Potential Commercialization of a Collagen-GAG Scaffold for Liver Regeneration

by

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Submitted to the Department of Materials Science and Engineering
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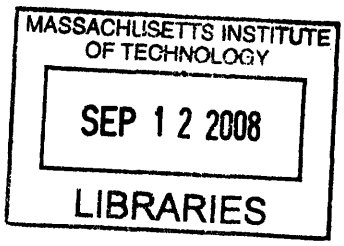

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ABSTRACT

The potential for commercializing a scaffold made of collagen and glycosaminoglycan to help regenerate cirrhotic liver was analyzed and a business plan and model were created. Using a lypholization technique, a bulk-sized and highly porous scaffold is created. It is then inserted into the hole created by the excised liver scar tissue. By blocking contraction of the wound and mimicking the natural extracellular matrix, the scaffold induces regeneration of normal liver tissue. The *in vivo* approach is compared to several other experimental treatments of cirrhosis found in the literature. The difficulties that need to be addressed are explained and potential solutions are given.

A cost model was created, incorporating equipment, labor, FDA, and raw material costs. This model was combined with information regarding the cost of current liver transplant procedures to create a profitable business plan based on the collagen-GAG scaffolds. A manufacturing and product sales business model was chosen due to the fairly low level of market competition and moderate barrier to entry. The intellectual property landscape is described and analyzed in terms of problematic existing patents and the potential for protecting the proposed scaffolds. A timeline for future research and development was created, with potential sources of funding during each phase. In addition to the current embodiment of the scaffold, possible changes to the scaffold properties and composition are proposed.

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To my parents, Thomas and Deborah Southworth. Everything I've accomplished can be traced back to you and the love and support you've given me. You've taught me to work hard, be responsible, and always strive to do my best, not just the minimum to get by. Thank you for always being there and giving me everything I needed, and more, to succeed.

To my brother, Kevin Southworth. Thank you for providing the perfect example and giving me someone to look up to.

To Professor Yannas, my thesis advisor, and Eric Soller. Thank you for sharing your fascinating research with me and for taking the time out of your busy schedules to help me with my thesis.

Chapter 1. Introduction

Liver cirrhosis is a deadly condition that is very prevalent in the United States. Every year there are over 500,000 hospital visits for chronic liver disease or cirrhosis and more than 27,000 deaths (1). Cirrhosis occurs when healthy liver tissue is replaced by fibrous scar tissue. The condition develops over many years as a result of chronic inflammation due to liver disease. The diseases that can lead to cirrhosis include hepatitis B and C as well as alcoholism. Alcoholism is responsible for about 40% of the deaths due to liver cirrhosis each year (2). There are almost 13 million "heavy drinkers" in the United States, of which 10 to 20 percent will develop liver cirrhosis at some point in their life (2, 3). This means that approximately 2 million people either have or will develop cirrhosis within the next 10-15 years.

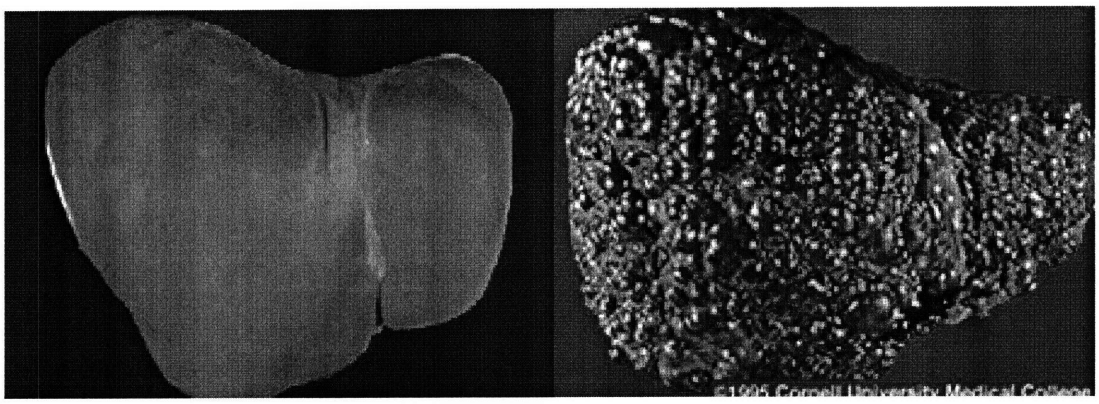

Figure 1. A healthy liver (left) compared to a cirrhotic liver (right) (4, 5).

Currently, a transplant is the only effective treatment for late stage cirrhosis. Liver transplants are extremely costly, and the supply of donors is limited and insufficient for the number of needy recipients. Some chemical and drug treatments are in the early research phase, but there is no cure in the foreseeable future. An alternative approach, taken by Dr. Yannas' lab, is to regenerate the scarred liver tissue using a tissue regeneration scaffold. This report will analyze the current state of the liver scaffold technology and expand on the technical hurdles still ahead before the technology can be considered ready for commercialization. Competing technologies will be described and briefly analyzed, with emphasis on liver transplant. The manufacturing process and costs

involved with the tissue scaffold will be detailed and the relevant FDA and intellectual property considerations will be described. Finally, a business plan will be proposed, including the envisioned supply chain, for turning the scaffold technology into a successful start-up company.

Chapter 2. The Liver

2.1 Anatomy

The liver is the largest visceral organ, weighing about 1.5 kg. It is located in the right side of the abdomen, beneath the diaphragm (6). A fibrous capsule and some peritoneum surround the liver (7). It is traditionally broken into four lobes: left, right, caudate, and quadrate. The right and left lobe are separated by the falciform ligament. The right lobe is larger than the left, comprising about 60% of the liver volume. The blood supply to the liver is composed of three major vessels. Oxygenated blood is supplied to the liver by the hepatic artery and blood from the digestive system is supplied by the hepatic portal vein. About 70% of the blood enters through the portal vein and the remaining 30% is from the hepatic artery (7). Once the liver has performed its many functions, blood is returned to the systemic circulation by the hepatic veins, which eventually empty into the inferior vena cava (6).

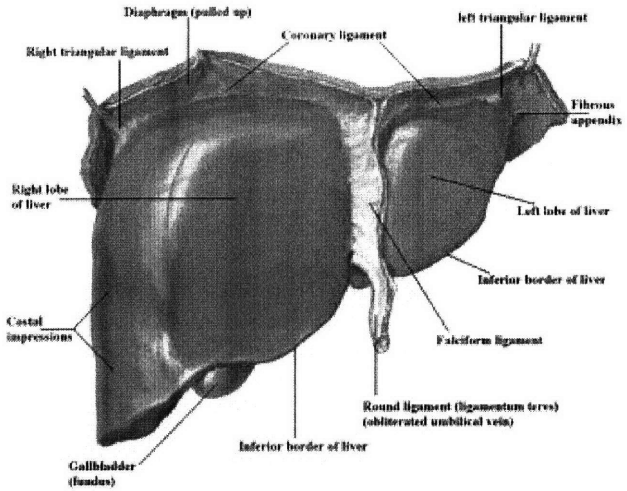

Figure 2. Anatomy of the liver (8).

2.2 Function

The liver has been associated with over 200 functions, which generally can be broken down into three categories: metabolic regulation, hematological regulation, and bile

synthesis and secretion (6). Metabolic regulation involves regulation of levels of carbohydrates, lipids, and amino acids in the body. All blood leaving the digestive system flows through the liver, where excess nutrients, toxins, and metabolic wastes are removed before the blood enters the rest of the body. If there are deficiencies, they are corrected by releasing stored reserves or synthesis of new substances. The liver is also where fat soluble vitamins are absorbed and stored (6).

Hematological regulation involves maintaining homeostasis in the blood. The liver is the largest blood reservoir in the body, accounting for 25% of the cardiac output at any given time. Phagocytic cells in the liver remove old and damaged red blood cells, cellular debris, and pathogens. The liver cells also synthesize new plasma proteins that help to regulate the osmotic concentration of the blood as well as transport nutrients and blood clotting agents (6).

Bile is synthesized by the liver and stored in the gallbladder. Bile consists mostly of water, with some ions, bilirubin (pigment), and lipids. The lipids are known as bile salts. Bile helps to maintain acid levels during digestion and assists in lipid breakdown (6).

2.3 Histology

The functional unit of the liver is called the lobule, and there are approximately 100,000 lobules per lobe. Lobules are hexagonal in cross section, with a central vein at the center. At each corner of the hexagon there is a portal area, also called the hepatic triad, which contains a branch of the hepatic portal vein, a branch of the hepatic artery, and a bile duct. Hepatocytes, the functional cell of the liver, are aligned in a spoke-like fashion around the central vein. Each "spoke" is only one cell wide, and the spokes are separated by sinusoids. Sinusoids are where the blood from the portal veins enters the lobule. As blood flows through the sinusoids it passes over the microvilli that line the exposed surface of the hepatocytes. These microvilli give the cell membrane a very high surface area, allowing diffusion of substances back and

forth between the cell and blood in the sinusoid. The sinusoid is lined by endothelial and Kupffer cells. Endothelial cells line all blood vessels and Kupffer cells are phagocytes that engulf pathogens, cell debris, and damaged red blood cells. The Kupffer cells also retain heavy metals like tin and mercury. To allow large substances to pass through, the sinusoids have openings called fenestrae. Once the blood has passed by the hepatocytes it empties into the central vein, which eventually connects back to the hepatic veins and then the inferior vena cava (6).

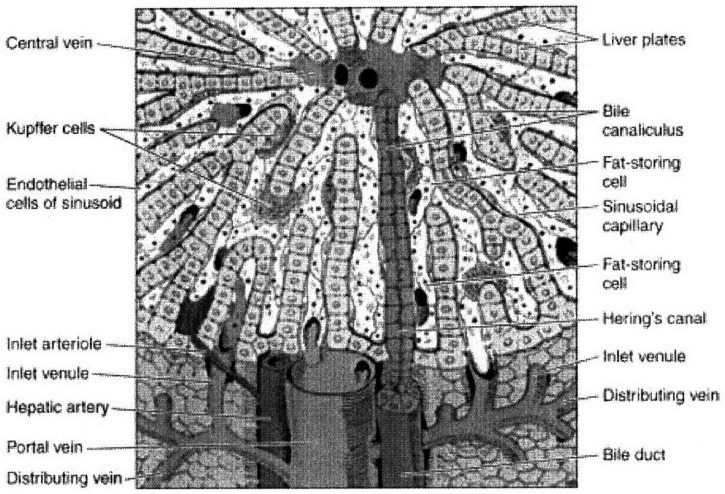

Figure 3. Schematic diagram of a liver lobule (9).

Chapter 3. Current Treatment

As mentioned previously, the only truly effective treatment for liver cirrhosis is a liver transplant. Liver transplants can be taken from recently deceased or living donors. Living donors can have one of the right or left lobes removed; typically the left is taken for a child and the right for an adult (10). Approximately 6,500 transplants are performed each year, but about 17,000 people remain on the waiting list at the year's end. The surgery can take between six and twelve hours, and requires a hospital stay of up to three weeks following surgery (2). The hospital stay could be shorter, from five to ten days, but frequent assessments have to be done for up to a month after the surgery. For this reason, it is recommended patients arrange for living accommodations near the hospital (10). Aside from having to take immunosuppressant drugs for the rest of their life, patients can return to a normal lifestyle after about six months. The five-year survival rate is about 75% (2).

Due to the time consuming donor-recipient matching process, organ procurement, complexity of the surgery, and extensive recovery time, liver transplants are extremely expensive. The table below breaks down the cost of each aspect of the transplant.

Evaluation	Procurement	Hospital	Physician	Follow-up	IS Drugs (first year)	Total
$25,900	$59,100	$248,100	$66,900	$88,500	$31,100 + ~$360,000 For 20 additional years	$519,600 $879,600 After 20 years

Table 1. Cost breakdown of each part of the transplant process (11)

The cost of the transplant and the associated fees and drugs for the first year is $519,600 (11). However, some of the immunosuppressant drugs must be taken for the rest of the patient's life. If a patient were to live 20 more years after the surgery, this would add up to an additional $360,000 (12), for a total cost of almost $880,000. Medical insurance coverage varies widely by policy, patient, and hospital, so a definitive breakdown of who will pay what costs is impossible to know. It is estimated, however, that private insurance will typically pay about 80% of the transplant costs. Patients who qualify for Medicare will have most of the bill paid, minus some deductibles and physician fees (12).

Chapter 4. Competing Technologies

Although a liver transplant is currently the only treatment used, combating cirrhosis is an active research field. Most approaches rely on proteins or peptides to either directly reduce the amount of fibrous scar tissue or to affect the processes that govern its formation and regression. An example of the former is the injection of collagenase directly into the portal vein, a study done by Jin et al. (13). Collagenase is an enzyme that breaks down collagen, the primary component of scar tissue. In the study, bacterial collagenase was delivered directly to the portal vein of CCl_4-induced cirrhotic livers in rabbits via a catheter.

The experiment consisted of four groups: a control group, a group that received CCl_4 for 12 weeks and collagenase for weeks 7-12, a group that received CCl_4 for 12 weeks followed by 12 weeks of collagenase, and finally a group given CCl_4 for 12 weeks followed by saline for 12 weeks. This allowed for analysis of the effect of collagenase with the cirrhosis stimulant still there (Hepatitis), and without it (a former alcoholic). The rabbits were given 6 mg of collagenase twice a week. The results of the study showed that simultaneous infusion of CCl_4 and collagenase resulted in less collagen formation than CCl_4 alone, although still above the control levels.

Collagen content was determined using a hydroxyproline assay. The group that received collagenase for 12 weeks after a full 12 weeks of CCl_4 showed a drastic reduction in liver fibrosus and was almost back to normal according to the histology. However, the group receiving only saline injections also showed a large reduction in fibrosus, although not as completely. The hydroxyproline levels of the group III rabbits returned to almost control levels, while the group IV rabbits receiving saline injections was still significantly above the control (13).

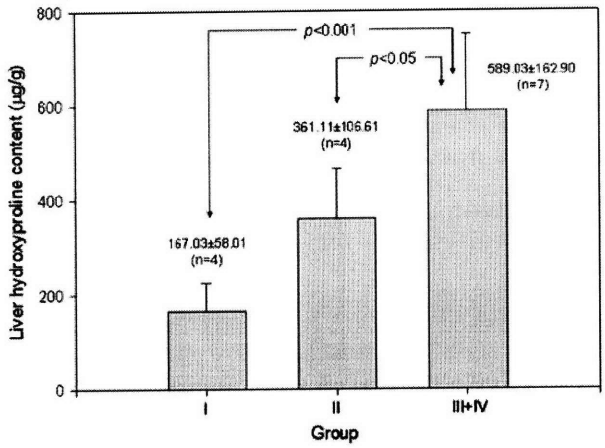

Figure 4. Hydroxyproline content after 12 weeks of CCl_4 (13).

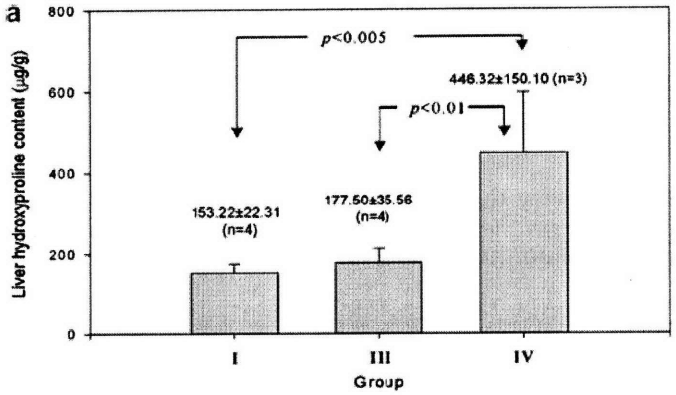

Figure 5. Hydroxyproline content after 12 weeks of CCl_4 and 12 weeks of collagenase or saline (13).

Overall, the results showed that portal collagenase injections can help slow cirrhosis development while given concurrently with a cirrhosis causing stimulant and can also cause regression of fibrosus when the stimulant is removed. While the study is encouraging, there are some notable risks and questions that remain. The first is that during the study, 46% of the rabbits died prematurely due to surgical complications. It is not believed that this had any effect on the results or that the collagenase treatment had anything to do with the high death rate; however it shows the potential for complications that may occur if the approach was translated to humans. The second concern is that while the collagenase did significantly accelerate the regression of fibrosus, the rabbits receiving saline injections also showed marked signs of regression. It could be hard to distinguish the effect of collagenase versus the

removal of the cirrhotic stimulant. Lastly, although tests that were done on the blood, kidneys, heart, and brain showed no adverse effects from the collagenase treatment, it is still likely that there could be some sort of long-term side effects of the treatment in humans. The collagenase injections were only done over a 12 week period, not long enough to ascertain any chronic toxicity or other long-term implications.

A second approach, published in 2002 by Vinokurov et al., was to use adrenoceptor agonists to reduce the effects of TGF-β in formation of liver cirrhosis. The specific adrenoceptor agonist used was dobutamine. The motivation for suppressing the effect of TGF- β is that it stimulates the formation of connective tissue and inhibits hepatocyte proliferation (14). Cirrhosis was induced in mice using CCl_4, administered twice a week for 30-60 days. The hepatocytes from the treated mice were then cultured with various combinations of EGF and dobutamine to determine their proliferative activity. The cells cultured with dobutamine showed a significantly higher proliferative activity than those with just EGF. The proliferative activity was maximized with a dobutamine concentration of 8 μg/ml (14). In *in vivo* tests, treatment with dobutamine 24 hours after a partial hepatectomy (HPE) showed an increase in proliferative activity compared to PHE alone. However, when combined with CCl_4, the proliferative activity was reduced to levels slightly above the control (14).

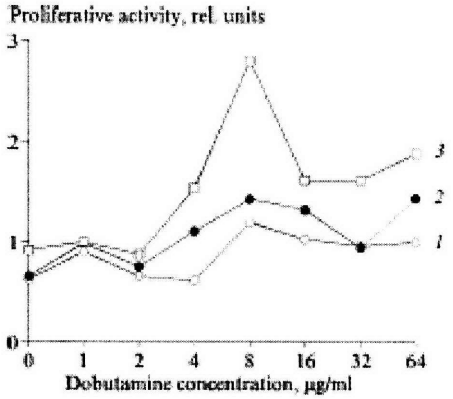

Figure 6. Proliferative activity of hepatocytes after culturing with dobutamine for 8, 16, and 24 hours (14).

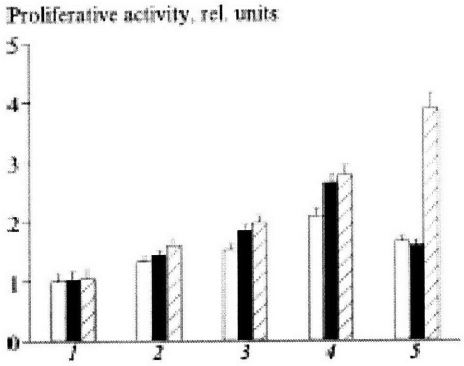

Figure 7. Proliferative activity of hepatocytes after 8, 16, and 24 hours (white, dark, shaded), when preincubated in EGF (2-4) or dobutamine (5) (14).

While this study was interesting, the results need to be examined critically. First, the initial studies were done *in vitro*, and proliferative activity may or may not be the same in culture as it is in the body. Second, the *in vivo* tests are done mostly with PHE, but the test with CCl_4 and dobutamine does not involve a PHE, making the results difficult to compare. Also, since the liver is able to partially regenerate on its own, conducting a PHE can make it difficult to separate the effects of the dobutamine from the natural proliferation activity of the body.

Another approach, published recently by Buck and Chojkier, is to block ribosomal S-6 kinase (RSK) activation using an inhibitory peptide (15). It was shown that activation of RSK caused phosphorylation of protein C/EBPβ on a gene in hepatic stellate cells (HSC) that leads to synthesis of excessive extracellular matrix (ECM). However, when a transgene, C/EBPβ-Ala217, replaced the normal version, fibrosis was not observed. The same result was achieved by treating the mice with a cell permeant RSK inhibitory peptide. When cirrhosis was induced using CCl_4 for 8 weeks, followed by treatment with the peptide for 4-8 weeks, apoptosis (cell death) of the HSC and regression of fibrosis was observed (15). A similar pathway to liver fibrosis exists in humans, leading the researchers to believe that this approach may be applicable to people. This is certainly another promising study in the fight against liver cirrhosis, but again must be examined critically. Currently gene therapy is very limited and experimental in humans, and getting FDA approval is extremely difficult.

This means that the cell permeant peptide would likely be the first form of this treatment to be viable in humans. Once again the treatment requires repeated dosing and the effects on the rest of the body are unknown as well as the appropriate delivery method.

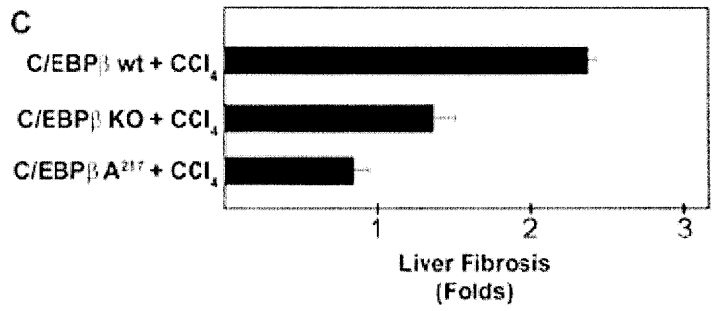

Figure 8. Mice with Transgene show 2.5X reduction in fibrosis (15).

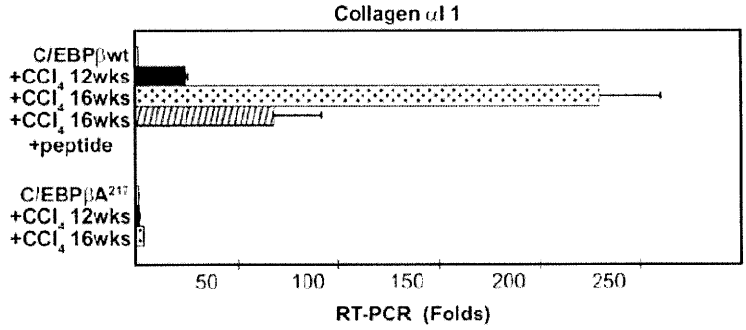

Figure 9. Mice treated with RSK inhibitory peptide show significant decrease in collagen (15)

Chapter 5. Proposed Technology

5.1 Overview

The technology proposed to produce liver regeneration is a collagen and glycosaminoglycan (GAG) scaffold. It is created by a lypholization procedure and is highly porous. The scaffold will be placed in the wound of the surgically excised scarred liver tissue. Due to certain size constraints, which will be described later, multiple scaffolds may be placed to achieve the desired total volume. The scaffold will then induce regeneration based on principles described by Dr. Yannas and due to the unique properties of the scaffold.

5.2 Materials

The primary component of the scaffold is collagen. There are many types of collagen in the collagen family, but the proposed scaffold uses type I. Collagen is the primary tensile load bearing structure in the extracellular matrix but also serves functions in tissue scaffolding, cell adhesion, cell migration, angiogenesis, and more (16). Collagen is composed of a triple helix of polypeptide (α) chains. The chains are arranged in a right handed super helix and each chain has a repeating amino acid sequence of Gly-X-Y, where Gly is glycyl and X and Y are often proline and hydroxyproline. The three chains are held together by hydrogen bonds (16). Each chain contains 1050 amino acid sequences and is 300 nm long and 1.5 nm in diameter (17). Many of the collagen triple helix molecules pack together side by side to create collagen fibrils. These fibrils are 50-200 nm in diameter and are several micrometers long. The collagen molecules come together in a staggered manner, with a periodicity of 67 nm. This results in a "banded" structure (17).

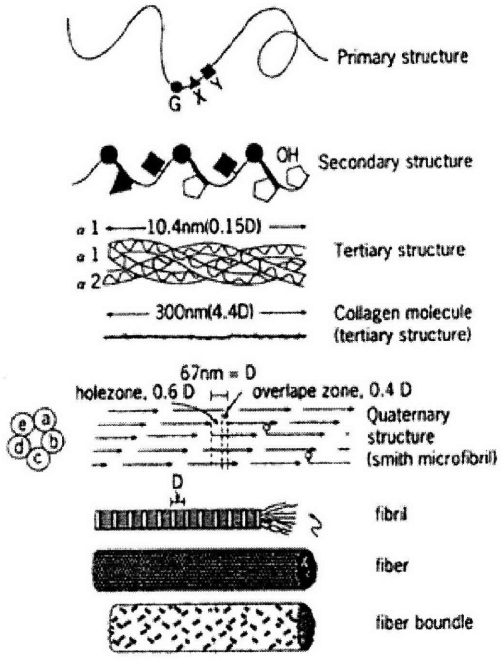

Figure 10. The structure of collagen molecules, fibrils, and fibers (18)

Collagen is resistant to most proteases (enzymes that break down proteins), but is susceptible to matrix metalloproteinases (MMPs), cysteine proteinases, and serine proteinases (16). The liver is only about 0.5% collagen by weight (wet), and most of the collagen is type I and III. In cirrhotic liver the amount increases about tenfold to 5% by weight and type I becomes more predominant (19).

The second component of the scaffold is glycosaminoglycan, or GAG. Glycosaminoglycans are polysaccharides with a disaccharide repeat sequence (20). One of the repeat units is an amino sugar and the other is usually an uronic acid. There are many types of GAG, with some of the most common being chondroitin sulfate, heparan sulfate, keratan sulfate, and hyaluronic acid. GAG molecules are often covalently attached to a core protein to create a macromolecule called a proteoglycan. GAGs help to localize proteins and enzymes at their point of function with cells and the ECM (20).

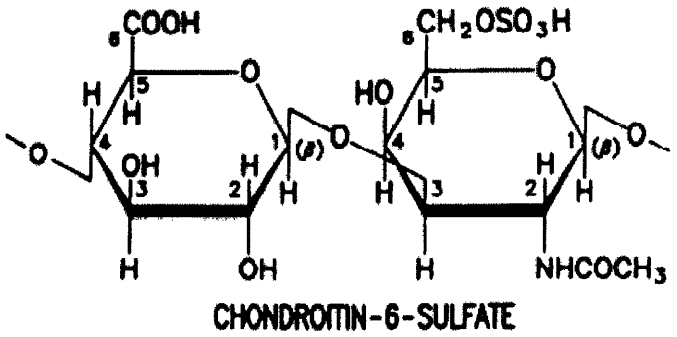

Figure 11. The chemical structure of chondroitin sulfate, one of the common GAGs (20)

5.3 Scaffold Properties

The tissue regeneration scaffolds created by Dr. Yannas' group are highly porous, with a pore volume fraction of over 99%. The scaffold is 0.5 wt% collagen type I and 0.05 wt% chondroitin 6-sulfate. The pore size can be tailored by the freeze-drying process, but is typically from 90-150 μm (21). The pores are equiaxed and fairly uniform in size. The pores are also interconnected throughout the scaffold, allowing cell and nutrient migration. The scaffolds currently being used in mice studies are cylindrical in shape with a radius of 1.5 mm and a height of 5 mm. This gives a volume of 35.3 mm^3, or 0.0353 cm^3. Using the liver to body weight ratios of mice and rats (5.8%), their average body weights (20 grams and 300 grams), and an empirical equation for rat liver volume for a given liver weight (eq. 1) an average mouse liver volume of 1.09 cm^3 was reached (22, 23).

$$L_v = 0.892 \times L_w + 0.8 \qquad (1)$$

Using these values, the current studies are being done with a scaffold that replaces approximately 3.2% of the mouse liver volume. An average human liver (men and women combined) is approximately 1470 cm^3 (24). In order to duplicate the proportional size and shape of the mouse scaffold, a human scaffold would therefore have a radius of 3.3 cm and a height of 5.5 cm and a total volume of 47 cm^3. To control the degradation rate of the scaffold, the molecular weight between crosslinks, Mc, can be altered. A

higher value of M_c results in faster degradation due to a lower crosslink density. The scaffold proposed likely will have an M_c of 5-15 kDa and should degrade in the body within about 40 days (25).

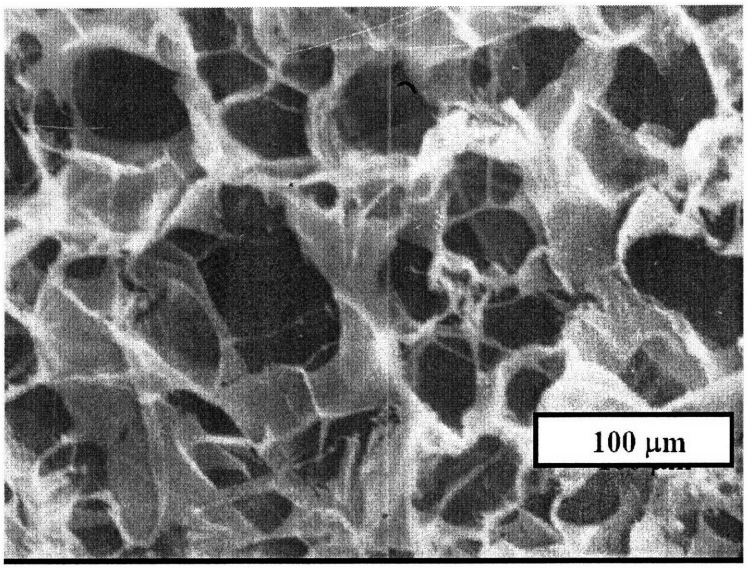

Figure 12. A SEM image of the collagen-GAG scaffold showing large, equiaxed pores (25).

The mechanical properties of the scaffold are also important. The Young's modulus of a scaffold has been shown to affect cell adhesion, growth, and differentiation (26). Cell migration speed is also influenced by substrate stiffness, showing a biphasic relationship with a maximum speed at intermediate stiffness. The elastic modulus of the collagen-GAG scaffolds is approximately 2,000 Pa while hydrated (26). The individual struts were shown to have a modulus of about 5.3 MPa while hydrated (26). The mechanical properties of the scaffolds as a whole do not vary with pore size, which is consistent with cellular solids theory that is used to model them (26). Increasing the crosslink density causes the strut modulus to increase. This can create large changes in the overall scaffold modulus, up to an order of magnitude (26).

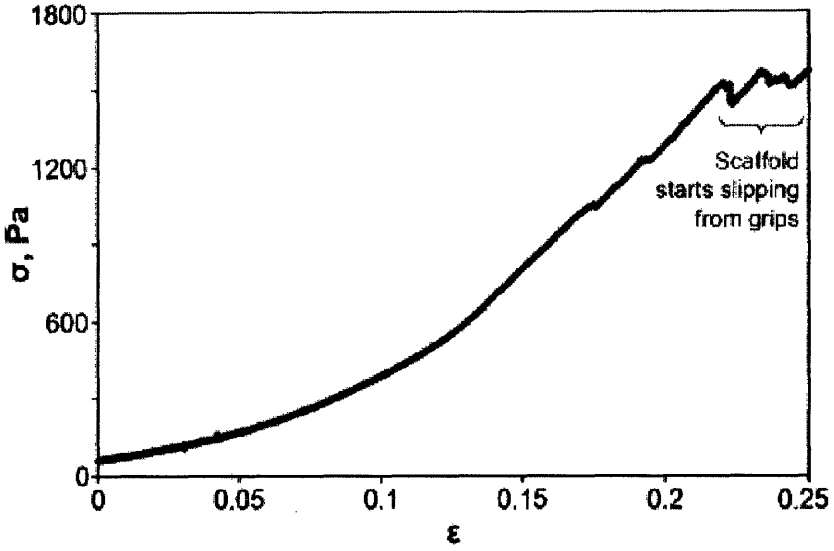

Figure 13. Stress-strain relationship of a hydrated collagen-GAG scaffold in uniaxial tension (26).

5.4 Scaffold Manufacturing

The collagen-GAG scaffolds are created using a lypholization technique, also called freeze-drying, where the collagen-GAG slurry is frozen and then put under vacuum to cause the ice to change to gas. When the ice crystals nucleate throughout the suspension they push the collagen and GAG aside, creating the 3D network of interconnected pores. When the ice undergoes sublimation, the water vapor can exit the scaffold without disrupting the newly formed pore structure.

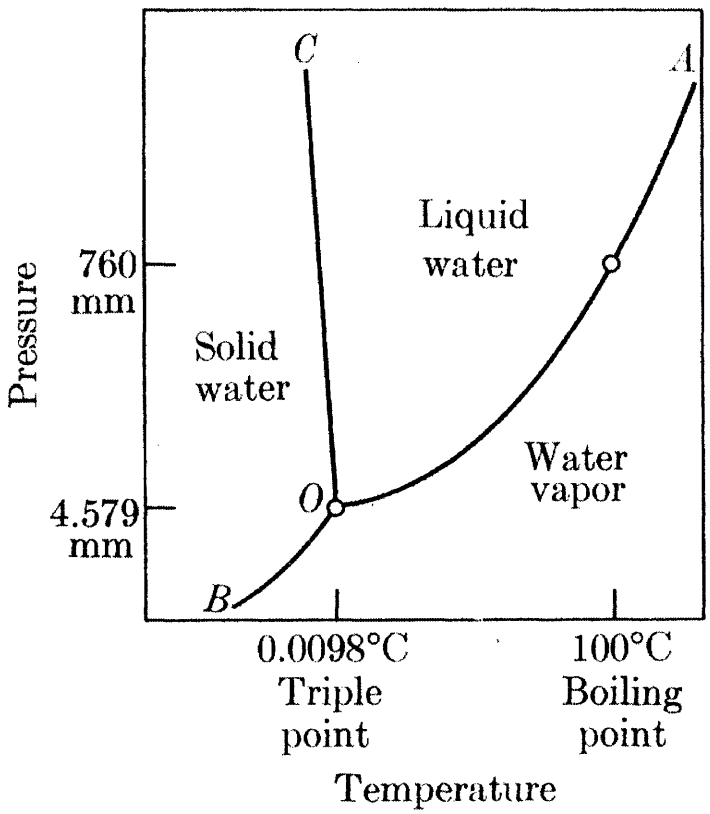

Figure 14. Phase diagram of water, showing solid to ice transition at low temperature and pressure (27).

First, microfibrillar type I collagen (typically from bovine tendon or rat tail) and chondroitin 6-sulfate (typically from shark cartilage) are blended in 0.05 M acetic acid of pH 3.2 at 15,000 rpm. The suspension is maintained at 4°C during mixing to prevent denaturation of the collagen. To achieve the afore mentioned weight percents of collagen and GAG, 3.6 grams of collagen and 0.36 grams of chondroitin 6-sulfate are used per 720 ml of acetic acid. To make one human-size scaffold, that equals 235 mg of collagen and 23.5 mg of GAG. After mixing the suspension, it is degassed under 50 mTorr vacuum for one hour to remove air bubbles (21). After degassing, the suspension is placed in stainless steel pans measuring 10.25 x 10.75 x 5.5 cm. Larger pans can be used, but it was found that smaller pans create a more uniform and equiaxed pore structure due to higher stiffness and better contact with the freeze-dryer shelf (21).

The pans are placed in a freeze dryer that begins at room temperature. The temperature of the shelves is then lowered at a constant cooling rate until the final temperature of -40°C is reached. The cooling rate can be adjusted to achieve a range of pore sizes. As the cooling rate increases, the pore size decreases due to increased nucleation and lowered growth of the ice crystals formed in the suspension (21). Smaller pore sizes have been shown to increase cell adhesion due to the increase in ligand density (28). Ligands are adhesion molecules that are recognized by cell membrane receptors (integrins) and allow cells to adhere and migrate across a surface. The pore size of the structure cannot be too small, however, because below a certain size cells and diffusing nutrients will not be able to easily fit through the openings in the scaffold (25). For this reason, an intermediate pore size, and therefore cooling rate, is needed. To achieve this, the cooling time should be between 60-90 minutes to reach -40°C. After the final temperature is reached, it is held for 60 additional minutes to allow for complete phase transition to occur. After cooling is completed, the suspension is sublimated under less than 100 mTorr for 17 hours at 0°C to remove the vapor.

Following the freeze-drying process, the scaffolds are dehydrothermally crosslinked and sterilized using a vacuum oven. The scaffolds are placed in the oven at a temperature of 105°C for 24 hours at a vacuum of 50 mTorr. The process introduces covalent crosslinks between the polypeptide chains of the collagen without denaturing it into gelatin (21).

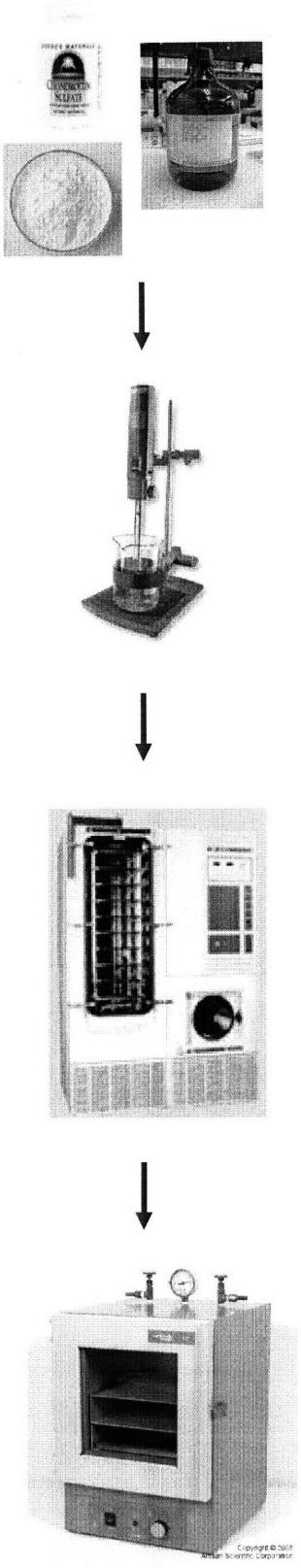

Figure 15a. Schematic of the manufacturing process with examples of the necessary equipment (29, 30, 31).

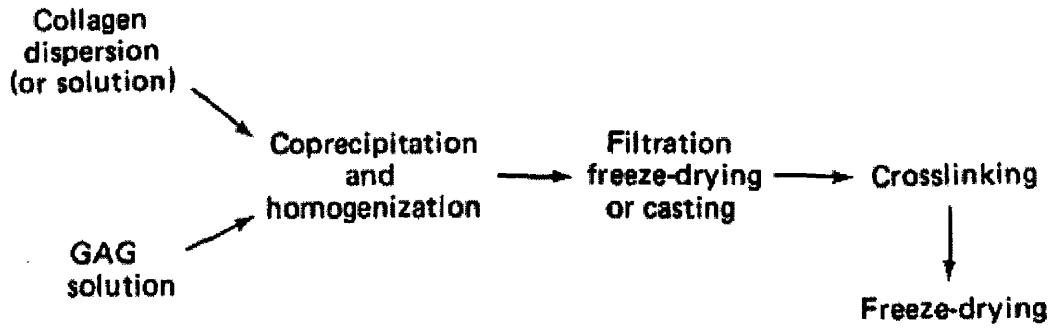

Figure 15b. Diagram of the scaffold manufacturing process (32).

Chapter 6. Organ Regeneration

Organ replacement has been addressed from multiple angles throughout medical history. The five approaches currently used or being researched are transplants, autografts, permanent implants, in vitro synthesis, and in vivo synthesis (25). As already mentioned, transplants are extremely expensive and there are not enough donors to satisfy the need. Autografts and permanent implants cannot be used for procedures as complicated as a liver replacement. That leaves in vitro and in vivo synthesis as the two possible approaches to replacing a failed liver. In vitro synthesis of a 3D organ is very difficult because it is not yet possible to create a tissue with a fully developed vasculature. Most in vitro tissues rely on diffusion of nutrients from culture medium and are therefore restricted to very thin cross sections for sustained life. In vitro synthesis is also difficult because there is not a way to truly recreate the body environment in the lab. It is possible to include some growth factors, control the pH level, and even simulate the extracellular matrix to a fairly high degree, but it is simply not possible to exactly copy the in vivo environment. This makes it extremely difficult to create an organ that will be physically and histologically identical to the natural tissue. Even if it were possible, there is a chance that by implanting it into the body, the change in environment would destroy it or alter its function.

It would seem, then, that in vivo synthesis is the best approach to replace a failed organ. The goal is to only implant what needs to be implanted and let the body supply the rest. It has been shown in the skin and conjunctiva that it is possible to achieve regeneration with only a scaffold similar to the one proposed here (25).

To be suitable as a regeneration template, a scaffold must meet four structural characteristics. First, it must have the appropriate chemical composition. This is necessary to ensure that the right ligands exist for the host cells to adhere to and migrate on the scaffold (25). Collagen and GAG are both naturally occurring in the body, and specifically the liver, and therefore the scaffold composition should be

acceptable. Second, the scaffold must contain the right pore structure and size. High pore volume fraction and smaller pore sizes both increase the ligand density in the scaffold because they both increase surface area (25). The collagen-GAG scaffold proposed has an extremely high pore volume fraction (>99%) and the ligand density for the pore sizes tested have shown to provide good cell adhesion (28).

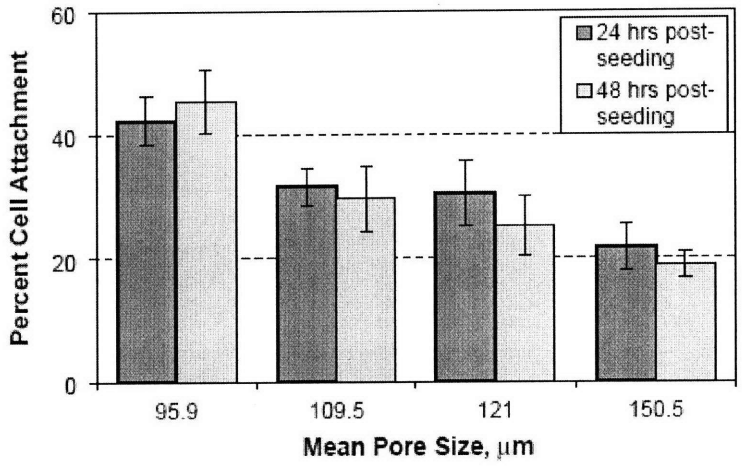

Figure 16. Effect of pore size on cell adhesion (28).

Third, the scaffold must have the correct pore orientation for the application. The orientation of the pores can dictate the cell migration and synthesis direction, therefore affecting the regeneration process (25). The liver is a bulk tissue with many small units called lobules that are roughly hexagonal in shape. An equiaxed pore structure should fit well with this morphology because there is no preferred axis of tissue growth. The shape of the pores does not precisely mirror the natural ECM, but enzymes will be able to remodel as necessary to attain the correct structure. Lastly, the macromolecular structure must be appropriate. This affects the degradation rate of the scaffold. It has been found that tissue regeneration occurs best when tissue growth occurs at the same rate as scaffold degradation (25). The current embodiment of the scaffold is meant to degrade in approximately 30 days. This is accomplished with a molecular weight between crosslinks, M_c, of 5-15 kDa. To increase the time of degradation, the M_c can be decreased to give a higher density of crosslinks and therefore a more resilient scaffold. As will be discussed later, the time to degradation

for this application likely will need to be on the order of 40 days, so an M_c of around 5 kDa may be the solution.

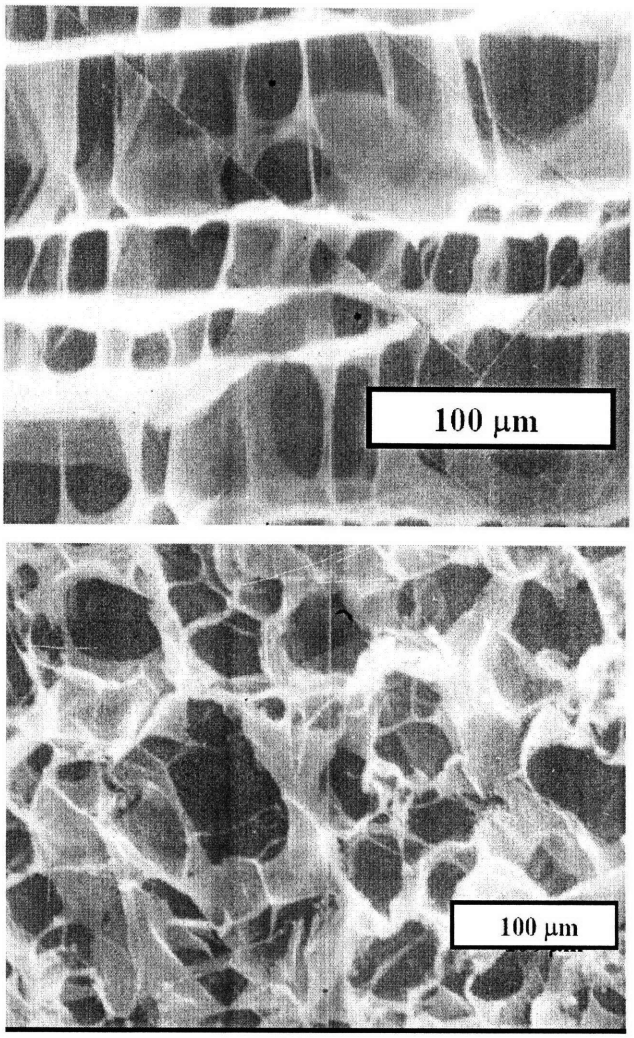

Figure 17. Axially aligned pore structure (top) versus equiaxed (bottom) (25).

In addition to having the above characteristics, the scaffold must be able to prevent the natural wound healing response from occurring. In humans, the ability to regenerate most tissues ends during the late stages of fetal development. Some tissues continue to regenerate naturally, such as bone and the epidermis of the skin, but others heal by contraction and scar formation. It is not completely understood why some tissues are able to regenerate and others aren't, but it is known that in order to induce regeneration, it is necessary to block the contraction that occurs in non-regenerating tissues (25). If contraction is blocked then scar tissue does not form, and

the wound is sometimes able to heal by regeneration. Blocking contraction is therefore necessary, but is not sufficient to achieve regeneration.

Contraction is blocked by reducing the number of contractile cells in the wound and by randomly orienting the contractile cells that remain. The first task is accomplished due to the unique characteristics of collagen precipitated using GAG from an acidic solution. When the collagen fibrils form under these conditions, they do not exhibit the characteristic "banding" structure nearly as much as seen in native collagen. The un-banded collagen retains its triple helical structure, however, so it is not turned to gelatin (25). The banding structure in collagen attracts platelet cells during blood clot formation following wound creation. With significantly less banding, there is severely reduced clotting and the cytokine TGB-β that is normally released during clotting is not as prevalent in the wound. TGB-β is known to encourage normal cells to turn into contractile cells, so with less of it in the wound there are less contractile cells as well (25).

The second task, randomly orienting the contractile cells that do arrive in the wound, is accomplished by the randomly oriented pore structure (Figure 10). When the contractile cells migrate into the scaffold they become oriented in the direction of the pore strut on which they are attached. When they contract as a whole, the effects are mostly cancelled due to the opposing alignments of the cells. Cells also synthesize matrix in the direction of their cell alignment. This means that instead of highly oriented scar tissue, as seen in most wounds, the synthesized ECM will be fairly isotropic as it was before the wound.

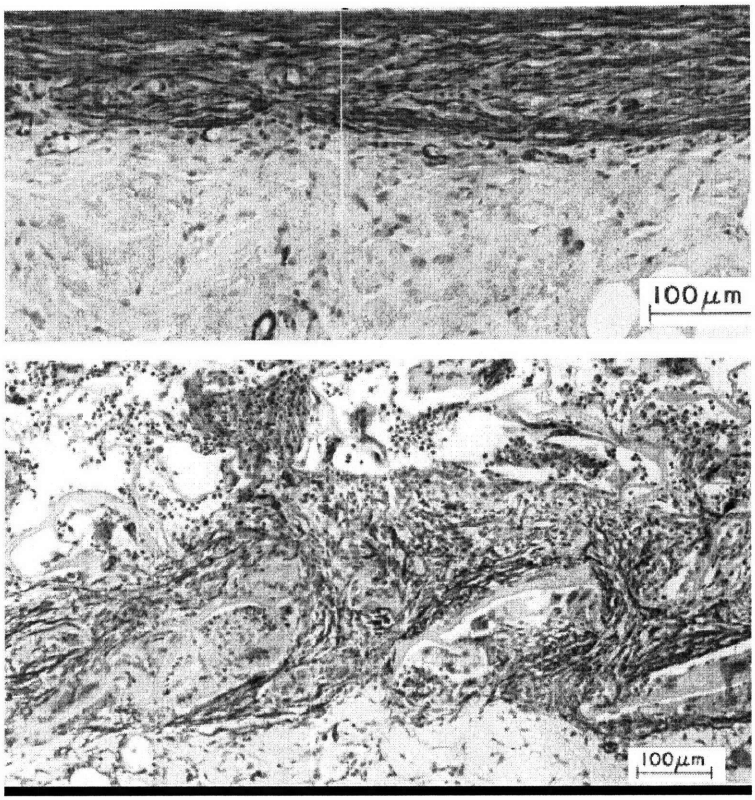

Figure 18. Scar formation in a preferred direction without a scaffold (top) and randomly oriented with a scaffold (bottom) (25).

Chapter 7. Technical Hurdles

At this point, the collagen-GAG scaffold has been shown to block contraction in a surgically created wound in a healthy mouse liver. The next step is to show that the same can be accomplished in a cirrhotic mouse liver, likely by giving the mice CCl_4. The scaffold has had success in the past with blocking contraction in scarred tissue, although not in the liver, so it is reasonable to expect that it will be able to do it on this small scale. The biggest obstacle, however, is the massive increase in size from a mouse to a human liver. As mentioned above, to replace 3.2% of the liver in a mouse requires a scaffold that has a volume of 0.0353 cm^3, but to replace the same relative amount of a human liver requires a volume of 47 cm^3. That is an increase of approximately 1330X.

The main problem with such a drastic increase in size is that oxygen and nutrient transport to the cells occurs by diffusion. The maximum thickness of a tissue is therefore only a few hundred μm to as low as 20-30 μm, depending on which study you look at (33, 34, 35). Even if cells do survive and proliferate into the scaffold, if the time for oxygen to reach them is too long then a hypoxic environment forms (36). In a hypoxic environment, cells convert glucose to lactic acid and necrosis starts to occur (36).

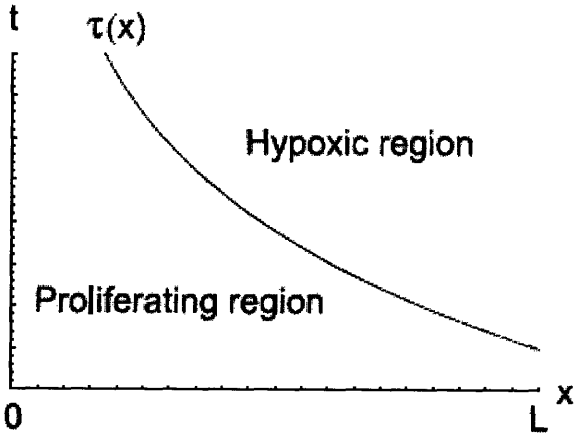

Figure 19. Schematic representation of the relationship between time and thickness of a scaffold in determining whether cells will proliferate or start to die (36).

In order to have tissue regeneration in a scaffold with a thickness of a few cm, extensive vasculature must be present within the scaffold before hepatocytes will be able to survive. It is therefore imperative that angiogenesis be as rapid and robust as possible. Endothelial cells migrate at about 0.4 mm/day (37). This means it will take approximately 40 days to reach the most central parts of the proposed scaffold, with a radius of 1.65 cm. This time frame may be too long and it may be necessary to develop ways to speed up the process.

One way to improve the blood vessel ingrowth may be to incorporate Vascular Endothelial Growth Factor (VEGF) into the scaffold using PLGA microspheres. In a study done by Kedem et al., a dramatic increase in density and size of capillaries was shown in a scaffold placed on the liver surface in rats by addition of VEGF microspheres (38). At the end of a two week period, the scaffold containing VEGF had an average capillary density of 220/mm^2 compared to 139/mm^2 for the control scaffold (38).

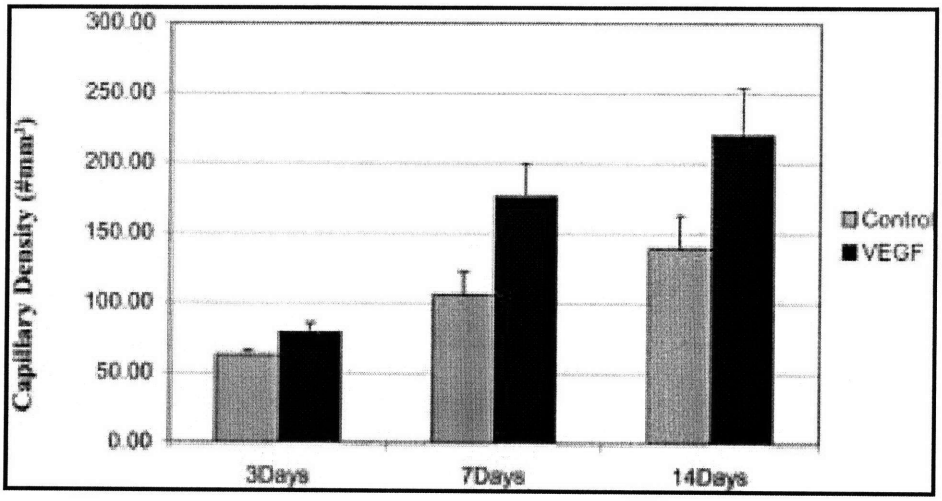

Figure 20. The difference in capillary density between scaffolds with and without VEGF (38).

Assuming a square array of capillaries, there would be a capillary every 67.4 μm for the scaffold with the VEGF and every 84.8 μm for the control. This translates to a maximum diffusion distance, for a cell located directly in the center of the square array, of 47.7 μm with VEGF and 60.0 μm without. The area of the scaffold

consumed by capillaries was not significantly different at 1, 7, and 14 days, so it does not appear that VEGF causes the capillaries to grow faster, just in greater numbers. The size distribution of the capillaries was significantly different, with the VEGF scaffolds having almost 40% of the capillaries with a diameter of over 16 μm as opposed to only 10% in the scaffolds without VEGF. The capillaries in the VEGF scaffold also stained positive for smooth muscle actin (SMA), which is an indicator that the vessels are mature and will not regress (38).

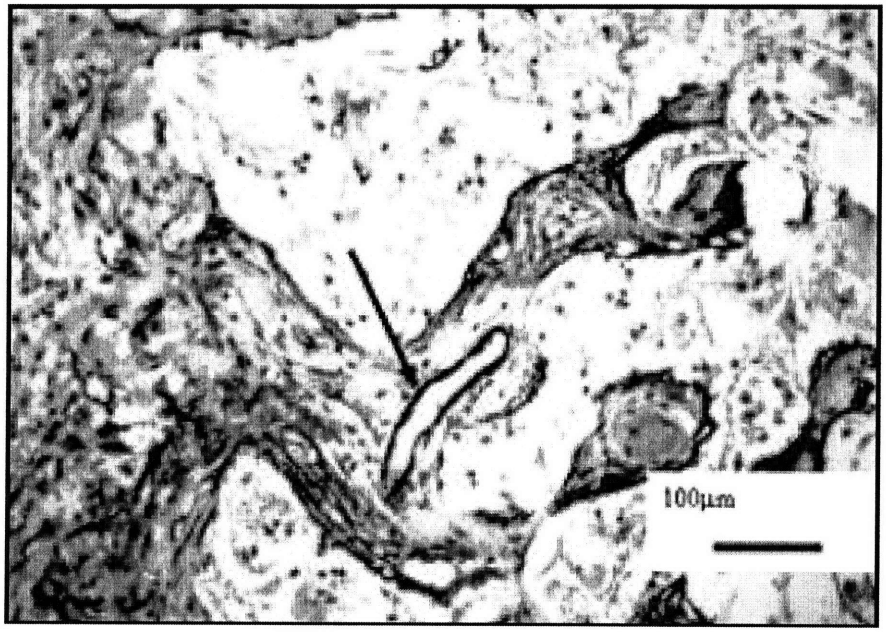

Figure 21. Histology of newly formed capillary in VEGF scaffold with arrow pointing to SMA around a large capillary (38).

The microspheres are created using a modified solvent evaporation method based on a double emulsion (39). First, 50 μl of aqueous solution containing 20 mg of BSA and 3.75 μg of human recombinant VEGF are mixed with 200 mg of PLGA dissolved in 0.5 ml of methylene chloride (38, 39). This mixture is then sonicated to form an initial emulsion. Then, 1 ml of 1% (w/v) aqueous PVA is added to further emulsify the mixture. The double emulsion is then poured into 50 ml of 0.1% (w/w) PVA solution and then stirred for 5 minutes before adding 50 ml of 0.1% (w/w) PVA containing 10% (v/v) 2-propanol (39). The solution is then stirred for 30 minutes and the microspheres are removed by centrifugation and then freeze dried. The

microspheres are typically between 65-70 μm in diameter. When used in a smaller scaffold by Kedem et al., the microspheres were incorporated at a ratio of 40 mg of microspheres per 0.16 cm^3 of scaffold (38, 39). The release rate was approximately 8-10 ng/day, which lasted for more than two weeks (38).

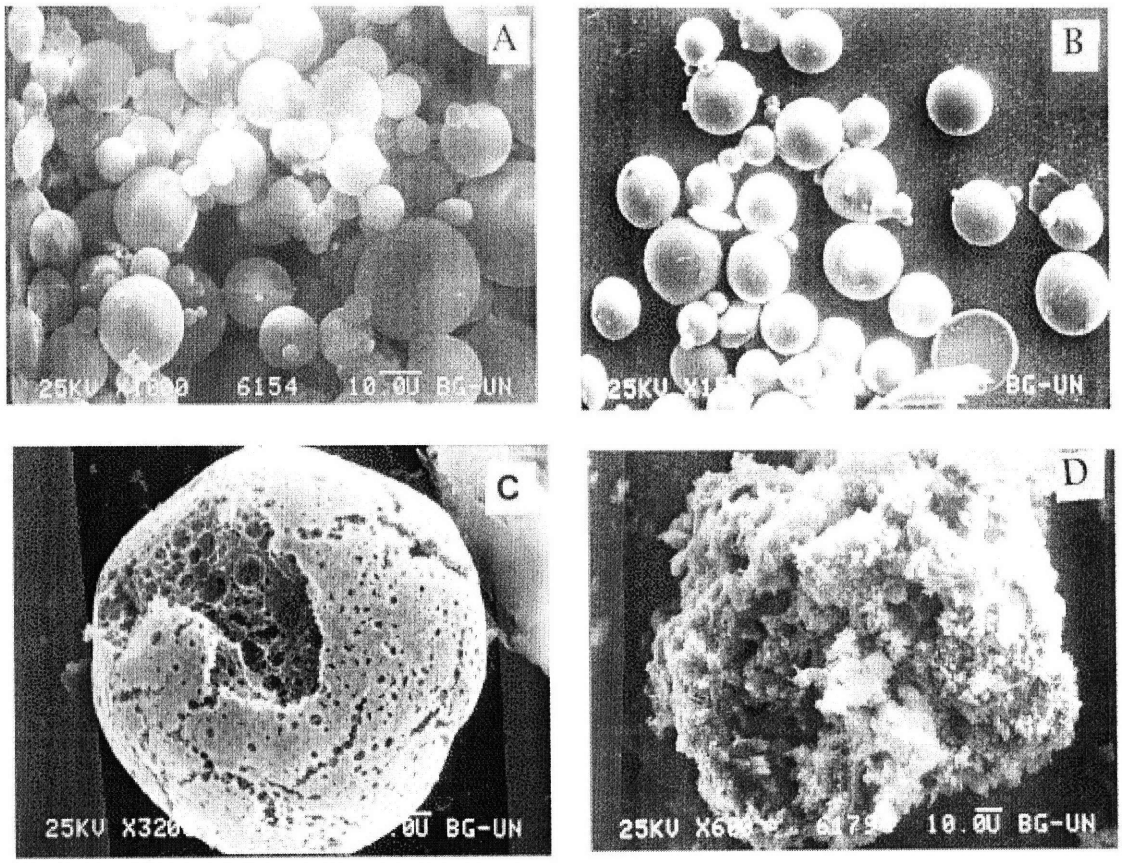

Figure 22. Microspheres as fabricated (A) and after 5 (B), 14 (C), and 30 (D) days in phosphate buffer at 37°C and pH 7.4 (39)

In addition to adding growth factors, such as VEGF, to increase vascularization, other approaches can be taken to improve nutrient diffusion and transport. Increasing the pore size has been shown to increase the permeability of tissue engineering scaffolds (40). Scaffold permeability is a function of porosity, pore size and distribution, pore interconnectivity, fenestration size and distribution, and pore orientation (40). It can be quantified using the fluid mobility, K, which is defined as the permeability divided by the viscosity of the fluid.

$$K = \frac{k}{\mu} \quad (2)$$

For the collagen-GAG scaffolds, the fluid mobility is on the order of 10^{-10} m^4/Ns, which compares favorably to some natural tissues, such as cartilage at 10^{-15} m^4/Ns, and is on par with some other synthetic scaffolds made of PLA and PGA. The fluid mobility can be almost doubled by increasing the pore size from 96 to 150 μm (40). This would likely increase the critical distance needed between capillaries and may allow for non-VEGF scaffolds to succeed.

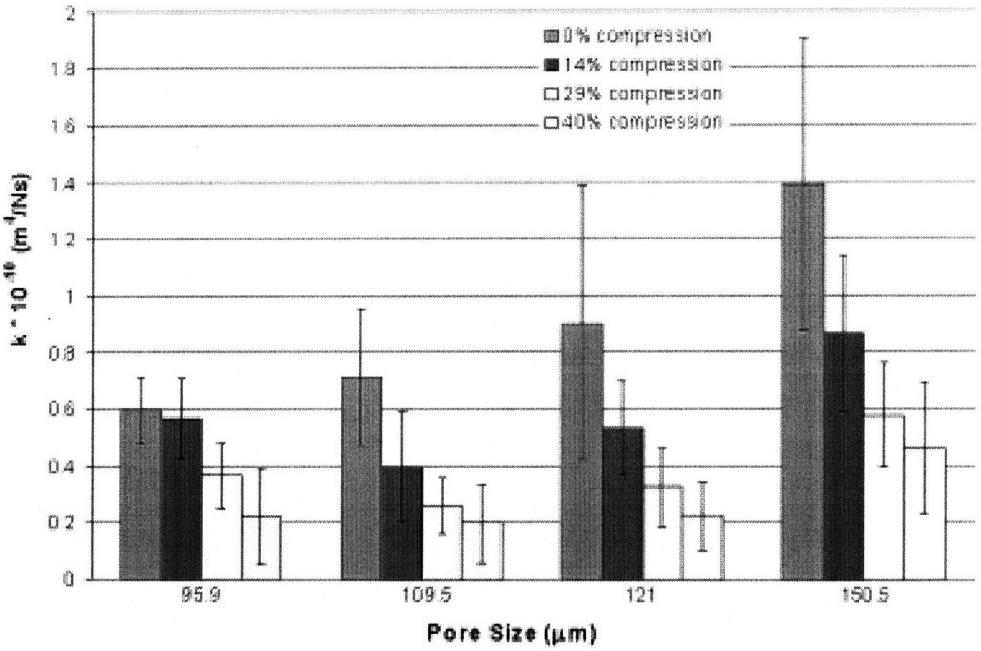

Figure 23. The effect of pore size on fluid mobility for collagen-GAG scaffolds (40).

TE Scaffolds	Author	Material	Porosity	Method	Permeability	Units
Ceramic and composites	Shibong et al. (2003)	Z-BCP; D-BCP; I-BCP	75; 74; 54	1	$0.01 - 0.35 \times 10^{-9}$	m^2
		Coral; HA-CAM; HA-50	–	1	$0.05 - 0.35 \times 10^{-9}$	m^2
	Haddock et al. (1999)	Coralline hydroxyapatite	–	1	$1.7 - 8.6 \times 10^{-10}$	m^2
Synthetic polymers	Beatty et al. (2002)	PGA-PLLA	77	2	$1.77 \pm 0.99 \times 10^{-15}$	m^4/Ns
	Spain et al. (1998)	PLA/PGA	61–71	1	$1.82 \pm 3.65 \times 10^{-9}$	m^4/Ns
	Agrawal et al. (2000)	PLA/PGA (salt:polymer) 3.5-14:1	80–92	1	$2 - 16.1 \times 10^{-9}$	m^4/Ns
Natural polymers	Ramamurjan et al. (2002)	Collagen gel	1–4.5% w/v	1	$1 \times 10^{-15} - 10^{-16}$	m^2
	Beatty et al. (2002)	SIS	–	2	$17.09 \pm 8.95 \times 10^{-15}$	m^4/Ns
	Grattoni et al. (2001)	Polyacrylamide gel	1.8–3% w/v	1	$0.004 - 0.013 \times 10^{-12}$	m^2

Table 2. Permeabilities and fluid mobilities for various synthetic and natural tissue engineering scaffolds (40).

As mentioned in Chapter 6, however, increasing the pore size can have an adverse affect on cell adhesion. The nutrient transport and vascularization problem is likely to be the most significant though, so sacrificing some cell adhesion may be necessary to achieve a viable scaffold.

If adding growth factors or altering pore size is not sufficient to get the necessary nutrient transport, a more extreme approach may be necessary. Recently, in an effort to solve the vascularization problem, studies have been done where arteriovenous (A-V) loops are inserted into a scaffold to encourage vessel growth (36). Briefly, vein grafts are used to re-route existing arteries into a chamber containing a scaffold (41). This allows vessel formation to occur from the inside, in addition to from the surface of the scaffold.

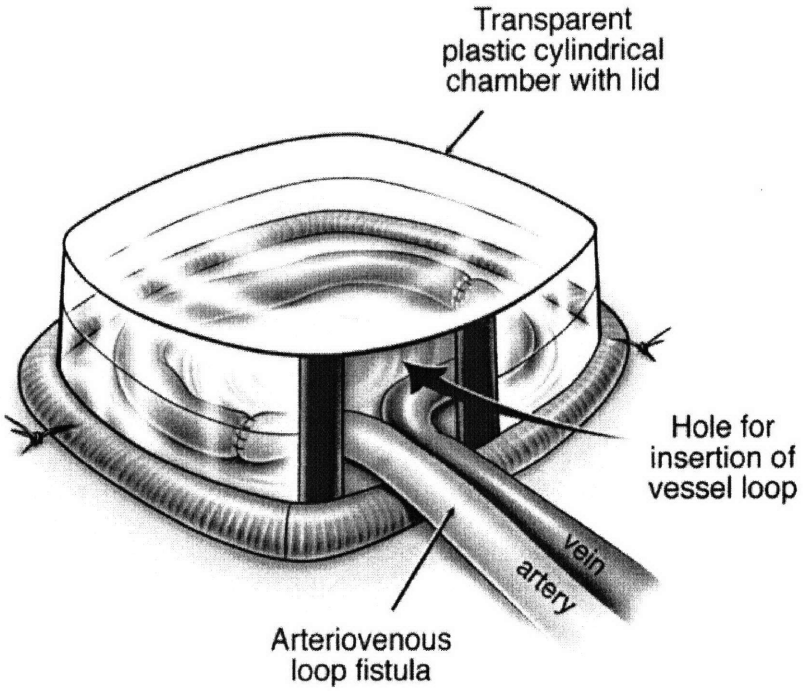

Figure 24. Schematic of an A-V loop used without a scaffold (41).

Incorporating the A-V loop strategy into the overall liver regeneration procedure would likely not affect the scaffold fabrication. Instead, it would probably be up to the surgeon performing the operation to cut the scaffolds in half, creating an upper and lower portion, and then re-route an artery or other existing blood vessel to form a loop that could be placed between the two halves. This may prove difficult, and could be unnecessary if the VEGF and pore size are effective. However, since portions of the liver are already being removed during surgery, there is likely a significant supply of grafting material that could be taken from the excised tissue. This would eliminate the need to find a donor site elsewhere in the body, reducing the risk of complications.

Chapter 8. FDA Considerations

All medical devices sold or used in the United States must be approved by the U.S. Food and Drug Administration (FDA). Devices are divided into three types: I, II, and III. Type I devices are the simplest and have the least possible health risk. Examples are bandages, surgical gloves, and simple hand tools. Type I devices must meet "General Controls," which include registering the device and its manufacturers, meeting Good Manufacturing Processes (GMP), labeling, and a premarket notification [510(k)] (42). The 510(k) is necessary to show that the product is substantially equivalent to a previously approved device in terms of the intended use, materials used, and safety and effectiveness.

Type II devices are subject to "General Controls" and "Special Controls," which include special labeling requirements, mandatory performance standards, and postmarket surveillance (42). They also require a 510(k). These devices are more complicated and pose a higher health risk. Examples include infusion pumps and tubes to reconnect damaged nerves. The highest risk, and therefore most regulated devices are Type III, and require a premarket approval (PMA). A PMA is necessary to prove or demonstrate safety and effectiveness of the new device, which is done through preclinical and clinical studies (42). The preclinical trials, using *in vitro* tests and animal studies, must show that the materials, chemical composition, and manufacturing process are biocompatible and safe. Biocompatibility tests include toxicity, irritation, implantation, and genotoxicity. Clinical trials in human patients must also be done for Type III devices to show that the device is not only safe, but effective. These trials can range from tens to hundreds of patients and generally include at least one control group for comparison.

Because there are no liver regeneration scaffolds on the market, or any similar products with the same function or intended use, the collagen-GAG scaffold would almost definitely receive Type III classification. From a device company's standpoint, Type III is the worst possible type due to the expense and time of

conducting the extensive tests and trials. The materials and chemical composition analysis have either been done already or could be completed fairly easily in the MIT labs, but the biocompatibility tests and clinical trials would be difficult or nearly impossible to conduct due to the extra equipment, labor, and time restrictions required. There are contract research organizations (CRO) that will conduct or organize these studies in their own labs. For a small startup company with very few employees and limited lab space/equipment, having a CRO do the preclinical and clinical trials is likely the easiest and most sensible route. The costs of these tests will be discussed in the cost model section.

It is important to note that the above information is for medical devices, which are defined as:

"an instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article, including a component part, or accessory which is:
– recognized in the official National Formulary, or the United States Pharmacopoeia, or any supplement to them,
– intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals, or
– intended to affect the structure or any function of the body of man or other animals, and **which does not achieve any of it's primary intended purposes through chemical action within or on the body of man or other animals and which is not dependent upon being metabolized** for the achievement of any of its primary intended purposes (42)."

The critical part of the definition, which is in bold above, is that devices do not rely on chemical action or being metabolized by the body to perform their intended purpose. This is an accurate description of the collagen-GAG scaffold in its current form. However, if VEGF microspheres are added to the scaffold then the classification could become fuzzy. The inclusion of VEGF could cause the device to be classified as a drug or biologic, which can have different (and typically more extensive) testing procedures and timelines. It is possible that the collagen-GAG scaffold would still be classified as a device, evidenced by the approval of the InFUSE Bone Graft/LT-CAGE Lumbar Tapered Fusion Device by Medtronic (43).

Chapter 9. Intellectual Property

At a minimum, any new device must not infringe on any existing patents without some sort of licensing agreement. Preferably, a new product would be patentable, therefore allowing the inventor/company to exclude others from producing it or selling the idea. A background patent search was done to assess the relevant IP in the liver regeneration scaffold field. There are a myriad of patents for scaffolds involving bone and cartilage repair, some of which include lengthy background sections that "teach" the use of collagen and polysaccharides (GAG) in scaffolds to induce regeneration. The extent to which these patents truly teach the collagen-GAG scaffold discussed here is very debatable. The body and the claims of the patents focus almost entirely on bone or soft tissue (cartilage, ligament, etc.) regeneration and make only fleeting references to visceral organs in the form of tables of possible materials and treatment sites. A list of the relevant patents, starting with the most recent, and a summary of the IP outlook is provided below.

1. US 7,241,316 – Devices and Methods for Treating Defects in the Tissue of a Living Being (bone oriented)
2. US 7,105,580 – Porous Structures Useful for Growing Living Tissue, and Methods of Manufacture (manufacturing methods)
3. US 6,969,523 Collagen/Glycosaminoglycan Matrix Stable to Sterilizing by E-Beam Radiation (manufacturing methods)
4. US 6,962,716 – Compositions and Methods for Biodegradable Microspheres as Carriers of Bioactive Substances (new microsphere method)
5. US 5,330,768 – Controlled Drug Delivery Using Polymer/Pluronic Blends (original microsphere patent)
6. US 4,947,840 – Biodegradable Templates for the Regeneration of Tissues (original Yannas patent)

The first patent, US 7,241,316, has an extensive background section that reviews the recent prior art for all types of tissue engineering scaffolds. The summary of the

invention describes a synthetic tissue substitute material that can be composed of polymer, ceramic, or metals. It will partially or fully resorb in the body. It may contain a depot of material to assist in the in-growth of cells, possibly cytokines or drugs. All of these are fairly generic to the tissue engineering scaffold field, and do not teach the specifics of the proposed scaffold. While specifying potential materials to be used, collagen, polysaccharides, PLGA, and VEGF are all included in very long tables as examples. Lypholization of collagen implants is also mentioned in the body, but this is a very old and common manufacturing method that is not patentable anyway. The use of microspheres is described in the body, but the specific use of the double emulsion process as well as the VEGF is not included. The liver is listed as a potential target tissue, however it is only present in a long list and there is no detailed mention of it in the body. Pore size and volume fraction are described as 25-1000 μm and over 50%. The values of the proposed liver scaffold are within these ranges, but the pore size range is extremely large and the proposed porosity is much more specific than what is cited. The claims of the patent do not pose any difficulties for the liver regeneration scaffold because they specifically mention the regeneration or repair of bone tissue.

The second patent, US 7,105,580, describes a scaffold that is fairly similar to the collagen-GAG scaffold proposed, and also its manufacture. The main difference, however, is that the patented scaffold includes amino acids in the lypholization mixture. The claims also do not mention the liver or overall porosity. There are claims that describe the pore size as 1-100 or 1-300 μm, which do overlap with the proposed scaffold. The patent also does not specify a method by which it can induce regeneration. Overall, the patent does not pose a significant challenge to the production of the collagen-GAG scaffold. It may, in fact, give hope that the manufacturing method of the liver scaffold could be patentable if small twists are added to distinguish it from existing lypholization techniques.

The third patent, US 6,969,523, is assigned to Integra LifeSciences, who produces a scaffold very similar to the proposed scaffold for skin defects. The product is based

off of Prof. Yannas' research on wound healing and treatment. The difference, however, is in the application and the scaffold geometry. Also, the Integra patent includes an extra component, a layer of silicone that covers one surface to prevent loss of moisture. The claims do not specify a tissue type, but the silicone layer makes the scaffold only applicable to surface wounds. The patent claims include the lypholization technique used for the proposed liver scaffold, but specify a different crosslinking and sterilization method. Overall, this patent should not preclude the liver scaffold from being manufactured, since there are clear differences in the composition and size/shape, but may cause some problems for attaining patents on the new scaffold. This is due to the potential for the USPTO to say that the use of the very similar scaffold in a new application and with a different size/geometry is "obvious" and therefore not patentable.

The fourth patent, US 6,962,716, details the production of microspheres that can release bioactive substances. The use of VEGF is specifically mentioned and described in some detail, although not explicitly for the liver. The claims of the patent, however, only specify a single emulsion process. The rest of the processes and components are very similar to the double emulsion process described in chapter 7. The claims include being biodegradable, including VEGF, emulsifying with PVA, and retrieving the microspheres with centrifugation. This patent only poses a difficulty if 1) VEGF microspheres are used in the scaffold and 2) if it is necessary to work around the fifth patent, which will be discussed next.

The fifth patent, US 5,330,768, is the patent for the exact double emulsion microsphere synthesis process that is described in chapter 7. The good news, however, is that it was filed in 1991, meaning it only has three years left of exclusivity. As the proposed timeline shows in the business model chapter, there will likely not yet be a commercial product in three years. This means that if VEGF microspheres are necessary there are a few options. The first is to work around the patented double emulsion process in a way that still yields effective microspheres. This is where the fourth patent, above, comes into play. The likelihood of finding a

way other than the single emulsion process described in that patent is probably fairly slim and the pursuit of finding it would sidetrack the entire focus of the liver scaffold. The second option is to license the technology for the next three years while waiting for the protection to expire. Since it is so close to expiring, the inventors may be willing to license the patent for fairly cheap since they won't be able to profit from it at all for much longer. Also, the inventors are from MIT, who is the assignee, so working out a deal would likely be quite straightforward. The last option is to just wait it out and let the protection expire in 2011. The reality is that lab tests with the scaffold alone will probably still be ongoing in 2-3 years, so waiting may be the most practical approach.

The sixth patent, US 4,947,840, is Prof. Yannas' original patent on tissue regeneration templates. It was filed in 1987, but received an extension on the patent term for 923 days past the original expiration date. This means that it still has approximately a year and a half left of protection. The patent outlines the procedures for making a scaffold for skin tissue regeneration that is very similar to the currently proposed scaffold for the liver. The body also contains information on the composition and purpose of the scaffold that are very close to the liver scaffold. Since Prof. Yannas is an inventor on the patent, it is unlikely that the patent would pose difficulties in producing the new liver scaffolds, but the potential for it to raise "obviousness" concerns by the patent office is quite high if the patent application was written as broadly as normal.

In summary, it should be possible to attain a patent on the collagen-GAG scaffold by making certain specifications in the claims that differentiate it from the currently patented scaffolds. Points of emphasis would likely include the large size of the scaffold, the targeted organ (liver), and the method by which it induces regeneration (blocking contraction). The high pore volume fraction and un-banded collagen fibrils are also important aspects of the scaffold that might strengthen the patent application.

As mentioned above, the manufacturing process of the scaffold, lypholization, is a very old and known technique and therefore not patentable. Specific alterations to the process have been patented, but they include steps or materials that are not used in the production of the collagen-GAG scaffolds for the liver. If VEGF was to be incorporated into the scaffolds, then the relevant IP would be significantly expanded. The VEGF itself is sold as a commodity by R&D Systems in Minneapolis, MN. The PLGA is also sold by various companies, including Sigma Aldrich (St. Louis, MO), which also supplies the collagen and GAG for the scaffolds. A table depicting the patentability outlook of the manufacturing methods and device itself for the different scaffold scenarios is shown below.

	Manufacturing	**Device**
Current Form	-	+
VEGF Microspheres	-	-
Scaffold w/ VEGF Microspheres		++

Table 3. Summary of IP outlook with negatives as (-), positives as (+) and unsure areas in gray.

In its current form, collagen-GAG only, the manufacturing process for the scaffold is unlikely to be patentable since lypholization has been around for many years. If along the way there were novel alterations to the process then the scenario would certainly change, but as of now it is not a new process. The device itself is likely to be patentable in the current form, but it will probably have to be written fairly narrow due to existing prior art containing collagen-GAG scaffolds. The VEGF microspheres themselves are currently already patented, so there is obviously no way of getting a new patent on that. It is marked as gray because of the possibility of creating a new way to synthesize the microspheres, which could potentially be patentable. Manufacturing the scaffold with VEGF microspheres is a gray area, the

first patent does teach incorporation of microspheres into a scaffold, but not with much specificity. It is also likely that the patent office may consider combining a scaffold with existing microspheres an obvious combination of manufacturing methods. A scaffold with VEGF microspheres, however, is probably quite patentable since most existing patents concerning collagen-GAG scaffolds do not include microspheres, particularly not with VEGF and intended for the liver. This would add novelty to the invention and make it easier to patent.

Chapter 10. Cost Model

To build an appropriate cost model, it first was necessary to determine the market size and estimated production numbers. As mentioned in the introduction, there are multiple causes for liver cirrhosis. Alcoholism is responsible for about 11,000 (40%) of the more than 27,000 deaths each year. Alcohol induced cirrhosis is likely the most feasible type to be treated with the collagen-GAG scaffold since its progression is typically halted once alcohol consumption stops. This means that if the scaffold is successful in regenerating healthy liver tissue, the treatment will be a success. Treating patients with hepatitis induced cirrhosis with the liver scaffold may be successful in the short term, but without treating the underlying disease the regenerated tissue may just become cirrhotic once again. For these reasons, the target market has been chosen as roughly double the number of alcohol related cirrhosis deaths per year, or 20,000 procedures.

It is difficult to attain incidence data (number of diagnoses per year) for alcoholic cirrhosis since it takes many years to develop and there is no clear-cut line when fibrosis turns to cirrhosis. However, with the estimated 2 million people that will develop cirrhosis due to drinking over the next 10-15 years, it would be reasonable to estimate that an additional 9,000 people will be in the near death stages of cirrhosis in addition to the 11,000 a year that do die from it. It has been shown that a healthy liver can regrow from an 85% resection, meaning that 15% is the critical limit for sustained life (7). Since each scaffold is 3.2% of the total liver volume, five scaffolds would be required per procedure to reach this critical size. It may be possible that more or less would be used per procedure based on the determined need, but an average of five scaffolds per procedure will be used in the cost model. At 20,000 procedures a year and five scaffolds per procedure, 100,000 scaffolds would be needed per year. For a slightly longer-term outlook, a five year (post FDA approval) model will be constructed, requiring 500,000 scaffolds. The assumed number of workdays in a year is 240, allowing for weekends, holidays, and two weeks of vacation.

The associated costs can be broken down into two overall categories, fixed and variable. Fixed costs are those that do not change based on the number of scaffolds produced, for example, lab equipment. Variable costs are those that scale with the amount of scaffolds produced, and consist predominantly of raw material costs. The fixed costs can be further broken down into general lab equipment, specific scaffold production equipment, labor, and FDA trials. The breakdown of the general lab equipment, scaffold equipment, and labor costs are listed below.

Lab Space	**Square Feet**	**Price Per sq ft**	**Subtotal**
	2,000	$30	$60,000
		5 Year Total	**$300000**
General Lab Equipment and Supplies	**Quantity**	**Price per Unit**	**Subtotal**
Refridgerators/Freezers	6	$7,000	$42,000
Centrifuge	3	$7,000	$21,000
Analytical Balances	2	$9,500	$19,000
General Lab Equipment	4	$2,500	$10,000
Autoclave	2	$7,000	$14,000
Deionized Water System	1	$22,000	$22,000
Chemical Fume Hoods	4	$6,000	$24,000
Chemical Storage	4	$2,000	$8,000
		Total	**$160,000**

Table 4. Cost breakdown for general lab equipment and supplies.

Scaffold Fabrication Equipment	Quantity	Price per Unit	Subtotal
Collagen Suspension Blending Equip.	3	$8,000	$24,000
Freeze Dryer	1	$120,000	$120,000
Vacuum Oven	7	$9,300	$65,100
Desiccators	14	$350	$4,900
		total	$214,000
	Total Lab Equipment Cost		**$374,000**

Table 5. Cost breakdown for scaffold production equipment.

Labor	Quantity	Cost per Year	Years	Subtotal
Technician	3	$50,000	5	$750,000
			Total	**$750,000**

Table 6. Cost of labor for 5 years.

The general lab equipment and scaffold fabrication equipment costs were calculated on the basis of 100,000 scaffolds being produced a year (240 work days). The bottleneck of the production is the freeze-drying process, since it is both time and capital intensive. As detailed in the manufacturing section, the entire freeze-drying process takes approximately one day. After freeze-drying, the scaffolds are crosslinked for a full 24 hours as well. This means that it takes two full days of preparation to produce each batch of scaffolds. In order to meet the goal of 100,000 scaffolds per year, the freeze-dryer capacity must be able to accommodate about 850 scaffolds per batch. There also must be enough vacuum ovens to handle that many scaffolds after they come out of the freeze-dryer.

The presence of scrap materials must also be accounted for in these calculations. Because the scaffolds are created in sheets with a thickness equal to the scaffold thickness, only two dimensional scrap exists. If the scaffolds are circles stamped out of a rectangular sheet, then they can be modeled as a simple cubic arrangement. This

is not the most efficient way, but is likely a good approximation for the actual efficiency that could be expected. Using this model, each sheet would have a yield of 78.5%, meaning that 21.5% of the area is wasted. The freeze-dryer that met this requirement is the VirTis Ultra Freeze Dryer, with 20.5 x 10.75 inch shelves that can be adjusted to fit different height clearances.

In order to transfer an entire batch of scaffolds from the freeze-dryer, seven vacuum ovens are necessary due to their substantially smaller interior volume. The vacuum oven model chosen was the Fisher Isotemp #282A. It is estimated that three skilled lab technicians would be necessary to prepare the scaffold suspension as well as troubleshoot any problems that may occur. Since each batch takes a day in the freeze-dryer, there would be considerable time during the day for the techs to mix the suspension for the next day. However, due to the small volumes and specificity of the ingredients necessary, it is likely that small amounts of suspension will be created at a time to maintain quality. This means that although the volume might indicate only one tech would be necessary, the exactness of the process necessitates more.

Preclinical and clinical FDA trials are difficult to build ground-up or top-down cost models for due to the large variability in the necessary tests for each device, number of patients needed to prove efficacy, the cost of doing trials in different locations, and the cost of doing surgical procedures and hospital costs for human patients to name a few reasons. Preclinical tests are a bit more standardized and a quote from Pacific BioLabs, a CRO, was received that outlined the costs per test, totaling about $165,000. The breakdown is given in the table below.

NO. OF TESTS	PBL TEST CODE	TEST: REQUIREMENT	TEST NAME	SAMPLE AMOUNT	TURNAROUND (IN WEEKS)	GLP UNIT PRICE
1	6521	Cytotoxicity	ISO MEM Elution	60 cm^2 ① x 2	3	$415.00
1	7503	Sensitization	ISO Maximization Test (Saline & Vegetable Oil)	60 cm^2 ① x 6	8	$8,245.00
1	7512	Sensitization	Murine Local Lymph Node Assay (Saline & Acetone/Olive Oil)	60 cm^2 ① x 4	5 – 6	$7,280.00
1	7925	Irritation	ISO Intracutaneous (Saline & Vegetable Oil)	60 cm^2 ① x 2	4 – 5	$780.00
1	7231	Systemic Toxicity	ISO Acute Systemic Toxicity (Saline & Vegetable Oil)	60 cm^2 ① x 2	4 – 5	$775.00
1	7200	Systemic Toxicity	Pyrogenicity ② (material mediated)	540 cm^2	3 – 4	$910.00
1	7259	Subchronic Toxicity	14 Day Subchronic Toxicity Study Mouse IV (ISO) (see page 2 for study design)	60 cm^2 x 28 pieces ①	12 – 14	$88,081.00
1	7934	Implantation (Subchronic)	ISO Implantation (90 Days) w/Histopathology	12 strips 1 x 1 x 10 mm	18 – 20	$5,000.00
1	7935	Implantation (Subchronic)	ISO Implantation (180 Days) w/Histopathology	12 strips 1 x 1 x 10 mm	8 months	$7,100.00
1	7931	Implantation (Subacute)	ISO Implantation (14 Days) w/Histopathology	15 strips 1 x 1 x 10 mm	9 – 11	$2,900.00
1	7936	Implantation (chronic)	ISO Implantation (365 Days) w/Histopathology	15 strips 1 x 1 x 10 mm	15 months	$13,970.00
1	Subcon-tracted	Genotoxicity	Ames Test (Saline & DMSO)	60 cm^2 ① x 2	8 – 9	$3,040.00
1	Subcon-tracted	Genotoxicity	Mouse Lymphoma Forward Mutation Assay (Saline & DMSO)	60 cm^2 ① x 2	15 – 17	$10,835.00
1	Subcon-tracted	Genotoxicity	Chromosomal Aberration (2 extracts)	60 cm^2 ① x 2	12 – 14	$15,180.00

Table 7. Costs of biocompatibility tests done by Pacific BioLabs (44).

The cost of clinical trials is much more variable, because the number of patients necessary to prove efficacy changes based on the expected effectiveness of the treatment. Some studies have only a dozen or two patients, others can have hundreds. If the scaffold does well in animal models before human testing, the expected difference between a scaffold-treated patient and a negative control should be quite high. Therefore, and abundance of patients should not be required to see a statistically significant improvement. Average costs of clinical trials in the literature range from $1.5 million to more than $5million in the literature (45, 46, 47). A rough quote, based on the limited knowledge of the trial size and design, was given by Regulatory & Clinical Research Institute, another CRO, of $2.5 million. This did not include some physician and hospital fees. Based on the estimated quote and the average costs

of trials in the literature, a cost of $3 million was assumed for the human clinical trials. A summary of the preclinical and clinical trial costs are listed below.

FDA Trials		
Pre-Clinical		$165,000
Clinical		$3,000,000
	Total	**$3,165,000**

Table 8. Estimated costs of preclinical and clinical trials for FDA approval (44, 48).

The variable costs, or the raw materials costs, make up the vast majority of the overall cost per scaffold. Collagen from rat tails costs $4.13 per mg and there are about 300 mg of collagen used per scaffold (including scrap). The GAG and acetic acid are extremely cheap relative to the collagen and add a negligible amount to the cost. If VEGF microspheres were added to the scaffolds then the cost would rise dramatically. VEGF purchased from R&D Systems costs $18,100 per mg and 0.225 mg are used per scaffold. The PLGA used to make the microspheres is considerably cheaper than the VEGF, at $0.034 per mg, but 12 grams are used per scaffold. The full cost breakdown of the raw materials per scaffold is given below.

Raw Materials	Amount per Scaffold (mg or ml)	Price per mg or ml	Cost per Scaffold
Collagen	$301.80	$4.1	$1,237.38
GAG	$30.20	$0.023	$0.69
0.05M Acetic Acid	$60.40	$0.00145	$0.09
PLGA	$12,000	$0.0341	$409.20
VEGF	$0.225	$18100	$4,072.50

Table 9. Cost per scaffold for each of the raw materials (49, 50).

With or without VEGF, the raw material costs dominate the cost per scaffold due to the fairly low equipment costs and high production volumes. The breakdown of the total cost to produce one scaffold is given below.

Cost Type	Total	Per Scaffold	% w/out VEGF	% w/ VEGF
Fixed - Lab Costs	$674,000	$1.35	0.11	0.02
Fixed - Other	$3,915,000	$7.83	0.63	0.14
Variable - Raw Materials w/out VEGF	$619,080,939	$1,238.16	99.26	
Variable - Raw Materials w/ VEGF	$2,859,930,939	$5,719.86		99.84
Total w/out VEGF	**$623,669,939**	**$1247.34**		
Total w/ VEGF	**$2,864,519,939**	**$5729.04**		

Table 10. Breakdown of where the costs per scaffold come from.

Chapter 11. Business Model and Supply Chain

The start-up company built around the collagen-GAG scaffold technology will be a product/manufacturing company. Good quality raw materials (collagen, GAG, etc.) are readily available in mass quantities so it would not be necessary or cost effective to produce them within the company. The materials can be purchased and the described manufacturing steps will be performed to create a finished product. The preclinical and clinical trials are not really part of the supply chain once they are completed, but during the early timeline they can be considered a horizontal part of the chain alongside the manufacturing of the scaffolds. After the scaffolds are created, they will be sold to hospitals on a semi-need basis. Hospitals prefer not to keep extra devices in their inventory due to lack of space and adequate storage facilities. Most storage of completed devices will therefore take place at the company's facility within desiccators that have a controlled atmosphere to prevent oxidation and other contamination. When the hospitals schedule a scaffold procedure, they will order the appropriate number of scaffolds. The patient and/or the patient's insurance will pay for the hospital fees and the scaffolds price for the implantation procedure. The supply chain therefore looks like this:

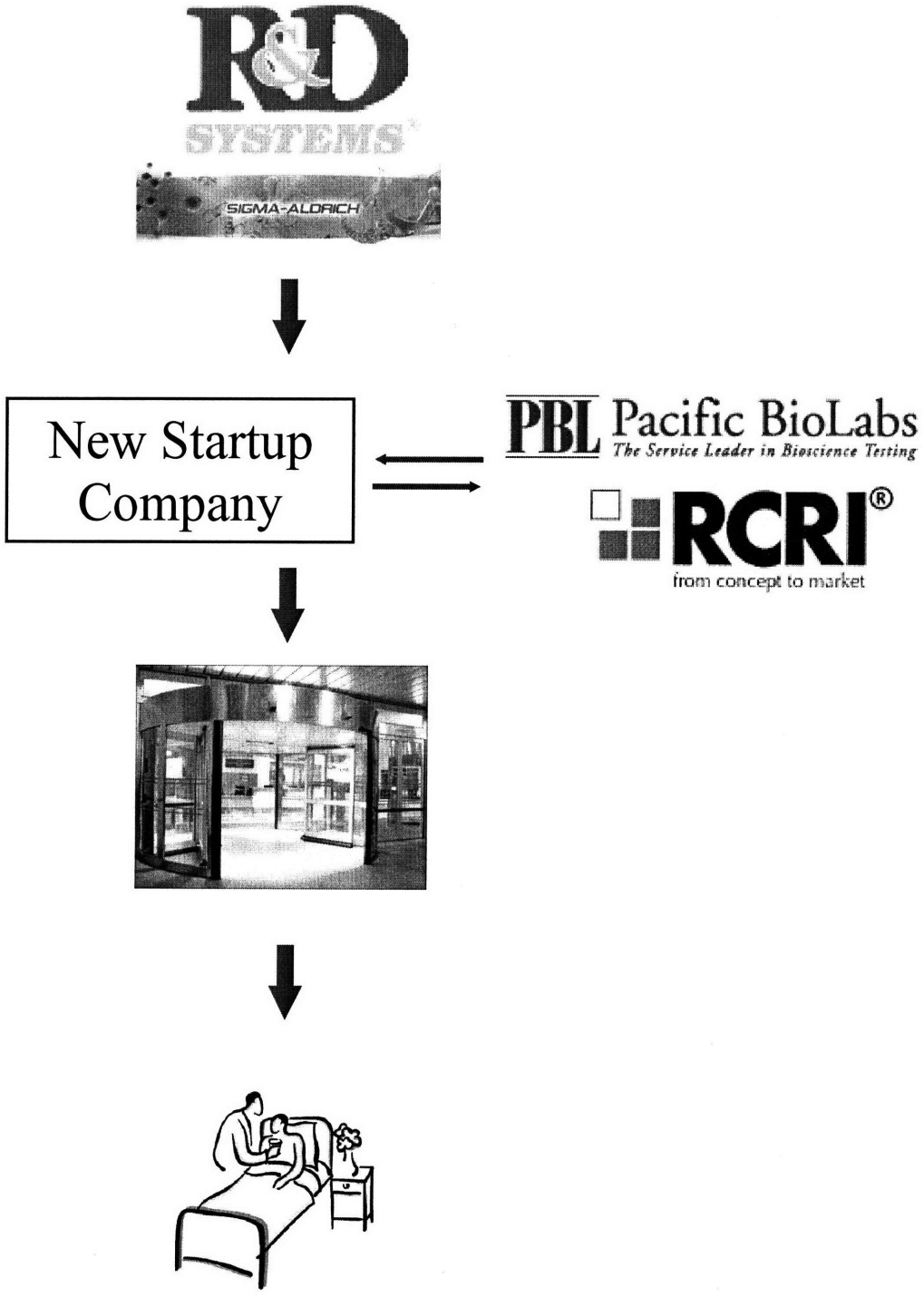

Figure 25. Supply chain for the commercialization of collagen-GAG scaffolds (44, 48, 49, 50, 51, 52).

For a new product to come in and successfully disrupt the current supply chain, in this case an organ transplant, it must be able to extract value for itself but not alienate the other members of the chain on which it depends. In this case, the procedural costs far outweigh the costs of the scaffold and are normally paid by the patient's insurance company. Insurance companies already do battle with patients on a day-to-day basis about how much they are willing to pay for many procedures. It is therefore unlikely that insurance companies will be willing to pay a total amount for the new procedures that is more than the total amount they are paying now for organ transplants, at least in the short term before the procedure becomes mainstream and costs are reduced.

To determine the price that can be charged per scaffold it is necessary to find the break-even point between the total cost of 6500 transplants per year for five years and 20,000 scaffold procedures a year for five years. As previously shown, liver transplants are extremely expensive. About half of the total cost comes from transplant-specific procedures or drugs and therefore would be eliminated with the scaffold procedure. In addition to these reductions, the cost of the hospital stay and testing (the largest single cost of the transplant) would be drastically reduced due to shorter recovery and follow-up time. Hospital stays can last for weeks following a transplant. However, it is conceivable that it would be only a matter of days for a scaffold, since once the surgical wound is closed the rest of the recovery occurs over time and should not require any active interference. For this reason, it has been assumed that the remaining costs associated with the transplant would be cut roughly in half. The costs of the transplant procedure and the estimated costs of the scaffold procedure are given below.

	Evaluation	Procurement	Hospital	Physician	Follow-up	IS Drugs (first year)	Total
Liver Transplant	$25,900	$59,100	$248,100	$66,900	$88,500	$31,100 + ~$360,000 For 20 additional years	$519,600 $879,600 After 20 years
Liver-specific costs		$59,100				$31,100 + ~ $360,000 for 20 additional years	$90,200 $450,200 After 20 years
Scaffold Procedure Costs	$12,950		$124,050	$66,900	$44,250		$248,150

Table 11. Estimated cost comparison between transplant and scaffold procedure.

According to this estimate, the procedure for implanting the new scaffolds would cost slightly under $250,000, or 72% less than the cost of a lifelong transplant patient. If the insurance companies were unwilling to spend a single dollar more than what they do now, then the price that could be charged per procedure would be the difference between the total cost of 6,500 transplants a year for five years and doing 20,000 scaffold procedures a year for five years using the estimated cost divided by the number of procedures (100,000). This would work out to be approximately $28.6 billion minus $24.8 billion divided by 100,000, which equals $37,500 per procedure. If five scaffolds were used for an average procedure, that means a price of $7,500 could be charged per scaffold. When compared to the cost per scaffold calculated in the cost model, there is an extremely high profit margin for the non-VEGF scaffold (~500%) and a very good

margin still for the VEGF scaffold (~31%). This means that there is room for some fluctuation in the price, while still making a profit, of the scaffolds if the number of procedures changes from the estimated 20,000 per year.

The timeline for starting a company to produce the collagen-GAG scaffolds is shown below. The yellow portion represents the time still needed in the university setting. During this time NSF/NIH funds will be used to continue the current mouse research and expand into cirrhotic mouse livers and possible other animal models. Once that phase is complete, after about a year and a half, a company will be formed and a lab will be set up off-campus as detailed in the cost model. During this time samples will be sent to a CRO, such as Pacific BioLabs, to begin the preclinical trials. The cost of the lab and preclinical trials are under a million dollars, which means that it is possible that the Small Business Innovation Research (SBIR) program is a potential source of funding while waiting for the technology to mature (53). After the preclinical trials are complete and the lab/manufacturing site is set up, venture capital will be needed to cover the high initial costs of clinical trials and raw materials for mass production. Approximately $35 million would be required to pay for clinical trials and stock three months of the necessary raw materials (assuming non-VEGF scaffolds).

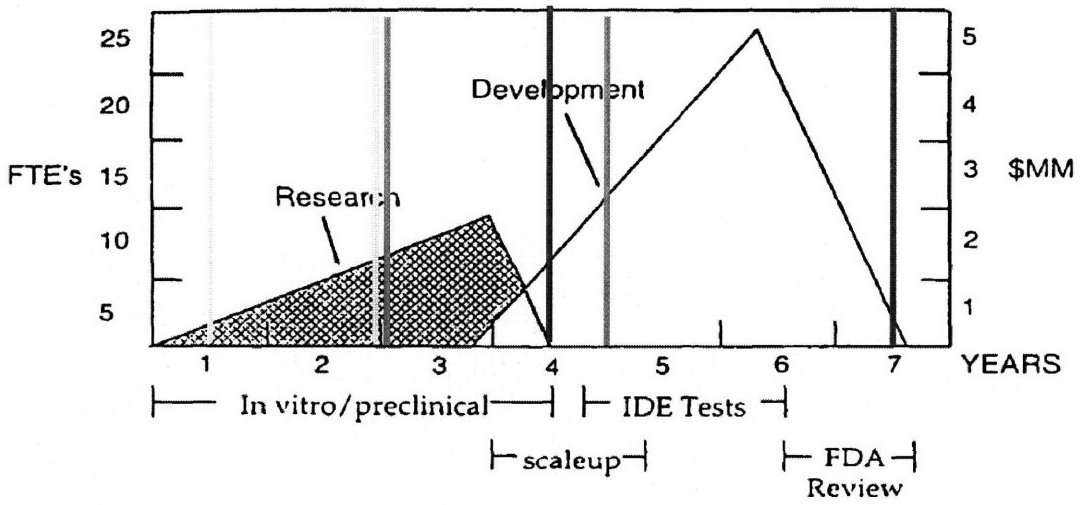

Figure 26. Projected timeline for product development and funding (47).

Chapter 12. Conclusion

The market for a solution to liver cirrhosis is very large, lucrative, and there for the taking if the right technology can be developed. Transplants cost almost a million dollars over the course of a patient's lifetime and hospitals, patients, and insurance companies would all like to see a better, less costly solution. Scaffolds made from collagen and glycosaminoglycan may be that solution. The scaffolds have been shown to prevent contraction, the root cause of scar formation, in healthy mice livers and show promise of being able to regenerate cirrhotic tissue. Numerous technical hurdles lie ahead, the largest of which is adequate blood vessel ingrowth, or angiogenesis. If these challenges can be overcome, then high margins are attainable in the marketplace with a product adoption of slightly less than double the current death rate due to alcohol induced cirrhosis. The technology is still at least five years from commercialization, possibly up to ten. The current estimate is that development will require at least another year and a half in the university before moving on to a small-scale manufacturing lab funded by the SBIR. After that, venture capital will be needed to pay for the high cost of FDA trials and raw materials. Overall, the company should be able to create a successful product that makes a profit while it simultaneously lowers individual patient medical costs, maintains current costs for insurance companies, and most importantly, helps to reduce hospital stays and save lives.

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