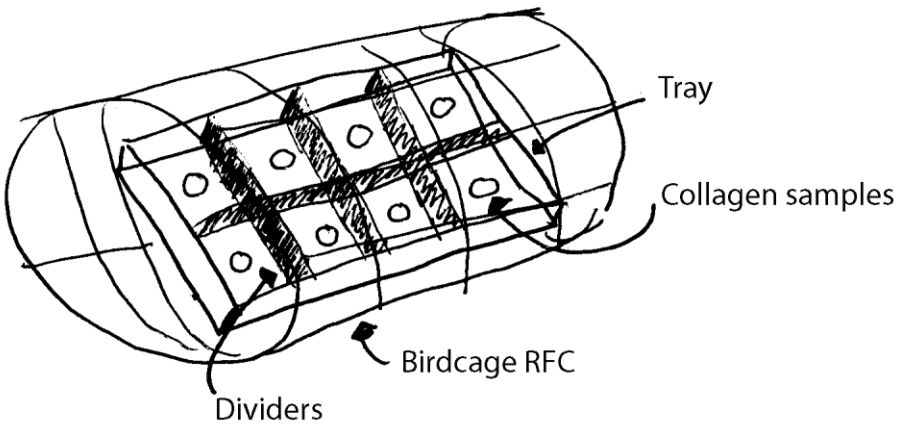

Figure 66: Tray with dividers and birdcage RFC

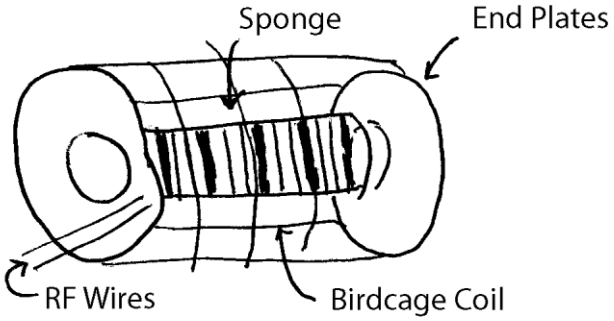

Figure 67: Concentric tube with birdcage coil

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