Development of a Bi-Layer Mineralized Bone and Cartilage Regeneration Template

by

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ABSTRACT

Porous collagen-glycosaminoglycan (CG) scaffolds have been studied extensively and proven to be capable of tissue regeneration in vivo for applications including skin regeneration templates, hollow nerve guides and conjunctiva regeneration. While the current CG scaffold has been thoroughly examined both mechanically and clinically, it has yet to prove appropriate for load-bearing applications. This study will investigate the mechanical properties of a mineralized CG scaffold and its application potential in a load-bearing environment. Through the introduction of calcium-phosphate mineral into the standard CG formulation the matrix analog will be available for bone regeneration. Utilizing a patented triple co-precipitation technique developed at Massachusetts Institute of Technology and Cambridge University, a homogenous mineralized scaffold will be manufactured. Comparison to healthy trabecular bone as well as the selection of the most appropriate extracellular matrix analog will be presented.

The key to commercial success is the introduction of a bi-layer bone and cartilage regeneration template to address concerns and difficulties in cartilage repair today. This dual combination is termed a *layered osteochondral scaffold.* The commercial viability of this product as well as the company founded on its inception, OrthoCaP, Inc., is delivered as a start-up venture over the next eight to ten years. With several key patents already filed, an extensive patent search was completed to establish leading competitors and technology in the marketplace. Although still in the primary phases of development, short-term profitability can be seen through licensing the technology to larger more secure firms. Long-term profitability is realized through a more scientific approach of broadening the technology to other areas of tissue regeneration and modifying the mechanical and material characteristics associated with collagen based templates.

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1 Introduction

According to the Center for Disease Control, arthritis and chronic joint symptoms affect nearly 70 million Americans, or about one of every three adults, making it one of the most prevalent diseases in the United States. As the population ages, this number will increase dramatically. Besides being the leading cause of disability in the United States, it leads to economic loses totaling over $82 billion annually.[1] Current research has successfully developed novel technologies involving polymeric scaffolds to aid in the regeneration of tissues such as articular and meniscal cartilage, diseased bone and severed peripheral nerves. Armed with this published information as well as a new layered osteochondral scaffold developed at Massachusetts Institute of Technology and Cambridge University, exploration into regenerated bone and meniscal cartilage by cell proliferation and scaffold mechanics will be addressed.[2,3]

The layered osteochondral scaffold represents the debut product from the start-up venture OrthoCaP, Inc. In addition to addressing the physiological requirements of the scaffold a complete patent search and manufacturing plan are discussed. An introductory business model and cost model are introduced at the end of this study to justify the time and effort invested in the commercialization of this technology. Although hypothetically profitable there are many trials in the research and development stage to conquer before FDA approval can be sought and full-scale manufacturing can begin.

1.1 Natural Bone Regeneration

Bone is a dynamic tissue that is constantly being resorbed and reformed by a particular group of cells in the body. There are three distinct ways that bone can be modified: osteogenesis, modeling and remodeling. These can differ depending on the person's age, type of bone being generated as well as the size of the defect or remodeling site. The layered osteochondral scaffold discussed in this study will support the regeneration of bone through the modeling process. After full degradation of the layered osteochondral scaffold, normal remodeling will occur as the body monitors the new bone tissue.

The human long bone has four surfaces on which new bone can be generated. These include the periosteal, the outer surface of all bones, the endosteal, the inner surface of cortical

bone, the Haversian, the inner surface of the Haversian canals, and the trabecular bone surface. Each of these surfaces can be modified. Figure 1 shows the structure of a long bone. The layered osteochondral scaffold will be implanted adjacent to healthy trabecular bone in order to take advantage of the ample supply of nutrients and bone remodeling cells present along the inner section of bone known as the marrow.

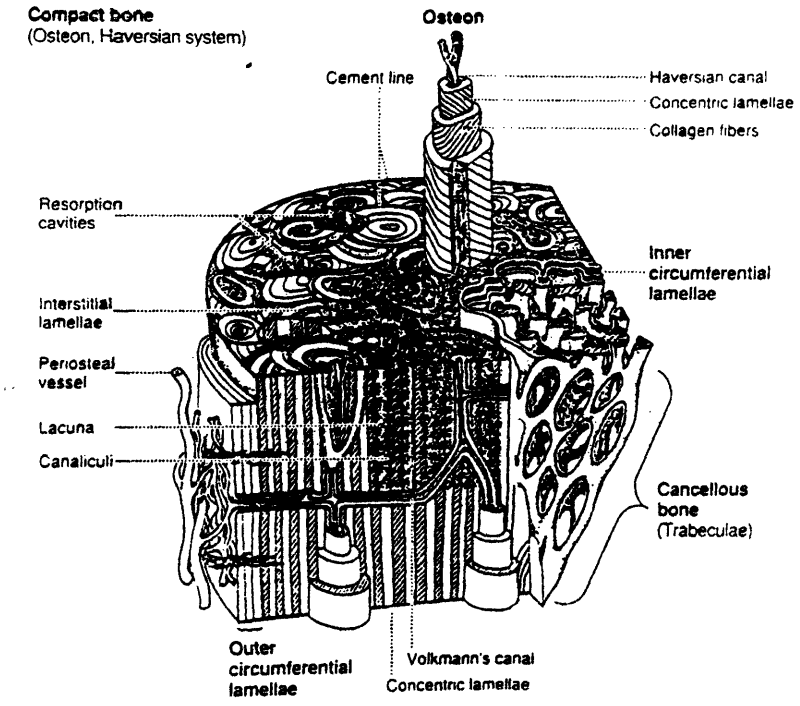


Figure 1 Long bone structure.
(Reproduced from Mow, 2004, Basic Orthopaedic Biomechanics & Mechano-Biology, 3rd Edition, Lippincott, Williams & Wilkins)

An embryo develops bone through the modification process of osteogenesis. This is the formation of bone on soft tissues, either fibrous tissue or cartilage. This process also occurs with the fracture of bones during adolescence. There are two sub-classes of osteogenesis: intramembraneous and endochondral ossification. Intramembraneous ossification occurs for flat bones such as the skull and mandible and does not readily apply to the goals of the layered osteochondral scaffold. Endochondral ossification is different from intramembraneous because it occurs where there is a cartilage base. Embryonic bones begin as cartilaginous tissue. This ossification process is responsible for long bone and vertebrae formation.

Endochondral ossification is comprised of 4 steps, each of which is outlined pictorially in Figure 2.[4]

1. Fig. 2.A: Swelling of the chondrocytes occurs; chondrocytes cease the production of Type II collagen and proteoglycan aggregates; chondrocytes begin production of Type X (ten) collagen and alkaline phosphates that will result in a matrix vesicle in which the chondrocytes will die.
2. Fig. 2.B: The cartilage matrix begins to calcify and harden; with the limited permeability of the new matrix oxygen, nutrients and waste cannot diffuse resulting in chondrocytes degeneration and death.
3. Fig. 2.C: Blood vessel invasion by a periosteal bud of blood vessels that penetrates the primary marrow cavity through the bone collar formed in step 1.
4. Fig. 2.D: A new bone matrix is laid down on the scaffolding provided by the calcified cartilage; osteoblasts formed from the periosteal bud.

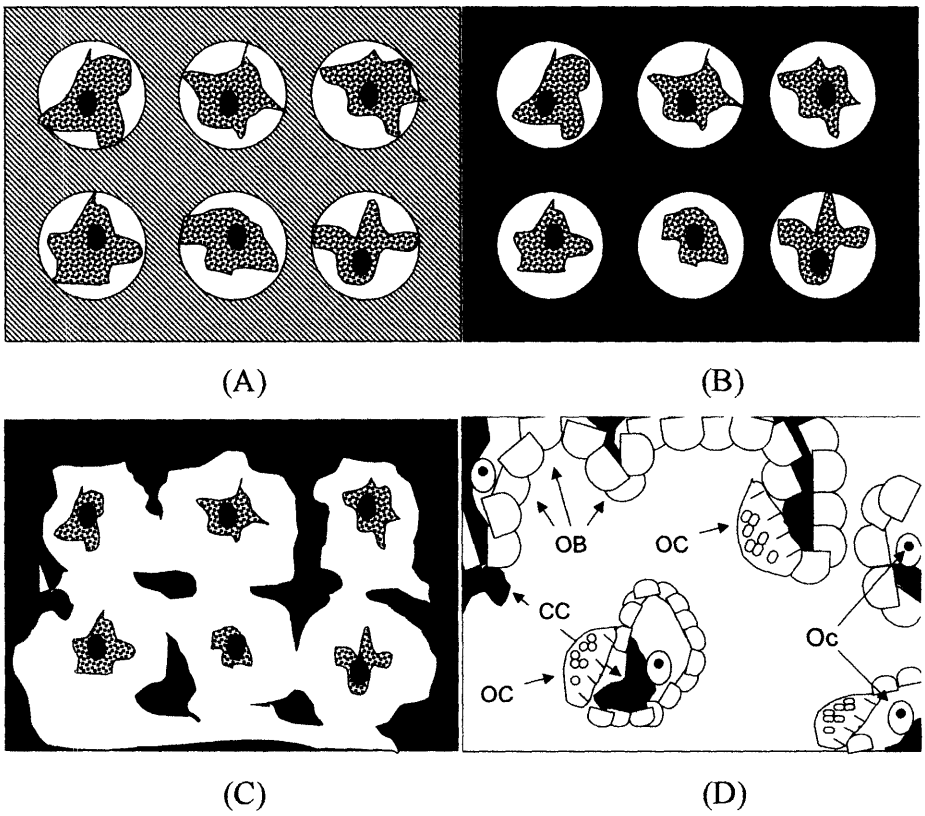


Figure 2 Endochondral Ossification (Fig D: OB = osteoblasts, OC = osteoclasts, CC = calcified cartilage, Oc = osteocytes)
(Reproduced from Mow, 2004, Basic Orthopaedic Biomechanics & Mechano-Biology, 3rd Edition, Lippincott, Williams & Wilkins)

During osteogenesis osteoblasts and osteoclasts are not connected in any way. They act independently from one another and are not controlled by a coupled feedback loop.

Bone modeling is the modification process most relevant to the success of the layered osteochondral scaffold. Because the calcified scaffold is provided at the implantation site osteoblasts cells need only relocate to this new region and begin depositing new bone. The key

part of this implantation is providing a blood supply and nutrients to the site. Necessary cells will be delivered with an adequate blood supply. This is achieved by performing a subchondral drill into the soft tissue of the bone. The soft tissue, or bone marrow, has an abundant blood supply and will be the resource during bone modeling.

Modeling is a way of depositing large amounts of healthy new bone. Again, as with ossification, osteoblasts and osteoclasts are not linked in any way during modeling. Only after sufficient amounts of new bone have been generated will a feedback loop become necessary. This process can take up to several weeks to complete; however, modeling will begin within days of the implantation of the layered osteochondral scaffold. As enzymes degrade the scaffold, new bone will be remodeled in its place resulting in a homogenous healthy bone structure.

Once the new bone has been modeled the maintenance period begins. Bone remodeling is an on-going process controlled by a feedback loop managing the deposition and resorption of bone. Research has shown that mechanical loading induces growth of long bone.[5] Therefore, with normal physical activity a bone is in constant remodeling phase. When bone are unloaded for extended periods of time, bone deposition lags the resorption rate resulting in lower bone mass. It is speculated that bone cells can sense a state of strain in the bone matrix around them and either add or remove bone as needed.[5] Certain research indicates that a piezoelectric stimulus is a part of the feedback loop to control remodeling cells.[6,7]

In addition to the bone regeneration layer of the layered osteochondral scaffold, a chemically bonded cartilage regeneration template will be included. The bone regeneration template will aid in the anchoring of the cartilage template within the joint. Upon the implantation of the layered osteochondral scaffold a blood supply and nutrients will be supplied to the cartilage regeneration template by way of the bone regeneration layer.

1.2 **Articular & Meniscal Cartilage**

Cartilage is a vital part of joints in the human body as it provides cushioning of bone on bone interactions as well as a smooth gliding surface that experiences little wear and has been nearly impossible to replicate with synthetic materials. The meniscal cartilage is C-shaped and aids in the distribution of sinovial joint fluid to the articular cartilage that covers the ends of both the tibia and the femur. Loss of the meniscal cartilage can lead to degenerative osteoarthrosis in the knee joint.[8] Articular cartilage possesses neither a blood supply nor lymphatic drainage. The

chondrocytes present in the extracellular matrix are essentially blinded to the immunological system of the human body and are ineffective when presented with injury.[9] Damage to joint cartilage, particularly in the knee joints, can range from small tears to complete degeneration resulting in a meniscectomy, or implantation of synthetic or allograft cartilage after removal of meniscal cartilage.

With the lack of vascularization and mobility of chondrocytes, articular cartilage is deemed irreparable under its own power. Regeneration can only occur if ample blood supply and nutrient is readily supplied throughout the growth phase. Scientists have experimented with cell seeding and growth factors to encourage regeneration, however, the aspect of load-bearing synthetic surfaces has not been adequately addressed.

1.3 **The Problem**

Several problems and deficiencies currently exist with current bone regeneration templates and cartilage regeneration scaffolds. Although bone is a highly researched area, the current technologies have not dealt with load-bearing applications. Many product lines boast mineralized scaffolds or regeneration templates, however, very little published data exists on the mechanical properties of these products. Most attention is spent on the biocompatibility and regeneration capability of these applications. In order to fully mimic the human long bone, the device must be able to bear loads of natural human activity such as standing over a period of time or walking short distances. By establishing mechanical data for the layered osteochondral scaffold we have provided a niche market into load-bearing applications such as those in the knee and hip joints as well as the market for general bone regeneration throughout the body.

Articular and meniscal cartilage tissue is unable to regenerate itself without aid from medical devices or arthroscopic surgery to relocate healthy cartilage tissue. Research for the solution to torn and degenerated cartilage has spanned a generation with many novel technologies uncovered. The current technique for treating small areas of missing or torn cartilage is to transplant healthy cartilage from other joints or areas adjacent to the affected area.

Bone marrow contains active stem cells that are able to deliver necessary chondrocytes to damaged areas of cartilage and subchondral bone. A common method, know as a subchondral drilling or microfracture involves the puncture of the cortical bone directly beneath the torn cartilage to release blood and cells from within the bone. Once punctured, a cartilage flap,

harvested from an adjacent area of the joint, is sutured over the hole. As the blood flows from within the bone, chondrocytes and nutrients are delivered to the cartilage flap, allowing proliferation of cells and successful acceptance by the body.[9]

There are three main problems with this method of subchondral drilling: 1. No extra cellular matrix (ECM) is provided for the repair of the drilled and damaged bone; 2. Transplanting healthy cartilage leaves a new area damaged and can result in donor site mortality; 3. Although adjacent to the flow of bone marrow cells and blood supply, the cartilage flap is not securely fixed to the adjacent bone like natural cartilage is. In a high wear environment such as the knee or hip joining, secure attachment and anchoring is necessary for a cartilage regeneration template. The solution of these problems must come in the form of a bi-layer physically bonded mineralized bone regeneration and cartilage regeneration template.

1.4 **The Solution**

With the development of polymeric scaffolds and collagen-based matrices, biocompatibility and the ability to control cellular ingrowth as well as mechanical strength have been investigated both in vitro and in vivo through animal modeling. Several clinical trials have been completed with results showing porous collagen matrices can support cellular ingrowth and matrix synthesis.[8] Several areas of improvement were noted and include the presence of collagen matrix particles within the joint, complete replacement of meniscal cartilage by porous collagen matrix and the ability of the scaffold to support progressive loading for long periods of time.[8,9] With these improvements in mind the layered osteochondral scaffold addresses each of the concerns and with continued development and clinical trials will provide a safe, reliable and long-term solution to the existing alternatives.

Through the successful mineralization of the collagen-glycosaminoglycan (CG) scaffold a template will be produced to sufficiently support the mechanical properties necessary for bone and cartilage regeneration. The layered osteochondral scaffold also improves the ease of implantation, as it is currently very difficult to integrate a scaffold without the use of sutures. Utilizing a 'plug' approach, the pliable matrix will be inserted snugly into a punched hole without the need for sutures or adhesives. Immediate bonding to the peripheral tissue would insure stability during the postoperative period.[8] With the development of the layered osteochondral scaffold, the subchondral drilling method would be improved.

Extending the successful collagen-GAG scaffold across two tissue types is an immense accomplishment. Increasing the mechanical strength of the bone regeneration scaffold would eliminate diseased bone tissue near the cartilage implant as well as support a higher healing rate and more efficient cell proliferation.[2,3] It seems perfectly natural to incorporate these two scaffolds as ultimately the cartilage requires the cells and nutrients supplied by the bone marrow. Also, by incorporating this bone scaffold, cells will be inclined to propagate into the cartilage regeneration scaffold more readily as they continue to establish an extracellular matrix spanning the transition length.

The difficulty lies in uncovering the optimum scaffold characteristics necessary to induce cell ingrowth in two very different matrices, while allowing degradation of the scaffold itself. A variable that will be investigated includes the percent mineralization of the bone scaffold. Pore size will have to be optimized to meet both mechanical requirements as well as allowing permeability to macromolecules, regeneration cells of bone and other nutrients necessary for cell mitosis and synthesis.

A need exists to reduce the trauma introduced into subchondral bone during routine arthroplasty procedures attempting to repair torn or degenerated meniscal cartilage. Development of a dual regeneration scaffold will not only solve the problem of damaged or diseased bone, but also provide a pathway for valuable cells and nutrients to proliferate into the cartilage matrix. Exploring variables such as mineralization and cross-linking density will result in an optimum model for bone regeneration.

2 Intellectual Property & Patent Filing

Intellectual property refers to creations of the mind: inventions, literary and artistic works, and symbols, names, images, and designs used in commerce.[10] Intellectual property is divided into two categories: Industrial property, which includes inventions or patents, trademarks, industrial designs, and geographic indications of source; and copyright, which includes literary and artistic works such as novels, poems and plays, films, musical works, artistic works such as drawings, paintings, photographs and sculptures, and architectural designs.[10] This IP study will focus predominantly on the first category of IP: industrial property. Copyright may come into play when OrthoCaP, Inc. has the ability to market a product. An appropriate search of trademarked names and logos would have to be completed.

Intellectual property is a way to describe ideas and processes that are unique and can be marketed, commercialized and sold for profit. Large corporations protect their intellectual property by having employees sign confidentiality agreements and keeping 'trade secrets' out of the press and product descriptions. However, for small start-up companies, especially those stemming from academic institutions, patent filing and trade marking is an absolute must to establish rights to technology.

It is also important to do a complete patent search for technologies relevant to the new product. The reason for this is two-fold. Firstly, infringement on filed patents can be very costly and in many cases can exploit all available funds on legal fees and possibly paying retribution on any profits earned from the infringing technology. Secondly, the descriptions of these products can be very deceiving in the patent language. Even slight differences in protocols and process variables can result in an approved patent. Language used in patents can be misleading and completing a patent search can enlighten the searcher of the ways to describe their product without causing infringement and create a niche for their particular invention.

Internationally, the policies toward intellectual property differ greatly. For example, in Asian countries such as India the IP belongs to the first person or group to file a claim, not necessarily the original inventor. This differs dramatically from the policies in the United States, where the IP rights fall to the original inventor as long as guidelines have been followed. This has a dramatic effect on the need for secrecy and an incredibly different timetable. In the less strict countries, it is literally a rat race to the filing office to establish rights. The United States

has much more stringent policies that honor the original inventor(s) and seek to grant IP to the correct institution or enterprise.

2.1 IP Study

This IP study investigated filed patents in both the Unites States and the United Kingdom. The search was limited to these two industrialized countries due to the intent to initially commercialize within them. The search itself was broken down into several parts. First, the areas of application had to be determined. This would eliminate irrelevant patent searches and would provide the best idea of how competing technology may be marketed and in what indication. Second, within the relevant patents particular attention is paid to several key areas of interest. Even in the slightest difference in manufacturing protocols or raw materials can be the deciding factor for infringement liability. Finally, close attention will be paid to the filing party. It is important to note whether individuals or enterprises hold the primary rights and to conduct parallel research on those companies.

2.1.1 Areas of Application

Before performing the patent search it is necessary to recognize areas of application. This makes the search more organized and allows one to analyze more relevant patents in this way. The search was focused on three areas, the first being collagen based biodegradable implants, the second being articular cartilage regeneration templates of various types of collagen and the last and most concentrated search was on mineralized Type I collagen templates utilized for either bone or articular cartilage regeneration. There was a vast amount of overlap, however, nearly 150 relevant patents were found. Nearly 50 of these were read thoroughly and trends were recognized. In doing the patent search for the mineralized scaffolds the following areas were investigated:

- 3D Biodegradable Matrices
  - Collagen/GAG Scaffolds
  - Surface Mineralization & Patterning
- Tissue Engineering
  - Bone Regeneration
    - Articular/Meniscal Cartilage
- Synthetic Mineralized Compounds

These areas are relevant applications for the mineralized bone scaffolds. The 3D biodegradable matrices are of interest because it is an area where the majority of relevant patents are located. In particular, a co-founder of the mineralized patents holds the three oldest patents for collagen-GAG biodegradable scaffolds that were developed at MIT. Tissue engineering is a very broad topic, but the search was limited to bone regeneration solutions, particular those relevant to the regeneration of articular and meniscal cartilage. Finally, we were interested in the competing process for the mineral components used in biotechnology. The two categories included fillers and compounds. These were predominantly bone cements used in hard tissue implants.

2.1.2 **Patent Search**

Approximately 25 relevant patents were selected from 150 patents found at the United State Patent Office website. Please see Appendix A for the complete list including brief abstracts. A brief overview of the patent holders and relevancy will be completed. It will be interesting to note the filing date and country of origin.

The patent holders cover three broad areas. These include research universities, which hold the oldest and most rooted technology patents. Research hospitals seem to hold patents in collaboration with research universities. On the industry side, it seems the companies that hold the most specific patents are from start-up organizations. These start-ups sometimes derive from academic institutions or individuals branching out from major corporations in the field. In this last instance, these patents hold remarkable profits when licensed to larger corporations with the means to commercialize new products. Lastly, there are a handful of brilliant scientists who have the means to patent novel technology on their own. This is a rare occurrence due to the large monetary commitment to file, however, it seems that this occurs more regularly overseas than in the United States.

2.1.3 **Specific Findings in Areas of Application**

2.1.3.1 **3D Biodegradable Matrices**

In terms of academic institutions MIT has really led the pack. Filing patents as early as 1975 (PN4060081), Professor I.V. Yannas and his team strategically developed consecutive improvements on the original technology of a multilayer membrane with a collagen-GAG based

matrix utilizing a polysaccharide (GAG) crosslinker. In 1987 (PN4947840), they filed a second patent building on the first by controlling the pore size and degradation rate. He made his third contribution to this area in 1994 (PN5489304) when a skin regeneration template was patented. This was done in conjunction with two research hospitals and the company *IntegraLife Sciences, Inc.* This patent was developed and commercialized and *IntegraLife Sciences* was able to profit from its self-named Integra Skin Regeneration Template®. Although these original patents have expired and many companies have made improvements or adjustments to the original technology, MIT has established the trend of subsequent filing.

With many novel ideas, small adjustments or improvements may call for multiple filings. With research and development in corporations or universities, continuous improvements are inevitable. These can each be filed separately and in greater detail than if filed all at once in a general patent. Professor Yannas was very strategic in filing his patents nearly a decade apart from one another. This would ensure his rights to the technology over a longer period of time, but still allowing substantial research and development to occur.

Another patent was found in the area of 3D biodegradable matrices (PN6187047). *Orquest, Inc*, holds this patent, which is located in Mountain View, California. This was also a collagen-based scaffold but with no mention of the polysaccharide crosslinker. It did, however, mention the inclusion of a calcium phosphate mineral component. There was also a crosslinking protocol provided to control degradability. Other patents in this area included one filed in 2002 (PN6858042) by *Osteobiologics* in San Antonio, Texas and another by *Osteotech, Inc.* from Eatontown, New Jersey in 2000 (PN6863694) and a subsequent one in 2001 (PN6808585).

Osteobiologics filed a patent describing the manufacture and use of a fiber-reinforced, porous, biodegradable and implantable device for the general purpose of tissue engineering. It is the goal of *Osteobiologics* to facilitate the regeneration of load-bearing tissues such as articular cartilage and bone. This is in direct competition with the layered osteochondral scaffold to be commercialized by OrthoCaP, Inc. This technology utilizes the formation of oriented fibers in a biodegradable polymer to make possible the load-bearing capabilities of the final scaffold.

Osteotech, Inc. has patent rights to an osteogenic implant derived from bone. The implantable bone-derived sheet is manufactured from allogenic donor bone that is shaped using biological adhesive binders that can be enzymatically degraded. It is claimed that the preferred fiber is collagen fibers and the preferred binder is glycolide-lactide copolymer. This sheet can be

incorporated into a mesh, ideally titanium mesh. This invention is of particular interest due to the subsequent patent filings on the same invention in 2000 and 2001. Another area of interest in the use of mineralized inorganic phases available as binders. This would be similar to the incorporation of the calcium-phosphate co-precipitate in the layered osteochondral scaffold to be commercialized by OrthoCaP, Inc.

There were several patents that utilized a collagen based scaffold or matrix for either the regeneration of articular cartilage or bone, but not both. These included patents filed from the following corporations: *Taipei Biotechnology Ltd, Inc.* in Taipei, Taiwan (PN6852331); *Osiris Therapeutics, Inc.* in Baltimore, Maryland (PN6835377); *DePuy Spine* and associated divisions in Raynham, Massachusetts (PN6764517, PN6884428, PN6896904); *Collagen Corporation* in Palo Alto, California (PN4789663). Drexel University in Philadelphia, Pennsylvania also filed a patent relating to a collagen matrix in 2001 (PN6753311).

Although each of the above patents has interesting points, the two most revealing patents were filed by *DePuy Spine*, a *Johnson & Johnson* company based in Raynham, Massachusetts and *Osiris Therapeutics*, Inc. located in Baltimore, Maryland. It was revealed in this patent search that DePuy had the most competing technologies. They filed a patent in 2004 (PN6896904) dealing with a collagen/polysaccharide bi-layer matrix. *Osiris* filed a patent in 1998 (PN6835377) dealing with a method to regenerate osteoarthritis cartilage, a main market OrthoCaP, Inc is looking to permeate.

DePuy Spine did an excellent job describing the vast applications of their bi-layer collagen/polysaccharide matrix. In general, they offer very broad recommendations on the components involved in the layers. However, collagen is included as the main component with chondroitin-6-sulfate constituting the polysaccharide. The manufacturing protocol is limited to the means of incorporating the proteins and polysaccharides. It is recommended that the first layer comprises two polysaccharides or proteins crosslinked to each other. It is also recommended that the first layer be attached to the second layer by chemical crosslinking with divinyl sulfone or by thermal crosslinking through DHT. OrthoCaP's layered osteochondral scaffold is composed of Type I collagen copolymerized with chondroitin-6-sulfate and then co-precipitated with mineral. This patent has some similarities including the raw materials used. The chemical crosslinking protocol however is missing the key mineral component that essentially sets OrthoCaP's osteochondral regeneration template apart from DePuy Spine products.

Although this particular report does not include the description of the articular cartilage regeneration template an appropriate patent search was conducted to search for competing technologies. *Osiris Therapeutics* was successful in filing a patent in 1998 describing the usage of human mesenchymal stem cells in a biodegradable collagen gel matrix to regenerate cartilage. Using chemically crosslinked collagen gel and fibrin glue the inventors were able to show how both shallow cartilage chondral defects and full thickness cartilage defects could be regenerated with this approach.

Specific patents dealing with the combination of bone and cartilage regeneration were more difficult to find, however, their presence indicated that this is a hot research topic and many companies have technology coming down the commercialization pipeline. These patents belong to the following organizations: *Zimmer Orthobiologics* in Austin, Texas (PN6858042); *IsoTis N.V.* in Bilthoven, Netherlands (PN6692761); University of Michigan, Ann Arbor (PN6767928) and *Regeneration Technologies* in Alachua, Florida (PN6893462). Each of these has made a contribution to the research area of bone and cartilage repair through the use of biodegradable matrices.

Zimmer Orthobiologics filed a patent in June 2001 that encompassed a solution for the regeneration of articular cartilage by anchoring the regenerating matrix to the adjacent bone. It is the goal of this patent to establish materials capable of providing load-bearing support after a minimally invasive surgery to implant said materials. The manufacturing process is not clearly outlined, however, the materials utilized in the matrix are listed as autologous tissue harvested from pigs and/or cattle. The tissue is immunologically deactivated by way of photo-oxidation. This is an interesting patent due to the exclusive use of harvested tissue and that subchondral drilling is utilized as an implant technique.

The University of Michigan has developed a novel method for patterning and or mineralizing biomaterial surfaces with a calcium-rich solution. Filed in 2000, this method utilizes a polylactic acid polymer based onto which mineral islands are homogeneously patterned. The manufacturing process includes a foaming procedure to create a porous, biodegradable matrix. A leaching process is used to deposit the calcium-rich solution. Although it results in a similar mineralized matrix the scaffold material and manufacturing process are decidedly different than those found in the process for the layered osteochondral scaffold.

There is a more comprehensive patent list included in Appendix A. The patents listed here cover the wide range of cartilage and bone regeneration solutions. It is evident through this search that the mineralized collagen-GAG scaffolds, that OrthoCaP Inc. will attempt to commercialize, were derived from the earlier skin wound regeneration templates developed at MIT. In addition, it is clear that there are many companies worldwide looking to solve this massive problem. Multiple findings establish continued improvement on many of these novel inventions and provide the necessary protection against infringement and unlawful use.

2.1.3.2 **Mineral compound**

The mineral component search revealed several relevant patents. Those that stood out included one from *Millennium Biologix, Inc.* located in Kingston, Canada, which was filed in 2002 (PN6846493). The patent describes a synthetic biomaterial compound of stabilized CaP phases. The process is comprised of three steps starting with a colloidal suspension of silica and CaP. The next step involves spraying this suspension into a powder and finally sintering the dried powder.

Etex Corporation, located in Cambridge, Massachusetts, filed a patent in 1996 (PN6117456), This patent describes an amorphous phase CaP mineral component utilized in hard tissue implants. This product is generally used as a filler between permanent hip and knee prosthetics and surrounding tissue. The calcium phosphate is developed from a mixture of Dicalcium diphosphate and water in specific ratios.

A very interesting patent concerning a nano-calcium phosphates and collagen based bone substitute material was filed by Tsinghua University in Beijing in May 2001 (PN6887488). This porous material is aimed at treating bone defect and bone fractures. The patent describes a collagen molecular and nano-calcium phosphate particle composite material. With alternating layers of mineral and collagen, the ultimate thickness of this composite is 5-50 microns thick. Type I collagen is used in conjunction with calcium and phosphate sources of calcium chloride and sodium phosphate, respectively. Dissolving the collagen and mineral sources in acetic acid leads to a coprecipitation. This solution is centrifuged and freeze-dried to remove all aqueous states. The resulting material is ground into powder and added to a set ratio of poly(lactic acid) (PLA) or poly(lactic acid-co-glycolic acid) (PLGA) dissolved in dioxane. The solution is then freeze-dried to result in an open porous structure with pore size ranging form 100-500 microns.

Ultimately, this is a patent that serves a roadblock to the layered osteochondral scaffold. Utilization of a co-precipitation process and lyophilization is a concern. However, a patent has already been filed and accepted protecting the triple co-precipitation method used in the layered osteochondral scaffold. Secondly, a polysaccharide copolymerizer is utilized in the layered osteochondral scaffold and there is no mention of such material in this patent. Overall, this patent could potentially produce a product that would be in direct competition with the scaffold investigated in this study.

2.1.4 **Impact on mineralized bone scaffolds**

It is no wonder that collagen is used in tissue regeneration templates as it is the most abundant protein in the body. Also, GAG is a naturally occurring crosslinker and would only make sense to incorporate this with the collagen. The triple co-precipitation and lyophilization manufacturing process is part of what sets the layered osteochondral scaffold apart from the rest and prevents infringement on other patents. Merely setting protocols such as ratios and temperature ranges signifies a difference between manufacturing process and ultimately the final product. We are able to carefully tailor the physical characteristics and final properties of the scaffold that are much different from anything else out there.

As for the calcium phosphate mineral component, a patent application describing the protocols of the triple co-precipitation has been filed. An original calcium source is included with a solvent relationship that allows for evenly distributed deposits within the scaffold. Most other designs depend on salts or colloids to obtain deposition. The layered osteochondral scaffold is in the clear with both aspects of our design due to correctly filed and novel patents. With such a thorough patent search it is clear that the layered osteochondral scaffold is not infringing on other technology and since we have already applied for a patent, we have insured that no other individual or organization can copy this technology.

2.2 **Patent Filing Process**

Three types of patents exist. These are utility, design and plant. Within utility patent applications there are two types: provisional and non-provisional. It is important to follow the patent rules in order to file the correct type of utility patent. In addition, when filing a patent it is necessary to understand the time frame of protection, or when the patent begins and ends. As of

June 8, 1995 the protection term changed. If an application was filed prior to June 8, 1995, the protection term is the later of (1) 17 years from the issuance date of the patent, or (2) 20 years from the first U.S. filing date for the patent.[12] A patent filed after June 8, 1995 received a protection period of 20 years.

As noted previously, it is necessary to understand the difference between a provisional and a non-provisional patent. A provisional application establishes a filing date but does not begin the examination. The inventor is provided a one-year period to further develop the invention, determine marketability and seek licensing agreements.[12] Within the one-year provisional period the inventor must file a non-provisional application in order to receive a patent. The non-provisional application is considered the true patent application.

In order for an invention to receive a patent it much pass four tests that have been put in place to ultimately determine if it is useful, novel and applicable. The first test is the assignment of the invention to one of five 'statutory classes' of things that are patentable. These classes are the following:[12]

1. Processes
2. Machines
3. Manufactured Items
4. Compositions of Matter
5. New Uses of Any of the Above

The second test establishes the usefulness of the invention and that it is not merely a theoretical phenomenon. The third test institutes a novel invention, one that has not been discovered or made by anyone previously. The fourth and final test is the trickiest and generally is the area that most disagreements occur. The invention must be 'nonobvious' to 'a person having ordinary skill in the art to which said subject matter pertains'.[12] This is most difficult to prove and to argue.

Once the four required tests have been adequately passed a provisional application can be filed. The filing itself has a monetary component and a time component. Although not overtly expensive, the cost can range from $3,000 to $5,000.[12] The commitment comes in the form of a valid and complete patent search. When filing, the inventor, or assigned patent agent, must complete a thorough patent search to ensure that the new invention does not infringe on any existing technology. This can be incredibly time consuming, but if not correctly completed, and a similar patent exists, the application will be denied.

The most important aspect of the patent application process is confidentiality. For example, if the product is placed on sale or advertised for sale, or sold in the U.S. and more than one year passes, the invention is no longer patentable and no protection can be provided. In addition, by introducing the invention into 'public domain' someone else has the opportunity to steal the idea of the invention and essentially patent the technology on his or her own. This is very risky and close attention must be paid to the one-year time allowance.

The writing of the patent is very complicated. Attention must be paid to the details, such as words used, definition of terms and claims made. It is essential that the invention be clearly identified and described to limit the interpretations of other inventors looking to patent competing inventions. The rejection rate for an application is upwards of 95%. A patent is a government-granted monopoly, and the nature of the public policy dictates that no monopoly may be granted unless it is truly warranted by the inventor's creativity. The major focus that the examiner looks at is to make the application as narrow as possible in order to comply with that policy.[12]

As for foreign patent applications, the U.S. does not discriminate based on the citizenship of the inventor. They are held to the same stipulations set forth for U.S. citizens. There are several requirements for foreign applications: the inventor must submit the application along with a signed oath or declaration; a U.S. patent can only be granted if the original foreign application was filed less than 12 months prior. These stipulations are based on the original design and only in the case of death (of the original inventor) can a second or third party file a patent on their behalf.

A truly novel invention will have to undergo the careful scrutiny imposed by the U.S. government, however, once granted, a patent supplies a legal monopoly on a technology that can be sold for profit for many years! The true test is the endurance the inventor can display. The patent filing process is incredibly time consuming and in many cases expensive. The cost of legal counsel or patent agents can nearly triple or quadruple the cost of the application itself.

2.2.1 **Public Domain**

Public domain is defined as: the status of publications, products, and processes that are not protected under patent or copyright.[13] Public domain is of high concern when dealing with patent filing or industrial trade secrets. It is considered any forum that releases the 'secrets' or

methodology of the invention. Many companies enforce confidentiality agreements when customers or non-employees enter facilities containing products or technology not protected by patents.

For small start-up companies a strict confidence must be achieved with investors or others affiliated with getting the business off the ground. Especially those that have not filed a non-provisional patent application for their technology. Confidentiality agreements are readily employed to limit the discussion or distribution of sensitive information to the public.

Trade secrets are defined as: a secret formula, method, or device that gives one an advantage over competitors.[13] Many companies insist on keeping protocols or manufacturing processes under wraps for fear that a competitor would be able to replicate that technology. By utilizing confidentiality and non-compete agreements with current and former employees a company can efficiently protect their innovative assets. Another measure regularly taken by research and development firms is the strict usage and documentation of experiments, discoveries and analysis. Through the use of a simple lab notebook a company can plead their case in court. The only requirement is the continued documentation and sign-off done by members of the company. This practice is often utilized in large established corporations, as opposed to start-up organizations based on only a few areas of technology.

2.3 **Layered Osteochondral Scaffold Patent**

There are two patent applications currently filed for the protection of the layered osteochondral scaffold. The first patent filed describes the design and development of a layered osteochondral scaffold. Specifics are given for various versions and outline the following topics. The patent was filed in January 2005 as part of the MIT-Cambridge alliance.

The layered osteochondral regeneration scaffold has a general application of bone and/or cartilage regeneration. The patent goes on to outline the mechanical mixing procedure including temperature, pH and the ratio of collagen to GAG. The manufacturing process is also detailed with regard to nucleation and growth parameters of the ice crystals. An annealing step is included purely to ensure the full crystallization of the water, but unique in that it wasn't mentioned in any other patent in the comprehensive search. Both chemical and physical crosslinking protocols are included as well. The wording is always interesting, especially in one portion of the patent where it says... "composed of... at least the following": Type I or II collagen

from bovine tendon and a calcium phosphate phase comprised of brushite, octocalcium phosphate and apatite.

The second patent application ensures the rights to the nucleation of calcium phosphate, the triple co-precipitation of CaP. This is a novel process utilizing several calcium sources dissolved in a phosphate solvent. This process is intended for use in conjunction with a basic collagen-GAG scaffold. The idea is to increase the mechanical strength of the basic scaffold and provide a more appropriate extracellular matrix analog for bone. The reagents are fully reacted to form CaP deposits on the collagen fibrils through heterogeneous nucleation. Suggested ratios are provided that are based on the mechanical properties of natural bone measured in situ.

3 Full-Scale Manufacturing of Layered Osteochondral Scaffolds

The objective of this study is to define the manufacturing process of the mineralized collagen-glycosaminoglycan (GAG) portion of the layered osteochondral scaffold through governing equations. Ultimately we will solve the defining equations for the process of lyophilization as well as the triple co-precipitation method. Calcium phosphate is allowed to precipitate over time at a controlled temperature and pH in order to increase the mechanical stiffness of the bone scaffold. The process time of the final scaffold is a function of the growth rate of the precipitated calcium phosphate.

Lyophilization, or freeze-drying, is the manufacturing process of choice. Through the freezing and sublimation of ice particles a porous scaffold is left behind. By controlling the freezing rate and CaP supersaturation of the slurry, the final scaffold is produced with optimal pore size, specific surface area and predetermined calcium-phosphate composition.

Mineralized bone scaffolds are designed to facilitate regeneration of healthy bone in vivo.[14] The mineralized layer of the osteochondral scaffold provides an anchoring mechanism to adjacent bone. This anchor will support the attached articular cartilage regeneration template. By providing a collagen-based matrix, bone-remodeling cells such as osteoblasts and osteoclasts can migrate through the open porous structure and synthesize new extracellular matrix (ECM). As new ECM is synthesized, the scaffold will be degraded at a complimentary rate by enzymatic reactions.

A porous structure is ideal for facilitating the proliferation of cells throughout the scaffold. Utilizing the principles of lyophilization, or freeze-drying, we are able to control the heat and mass transfer of both the water molecules and particulates present in our system. A second consideration is the inclusion of calcium-phosphate particles that will be introduced in a constant co-precipitation method and with the management of temperature and exposure time can be tuned to a desired phase within our final system.

3.1 Lyophilization

The original application of this process was the preservation of biological materials. Employed because of its ability to preserve without injury, it involves the freezing of water

particles and subsequent sublimation of ice crystals. The ability of water to undergo phase change rather readily is a natural process that can be easily controlled with temperature and pressure.[15]

There are three broad categories of biological preservation involving freeze-drying procedures:[15]

1. Non-living matter such as blood plasma, serum, hormone solutions and foodstuffs.
2. Surgical transplants which are made non-viable so that the host cells can grow on them as the skeleton. Examples include artery, bone and skin.
3. Living cells destined to remain viable for long periods of time. Examples include bacteria, yeasts and viruses but not mammalian cells such as spermatozoa.

Utilizing lyophilization in the food industry is important because it stops the advent and growth of microorganisms and allows for long-term storage and transportation of otherwise perishable food.[16] This biological application has been utilized in our study to create a porous structure. This process has been employed for regeneration templates at Massachusetts Institute of Technology for more than 20 years as well as numerous research laboratories around the world. Several patents[17] are in existence whereby lyophilization is readily used as the chosen manufacturing process to produce porous scaffolds for use in tissue engineering research.

There are several competing manufacturing processes that can yield similar end results, but prove to be more difficult to control or alter. These include stereo-lithography and solid free-form fabrication, convection injection molding and sintering.[18] Details of these processes will not be described comprehensively, but can provide porous structures comparable to those for our mineralized bone scaffolds. In most cases, these processes are not economically feasible at the large-scale manufacturing levels due to time and labor costs.

3.2 **Triple coprecipitation Method**

The mineralized component of our scaffolds is derived from the inclusion of calcium-phosphate particles. These particles serve two purposes for bone regeneration:

1. Provide a scaffold with superior mechanical properties to those of unmineralized porous scaffolds. This property deems them load bearing for particular use in joint applications.
2. Calcium phosphate is readily absorbed by newly synthesized bone matrix. This uptake encourages the bonding of bone to the scaffold (or adjacent devices) and increases the rate at which healthy bone reaches its mature state.

The calcium-phosphate (CaP) component is introduced using standard supersaturation methods. Increasing the aqueous medium content or adjusting the pH of the solution may induce supersaturation of the solvent.[19] Once supersaturation has been established the CaP begins to nucleate. Once the nuclei reach a critical size, growth begins and crystals will form. The composition of these crystals is highly dependent on the chemical makeup of the initial solution. In our process care is taken to introduce calcium and phosphate components in the correct balance to ensure the proper phase as well as to regulate the pH of the solution prior to and during supersaturation. This method of individual introduction of calcium and phosphate is referred to as co-precipitation. The associated chemical reactions are shown in Figure 3.

Reaction Equations

Dicalcium Phosphate, DCP

Calcium Hydroxide Reaction

$$Ca(OH)_2 + H_3PO_4 = CaHPO_4 + 2H_2O$$

Calcium Nitride Reaction

$$2Ca(NO_3)_2 + 2H_3PO_4 = 2CaHPO_4 + 2H_2O + 2N_2 + 5O_2$$

Figure 3 Reaction equations for the calcium phosphate triple co-precipitation method.

This process is used for various applications including water processing to remove phosphate impurities from sewage or wastewater.[19] The introduction of calcium into the process results in supersaturation and the subsequent formation of co-precipitates that are less harmful to the environment and the water system.

3.3 Quantitative Analysis of Manufacturing Process

Each manufacturing process will be dealt with using families of equations that will fully describe the boundary conditions, process parameters and final product specifications. Please see Figure 4 for a brief introduction to the process.

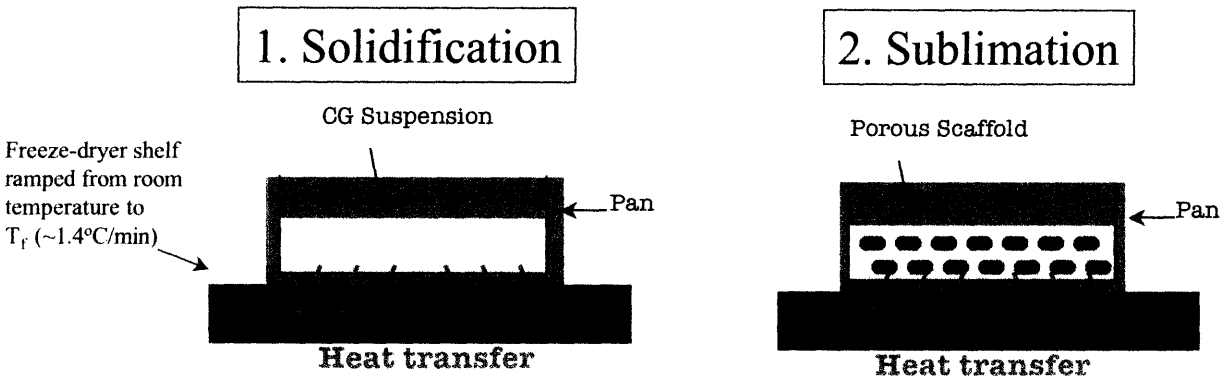


Figure 4 The first step of the lyophilization process is the freezing of the slurry suspension. This is done at a constant ramping rate to a predetermined undercooling of -40°C. After a 60-minute annealing period the pressure is dropped and temperature increased (all below the triple point) to induce evaporation of the ice crystals.

3.3.1 Lyophilization

The basic principles of lyophilization include the freezing and sublimation of ice particles, the transition from ice to vapor without melting. The water phase diagram (Figure 5) graphically shows the phase dependence on temperature and pressure. The triple point of water is defined according to the International Temperature Scale of 1990 (ITS-90). Triple point values: T= 273.15K, P= 611.657 Pa = 4,587.804 mTorr.

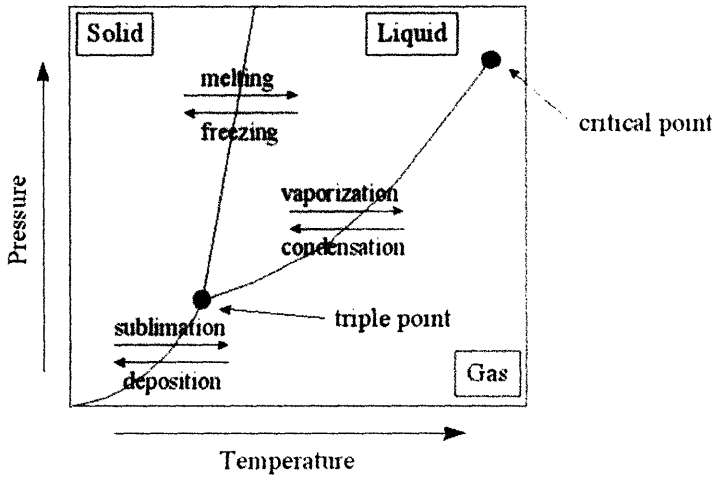


Figure 5 Water Phase Diagram: Graphical explanation of phase dependence on temperature and pressure[20].
(Reproduced from Brown, et al, 2003, Chemistry: The Central Science, 9th Edition, Prentice Hall)

The boundary conditions for this process are shown in Table 1. These boundary values will be used throughout this paper to quantitatively define this particular process.

Table 1 Boundary Conditions for lyophilization process including freezing and sublimation steps.

Process Step	Initial Temperature T_i (°C)	Final Temperature T_F (°C)	Pressure
1. Equilibration	293	273.15	1 atm
2. Freezing	273.15	233	1 atm
3. Sublimation	233	298	300 mTorr
4. Removal	298	293	1 atm

Also, it is important to define constant values that are associated with the kinetic and thermodynamic equations. These are included in Table 2 for reference.

Table 2 Thermodynamic & Kinetic Constants for the Formation of Ice Crystals during Freezing[25, 26]

Property of Water	Value
Surface Tension of Water, γ_{H2O}	73 mN/m or 73 dynes/cm
Dipole Moment of Water*, μ	6.471×10^{-30} cm
Molecular Density of Water**, n	3.35×10^{28} m^{-3}
Specific Latent Heat of Fusion of Ice	334.72 kJ/kg
Molecular Radius of Water Molecule	0.9584Å
Molar Volume of Water (unitless value)	1.093
Change in Temperature, ΔT	60°C or 60K
Viscosity of Water, υ	1.10×10^{-3} N•s/m^2
Induction Time Constant, α	2.08×10^{10} s
Induction Time Constant, G	1.56×10^{-8} m/s

** Dipole moments result from an unbalance in positive and negative charges of a molecule. These molecules are termed polar because they possess permanent dipoles. The asymmetry of the water molecule leads to a dipole moment. The value μ refers to the effective separation of the negative and positive charge centers. The polar nature of water and molecules allows them to bond to each other and is associated with high surface tension of water.*

*** Molecular Density of Water: The number of water droplets per area volume. Value was taken directly from published data. However a rough estimate can be made using the molecular radius of a water molecule and calculating the volume. Dividing a know volume, say one meter cubed, by the volume of the water molecule to solve for the total density of molecules in a set volume.*

3.3.1.1 Gibbs free energy of Reaction & Determination of Critical Radius

We will analyze the heterogeneous formation of ice crystals. The role of Gibb's free energy will be examined from which a critical radius will be determined and further growth will be calculated based on the variables of time and final temperature.

The crystal structure of ice will become important during heterogeneous nucleation. At normal atmospheric pressures and temperature above -100°C, ice will form a hexagonal-like structure as shown in Figure 6.

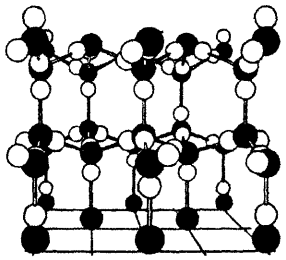


Figure 6 An expanded model of the structure of ordinary ice. Black spheres represent oxygen atoms and white spheres hydrogens, of which there are two attached to each oxygen. The rods represent hydrogen bonds.[23]
(Reproduced from WR Cotton, 2004, Atmospheric Thermodynamics & Microphysics of Clouds, Academic Press)

Heterogeneous nucleation occurs in the presence of a foreign particle. In this study, we will assume the collagen fibers will provide the nucleation sites for ice crystal formation. Physical parameters such as surface tension and lattice structure will be estimated for use in the following equations.

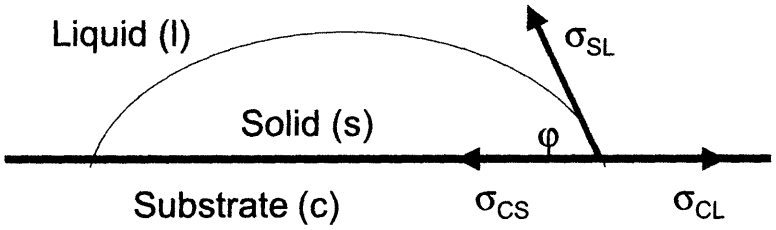


Figure 7 Balance of forces in order to solve for the contact angle between the substrate and the solid ice crystal.[23]
(Reproduced from WR Cotton, 2004, Atmospheric Thermodynamics & Microphysics of Clouds, Academic Press)

$$\sigma_{SL}\cos\varphi + \sigma_{CS} = \sigma_{CL} \tag{1.1}$$

We will have to examine the nucleation of the ice crystal at the microscopic level, including the lattice structures of the ice and the substrate. During a standard contact angle measurement the solid will form on the planar substrate at an angle, labeled φ. This angle is a

function of the surface tensions of both the solid ice crystal and the substrate, in our case, the collagen fibers. The forces will balance based on these surface tensions and the angle created by the solid droplet.

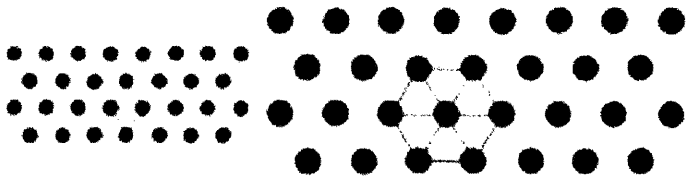


Figure 8 The central and peripheral collagen fiber matrix. Lattice parameter is on the order of 15 Angstroms (Å). (Reproduced from WR Cotton, 2004, Atmospheric Thermodynamics & Microphysics of Clouds, Academic Press)

Examining equation 1.1, by increasing the value of σ_{cs}, the force between the substrate and the solid, the contact angle $\cos\varphi$ will decrease as φ increases. We will assume an angle of 55° is created between the surfaces of the collagen and liquid and the collagen and solid ice crystal. This angle is based on discussions between members of this research group, although an actual measurement has not been made. The shape factor[23], $S(\theta)$, is defined as the shape of the crystal. It is described using the contact angle between the solid and the substrate collagen. Equation 1.2[23] and 1.3 shows the shape factor equation. Using $\varphi = 55°$ we are able to solve for the shape factor of the ice crystal. This will be used later in the calculation of the Gibb's free energy.

$$S(\varphi)=\frac{(2+\cos\varphi)(1-\cos\varphi)^2}{4} \tag{1.2}$$

$$S(\varphi)=0.1169 \tag{1.3}$$

The next step is to determine the surface energy between the collagen and the liquid water to determine the force necessary to nucleate ice. This will be taken into consideration during the calculation of Gibbs Free Energy. The surface energy[24] equation uses the surface tension, the dipole moment as well as the molecular density of water. These values can be found in the table of properties for liquid water and ice.

$$\sigma_{CL}=\frac{\mu^2 n}{4\pi\varepsilon_o}(n^{2/3})=130\frac{mJ}{m^2} \tag{1.4}$$

We can use this information to find the change in Gibbs Free Energy (ΔG) for nucleation on the collagen substrate. We will be calculating the Gibbs Free Energy for the reaction of liquid water to solid ice.

$$H_2O_{(l)} \leftrightarrow H_2O_{(s)}$$

The driving force for nucleation[25], ΔG_v, can be defined using the latent heat of fusion for water, the change in temperature of the solidification process and melting temperature of ice. These values are all given in table 1 and 2.

$$\Delta G_v = \frac{L_F \Delta T}{T_m} \tag{1.5}$$

From this driving force, we are able to solve for the heterogeneous Gibbs free energy change at the critical radius. This is shown in equation 1.6.

$$\Delta G^*_{het} = \frac{16\pi\gamma^3_{SL} S(\theta)}{3\Delta G_V^2} \tag{1.6}$$

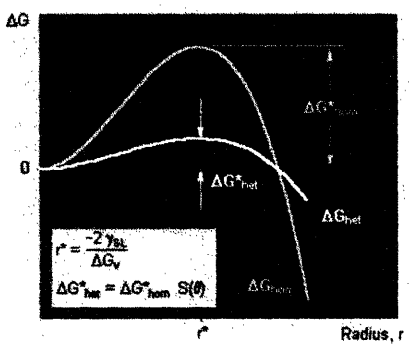


Figure 9 Gibbs free energy diagram comparing the free energies of heterogeneous and homogeneous nucleation at the critical radius, r*.[25]
(Reproduced from Porter & Easterling, 1992, Phase Transformation in Metals and Alloys, 2nd Edition, CRC Press)

The Gibbs free energy provides an idea of the tendencies of the reaction as well as supplying a comparison of homogeneous and heterogeneous nucleation. In Figure 9, a standard Gibbs free energy versus critical radius diagram illustrates the profound difference in energy required to reach the critical nucleus size. The Gibbs free energy for homogeneous nucleation is much greater than its corresponding heterogeneous energy. It is much more difficult for a particle to nucleate spontaneously with other molecules to form a critical sized particle than it is for molecules to find a new surface or established foreign particle by which it can latch on and join other particles. Although the same critical radius must be achieved to begin growth, introducing surfaces for nucleation can reduce the energy required. The critical radius equation is shown in equation 1.7.

$$r_{het}^* = \frac{-2\gamma_{SL}}{\Delta G_{het}} \tag{1.7}$$

It is dependent on the Gibbs free energy of heterogeneous nucleation and the surface tension between the solid and liquid surfaces. We are assuming that this value, γ_{SL}, is approximately 73 dynes/cm as it is listed in Table 2.

In order to understand the wide variations of pore size available within this process, Figure 10 is provided to show the dependence of the critical radius on the heterogeneous Gibbs free energy.

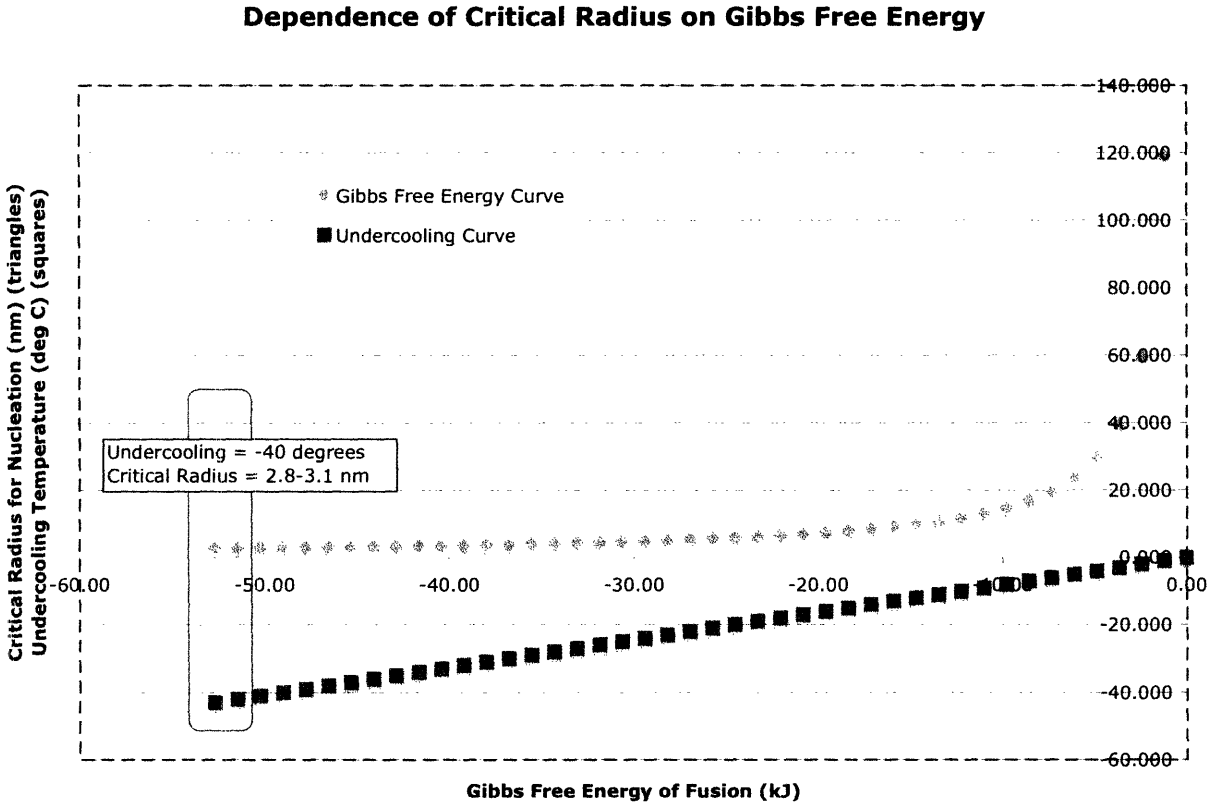


Figure 10 Gibbs free energy of heterogeneous nucleation. Triangle data points indicate the critical radius of nucleation as it depends on ΔG. In order to solve for a known process an undercooling curve (squares) is provided to gauge the correct critical radius.

It is important to notice that the critical radius increases with decreased undercooling. This makes sense, as it is easier to nucleate ice crystals far below the natural freezing temperature. Also, as the Gibbs free energy becomes more and more negative the radius continues to decrease. This would indicate that the reaction is favoring the formation of ice crystals and requires much less energy to complete the transformation.

By examining the chart and overlapping the critical radius with our desired undercooling temperature of -40°C we can see that our critical radius is between 2.8 and 3.1 nm. For calculation purposes we will assume that the average radius is 3.0nm. It would be interesting to figure out how many molecules of H_2O are contained within a particle of roughly 3.0nm.

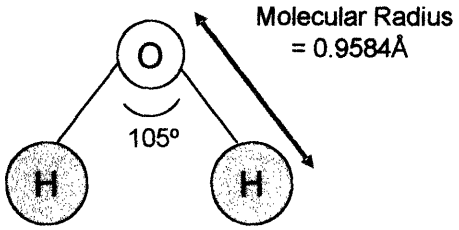


Figure 11 Water molecule and its associated molecular radius.

The volume of a sphere is given in equation 1.8.

$$V_{sphere} = \frac{4}{3}\pi r^3 \tag{1.8}$$

Solving by setting r = 3.0nm we are able to find the volume of the nucleated particle to be V_{sphere}= 1.13E-25 m^3. The molecular radius of a water molecule is roughly 0.9584 Å. The volume a single water molecule is V_{H2O}=3.687E-30 m^3. Dividing the volume of the sphere by the volume of the individual water molecule will give us a rough estimate of how many molecules have to spontaneously gather to form a particle of critical size.

$$\frac{V_{sphere}}{V_{H2O}} = \frac{1.13E-25}{3.687E-30} = 30,699 molecules \tag{1.9}$$

So, approximately 30,000 water molecules must come together to form a particle of critical size. The next step in quantifying the lyophilization process is determining the nucleation rate and growth rate of the particles once they have reached critical size.

3.3.1.2 **Nucleation & Growth Rate**

The nucleation rate describes the number of molecules forming per meter cubed per second onto the nucleation surface. This number is important because it can determine the induction time needed to reach the critical radius size for continued development controlled by the grow rate. Equation 1.10 shows the general form of the nucleation rate.[25]

$$C^* = C_o \exp[\frac{\Delta G}{k_b T}] \tag{1.10}$$

However, because we do not know the exact value of the correction factor, C_o, we must estimate the nucleation rate based on published data[8]. We will assume that the nucleation rate is approximately: $C^* = 0.01\ nm^{-3}s^{-1} \approx 10^{25}\ m^{-3}s^{-1}$. This is a very high nucleation rate, but due to the high surface area of the collagen fibrils, the availability of nucleation sites is increased. In addition, the large undercooling will promote a high rate of nucleation because the reaction will tend to move toward crystallization instead of remaining in the liquid phase.

The induction time refers to the amount of time, in seconds, for molecules of water to arrange themselves in such a way as to nucleate a particle of critical radius. Equation 1.11 shows the dependence of this time on the nucleation rate as well as two constants G and α, whose values are included in Table 2.[24]

$$t = \left(\frac{3\alpha}{\pi G^3 C^*}\right)^{\frac{1}{4}} \tag{1.11}$$

When all constants are inserted and the calculation is complete, the induction time is approximately 151 seconds or 2.5 minutes. This seems very reasonable; previous research shows that time to completely freeze the bone regeneration scaffold is approximately 8 minutes.[14] We will further quantify the freezing time during the discussion of growth rate.

The growth rate of the molecules will be very important to determine the length of freezing time in the freeze-dryer as well as to derive an appropriate economic analysis based on time to manufacture these scaffolds. Because we are undercooling to a very low temperature of -40°C, the growth rate must be carefully controlled to regulate the final size of the particles. For a large undercooling the nucleation rate dominates over the growth rate. The time for growth to occur is limited due in part to the ramping rate of the freezer as well as the fact that when undercooling is very high, nucleation of particles is more favored over growth. However, we are sure to provide enough time during the annealing procedure for adequate coarsening and ripening to occur.

The growth rate of our system is given by the expression in equation 1.12 and is measured in meters per second.

$$\frac{\Delta r}{\Delta t} = x\frac{m}{s} \tag{1.12}$$

In order to find the growth rate of our process several assumptions must be made. The first is that the mean pore size of our final scaffold is 125μm with an average radius of 62.5μm.

We will denote this as the final radius, r_F. We will denote our critical radius as our initial radius and label it r_C. The next assumption is that the final radius is reached in a fixed amount of time programmed into the freeze dryer. This time is based on two factors:

1. The change in temperature during the freezing portion is 60°C, from 20°C to -40°C.
2. The ramping rate of the freezer over this temperature range is -0.9°C/min.

Delta t can be determined from equation 1.13.

$$dt = \frac{\Delta T}{\frac{^\circ C}{\min}} = x \min. \tag{1.13}$$

We can find that the time over which freezing occurs is approximately 66.67 minutes or 4000.2 seconds. By dividing our change in radius over our change in time we are left with an approximate growth rate for our ice particles. (Eq. 1.14)

$$\frac{dr}{dt} = \frac{(r_F - r_C)}{4000.2s} = \frac{62.497\mu m}{4000.2s} = 0.0156\frac{\mu m}{s} \tag{1.14}$$

Growth rate can be controlled by either diffusion or reaction limited growth. Equation 1.15 shows the mathematical expression for diffusion limited growth:[25]

$$\frac{dr}{dt} = \frac{DV_M(C_o - C_i)}{r_F} \tag{1.15}$$

The variables include D, the diffusion coefficient, V_M, the molecular volume and the change in concentration around the particle of known radius. Molecular volume is calculated by dividing the atomic volume by the molecular volume. The value used here is V_M=1.093 and is a unitless number. This equation has two unknowns, both the diffusion constant and the concentration gradient. We will have to use a second equation to solve for one of these unknowns. Equation 1.16 shows the expression for the diffusion coefficient, D.

$$D = \frac{k_b T}{6\pi v r_F} = \frac{(1.3E-23 \times 233.15K)}{6\pi(1.10E-3\frac{Ns}{m^2} \times 62.5\mu m)} = 2.4828E-15\frac{m^2}{s} \tag{1.16}$$

In order to solve for the value of the reaction limited growth we are going to have to make an assumption about the concentration gradient, ΔC. We can assume that the gradient is approximately one and ignore it from both equations. Reaction limited growth is expressed in terms of $k_d r$ and is shown in equation 1.17.[25]

$$\frac{dr}{dt} = k_d V_M(\Delta C) \tag{1.17}$$

Substituting known values we are able to determine the k_d is equal to

$$0.0156\frac{\mu m}{s} = k_d(1.093)(1) \rightarrow k_d = 1.43E-8\frac{m}{s} \tag{1.18}$$

To determine which kind of growth we have, either diffusion or reaction limited, we must compare the values. The reaction limited growth variable must be multiplied by the radius of the final crystal. The following law applies: when...

$D \ll k_d r$ Diffusion Limited Growth

$D \gg k_d r$ Reaction Limited Growth

The value of $k_d r$ is shown in equation 1. 19.

$$k_d r_F = (1.43E-8\frac{m}{s})(62.5E-6m) = 8.92E-13\frac{m^2}{s} \tag{1.19}$$

Comparing the values of D and $k_d r$, it is obvious that $k_d r$ is much larger lending itself to the conclusion that the growth rate is limited by diffusion. ($D \ll k_d r$) Diffusion limited growth is shown in Figure 12 with a brief explanation of why this would make sense.

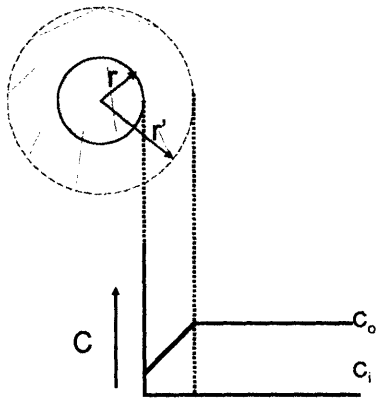


Figure 12 Diagram showing the diffusion limited growth of the ice particle. The concentration of the surrounding solute increases with the growth of the ice crystal.[25]
(Reproduced from Porter & Easterling, 1992, Phase Transformation in Metals and Alloys, 2nd Edition, CRC Press)

The solute surrounding the nucleated ice particle contains impurities such as unbound ions, collagen particles and polysaccharide molecules. As the particle grows and water particles are absorbed from its surroundings, a highly concentrated solute is left behind. As the nucleated particle grows and the concentration becomes greater, the ability of new water molecules to reach the surface of the ice crystal decreases, ultimately leading to diffusion-limited growth. Reaction limited growth would imply that the environment of the ice particle favored

dissociation of the particle, or melting, instead of crystallization. Due to the high undercooling experienced by this solution it makes sense that the reactions between the water molecules would favor crystallization.

3.3.1.3 **Process of Sublimation**

The process of sublimation constitutes the transition of a material from the solid phase to the gas phase without undergoing melting. As shown in Figure 5, sublimation occurs below the triple point, at very low pressure. The temperature can be increased past the equilibrium melting temperature of 273K due to the phase change below the triple point.

It is important to show that the pressures we are reaching during sublimation are sufficient to fully evaporate or sublimate the total amount of ice in the scaffold. Using the Clausius-Clapeyron equation and some known points, we are able to derive a function to not only establish that we have met the pressure requirements, but to solve for the enthalpy of vaporization.[26]

$$P = A\exp[\frac{\Delta H_{vap}}{RT}] \tag{1.20}$$

Pressure, P, is measured in mTorr and temperature, T, in Kelvin. The variable A is a correction constant and will be eliminated with the following derivation. If we have two known endpoints, T_1 and T_2, with their associated pressures, P_1 and P_2, we are able to take the integral of equation 20 and use these points to integrate over.[26]

$$\Delta H_{vap} = \frac{R\ln[\frac{P_1}{P_2}]}{(\frac{1}{T_1}-\frac{1}{T_2})} = \frac{R\ln[\frac{2965mTorr}{4560mTorr}]}{(\frac{1}{268}-\frac{1}{273})} = 52.3\frac{kJ}{mol} \tag{1.21}$$

Seeing that our freeze-dryer sublimates at less than 1000 mTorr, we can say it is more than sufficient to complete sublimation. The required pressures at temperatures as low at 268K only require 2965 mTorr, nearly seven times what we are capable of reaching.

3.3.2 **Triple Co-precipitation Method**

Calcium phosphate particles will be synthesized within the slurry solution. By controlling the supersaturation of the solution many factors of the nucleation and growth process can be calculated. Using a supersaturation method we will outline the steps necessary to solve for the final time requirement for this section of the process as well as the effects of calcium to

phosphorous ratio. The supersaturation of the solution can be controlled by temperature and molarity and will dictate the stability of the system as well as the ability of the CaP particles to nucleate and grow. A diffusion reaction equation will be introduced to help solve the growth rate, while mass transport equations will determine the rate of crystallization.

3.3.2.1 **Supersaturation of Solution**

Supersaturation is defined as: to cause a chemical solution to be more highly concentrated than is normally possible under given conditions of temperature and pressure.[22] In our case we will be supersaturating an aqueous solution with calcium and phosphate ions. These ions will move by diffusion and mass transport to the substrate, collagen fibers, and nucleate on the surface. We will assume in this process that the glycosaminoglycan molecules are significantly smaller than the collagen fibers and have no effect on solute formation.

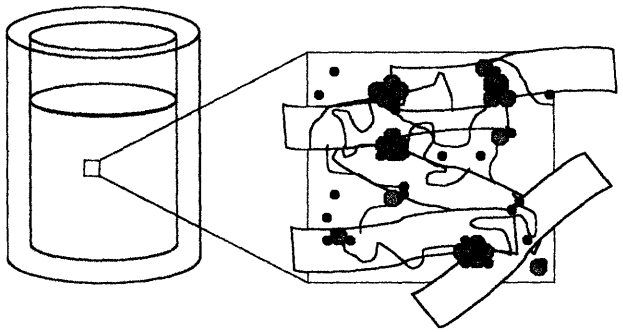


Figure 13 Cartoon expressing the nucleation of the calcium and phosphate ions nucleating in clusters on the collagen. (Larger Circles = calcium, Small Circles = phosphate, Thin Line = glycosaminoglycan, Bars = Type I collagen fibrils)

Supersaturation can be achieved in one of two ways: 1) increasing the calcium content of the solution or 2) increase the pH. As seen in Figure 14, depending on the molarity of calcium within the solution and the environmental pH, the phase formation of calcium-phosphate components can vary drastically. These curves are plotted as solubility isotherms at 25°C. The yellow highlighted area indicates the working zone in terms of pH. The molarity of the solution presented in this study is approximately 10^{-1} to 10^{-2}M.

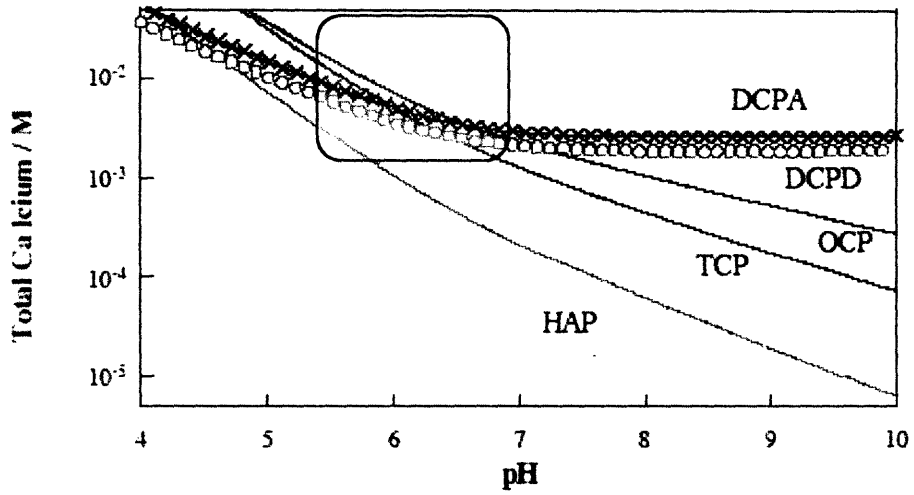


Figure 14 Phase curve derived from calcium supersaturation and environmental pH.[19]
(Reproduced from PG Koutsoukos, 2001, Current Knowledge of Calcium-Phosphate chemistry, 2nd International Conference on the Recovery of Phosphorus from Sewage and Animal Wastes)

At very high supersaturation calcium phosphate precipitates spontaneously, a feat demonstrated by the formation of cloudiness in the aqueous phase upon raising the supersaturation. Before reaching the region however, it is possible to prepare solution supersaturated with respect to calcium phosphate, but the precipitation takes place past the lapse of measurable induction times, following the establishment of the solution supersaturation.[27] The stability of these solutions will be discussed in depth in the following section.

The phases present in the above diagram include, dicalcium-phosphide (DCPD) and dicalcium-phosphate, as well as tri-calcium phosphate (TCP) and hydroxy-apatite (HAP). We are most interested in the TCP, DCPD and HAP phases that, fortunately, are the easiest to precipitate and remain the most stable across many environmental conditions. Our limitations also exist within the pH of the overall solution. Because we are working with organic materials such as collagen and GAG, it is important to prevent the pH from straying too far from the neutral position. We can easily control that by altering the calcium to phosphate ratio in our solution.[19] By examining Figure 14 we can determine the molarity of calcium required in our solution to be less than 10^{-3} Molarity or moles/Liter.

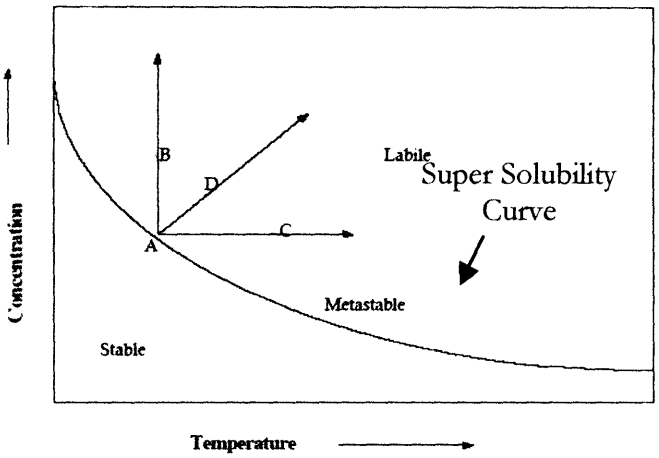


Figure 15 Solubility diagram of a sparingly soluble salt with inverse solubility.[19]
(Reproduced from PG Koutsoukos, 2001, Current Knowledge of Calcium-Phosphate chemistry, 2nd International Conference on the Recovery of Phosphorus from Sewage and Animal Wastes)

Supersaturation is the driving force behind the nucleation and growth processes; therefore, we are spending several sections on this topic. It is also important to understand the stable, metastable and labile regions of the salt solution in Figure 15. This figure assumes a homogeneous environment lacking suspended particles, agitation and maintains a controlled temperature.

If the stable region is deviated from by either an increase in temperature or the concentration of ions, precipitation will occur in order to equilibrate the solution back to the solubility curve. The precipitation in this region will correspond to the induction time calculated. The supersolubility curve is the point at which spontaneous precipitation occurs without the induction time preceding precipitation. At concentration and temperature points within the stable region dissolution will occur should any salt crystals be present. (CaP is referred to here as a salt due to its predisposition to ionize in solution)

For this discussion two variable calcium phosphate conditions will be tested: Constant Collagen Content Formulation & Constant Overall Density Formulation. The definitions of these conditions are below.[14]

Constant Collagen Content Formulation

Tissue regenerating scaffold prepared with a set density (0.0143 g/mL) of Type I collagen and glycosaminoglycan (GAG). An inorganic (calcium phosphate) component ranges in density from 50-75% of the total scaffold density. The overall density of the scaffold will increase as the density of CaP is increased.

Constant Overall Density Formulation

Density of the tissue regeneration scaffold is kept constant at 0.04200 g/mL. The ratio of organic to inorganic phase is balanced as the inorganic (CaP) weight percent is increased. As the CaP weight percent increases (50-75%) the organic collagen-GAG density is decreased.

3.3.2.2 **Rate of Growth & Crystallization**

It was mentioned earlier that by determining the supersaturation of the solution, many other parameters of the manufacturing system could be calculated. The supersaturation of the solution can be expressed in terms of the ion activity product (IAP), which takes into account the chemical activities of the calcium and phosphate ions free within the solution. The other term, $K_S°$, the thermodynamic solubility product, is used to further define the separate solubility of both the calcium and phosphate.[19,22]

$$S = \frac{IAP}{Ks^{\circ}} \tag{1.22}$$

The Ion Activity Product (IAP) is calculated using equation 1.23.[27]

$$IAP = [Ca^{+}]f \tag{1.23}$$

The activity coefficient is represented by f. Table 3 shows the values for the variable conditions investigated during this quantitative analysis.

Table 3 Ion Activity Product

Sample ID	Molarity Ca^{2+}	Activity Coefficient[28], f	IAP
CC50%	0.08353 (5)	0.436	0.0364 (5)
CC75%	0.22145 (1)	0.425	0.0947 (1)
66%	0.14869 (3)	0.434	0.0645 (3)
CD50%	0.11297 (4)	0.435	0.0493 (4)
CD75%	0.1710 (2)	0.431	0.0737 (2)

Solving for the thermodynamic solubility product involves multiplying the molarities of both the calcium and the phosphorus in the solution. See equation 1.24.[27]

$$K_S^{\circ} = [Ca^{+}][P^{-}] \tag{1.24}$$

The results for the thermodynamic solubility product are shown in table 4.

Table 4 Thermodynamic Solubility Product

Sample ID	Molarity Ca^{2+}	Molarity P^{-}	K_S°
CC50%	0.08353 (5)	0.105	8.77E-3 (5)
CC75%	0.22145 (1)	0.254	5.62E-2 (1)
66%	0.14869 (3)	0.1785	2.65E-2 (3)
CD50%	0.11297 (4)	0.1383	1.56E-2 (4)
CD75%	0.1710 (2)	0.2041	3.49E-2 (2)

Plugging the IAP and thermodynamic solubility product into equation 1.22, we are able to solve for the supersaturation of the five samples.

Table 5 Supersaturation of five solutions

Sample ID	IAP	K_S°	S
CC50%	0.0364	8.77E-3	3.19E-4 (5)
CC75%	0.0947	5.62E-2	5.32E-3 (1)
66%	0.0645	2.65E-2	1.71E-3 (3)
CD50%	0.0493	1.56E-2	7.69E-4 (4)
CD75%	0.0737	3.49E-2	2.57E-3 (2)

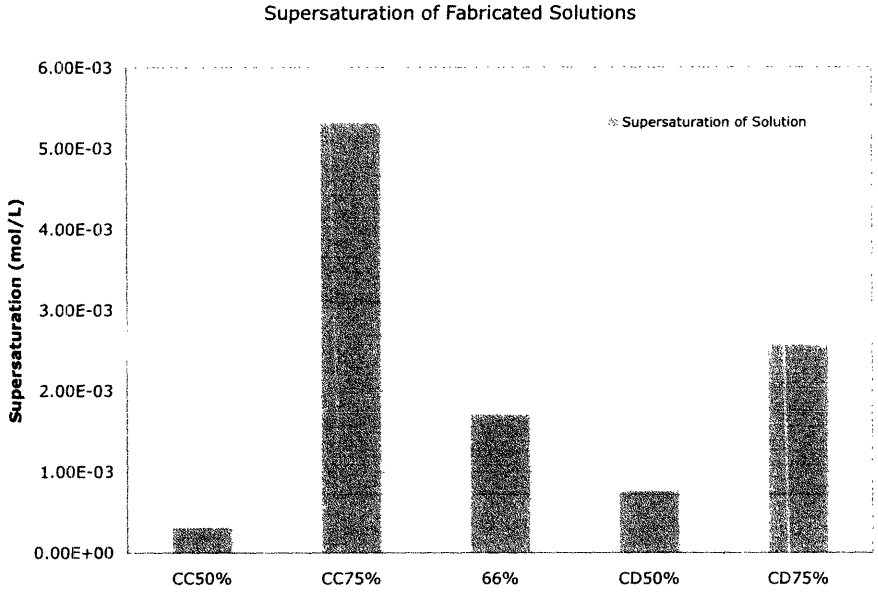


Figure 16 Supersaturation of samples. All samples have supersaturation of the same order of magnitude.

The supersaturation value will help determine the induction time. Induction time refers to the time corresponding to the development of supercritical nuclei, or nuclei that have reached the

critical radius for growth and crystallization. Equation 1.25 shows that the induction time is a function of the supersaturation of the solution and the temperature.[21]

$$\log t = A + \frac{B}{(T)^3(\log S)^2} \tag{1.25}$$

There are constants present in this equation (A & B) that are relative to the calcium-phosphate final phase component. Although A is not able to be determined, the constant B can be solved for using equation 1.26.[21]

$$B = \frac{\beta\gamma^3 V_m N_A f(\theta)}{(2.3R)^3} \tag{1.26}$$

See table 6 for the values of variables used to solve for the constant B.

Table 6

Property	Value
β, Shape Factor	16π/3 = 16.755
γ^3, Surface Tension	0.13 J/m^2
f(θ), Shape Correction Factor for Heterogeneous Nucleation	0.01
V_M, Molecular Volume	61.5 cm^3/mol
T, Temperature (K)	277K
R, Gas Constant	8.31451 J/K·mol
N_A, Avogadro's Number	6.02214E23 mol^{-1}

$$B = \frac{(16.755)(0.13\frac{J}{m^2})^3(61.5\frac{cm^3}{mol})^2(6.022E23\frac{1}{mol})(0.01)}{(2.3(8.31451\frac{J}{mol \bullet K}))^3} = 1.198E8°K^3 \tag{1.27}$$

Table 7 Induction time for supersaturated solutions. (Supersaturation 1-5 where 1 is the highest supersaturation) (Induction Time 1-5 where 1 is the longest time)

Sample ID	Supersaturation	Induction Time (s)
CC50%	0.000319 (5)	0.4611 (5)
CC75%	0.005320 (1)	1.0899 (1)
66%	0.001710 (3)	0.7362 (3)
CD50%	0.000769 (4)	0.5812 (4)
CD75%	0.002570 (2)	0.8402 (2)

It is interesting that the supersaturation is directly proportional to the induction time. Meaning that the higher the supersaturation the longer it takes to create a particle of critical radius. It would have made more sense that the higher the supersaturation the shorter the induction time due to the theory that the solution would want to move to a more stable state quickly. The potential to do this would seem obviously higher in a greatly supersaturated solution.

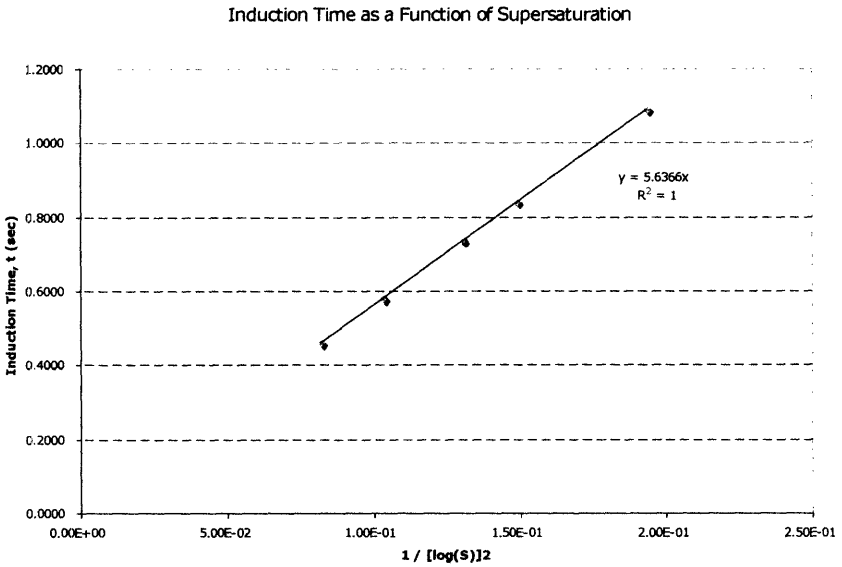


Figure 17 Induction time (sec) vs. 1/(logS)^2. Induction time varies linearly to the supersaturation rate. As supersaturation increase, induction time also increases.

From the induction time, t, we will be able to determine when the growth and nucleation begins and calculating the number of nucleation sites and the molarity of deposits, we will be able to determine the necessary refrigeration time.

3.3.2.3 Nucleation & Growth Rate of Mineral Component

The nucleation rate describes the number of molecules forming per meter cubed per second onto the nucleation surface. For the nucleation of calcium phosphate mineral we will use equation 1.28 shown below.[21]

$$J_s = F\exp[\frac{-\beta\gamma^3 V_M{}^2 N_A f(\theta)}{(RT)^3(\ln S)^2}] \quad (1.28)$$

F is a frequency constant known as a pre-exponential factor. It has a theoretical value of 10^{30} nuclei/cm^3.[21] Solving using the above known variables, the nucleation rate is equal to:

Table 8 Nucleation rate for mineralized scaffold samples. Nucleation rate corresponds to the induction time and supersaturation rate.

Sample ID	Supersaturation	Induction Time (s)	Nucleation Rate, J_S
CC50%	0.000319 (5)	0.4611 (5)	5.2027E+33 (5)
CC75%	0.005320 (1)	1.0899 (1)	2.1881E+36 (1)
66%	0.001710 (3)	0.7362 (3)	9.3638E+34 (3)
CD50%	0.000769 (4)	0.5812 (4)	1.9646E+34 (4)
CD75%	0.002570 (2)	0.8402 (2)	2.4133E+35 (2)

It seems as though there is a trade off between induction time and nucleation rate, so that with a lagging induction time, the nucleation rate increases. It may take longer for the particle to nucleate, however, once it does, the highly supersaturated solutions more rapidly solidify.

We have yet to determine the critical radius for nucleation of the mineral component. This turned out to be a very interesting section. The critical radius is calculated to be approximately one molecule of the desired mineral phase. This is very convenient for further calculations and assumptions.

The critical radius can be found in two steps. The first is using the nucleation rate, the theoretical constant F, and known temperature of reaction and substituting into equation 1.29. Equation 1.30[21] details the way to find the free energy barrier, ΔG_{CR}.

$$J_s = F\exp[\frac{-\Delta G_{CR}}{kT}] \quad (1.29)$$

Substituting in the known values for Boltzmann's constant, temperature (K) and constant F and associated values for the nucleation rate, J, we are able to accurately measure the free energy barrier, ΔG_{CR}.[21]

$$\Delta G_{CR} = \frac{4}{3}\pi r^2 \gamma \tag{1.30}$$

Table 9 Determination of critical radius of mineral cluster. Calculates to approximately 1 molecule of CaP. (CaP molecular radius = 0.290 nm)

Sample ID	ΔG_{CR}	Critical Radius (nm)
CC50%	3.27E-20	0.245088
CC75%	5.58E-20	0.320124
66%	4.38E-20	0.283474
CD50%	3.78E-20	0.263430
CD75%	4.74E-20	0.294963

The growth rate of the mineral component is handled as a molar rate deposition. We will approach this similarly to the way we handled the ice crystal nucleation. We will calculate the change in molarity of the solution from start to finish based on the limiting agent. In all cases this is the phosphate ion. Table 10 shows the ratio of calcium to phosphorous.

Table 10 Ratio of calcium to phosphate molarity.[21]

Sample ID	Ratio Ca:P
CC50%	1:0.27
CC75%	1:0.29
66%	1:0.31
CD50%	1:0.32
CD75%	1:0.31

Equation 1.31[29] is the rate of growth for the CaP mineral. Being a function of the surface area of deposition (A) as well as the molar rate deposition (dm/dt), the rate of growth (R_g) can be monitored and ultimately controlled.

$$R_g = \frac{1}{A}\left(\frac{dm}{dt}\right) \tag{1.31}$$

First we will determine the molar rate of deposition based on the molarity of phosphate in the samples as well as the change in time, which varies with each sample. The time shown in Table 11 reflects the current laboratory protocol for mineralized CG scaffolds. See table 11 for these values.

Table 11 Growth rate and associated variables for mineralized scaffolds.

Sample ID	ΔM, Molarity	Δt (min)	dm/dt (M/min)	1/SA ($1/nm^2$)	R_g ($M/min \cdot nm^2$)
CC50%	0.27	720	0.00038	1.32478383	5.03E-04
CC75%	0.29	30	0.00967	0.77652128	7.51E-03
66%	0.31	360	0.00086	0.9902942	8.52E-04
CD50%	0.32	1440	0.00022	1.14672283	2.52E-04
CD75%	0.31	30	0.01033	0.91465026	9.45E-03

We are going to assume several aspects of the calculation:

1. The surface area calculated will be based on the nucleated radius found in table 9.
2. The reaction is 100% complete when the limiting factor (P) is completely deposited.
3. Phosphate ions (P) are the limiting agent.

Now knowing that the critical radius is roughly equal to the molecular radius we will compare our calculated values with the actual lab values allowed for growth.

Table 12 Comparison of calculated time for reaction to the allowed laboratory time (min).

Sample ID	Time Required for 100% Reaction (min)	Laboratory Time Allowed (min)
CC50%	50	720
CC75%	8.6	30
66%	54	360
CD50%	142	1440
CD75%	5.6	30

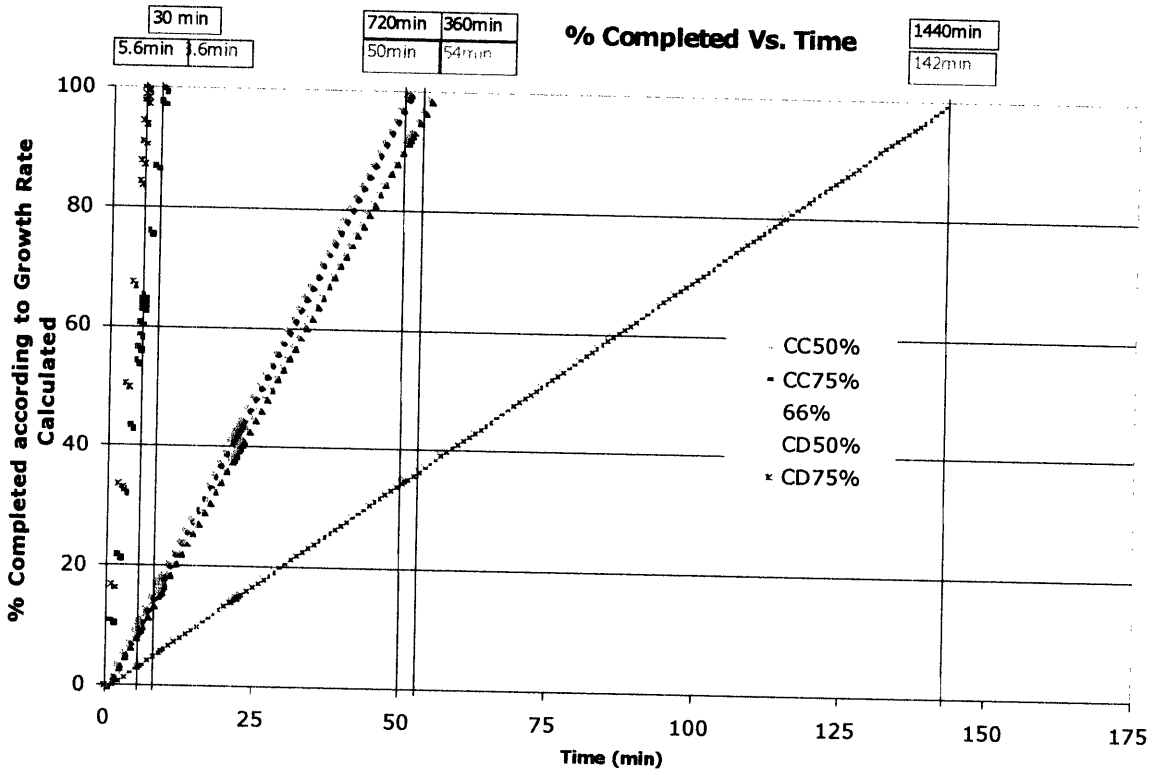


Figure 18 Above chart shows the characteristic time associated with 100% reaction for the mineralized scaffolds. The times in the black boxes correspond to the lab times allowed for this reaction.

So, what is so interesting about these findings? Well, the fact that all of the previous cost models took into account these long reaction times it had a great effect on the cost per sheet. The calculated growth times are nearly 90% less than those currently used. It will be interesting to see the effect during the cost modeling of this process.

3.4 Control of Degradability

As with any biodegradable material, the degradation and erosion rate must be controlled. The process of 'degradation' describes the chain scission process during which polymer chains are cleaved to form oligomers and finally to form monomers. 'Erosion' designates the loss of material owing to monomers and oligomers leaving the polymer.[30] Figure 19 shows a schematic representation of the differences between surface erosion and bulk erosion. The degradation rate refers to the time period or rate of biological breakdown of the scaffold as the body regenerates or secrets enzymes in the environment surrounding the implant. Allowing degradation to occur prevents the need to reenter the wound site and remove the device. There are several ways to

control the degradation rate of collagen based regeneration templates: photo-, thermal-, mechanical and chemical degradation.[31]

Polymer degradation occurs during the cleavage of bonds through two mechanisms: passively by hydrolysis or actively by enzymatic reaction.[34] Once implanted, the mineralized collagen-GAG scaffold is subject to both passive and active degradation. Being a natural polymer it is very susceptible to enzymatic degradation as it contains polysaccharides and proteins. It will be difficult to quantify how much of each degradation mechanism adds to the entire process, so each will be explained. There are several factors that manipulate the rate of degradation. These include the types of chemical bonds present along the backbone of the polymer as well as the functional groups, the pH of the surrounding environment, copolymer composition and water uptake of the polymer.[31]

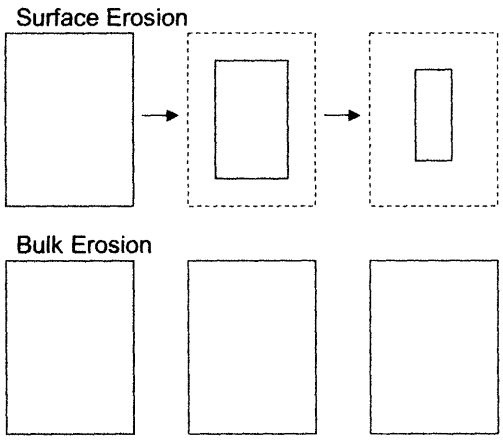


Figure 19 Schematic representation of the differences between surface erosion and bulk erosion.[31]
(Reproduced from A. Gopferich, 1996, Mechanisms of polymer degradation and erosion, *Biomaterials*)

Components of the bones' natural extracellular matrix (ECM) have been artificially combined in order to encourage the naturally regenerating process to occur efficiently and uninterrupted. For that reason a mineralized version of the collagen-GAG matrix was developed. The major constituents of ECMs in various organs are the collagens, a family of fibrous proteins that account for about one-third of the total protein mass in vertebrates. Members of the collagen family show tissue-specific differences in amino acid composition and occasionally in higher levels of structural order; at least 19 unique gene products or types of collagen have been described.[35] Type I collagen has been found most abundantly in the body in tissue such as bones, tendons and skin. An unusual amino acid composition and a characteristic wide-angle x-ray diffraction pattern, reflecting a triple helical structure, distinguish collagen from other tissue

components.[35] In addition to the collagen a natural polysaccharide crosslinker is incorporated. This study focused on the use of glycosaminoglycan, specifically chondroitin-6-sulfate.

There are a variety of GAG's available, including the currently used chondroitin-6-sulfate, heparan sulfate, haparin, dermatan sulfate and keratan sulfate. GAG chains are copolymerized to the collagen fibers during the triple co-precipitation method mentioned above. With the addition of GAG to the collagen in a slightly acidic solution (ex. phosphoric acid) covalent bonds are formed. This precipitation process is dependent on the presence of sulfate groups on the GAG of choice. For this reason, a popular GAG, hyaluronic acid, is not used due to its lack of sulfate groups. Both collagen and chondroitin-6-sulfate are incredibly soluble under the environmental conditions of implantation and must be crosslinked to control their biodegradability.

The disadvantages of using collagen as a biomaterial for tissue repair are its low biomechanical stiffness and rapid biodegradation.[32] Collagenous matrices are usually stabilized by crosslinking to maintain their stability during implantation.[33] All biodegradable polymers contain hydrolysable bonds. Their most important degradation mechanism is, therefore, chemical degradation via hydrolysis or enzyme-catalyzed hydrolysis.[31] Therefore, it is necessary to crosslink the collagen-GAG scaffold by either physical or chemical means. Sterilization must also be completed by γ-sterilization, after which a significant loss of molecular weight can be observed.[31]

Physical crosslinking refers to dehydration through the exposure of the scaffold to high heat and vacuum. This is referred to here as DHT (dehydrothermal crosslinking). It has been shown that by exposing the scaffold to temperatures equal to or exceeding 105°C at atmospheric pressure for a time period of at least 4 hours or at high vacuum at a temperature of 25°C, crosslinking will result in creating average molecular weights between crosslinks of 2.5 to 25kDa.[34] By removing the majority of water in this way the average molecular weight between crosslinked sections is increased. Molecular weight is a function of the polymer length and the individual atomic weights of the molecules making up the polymer chain. Removal of water below one weight percent causes the coprecipitate to become insoluble by promoting a condensation reaction to form inter-chain amide links.[35] Physical crosslinking protocols are carefully controlled due to the sensitive nature of the collagen.

Chemical crosslinking is achieved through the use of aldehydes and the covalent bonding that occurs between the lysine side chains.[33] Research has been completed on several methods of crosslinking for collagen-based tissue engineering scaffolds. These include the use of glutaraldehydes (GTA) and glycation with ribose, EDC/NHS (1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide and N-hydroxysuccinimide), diimidoesters such as dimethyl suberimidate (DMS) and dithiobispropionimidate (DTBP). [33, 36, 37] Although GTA is able to induce the maximum number of crosslinks it induces cytotoxicity from solution remaining and leaching out of the scaffold post implantation.[33] The mineralized collagen-GAG matrices utilized the EDC/NHS crosslinking method to covalently bind chondroitin-6-sulfate to the Type I collagen. The crosslinking solution is easily removed and neutralized within the scaffold to prevent leaching after implantation. It also allows for adequate molecular weight distribution between crosslinks. Degradation and erosion can be controlled in this way.

3.5 Mechanical Properties

3.5.1 Compression Testing Data

A displacement controlled compression test was preformed. Displacement rate was calculated at 1% deformation of the scaffold height per second or 0.0003 meters per second. As with any compression test, as the testing specimen was compressed at a set rate, the associated force required was recorded. A standard stress versus strain graph was obtained from the collected data. Engineering stress (1.32) and strain (1.33) were calculated with the following equations, where h_o is the original height of the scaffold and h is the height at time t, corresponding to a particular force data point. It is assumed that the area of the scaffold face is constant during compression. More detailed experiments can be completed to account for Poison's Ratio.

$$\sigma = \frac{Force(Newtons)}{Area(m^2)} \tag{1.32}$$

$$\varepsilon = \frac{h_o - h}{h_o} \tag{1.33}$$

After data has been manipulated to solve for stress and strain these data points are graphed against each other to get an informative chart from which the associated Young's modulus,

collapse plateau modulus and ultimate yield or collapse stress for each scaffold are obtained. An example stress-strain graph is shown in Figure 20.

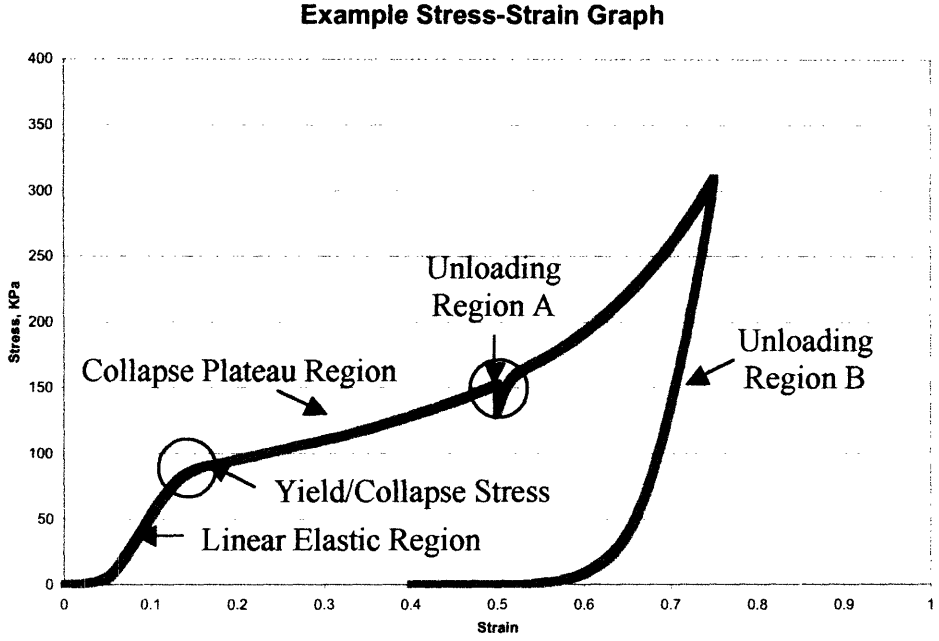


Figure 20 Stress-strain curve for a scaffold

The linear elastic region represents the mechanical interactions at the atomic level of the collagen and the calcium-phosphate mineral component. Under the linear elastic conditions of stress and strain the inter-atomic distances are being compressed and stressed. This region is considered elastic because when the force is released the atoms and molecules will return to their original position and orientation. The collapse stress represents the failure of the collagen struts and the point at which the open porous structure begins to collapse. The mineral component may be breaking away from the collagen struts at this point in mechanical failure. The collapse plateau region represents plastic deformation of the scaffold. In other words, with the removal of the force, the scaffold will not return to its original shape or orientation, it has been permanently deformed.

The slope of the linear elastic region is known as the elastic modulus or the Young's modulus. It is measured in Pascals or Newtons per meter squared. The collapse stress is also measured in Pascals, as is the collapse plateau modulus. These values will be measured for each of the conditions and compared.

3.5.1.1 **Results by Calcium-Phosphate content formulation**

The variable conditions being tested in this study span two issues: mineral content ratio to collagen-GAG content and the effects of crosslinking. There have been two conditions introduced: Constant Density Scaffolds and Constant Collagen Scaffolds. These have been previously defined (page 40-41). A simple testing matrix is supplied in Table 13. The 66% calcium-phosphate content scaffold has the same formulation for both conditions of constant density and constant collagen; the data will be stated only once.

Table 13 Testing Matrix for Mineralized Collagen-GAG Scaffolds

Sample ID (Condition & Percent CaP Content)	Crosslinked	Non-Crosslinked
	Constant Collagen 50%	Constant Collagen 50%
	Constant Collagen 75%	Constant Collagen 75%
	Mineral Content 66%	Mineral Content 66%
	Constant Density 50%	Constant Density 50%
	Constant Density 75%	Constant Density 75%

The constant collagen data will be introduced first, followed by the constant density data. Modulus calculations will be compared as well as the overall trends in the graphs. Comparison between the crosslinked and non-crosslinked data will be completed within each subset of formulation conditions. All the data will be compared to natural healthy trabecular bone data in the next section.

3.5.1.2 **Constant Collagen Mechanical Testing**

Constant collagen scaffolds were manufactured using a slurry fabrication method followed by lyophilization with a final temperature of -40°C. Compression testing was completed with the methods outlined above on at least four dry, 8mm punch samples extracted from the final scaffolds.

Figure 21 shows two columns of stress-strain graphs. The left column is the non-crosslinked versions of the constant collagen formulations. The EDC/NHS chemically crosslinked scaffolds are shown in the right. Figure 21.a shows a beautiful representation of the compression testing of the non-crosslinked constant collagen 50% CaP content scaffolds.

Obvious linear elastic regions exist at strains between 5-20% with associated collapse stresses within error of 85kPa. The collapse plateau regions are consistent between scaffolds. The slight toe region present from 0-5% is due to the uneven top surface of the tested specimen.

Figure 21.c is also a reasonable representation of the 66% calcium-phosphate content scaffolds. With relative reproducibility between specimens, the linear elastic region is again located between 5-20% strain with associated collapse stresses averaging 90kPa. The collapse plateau region is easily recognized with a slight shift in a single testing specimen. A loading-unloading step was included in several of these tests to compare with the loading modulus of the linear elastic region.

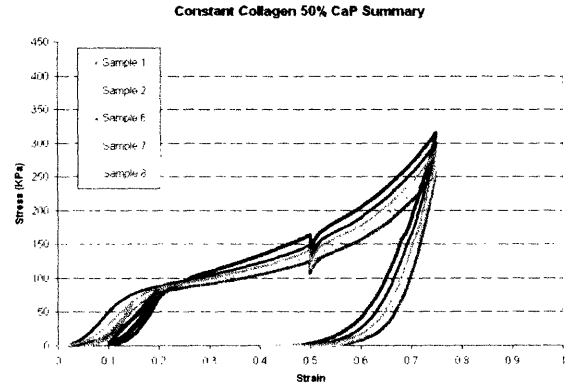
Figure 21.e represents the 75% CaP constant collagen compression testing. The stress-strain graphs produced here do not clearly display a linear elastic, collapse stress or collapse plateau region. It is presented here to provide complete data on all tested specimens but may be considered a 'poor' test result that may have been due to non-parallel surfaces or increased scatter in the microstructure of the scaffold.

Comparison of the crosslinked samples to the non-crosslinked samples yields a very important difference. The Young's modulus or linear elastic modulus is significantly less, in some cases nearly 80% lower, in the crosslinked samples. This lower modulus indicates a less stiff material. The higher the Young's modulus the stiffer the material is. The linear elastic region has been explained as the recoverable region of strain or displacement. This region is the most important in a load-bearing scaffold as this is the operating region for support. The collapse plateau region moduli data are not as drastically different and the data indicates that the collapse of the porous structure is similar in both the crosslinked and non-crosslinked samples. Ultimately, the chemical crosslinking process compromises the overall strength of the scaffold and a detrimental change is observed in the average collapse stress or peak stress.

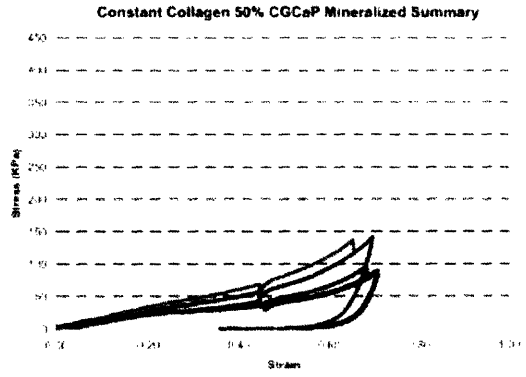
The collapse stress indicates the largest load that can be applied to the scaffold before permanent deformation and pore collapse occurs. It was expected that the crosslinked samples would display a much higher collapse stress than the non-crosslinked samples. However, the data shows that the in the 66% and CC75% samples the collapse stress is not nearly as high as needed for this type of load bearing environment. Although conclusive tests have not been completed, a possible explanation for the drastic decrease in collapse stress is the length of time that elapsed between initial fabrication and crosslinking. Although the scaffolds were kept in an operational

dessicator for approximately three months prior to crosslinking, the exposure to air and humidity while handling may have compromised the chemical bonding between the collagen fibers and chondroitin-6-sulfate, resulting in a ultimately weaker scaffold. In future batches the chemical crosslinking process should occur as quickly after fabrication as possible.

Some general observations reveal that each of the tested specimens exhibited a characteristic stress-strain curve. Independent of the actual values, each curve has representative regions of the expected stress-strain curve. Each crosslinked sample had a linear elastic region located between 5 and 25% strain. The collapse plateau region showed a comparatively lower modulus than the linear elastic region. The unloading curves in each of the samples exhibited linearity and the reproducibility of the plots lends itself to consistent testing and manufacturing methods. The results of the crosslinked samples are not what was expected, however, there are several variables that cane be modified to continue the investigation of load-bearing mineralized CG scaffolds. These include the method of crosslinking and density of scaffolds. Further investigation of crosslinking methods will be carried out in the coming months. It will be determined if the chemical crosslinking procedure used on these scaffolds was detrimental to the calcium phosphate deposits and their associated mechanical properties.



(a)



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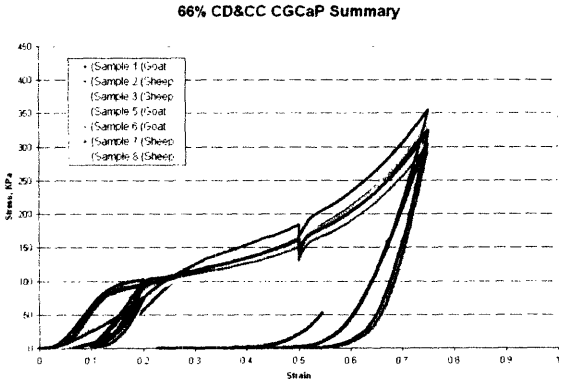

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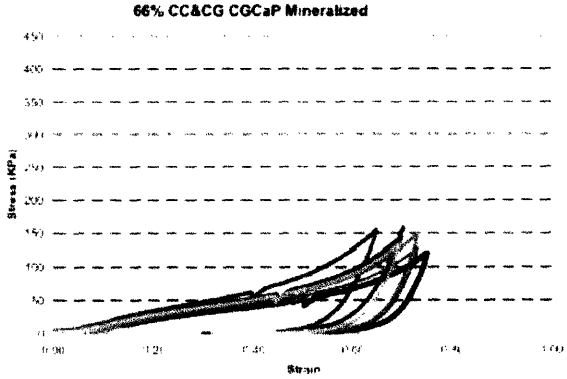

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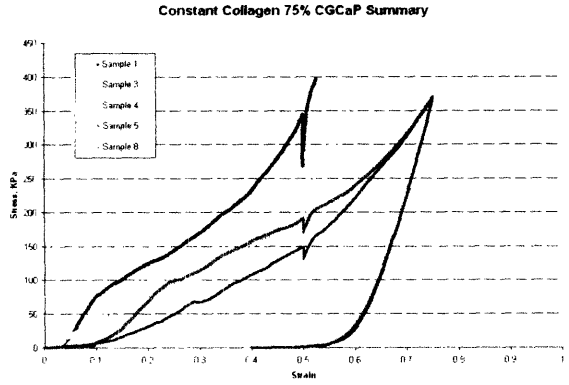

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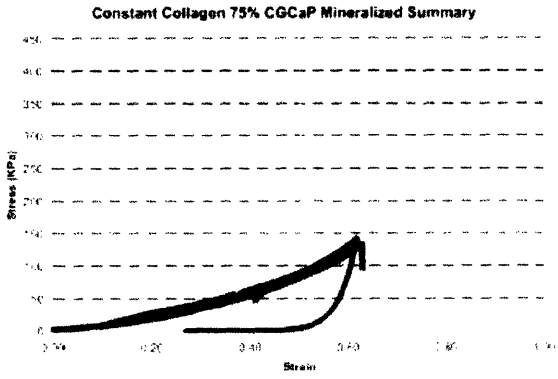


(f)

Figure 21 Constant Collagen (CC) Stress-Strain Graphs

Table 14 Loading Moduli & Collapse Stress

	Average Collapse Stress (kPa)		Young's Modulus (kPa)		Collapse Plateau Modulus (kPa)	
	Crosslinked	*Non-Crosslinked*	*Crosslinked*	*Non-Crosslinked*	*Crosslinked*	*Non-Crosslinked*
CC50%	26	85	157	798	90	173
CC66%	22	90	164	790	107	217
CC75%	15	87	126	579	141	488

Unloading moduli for the hold at 50% strain and the release of load at the end of testing are also reported in Table 15. Unloading moduli play a large part in explaining the relaxation characteristic of the scaffold. These regions are described graphically in Figure 20 on page 52. The moduli data revealed in Table 15 indicate that unloading modulus in region A is very linear and associated R^2 values support that observation. Values of R^2 that approach zero indicate a vertical slope. In terms of these unloading moduli, particularly region A, the low R^2 values indicate that the scaffold is not expanding as the force is relieved during that hold period. The scaffold has entered into the plastic deformation region of its mechanical properties and the displacement induced during the compression test is not recovered, leading to a vertical unloading curve. The negative modulus numbers here indicate that as the strain increases the force decreases. This is a phenomenon encountered with scaffold during the plastic deformation region while unloading occurs.

Unloading region B is the final portion of the test and is the displacement controlled return to a predetermined deformation of 40% strain. The long slow curve is generally linear in over a strain of 75-65%. The slope of this region indicates that the modulus remains high and stiff in this region. The R^2 value reflects linearity and consistency between the samples.

Table 15 Unloading Moduli (Region A & B)

	Unloading Modulus Region A (kPa)			
	Crosslinked	*R^2 Value*	*Non-Crosslinked*	*R^2 Value*
CC50%	-4767	0.0351	-5334.7	0.021
CC66%	26571	0.0094	28910	0.077
CC75%	-28286	0.0385	20873	0.016

	Unloading Modulus Region B (kPa)			
	Crosslinked	R^2 *Value*	*Non-Crosslinked*	R^2 *Value*
CC50%	1150	0.9875	2428	0.987
CC66%	1205	0.9877	2759	0.996
CC75%	469	0.4964	-	-

3.5.1.3 **Constant Density Mechanical Testing**

Figure 22 shows two columns of stress-strain graphs. The left column is the non-crosslinked versions of the constant density formulations. The EDC/NHS chemically crosslinked scaffolds are shown in the right. Figure 22.a shows two poorly characterized scaffolds. With no clearly defined linear elastic or collapse plateau region we would be unable to accurately compare this formulation to others in this set. We will show it here as an example of a poorly characterized mineralized scaffold.

Figure 22.c and Figure 22.d are the same representation of the 66% calcium-phosphate content scaffolds as shown in Figure 21.c and 21.d. Figure 22.e represents the 75% CaP constant density compression testing. The linear elastic region exists is strains ranging from 5-15%. The average peak stress of these specimens is 70kPa. This is relatively less than the 66% CaP content scaffolds. The collapse plateau modulus is easily identified, although maxes out the load cell at approximately 400 kPa. The collapse modulus of these scaffolds will be calculated over the most linear region of the slope, roughly 20-40% strain.

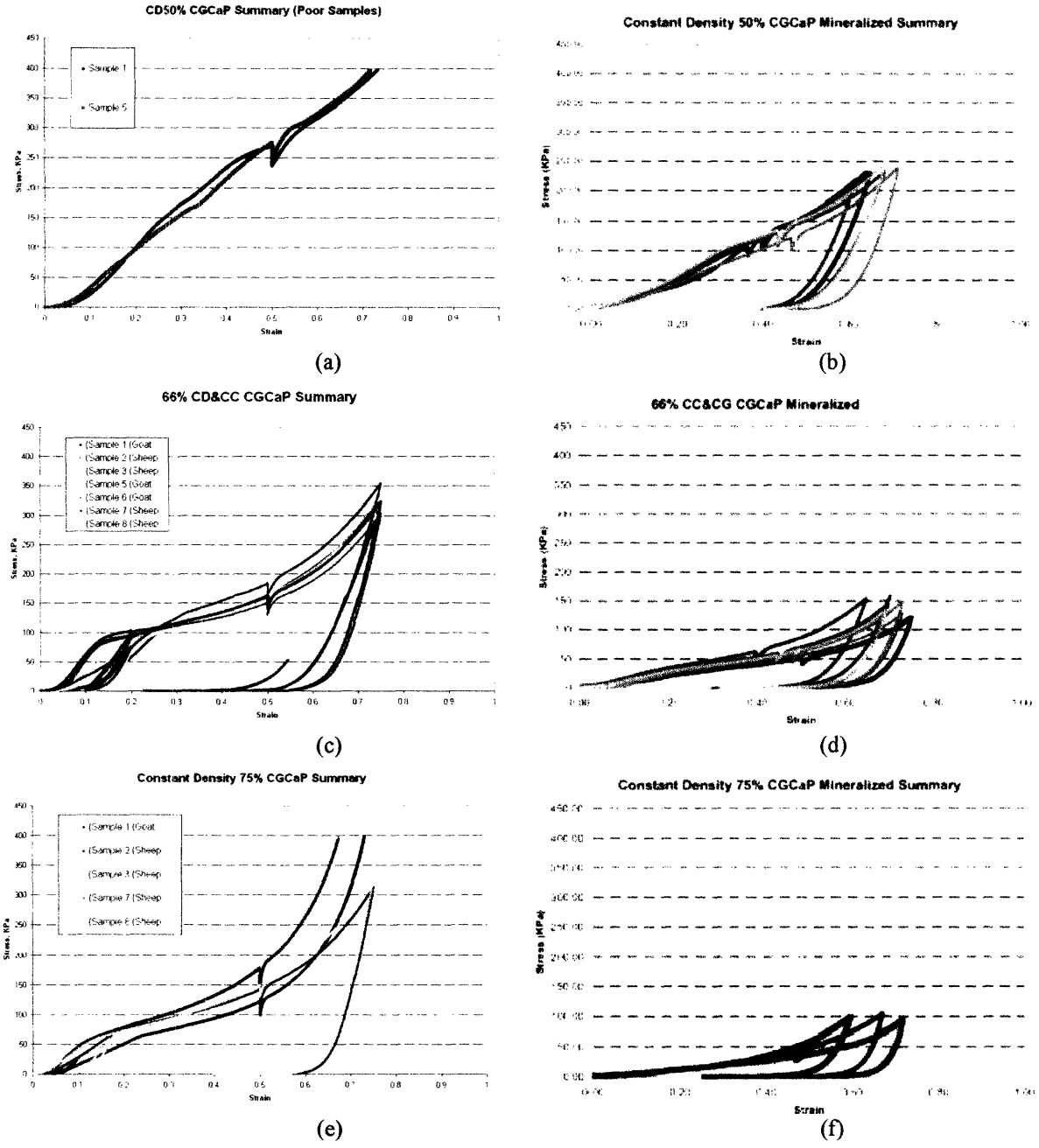


Figure 22 Constant Density (CD) Stress-Strain Graphs

Table 16 Loading Moduli & Collapse Stress

	Average Collapse Stress (kPa)		Young's Modulus (kPa)		Collapse Plateau Modulus (kPa)	
	Crosslinked	*Non-Crosslinked*	*Crosslinked*	*Non-Crosslinked*	*Crosslinked*	*Non-Crosslinked*
CD50%	87	156	286	730	331	637
CD66%	22	90	164	790	107	217
CD75%	14	70	68	505	84	242

As observed in the constant collagen samples the average collapse stress is remarkably lower in the all of the samples. The CD50% sample exhibits the highest collapse stress within each of the crosslinked and non-crosslinked subsets. This trend continues between the CD66% and CD75% indicating that the chemical crosslinking process affected each of the sample sets in the same way, compromising their overall mechanical properties to the same degree. The Young's modulus is again much less stiff in the crosslinked samples and in the collapse plateau region. Overall, the samples mechanical properties did not follow the expected hypothesis: that with increased mineralization the stiffness would increase.

Table 17 Unloading (Region A & B)

	Unloading Modulus Region A (kPa)			
	Crosslinked	*R^2 Value*	*Non-Crosslinked*	*R^2 Value*
CD50%	18358	0.1015	47785	0.131
CD66%	26571	0.0994	28910	0.077
CD75%	-15983	0.1314	17763	0.046

	Unloading Modulus Region B (kPa)			
	Crosslinked	*R^2 Value*	*Non-Crosslinked*	*R^2 Value*
CD50%	1711	0.9877	2279	0.998
CD66%	1205	0.9962	2759	0.996
CD75%	1443	0.9911	-	-

The unloading moduli for the constant density scaffolds show the same trends observed for the constant collagen scaffolds. Unloading region A and unloading region B are graphically illustrated in Figure 20 on page 52. Region A has the characteristic R^2 values approaching zero, indicating that the scaffold exerts little to no stress on the load cell and continues to remain compressed throughout the load hold. The negative slope value measured for the CD75% sample indicates that the measured strain increases slightly while a decrease in force was calculated. Although this data was measured during the test, because it occurred over a significantly small strain range (<1% strain) this phenomenon can be attributed to sensitive testing equipment and not a poor scaffold.

Some comparison charts have been put together including error bars to show the difference of the crosslinked and non-crosslinked samples graphically. Figure 21 is comparison of the calculated linear elastic modulus for the tested scaffolds. The error is calculated as a standard deviation of the collected data. It can be observed that for the CC50%, 66% and CC75% scaffold the data points for the crosslinked and non-crosslinked samples are within error of each other. The CD50% and CD75% data points are slightly higher for the non-crosslinked samples. Although unexpected values, we have attributed this decrease in modulus to the environmental factors of storage and crosslinked procedure. All data points are within the same order of magnitude.

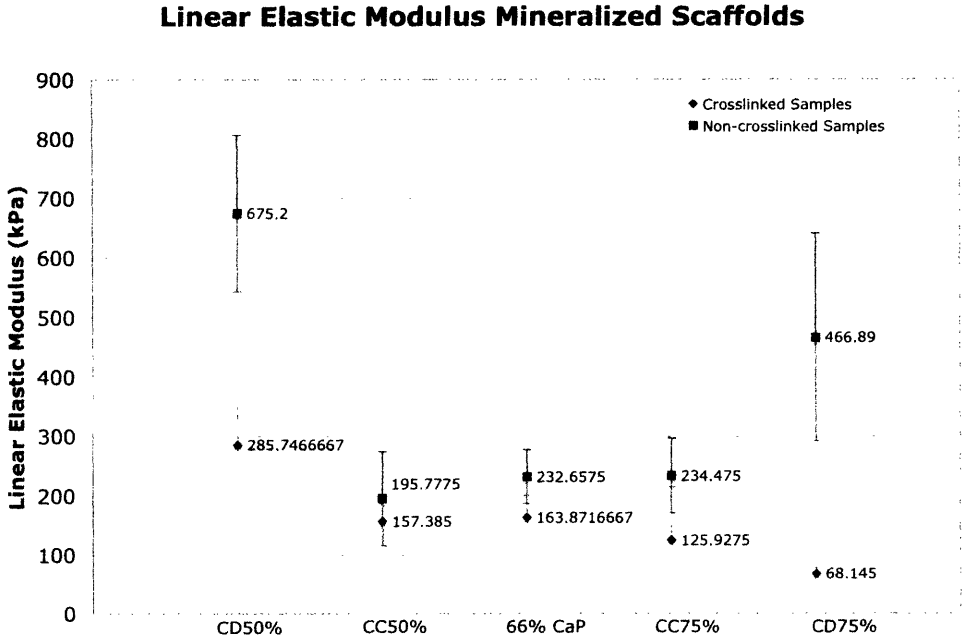


Figure 23 Linear Elastic Modulus of Mineralized Scaffolds

A second comparison chart for the collapse stress is shown in Figure 24. Here again it can be observed that the crosslinked samples show a slight decrease in the collapse stress, or the transition stress observed from the linear elastic region to the plastic deformation region. In four of the five sample groups the observed values are within error of each other and continue to exist in the same order of magnitude. It was hypothesized that an increase of at least one or two orders of magnitude would be observed for the crosslinked samples. The CD75% sample shows a negative collapse stress. This is due to the limited and poor data available for this sample group, and is further supported by a large associated error.

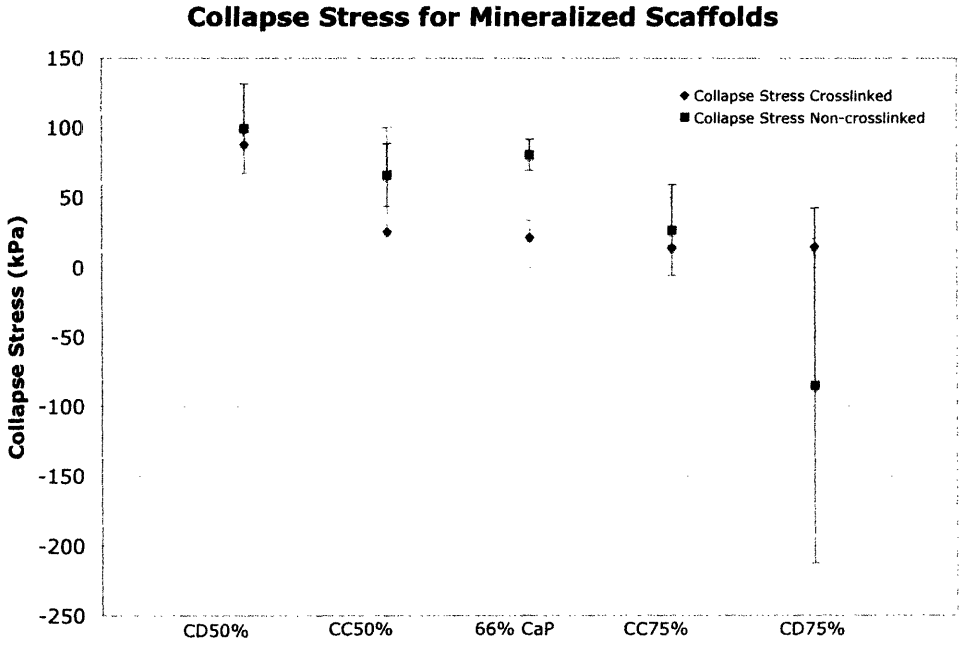


Figure 24 Collapse Stress of Mineralized Scaffolds

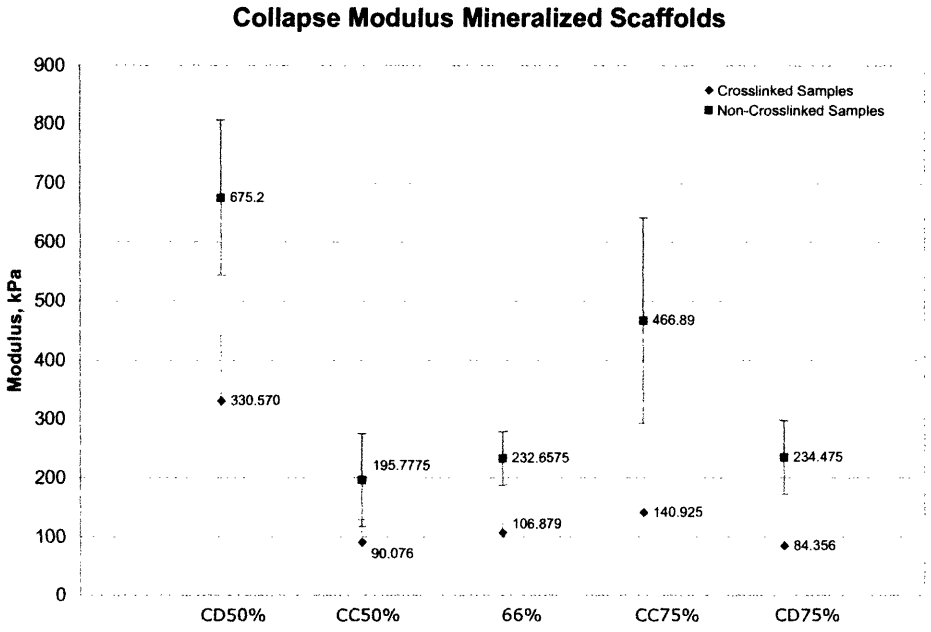


Figure 25 Collapse Modulus of Mineralized Scaffolds

Finally, a comparison of the collapse moduli for the tested samples is shown in Figure 25. As with the other comparison charts, there is not significant difference between the crosslinked and non-crosslinked samples. Again, the crosslinked samples have a slightly lower collapse modulus than its non-crosslinked counterpart. Error bars are included for reference and all data points are within the same order to magnitude, typically one to two orders lower than needed for an appropriate hard tissue analog. In the next section these data points will be compared to actual data gathered from wet and dry bovine bone samples.

3.5.2 Comparison to natural trabecular bone

The ultimate goal of this study is to establish that these mineralized collagen scaffolds can mimic natural bone upon implantation. Although only dry compression testing was completed and implantation will occur in an aqueous environment, we are able to accurately compare this data to published data from compression tests on natural healthy trabecular bone. The natural trabecular bone data was reproduced from: *Cellular Solids: Structure and Properties* by Lorna J. Gibson and Michael F. Ashby.[38]

The linear elastic or Young's modulus was plotted versus density on a log-log plot of the scaffold/bone. The triangle points on the uppermost trend line represent the reproduced bovine bone moduli. The lower square and diamond data points represent the various formulations of

the mineralized collagen-GAG scaffolds investigated in this study. It can be estimated that the moduli of the mineralized scaffolds is two orders of magnitude lower than the natural trabecular bone. However, the density of the scaffold is also lower by one to two orders of magnitude as well. It can be estimated that by either increasing the density or mineral content, or both, that the mechanical properties of the mineralized collagen-GAG scaffold can more precisely mimic that of natural bone.

After testing the crosslinked samples it was obvious that the chemical crosslinked process did not have the desired effects on the linear elastic modulus. There seemed to be a slight decrease in the Young's modulus with density held constant for our study. The next logical step would be to drastically increase the density of the mineralized scaffolds. Then there may be an observed increase in modulus that would more closely mimic that of healthy trabecular bone.

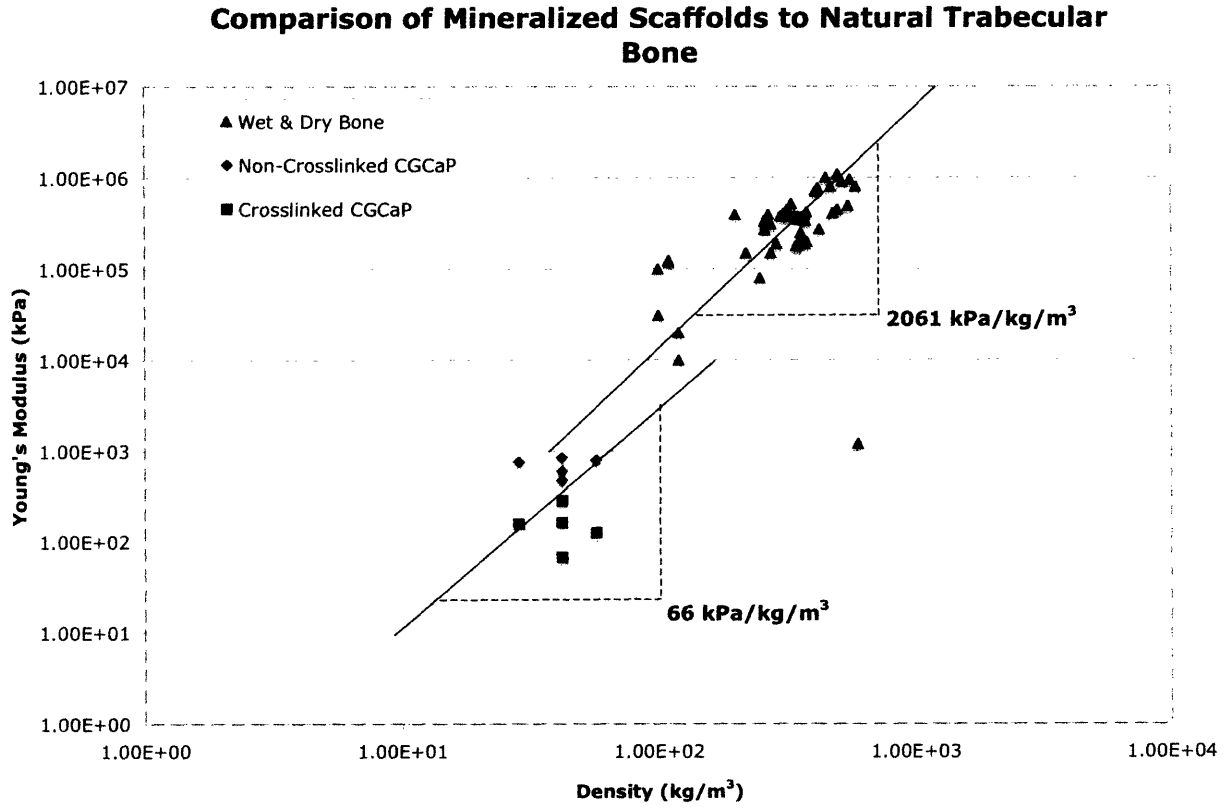


Figure 26 Comparison of Mineralized Scaffolds to Natural Trabecular Bone

3.5.2.1 SEM Micrographs

Scanning electron microscopy (SEM) was utilized to gain an understanding of the microstructure of our manufactured scaffolds. Some points of interest included homogeneity of

the scaffolds in terms of pore size and mineral dispersion as well as identifying particular mineral phases of the calcium phosphate mineral. By magnifying these samples at 50x, 250x and 500x it was possible to get a clear understanding of the trends for each formulation as well as drawing conclusions about mineral dispersion and its effect on the associated mechanical testing.

It has been established that the process of lyophilization, when accurately controlled, provides a porous scaffold with pores of equiaxed size and homogeneous distribution of pores throughout the bulk. This is clearly illustrated in Figure 24 with the 50% constant collagen and 66% CaP content scaffold at a magnification of 50x showing an equiaxed pore structure.

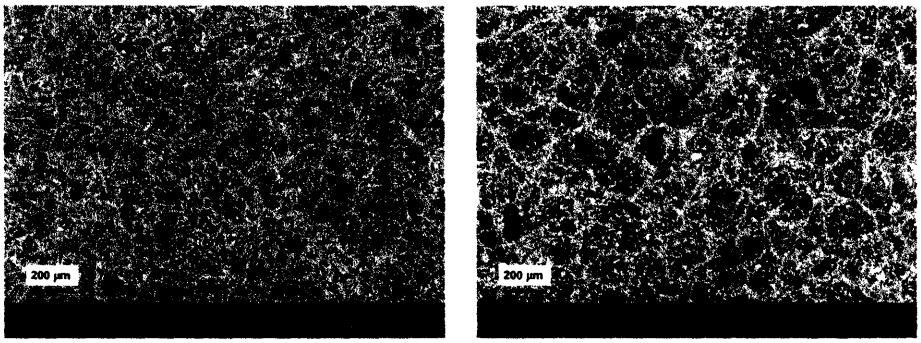


Figure 27 SEM Micrographs of Mineralized Bone Scaffolds. (Left) 50% CaP and (Right) 66% CaP content scaffold shows equixed pore structure.

Figure 25 shows a side view of the same scaffolds. A clearly homogeneous distribution of pores exists throughout the bulk. It should be noted that these scaffolds were cut using a rough method of biopsy punching which may have altered the exact microstructure of the scaffold.

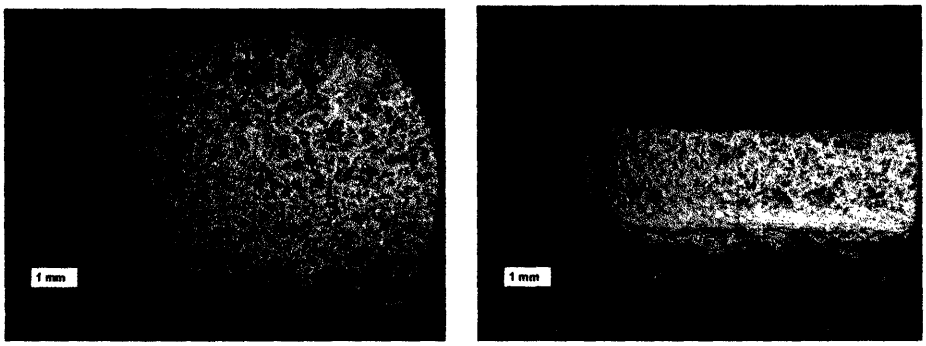


Figure 28 SEM Micrographs of Mineralized Bone Scaffolds. (Left) 50% CaP and (Right) 66% CaP content scaffold show equiaxed pre structure from a side view.

Another point of interest is the calcium-phosphate mineral component. An investigation of the existing phases, distribution and general characterization was also completed utilizing the SEM. The goal of the triple co-precipitation method is to introduce several phases including brushite, tricalcium phosphate, octocalcium phosphate and apatite. These phases represent

mineral orientation and chemical compositions that enrich the natural regeneration process of trabecular bone. The necessity remains for regeneration to occur as efficiently as possible and these mineral phases will encourage regeneration cells to infiltrate the scaffold and synthesize new ECM.

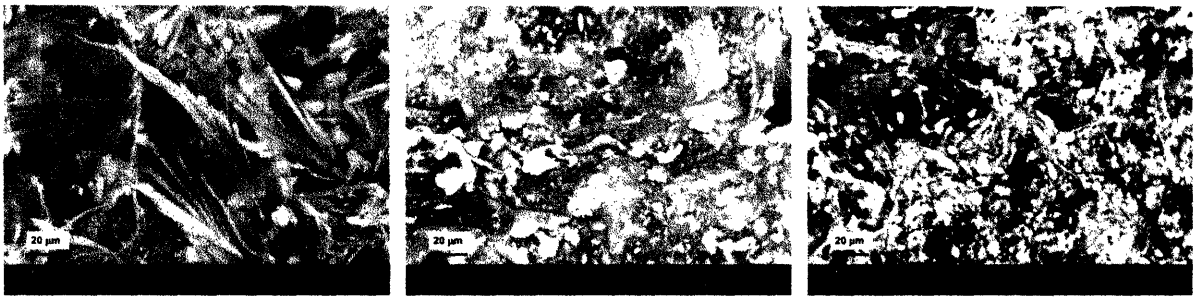


Figure 29 SEM Micrographs of Mineralized Collagen-GAG Scaffolds. (Left) Constant Collagen 50% CaP (Center) Constant Density 50% CaP (Right) Constant Collagen 75% CaP.

Brushite has characteristic feather-like structures. In Figure 26 the left most micrograph was taken at 500x magnification of the 50% CaP constant collagen scaffold. There is an obvious feather-like structure in this micrograph and can be assumed to be brushite, one of the desired phases for these scaffolds. The center micrograph was taken of the constant density 50% CaP scaffold. It can be seen that a layering effect occurs and this may be due to the high ratio of CaP:collagen. The large amount of calcium-phosphate mineral may result in layering and ultimately the poor mechanical results shown in earlier sections. The left most micrograph displays the flaky particles of CaP that many of the micrographs have shown. These flakes have not been distinguished as a particular phase, but the associated mechanical testing has shown promising results and reproducible data.

4 Business Model: OrthoCaP, Inc

This business model will outline the commercial viability of the layered osteochondral bone scaffold and its associated business venture: *OrthoCaP, Inc.* A thorough market analysis will be researched and explained. It will include a theoretical approach as well trends found in applicable ventures. The marketing channels will be examined and a plan of action will be provided. A cost optimization model will be outlined and precisely tailored for the appropriate production volume and consumer needs. The future growth of *OrthoCaP, Inc* will be addressed using information gained through several courses taken as part of the Master of Engineering curriculum. A hypothetical time line and managerial flow chart will also be presented.

4.1 Market Analysis

Many industries have developed marketing channels in order to appropriately introduce new products. There is a basic healthcare marketing channel that is used here to guide the commercialization and acceptance of the layered osteochondral scaffold.

Figure 30 Basic Healthcare Marketing Channel[39]
(NJ Kroloff, 1990, Healthcare Marketing Challenge: Launching new products which replace surgery, MIT Thesis)

Providing various market entrances allows for platforms of an application to be established. For example, by providing doctors immediate access to new products and their development, they may be more willing to incorporate them once on the market into their routine treatments. There is also a method of introducing the treatment to the patient directly through pamphlet initiatives, advertisements and paid clinical trials. These two methods are referred to as 'push' and 'pull' strategies.[39]

The 'push' strategy refers to the introduction of a new product at the beginning of the chain through the sales reps and doctors. This strategy is intended to expedite the movement of a product down this chain to the end-user or patient through proven acceptance by manufactures, sales representatives and doctors. The professionals associated with the healthcare chain prefer this method. Doctors prefer to test and evaluate new products on their own, without interference from outside influences such as those trying to make a quick turn around on a product.

The 'pull' strategy involves the introduction of products at the patient or end-user stage. Marketing strategies are used that instigate an end-user demand that is not normally found with unsolicited products. As noted before, this is very unusual in doctor administered products such as the layered osteochondral scaffold. Although patients are generally more educated these days about their treatment, it is unlikely that a treating surgeon or doctor would administer care based on a patients' request. Physicians are primarily interested in providing the best care available and generally react negatively to attempts of interference with the physician-patient relationship.[39]

The layered osteochondral scaffold will be introduced in a 'push' strategy for two reasons. Extensive clinical trials must be completed with as much input from participating surgeons and physicians as possible. The value of their input lies in two areas: the quality of the developed product and the ability of the surgeon to prescribe a treatment with sufficient knowledge of its ability to provide relief of pain and discomfort. This value is not achieved when introducing a product in a 'pull' marketing strategy. Having the backing of surgeons and prescribing physicians is invaluable when dealing with a surgical procedure.

A second consideration for healthcare product manufacturers is the patient referral chain.[39] This chain describes the process by which a patient either receives treatment or is referred to a specialist for continued treatment or more serious treatment. The patient referral chain is shown in Figure 28.

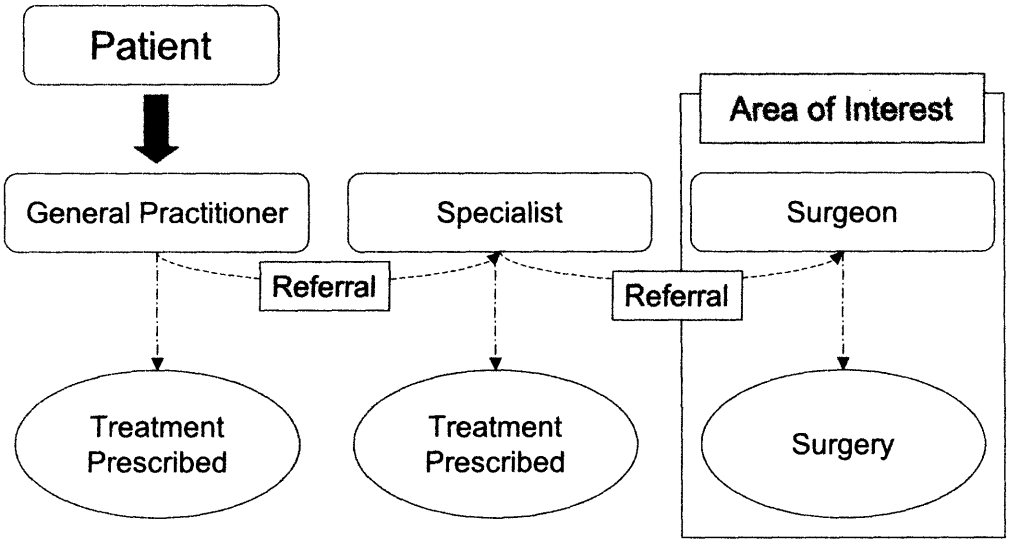


Figure 31 Patient Referral Chain[39]
(NJ Kroloff, 1990, Healthcare Marketing Challenge: Launching new products which replace surgery, MIT Thesis)

The patient referral chain can result in tremendous marketing implications for the healthcare product manufacturers.[39] In order to gain as much market share as possible, the product distributor must understand who will control the patients' movement down the chain of referrals. For example, although a specialist will not perform a surgical implantation, their knowledge of an existing technology will prompt them to refer the patient for further treatment with a surgeon who will, no doubt, take the specialists' comments and recommendations into consideration. Therefore, the individual who prescribes the final treatment has the most significant impact on the success of the product.

The treatment will ultimately be prescribed and completed by the attending surgeon. It is in our best interest to address the issues associated with alternative treatment, as is the case for the layered osteochondral scaffold. The three key advantages associated with this treatment include, ease of implantation as compared to a multi component knee arthroplasty, lower device cost and faster recovery for the patient.

The layered osteochondral scaffold will be marketed to surgeons, but with sufficient support from referring specialists. These specialists should be involved in our key areas of interest including rheumatology, osteoporosis and orthobiologics. These specialties are of particular interested due to the intended application of the layered osteochondral scaffold in diseased joints. With a rapidly ageing population in the United States as well as the extended lives people are leading, the market for joint disease is expanding.

Rheumatoid arthritis and osteoarthritis afflict older generations and more and more people insist on remaining active in their older years. The same situation exists for individuals afflicted with osteoporosis. Although normally associated with women, this disease affects men as well. With a decrease in bone mass due to an unbalanced amount of bone resorption to deposition, fractures and bone weakness are common symptoms. Specialists in this area insist on using growth hormones or even pharmaceutical drugs to suppress bone resorption to increase bone mass. The layered osteochondral scaffold would aid them in the case of severe fractures where natural bone is unable to heal itself. By providing a load bearing scaffold, the patient would be able to remain active, assured that the scaffold will not fail.

The orthobiologics market consists of bone graft substitutes, allograft distribution/processing, autogenous bone and soft tissue replacement. These are direct competitors of the layered osteochondral scaffold. These specialists can give insight into the

needs of the market as well as improvements to be made to the layered osteochondral scaffold. This product would be of particular interest to them due to the ease of implantation, its load bearing capabilities and affordable price. Their knowledge would also be beneficial in attempts to seed the scaffold with cells and particular growth factors. This is a readily employed step in many competitive technologies. In general, most growth factors and cell seeding can only improve the biocompatibility and regeneration capability of the device.

This marketing strategy will be supported quantitatively in the next section.

4.1.1 Current Value of Orthobiologics Market

Market research was prepared in the area of orthobiologics, which includes bone graft substitutes, allograft and autograft bone, soft tissue replacement products and viscoelastics.[40] Viscoelastic biomaterials used for orthopedic problems have specifically defined mechanical properties. While bone has natural viscoelastic behavior, or a time dependent stress-strain relationship, some biomaterials exhibit similar characteristics to more adequately mimic replaced tissue. Data was gathered from 2000-2004 and will be reported as a general overview of the trends, value and future of the orthobiologics market.

Table 18 2001 Worldwide Orthopedic Product Sales ($$Billions)[40]

Product Segment	U.S. Market	Non U.S. Market	Total	Change vs. 2000
Reconstructive Devices	$2.8	$2.6	$5.4	12%
Fracture Fixation	$0.8	$0.8	$1.6	9%
Spinal Implants/Instrumentation	$1.3	$0.6	$1.9	23%
Arthroscopy/Soft Tissue Repair	$0.9	$0.4	$1.3	12%
Orthobiologics	**$0.9**	**$0.3**	**$1.1**	**13%**
Other Products	$2.3	$1.0	$3.3	9%
Total Market	**$8.9**	**$5.7**	**$14.7**	**13%**

In 2001, the orthobiologics market experienced a 13% gain from 2000 (Table 18). This resulted in a gross earning of nearly $1.1 billion dollars. If we conservatively project this growth

as roughly 10% each year, by 2006 the orthobiologics market will exceed $1.7 billion dollars. (Table 19) Additionally, we have the opportunity to gather some percentage of the arthroscopy and soft tissue repair market. Since the layered osteochondral scaffold is derived from a novel tissue-engineering matrix, further research and development could open up many other markets. However, as the current layered osteochondral scaffold has the potential ability to regenerate meniscal and articular cartilage, we will estimate a small percentage of the soft tissue and arthroscopy market can be gained upon commercialization.

In 2000, artificial skins and cartilage replacements had combined sales of $49 million dollars. The worldwide market for surgical procedures relating to cartilage repair is estimated at $1.2 billion annually.[41] There are 500,000 cartilage repair procedures in the US annually.[50] The major competition in these areas lies with several large companies including, Genzyme Biosurgery, Biomatrix, Bio Tissue Technologies, Integra LifeSciences, Advanced Tissue Sciences, ReGen Biologics and Osiris Therapeutics. These companies control 85% of the revenues.[41]

Table 19 Expected Market Share 2006
(*Total Market Share: Based on 10% growth per year of each market segment)

Product Segment	Market Share Expected 2006*	Market Value 2006*	Annual Expected Growth
Orthobiologics	5%	$85 Million (Total Market $1.7 Billion)	7%
Arthroscopy/Soft Tissue Repair	1%	$12 Million (Total Market $1.2 Billion)	10%
Total Value	≈ $97 Million Revenues		

The expected market share of $97 million (Table 19) is a conservative value and based on possible commercialization of the layered osteochondral scaffold in 2006. The future of the market is very dynamic and projections farther than 2006 may be skewed by undiscovered technology that will directly replace the current technology available.

4.1.2 **Production Volume & Value**

The production volume of the manufacturer must be based on the expected market share. For the orthobiologics product segment an estimated 5% of the market can be claimed. The value is based on approximately 21.6 million treatments including joint replacement, fracture fixation, spinal implants, instrumentation and bone grafting.[40, 42] Considering orthobiologics constitutes approximately 7.5% of the 21.6 million, a possible market share of 1.5 million applications is possible. Calculating OrthoCaP Inc.'s 5% share of 1.5 million equals approximately 75,000 applications. There are approximately 500,000 cartilage repairs annually in the US. One percent of this market is only 5,000 applications. Therefore, we will estimate that our total production volume, for both orthobiologics and cartilage repairs, should be 100,000 to allow for scrap and error in production. Our revenues on this production volume will be calculated later with the cost model.

4.1.3 **Competition & Market Strategy**

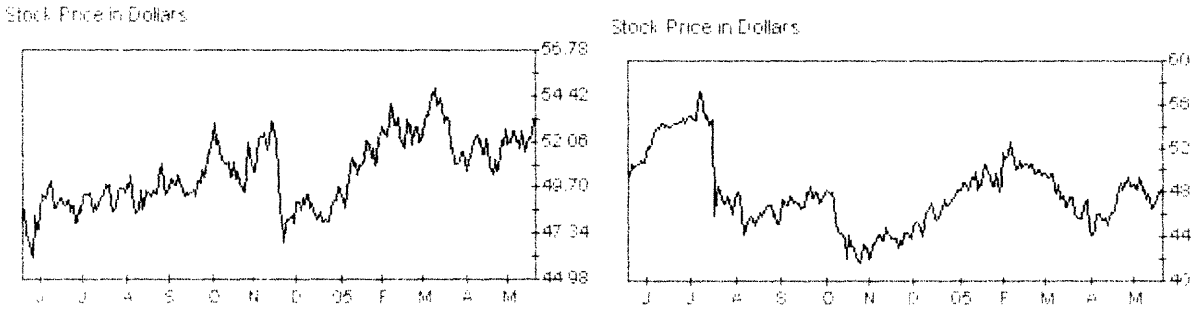
Being a start-up company it is necessary to look at the financial position of the largest competitors in the market. This involves distributors for autograft and allograft bone substitutes as well manufacturers of bone fillers and compounds. A more in depth analysis will be done on regeneration templates for articular cartilage and bone regeneration templates.

4.1.4 **Publicly Traded Companies**

There are two divisions of health technology of the New York Stock Exchange: Advanced Medical Devices and Medical Supplies. After examining the companies listed under both divisions it was clear several had interests in competing technologies. Large, well-established companies will be discussed first as a benchmark for future growth. Start-up companies will then be examined with particular interest in product lines and revenues. All the companies discussed in this study are U.S. based, however, many of them have international offices and distribution centers. This information will be used to make estimates about the *OrthoCaP, Inc.* business model and future success.

Medtronic, Inc and *Stryker Corporation* are two of the largest orthopedic solutions manufacturers. Their interests lie in the design, development and commercialization of bone stabilization implants and bone grafts, particularly allografts. Medtronic, Inc. went public in 1977 with only a handful of products in the pipeline. They have grown over the last 30 years to

revenues of nearly $1.9 billion.[43, 52] Although much of their revenues come from non-orthopedic products, they were founded on stabilization devices and bone grafts. Stryker Corporation went public in 1997, but has been incredibly successful reaching a value of nearly $435.5 million.[53] They are involved in many divisions of the orthopedic market, but have two specific divisions: Orthopedic Implants and MedSurg Equipment. The main competition lies with the Orthopedic Implants, which specializes in orthopedic reconstructive (hip, knee and shoulder), trauma, spine and micro implant systems, bone cement and the bone growth factor OP-1.[44] Stock prices over the last 12 months are shown in Figure 29. Their value is approximately $50 per share.



(a) (b)

Figure 32 (a) Medtronic Stock Ticker Volume: 1.374 Million (Last 12 Months)[43]
(b) Stryker Corporation Stock Ticker Volume: 0.556 Million (Last 12 Months)[44]

These companies have been around for two very different time periods and are both very successful. While Medtronic has many successful products, Stryker has been incredibly successful with the few products commercialized to date. For short-term goals, the layered osteochondral scaffold is a promising product launch and could lead the way to future successes for the commercializing company. The layered osteochondral scaffold may present itself as the initial product line, but continued research and development may yield the success of Medtronic or Stryker. Continuously improving and introducing new products is a sure way to build revenues. Our cost model will break down pricing and expected revenues for two periods of development, each spanning 3 years each.

The stock exchange also has its share of small start-up companies with enough success to go public in the last 5 years. These companies include *dj Orthopedics, Inc.*, *Cryolife, Inc.*, *Smith & Nephew*, *Symmetry Medical Inc.* and *Zimmer Holdings, Inc.* Table 27 shows the stock ticker value and volume of each of these companies as well as publicly listed date.

dj Orthopedics, Inc. went public in November of 2001. They specialize in rehabilitation and regeneration products for the non-operative orthopedic and spine markets.[45] They have about 600 rehabilitation products that are currently on the market. In November 2003, dj Orthopedics, Inc. acquired Orthologic Corporation. This acquisition began their involvement in the regeneration market. Their products specifically target the spine and provide a therapy that accompanies spinal fusion therapy. The value is nearly $1.45 million after approximately 5 years of business. The success of dj Orthopedics is encouraging as there short-term profits are analogous to our cost model.

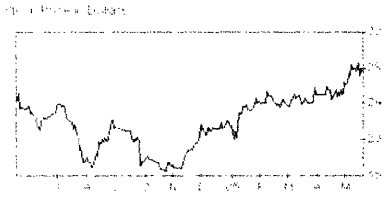
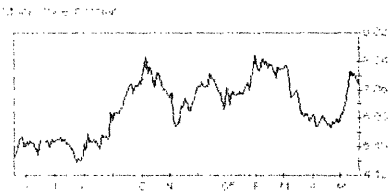
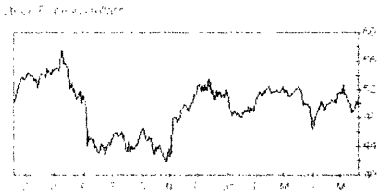
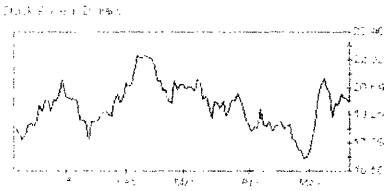
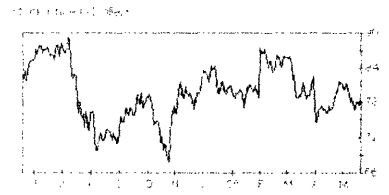
Cryolife, Inc. is the oldest company in our start up list, going public in 1997. With public trading value of nearly $5.7 million, it has the most closely associated product line to our layered osteochondral scaffold. They are involved in the preservation and distribution of various human tissues including cardiovascular, vascular and orthopedic transplant applications.[46] They are leading suppliers of allograft bone substitutes.

In review of the financial histories of *Smith & Nephew* and *Symmetry Medical, Inc.*, it was apparent that success could be attributed to the acquisition of smaller companies with niche technologies that can be a value-added commodity when introduced into the product lines of an established company. Smith & Nephew, Inc is an independent provider of implants, related instruments and cases to orthopedic device manufacturers. In March 2004, Smith & Nephew acquired Midland Medical Technologies (MMT) another orthopedic device manufacturer.[47] Symmetry Medical, Inc. is a provider of implants for orthopedic device manufacturers. Symmetry acquired *Mettis* in June of 2003. Mettis was a manufacturer of forged, cast and machined implants for the global orthopedic device market. These devices include hip, knee and joint replacement devices as well as screws and other fixation treatments. Symmetry has a market value of approximately $300,000 dollars, far less than other competition.[48]

Reviewing the success of publicly traded companies can give a realistic look at the type of approach needed for a small start-up company. The ones investigated here specialize in orthopedic implantation and regeneration devices and have all started from a handful of niche technologies and grown into full-fledged businesses. In addition to introducing a new product to consumers, the reality of acquisitions provides another aspect of revenue and continued growth. A shortcut to growth can also include the purchase of rights and license to patents and other technology owned by smaller companies or individuals.

A brief business model will now be introduced for *OrthoCaP, Inc* that will describe four areas: Business Description, Management & Time Line and a Cost Model.

Table 20 Stock Exchange information for small, start-up companies with competitive products and technology.[43, 44, 45, 46, 47, 48, 49, 50]

Company Name & Stock Ticker	Symbol	Value/Volume (5/20/2005)	Listing Date
dj Orthopedics, Inc. 	**DJO**	**$27.56 52,5000 shares ($1.45 Million)**	**November 15, 2001**
Cryolife, Inc. 	**CRY**	**$7.34 77,000 shares ($5.65 Million)**	**July 15, 1997**
Smith & Nephew 	**SNN**	**$49.64 9,000 shares ($0.45 Million)**	**November 16, 1999**
Symmetry Medical, Inc. 	**SMA**	**$19.90 18,600 shares ($0.37 Million)**	**December 9, 2004**
Zimmer Holdings, Inc. 	**ZMH**	**$78.17 426,600 shares ($33 Million)**	**July 25, 2001**

4.2 Business Description: OrthoCaP, Inc.

OrthoCaP, Inc. is an orthopedic medical device company specializing in regeneration templates for use in load bearing applications such as articular and meniscal cartilage repair and bone regeneration within the knee and hip joints. Two patents have been filed in order to protect our developments in this area and have been established by the MIT-Cambridge Alliance. OrthoCaP, Inc. projects earnings of nearly $1 million by end of year one and expected profits of nearly $15.2 million by period 2. Period 1 reflects the first three years of development while period two earnings reflect efforts during years four through six.

Our timeline began in 2001 with the inception of this layered osteochondral scaffold in the labs at Massachusetts Institute of Technology. There was intense research completed during 2002 through 2004 to establish the now patented mineralized scaffold protocols. Now, in 2005, the first written document determining the business possibilities of the mineralized scaffold has been written. In vivo animal studies have begun at the Addenbrooke's Hospital in Cambridge, UK and are about to begin at the VA Hospital in Boston, Massachusetts. Human trials may be scheduled as early as 2008 with completion in 2010. As the animal testing is completed the steps toward FDA approval will be taken. This is a lengthy and expensive process that will hopefully be covered by academic grant resources. However, it is important to solicit and secure funds so that research can continue after academic grants expire. The founding members of OrthoCaP, Inc. have started soliciting for monetary funds. Once funding has been ascertained we can look forward to gaining FDA approval in 2013 with the first product leaving the manufacturing area in late 2014.

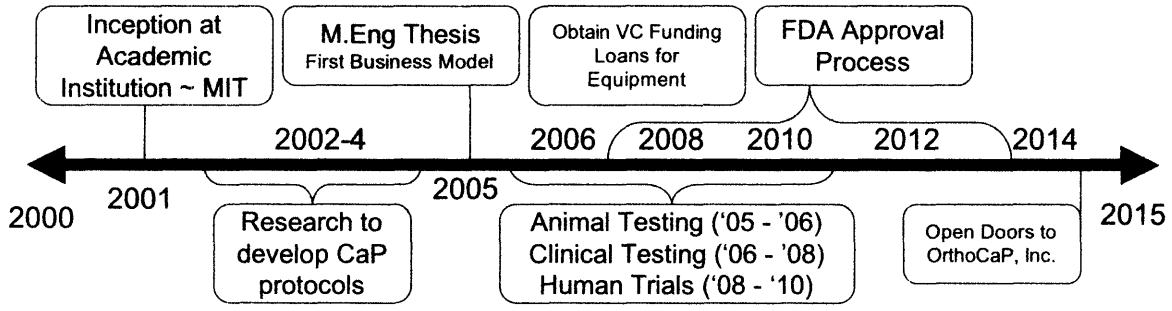


Figure 33 Business Timeline for OrthoCaP, Inc.

4.3 **Management**

The business model for OrthoCaP, Inc. also must address upper management of the organization. The management team is responsible to the bank and investors for generating revenue. In this case, a Board of Directors (BOD) is put in place and consists of the CEO and four investor chairs. We will also allocate 4 chairs to the investors responsible for putting OrthoCaP, Inc. in business. We will have a Chief Technology Office (CTO) and a Chief Financial Officer (CFO) each reporting directly to the CEO.

The management model presented in Figure 31 is representative of OrthoCaP, Inc. after 5 years of business. Funds will be allocated for three customer service representatives to support four sales personnel. The Sales Force will each work off of a draw of $60,000 plus will be eligible for 1.5% commission on all sales over $100,000. The CFO will be responsible for the management of the customer service and sales force. The CTO will have the responsibility of overlooking the manufacture and quality assurance involved with the production. In addition, it makes sense to continue thinking up large ideas, so an investment will be made to employ several individuals in the area of Research & Development. This includes a director, a lead technician, a lead engineer and a lab technician. The total salary overhead, not including the board of directors, is roughly $965,000/year.

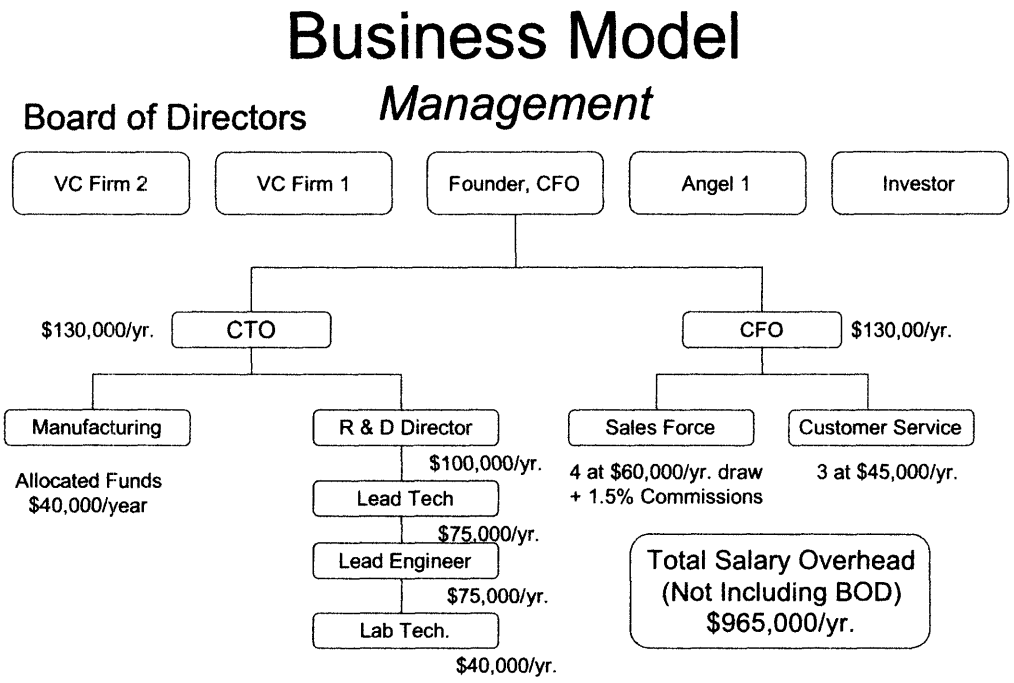


Figure 34 Business Model: Management Hierarchy

4.4 *Cost Model & Financial*

The cost model for the layered osteochondral scaffold will address two financial issues. The first issue is the calculation of the cost to manufacture the scaffold as well as the necessary mark-up to make a profit. The second issue is to calculate the expected profits and subsequent expansion of manufacturing volume. It was clearly shown in the manufacturing section that the existing manufacturing protocol time is nearly 90% longer than necessary. Two cost models will be presented to show the impact of the extended manufacturing time on overall profits.

The cost model used here was developed by Gabrielle Gaustad of the Materials Systems Laboratory of the Department of Materials Science and Engineering at Massachusetts Institute of Technology.[54] After tediously researching and documenting the standard operation procedure of the manufacturing process, accurate cost factors were incorporated into the complex model. Overall cost is composed of variable costs and fixed costs. Variable costs depend on the volume of product produced. Fixed costs are independent of the production volume and exist if either one or a million products are manufactured. These fixed cost factors include operational costs, equipment costs and exogenous cost factors. Exogenous cost factors include expected equipment life, overhead costs such as building and electricity charges as well as discount rates and tooling. A brief overview of these fixed costs is included in Table 21.

These costs are included in the cost per unit and are essentially divided by the volume of pieces produced each fiscal year. The operation factors section is most concerned with labor costs. Because these processes are not completely automated yet, every hour of the fiscal year must be accounted for and the correct value of time must be included in the final piece price. This can be very expensive if the labor is not utilized correctly. For a start-up such as OrthoCaP, Inc. the labor section may be trimmed and fewer employees hired to cut down on costs. Employees may be required to wear many hats and distribute their time across different functions. The final section, labeled exogenous cost factors, include things such as building rent, electricity, maintenance and equipment life. These factors are commonly calculated as percents of overall fixed costs due to the difficulty of valuing elements that are dependent on uncontrollable factors. These fixed cost factors are shown in pie-chart format in Figure 32.

Variable cost factors are dependent on aspects of manufacturing that can be controlled, such as material costs and product volume, labor costs and energy costs. A breakdown of the variable costs will show that the labor costs for the osteochondral scaffold are nearly 98.6% of

the variable costs, while material and energy costs account for 0.9% and 0.5% respectively. In other words, the labor required to produce a quality scaffold far outweighs the raw materials to produce it. Costs can be saved by allocating the correct amount of efficient labor and ensuring that accurate scheduling occurs and maintenance is performed routinely.

Table 21 Cost Factors

Main Equipment	Capacity mL	Cost $/each	Power Rating kW-hr	Area m^2
Blender 1	1000	$18,000.00	0.746	0.9
Blender 2	1000	$18,000.00	0.746	0.9
Blender 3	1000	$18,000.00	0.746	0.9
Liquid Nitrogen Tanks	3500	$1,349.00	2.2	0.01
Holding Tanks	5000	$780.00	0.5	0.5
Holding Tanks (feed)	5000	$780.00	0.50	0.5
Freeze-dryer	500	$45,278.00	5.00	1.13
Vacuum Drying Oven	500	$4,199.00	2.30	2
Pumps	9 GPM	$4,900.00	2.20	1
Dessicator		$11,633.00	2.20	0.7

Operational Factors		
Operating Days	300	days/yr
Numbers of Shifts	2	(1-3)
Hours in Shift	8	Hours
Downtime-unpaid	8	hrs/day
Downtime-paid	3	hrs/day
Maintenance (paid)	2	hrs/day
Unplanned Breakdowns	1	hrs/day
Wage (including ben.)	20	$/hr
Unplanned Breakdowns	0.5	hrs/day

Exogenous Cost Factors		
Discount Rate	10.00%	%
Equipment Life	10	Years
Fixed Overhead	50.00%	%
Building Costs	1000	$/m^2
Building Life	30	Years
Auxiliary Equipment	15.00%	% of equip
Tooling	5.00%	% of equip
Equip and Build Maintenance	15.00%	% of fixed
Tooling Maintenance	5.00%	% of fixed
Electricity	$0.08	$/kW-hr

The cost model was broken down into five sections of the manufacturing process:

1. Collagen Blending
2. Slurry Blending
3. Calcification
4. Freeze-Drying
5. Crosslinking

Each section was assigned the appropriate variable and fixed cost factors and the entire price was calculated per section. The overall price to manufacture was the sum of the five

sections. Again, two cost models were developed: one dependent on the existing manufacturing protocols and the second based on the calculated manufacturing times from this study.

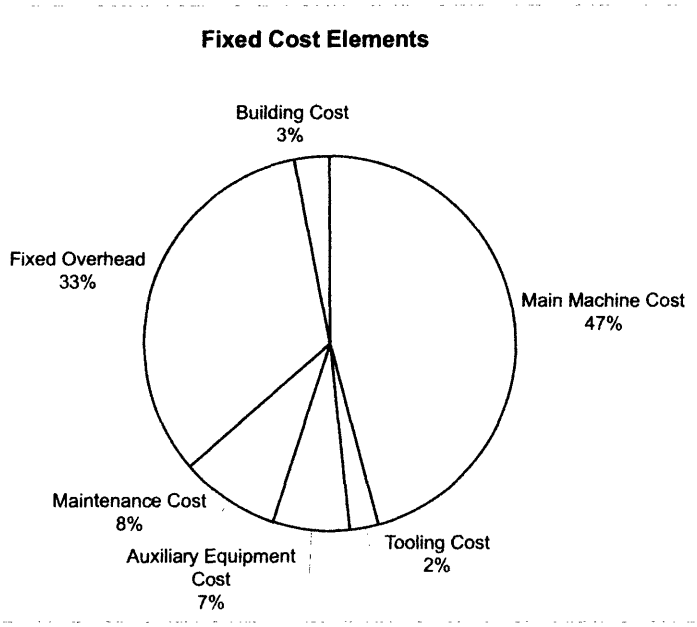


Figure 35 Fixed Cost Elements ~ Layered Osteochondral Scaffold

There are several variables that must be defined prior to calculating the manufacturing price. These include the production target volume including scrap and testing samples, the size of the final scaffold and the calcium content of the mineralized scaffolds. Table 22 outlines the fixed values that will be used from this point on. These values will remain constant for each of the two cost models.

Table 22 Production Variables

Production – Target	85000	Units/year
Scrap Rate	10%	%
Testing	5%	%
Total Product – Actual	97750	Units/year
Unit Volume	78.125	cm^3
Pore Size	92	Micron
CaP Solids Loading	66	%

The main difference between the two cost models is section three, the time required for calcification to occur to 100%. The current laboratory protocol specifies 360 minutes as the time

required for completion of section three. After careful calculations it was concluded in the manufacturing section that the total calcification reaction takes place in about 54 minutes, nearly 85% less time. This difference will be evident in the labor and energy costs for the individual section of calcification. This factor was changed for the two cost models and although it is just a single step in the process, the cost differed by several percent.

Table 23 Cost Summary for Protocol Manufacturing Time

VARIABLE COST ELEMENTS	per unit	per year	percent	
Material Cost	$5.587	$474,923.99	0.9%	
Labor Cost	$614.426	$52,226,197.03	98.6%	
Energy Cost	$2.931	$249,095.58	0.5%	
Total Variable Cost	**$622.944**	**$52,950,216.59**	**100.0%**	
FIXED COST ELEMENTS	**per unit**	**per year**	**percent**	**investment**
Main Machine Cost	$97.784	$8,311,636.74	46.0%	$51,071,410
Tooling Cost	$4.889	$415,581.84	2.3%	$2,553,570
Auxiliary Equipment Cost	$14.668	$1,246,745.51	6.9%	$7,660,711
Maintenance Cost	$18.069	$1,535,905.93	8.5%	
Fixed Overhead	$70.896	$6,026,166.68	33.3%	
Building Cost	$6.382	$542,463.35	3.0%	$3,659,418
Total Fixed Cost	**$212.688**	**$18,078,500.05**	**100.0%**	
Total Fabrication Cost	**$835.632**	**$71,028,716.65**	**100.00%**	

Table 24 Cost Summary for Calculated Manufacturing Time

VARIABLE COST ELEMENTS	per unit	per year	percent	
Material Cost	$5.587	$474,923.99	0.9%	
Labor Cost	$585.834	$49,795,925.24	98.6%	
Energy Cost	$2.881	$244,926.97	0.5%	
Total Variable Cost	**$594.303**	**$50,515,776.20**	**100.0%**	
FIXED COST ELEMENTS	**per unit**	**per year**	**percent**	**investment**
Main Machine Cost	$96.444	$8,197,716.73	45.9%	$50,371,421
Tooling Cost	$4.822	$409,885.84	2.3%	$2,518,571
Auxiliary Equipment Cost	$14.467	$1,229,657.51	6.9%	$7,555,713
Maintenance Cost	$17.836	$1,516,017.98	8.5%	
Fixed Overhead	$69.977	$5,948,030.88	33.3%	
Building Cost	$6.386	$542,783.69	3.0%	$3,620,652
Total Fixed Cost	**$209.931**	**$17,844,092.64**	**100.0%**	
Total Fabrication Cost	**$804.234**	**$68,359,868.84**	**100.00%**	

The two costs have been calculated to $835.63 for the protocol time and $804.23 for the calculated time. This represents a 3.8% difference in price. The real difference will come during the pricing. To be competitive in the market, we will assume that our product must be priced around $1000 per sheet. Therefore, with a set market value the real profits come in the form of reduced manufacturing cost. Another aspect is the production volume. Although fixed costs are distributed evenly among produced product, by merely increasing the product volume, thus decreasing cost per sheet, we run the risk of having unsold product and storage fees for overstock. So, the production volume will follow what the market allows and for the first year of business will remain at 85,000 sheets.

Protocol Time

Part Cost	$835.632	
Part Price	$1,000.000	16.44% Markup
Production Volume	85000	
Revenue	$85,000,000.00	
(Cost)	$71,028,716.65	
Profits	$13,971,283.35	

Calculated Time

Part Cost	$801.42	
Part Price	$1,000.00	19.86% Markup
Production Volume	85000	
Revenue	$85,000,000.00	
(Cost)	$68,120,941.90	
Profits	$16,879,058.10	

It becomes obvious after the profit calculation is complete that with only a 3.8% difference in cost, comes a 17.2% increase in profits. This kind of difference could make or break a company. The turn of profits would be a welcomed sign of success to investors and employees. The next step in continuing this success is to market the current products successfully and incorporating ongoing research into the product lines available. Following the expected business plan with an average annual production volume increases of 8% each fiscal year over the next six years the following table shows the expected revenues and associated profits with a constant retail price for each layered osteochondral sheet.

Table 25 Expected Profit for Period 1 and Period 2 for OrthoCaP, Inc.

Period	Year	Target Production Volume (sheets/year)	Revenues $US	Profits $US
Period 1	1	85,000	$85MM	$16,640,131.16
	2	91,800	$91.8MM	$18,039,420.00
	3	99,144	$99.1MM	$19,550,651.94
Period 2	4	107,075	$107MM	$21,182,675.42
	5	115,641	$115MM	$22,945,367.78
	6	124,892	$124MM	$24,849,017.90

These profits are based on the market share of a single product, the layered osteochondral scaffold. It would have to be assumed that OrthoCaP, Inc would continue its Research & Development initiative with a great majority of its profits in order to secure its success long-term. Continuing improvements on the layered osteochondral scaffold and additional tissue regeneration templates would be the best way to increase market share and create a trusted brand name in the industry of medical device manufacturers.

5 Conclusion

Standard CG scaffolds have shown impressive results in industry and laboratory settings and have become successful in the market of tissue regeneration specifically targeted at skin wounds, nerve regeneration and repair of the conjunctiva. With this successful research in hand new markets and applications were explored particularly in the area of bone remodeling. Taking this novel idea and developing it into a commercialized product was the main focus of this study.

With a lack of load-bearing polymeric scaffolds available in the marketplace, an opportunity existed to attack a niche market valued at nearly $97 million dollars. Completing a thorough patent search and IP investigation two successful patents were filed and ensured the rights to this novel technology to MIT and Cambridge University. A main barrier at this juncture was the lack of mechanical data required to support further development. With laboratory manufacturing protocols in place, samples were fabricated and then characterized using standard compression tests. Although promising, the final results showed room for improvement, specifically in the areas of collapse stress and the linear elastic modulus. Crosslinking the samples is a mandatory process but results showed that the EDC/NHS process may not be the best choice. With current data gathered, the next steps for development can take place.

From a business perspective, the layered osteochondral scaffold will be a lucrative product line for OrthoCaP, Inc. This start-up company has the opportunity to profit substantially if resources and the market play out as expected. Although a promising product line, OrthoCaP, Inc. must foster an environment of continued research and development to consistently stay ahead of competitors and hold onto the niche market of hard tissue regeneration. The full commercialization of the layered osteochondral scaffold is still many years away, however, with the continued dedication of the faculty and staff associated with this project, it is sure to make its debut on the market in the next 10 years. With its introduction will come a quality of life for those desperately in need of relief from pain and discomfort as well as a possibility of an active lifestyle well into the future.

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7 Appendix A

Patent Number & File Date	Patent Inventor(s)/Assignee	Abstract
4,060,081 July 15, 1975	Yannas; Ioannis V., Burke; John F., Gordon; Philip L., Huang; Chor Assignee: Massachusetts Institute of Technology (Cambridge, MA)	A multilayer membrane, which is useful as synthetic skin, is disclosed herein. A first layer is formed from a material which does not provoke an immune response and which is also insoluble and nondegradable in the presence of body fluids and/or body enzymes. Preferred materials for the first layer are crosslinked composites of collagen and a mucopolysaccharide. A second layer is formed from a nontoxic material which controls the moisture flux of the overall membrane to about 0.1 to 1 mg./cm.sup.2 /hr. Suitable materials for the second layer include synthetic polymers such as silicone resins, polyacrylate or polymethacrylate esters or their copolymers, and polyurethanes.
4,789,663 July 5, 1985	Wallace; Donald G., Smestad; Thomas L., McPherson; John M., Piez; Karl A., Seyedin; Saeid, Armstrong; Rosa Assignee: Collagen Corporation (Palo Alto, CA)	A method of repairing bone defects by use of suspensions containing purified atelopeptide, reconstituted, fibrillar skin collagen or bone collagen powder or mixtures thereof is disclosed. The suspensions provide matrices for conductive growth of bone into the defect. The skin collagen my also be lyophilized and use din the form of mats.
4,947,840 August 21, 1987	Yannas; Ioannis V., Lee; Elaine, Ferdman; Ariel Assignee: Massachusetts Institute of Technology (Cambridge, MA)	This invention relates to porous, biodegradable materials in which the pore size, biodegradation rate, and pore volume fraction are controlled and within values at which skin contraction rates around an implant-containing wound are delayed or slowed.
5,489,304 April 19, 1994	Orgill; Dennis P., Butler; Charles E., Barlow; Mark Ritterbush, Scott, Yannas; Ioannis V., Compton; Carolyn Assignee: Brigham & Women's Hospital (Boston, MA); Shriners Hospital for Crippled Children (Tampa, FL); Massachusetts Institute of Technology (Cambridge, MA); Integra LifeSciences, Corporation (Plainsboro, NJ)	The present invention relates to a method of skin regeneration of a wound or burn in an animal or human. This method comprises the steps of initially covering the wound with a collagen glycosaminoglycan matrix, allowing infiltration of the grafted GC matrix by mesenchymal cells and blood vessels from healthy underlying tissue and applying a cultured epithelial autograft sheet grown from epidermal cells taken from the animal or human at a wound free site on the animal's or human's body surface. The resulting graft has excellent take rates and has the appearance, growth, maturation and differentiation of normal skin.

5,626,861 April 1, 1994	Laurencin; Cato T., Devin; Jessica, Attawia; Muhammed Assignee: Massachusetts Institute of Technology (Cambridge, MA)	A method for the fabrication of three-dimensional macroporous polymer matrices for use as bone graft or implant material was developed. The composites are formed from a mixture of biodegradable, biocompatible polymer and hydroxyapatite (HA), a particulate calcium phosphate ceramic. The method leaves irregular pores in the composite between 100 and 250 microns in size. In a preferred embodiment, implants are composed of a 50:50 poly(lactide-co-glycolide) (PLGA) polymer and reinforced by hydroxyapatite. Mechanical and histological analysis showed the matrix fabricated by this method to be structurally and mechanically similar to cancellous bone.
5,882,929 April 7, 1998	Fofonoff; Timothy W., Bell; Eugene Assignee: Tissue Engineering, Inc. (Boston, MA)	Apparatus and methods are disclosed for maturing a biopolymer tissue construct in vitro prior to use as a replacement construct in vivo as, for example, a graft, implant, or prosthesis. The tissue is seeded with specific cells, exposed to a maturation fluid, such as a synovial-like fluid containing hyaluronic acid, and subjected to selected conditioning and maturation forces, which can include frictional forces, shear forces, and compressive pressure. The tissue is mounted on a first support element and a second surface applies a selected force to the tissue. This maturation process occurs within a maturation chamber. The resultant matured replacement tissue construct is intended to provide a replacement tissue that is more readily integrable in vivo to produce a more durable and functional replacement tissue.
6,117,456 October 16, 1996	Lee; Dosuk D., Rey; Christian, Aiolova; Maria Tofighi; Aliassghar Assignee: Etex Corporation (Cambridge, MA)	The present invention provides a novel process for producing a calcium phosphate cement or filler which hardens in a temperature dependent fashion in association with an endothermic reaction. In the reaction a limited amount of water is mixed with dry calcium phosphate precursors to produce a hydrated precursor paste. Hardening of the paste occurs rapidly at body temperature and is accompanied by the conversion of one or more of the reactants to poorly crystalline apatitic calcium phosphate. The hardened cements, fillers, growth matrices, orthopedic and delivery devices of the invention are rapidly resorbable and stimulate hard tissue growth and healing.
6,187,047 July 7, 1998	Kwan; Michael K., Pacetti; Stephen D., Yamamoto; Ronald K. Assignee: Orquest, Inc. (Mountain View, CA)	A porous three-dimensional bone grafting matrix is provided which is biodegradable. The matrix is preferably formed from mineralized collagen where the mineral comprises particulate calcium phosphate immobilized in the matrix and having a particle size of 5 microns or less.

6,228,117 July 15, 1998	De Bruijn; Joost Dick, Bovell; Yvonne Pearl, Van Den Brink; Jennigje, Van Blitterswijk; Clemens Antoni Assignee: IsoTis B.V. (Bilthoven, Netherlands)	A device for bone tissue engineering is described which comprises a scaffold material consisting of a bioactive, osteoconductive and bone-bonding segmented thermoplastic copolyester and cultured osteogenic or osteoprogenitor cells, especially bone cells. The copolyester consists essentially of a multiplicity of recurring long-chain ester units and short-chain ester units, the long-chain ester units comprising from 35 to 80% by weight of the copolyester and being represented by the formula --OLO--CO-- or --OLO--CO--R--CO-- and the short-chain ester units being represented by the formula --OEO--CO--R--CO-- and/or --O--Q--CO-- wherein L is a divalent group remaining after removal of terminal hydroxyl groups from a poly(oxyalkylene) glycol with an average molecular weight of between 300 and 3000; R is a divalent group remaining after removal of carboxyl groups from a dicarboxylic acid having a molecular weight of less than 300; Q is an alkylene group having 1-6 carbon atoms and/or a cyclohexylene of phenylene group; and E is an alkylene group having 2-6 carbon atoms.
6,585,992 October 4, 2001 6,846,493 May 10, 2002	Pugh; Sydney M., Smith; Timothy J. N., Sayer; Michael, Langstaff; Sarah Dorthea Assignee: Millenium Biologix, Inc. (Ontario, CA)	The present invention is directed to a synthetic biomaterial compound based on stabilized calcium phosphates and more particularly to the molecular, structural and physical characterization of this compound. The compound comprises calcium, oxygen and phosphorous, wherein at least one of the elements is substituted with an element having an ionic radius of approximately 0.1 to 1.1. ANG. The knowledge of the specific molecular and chemical properties of the compound allows for the development of several uses of the compound in various bone-related clinical conditions.
6,692,761 September 5, 2001	Mahmood; Tahir, Riesle; Jens Uwe, van Blitterswijk; Clemens Antoni Assignee: IsoTis N.V. (Bilthoven, Netherlands)	A biodegradable, biocompatible porous matrix as a scaffold for tissue engineering cartilage is formed of a copolymer of a polyalkylene glycol and an aromatic polyester such as a polyester such as a polytheylene glycol/polybutylene terephtalate copolymer. A ceramic coating such as a calcium phosphate coating may be provided on the scaffold by soaking the scaffold in a solution containing calcium and phosphate ions.
6,753,311 June 28, 2001	Fertala; Andrzej, Ko; Frank Assignee: Drexel University (Philadelphia, PA)	Tissue Engineering scaffolds comprising collagen or a collagen-like peptides incorporated within or between polymeric fibers and methods for their production are provided.
6,764,517 February 22, 2002	Yamamoto; Ronald K., Kwan; Michael K., Pacetti; Stephen D. Assignee: DePuy AcroMed, Inc. (Raynham, MA)	A porous three-dimensional tissue repair matrix is provided which is biodegradable. The matrix is preferably formed from mineralized collagen where the mineral comprises particulate calcium phosphate immobilized in the matrix.

6,767,928 March 17, 2000	Murphy; William L., Peters; Martin C., Mooney; David J., Kohn; David H. Assignee: The Regents of the University of Michigan (Ann Arbor, MI)	Disclosed are advantageous methods for patterning and/or mineralizing biomaterial surfaces. The techniques described are particularly useful for generating three-dimensional or contoured bioimplant materials with patterned surfaces or patterned, mineralized surfaces. Also provided are various methods of using the mineralized and/or patterned biomaterials in tissue engineering, such as bone tissue engineering, providing more control over ongoing biological processes, such as mineralization, growth factor release, cellular attachment and tissue growth.
6,783,712 November 4, 2002	Slivka; Michael, Niederauer; Gabriele G., Kieswetter; Kristine, Leatherbury; Neil C. Assignee: Osteobiologics, Inc. (San Antonio, TX)	A fiber-reinforced, polymeric implant material useful for tissue engineering, and method of making same are provided. The fibers are preferably aligned predominantly parallel to each other, but may also be aligned in a single plane. The implant material comprises a polymeric matrix, preferably a biodegradable matrix, having fibers substantially uniformly distributed therein. In preferred embodiments, porous tissue scaffolds are provided which facilitate regeneration of load-bearing tissues such as articular cartilage and bone. Non-porous fiber-reinforced implant materials are also provided herein useful as permanent implants for load-bearing sites.
6,835,377 May 13, 1998	Goldberg; Victor M., Caplan; Arnold I., Barry; Francis P, Fink; David J., Marshak; Daniel R., Burnes; James S. Assignee: Osiris Therapeutics, Inc. (Baltimore, MD)	For repair of cartilage damaged as part of the degenerative effects of osteoarthritis, the inventors have found that the human mesenchymal stem cell approach makes it possible to: 1. Regenerate both shallow cartilage chondral defects and full thickness cartilage defects; 2. Broaden the suitable clinical population to routinely include the middle-aged patients; 3. Eliminate the use of autologous tissue grafts to repair an articular cartilage injury; 4. Regenerate other types of injuries cartilage such as patellar bone and spinal disk cartilage; 5. Regenerate articular joint cartilage in older patients with osteoarthritis; 6. Form new cartilage and subchondral bone which full integrate into the adjacent normal tissue.
6,858,042 June 21, 2001	Nadler; Daniel, Bittmann; Pedro, Akens; Margarete, Rechenberg; Brigitte, Auer; Jorg Assignee: Zimmer Orthobiologics, Inc. (Austin, TX)	Repairs of cartilage defects or of cartilage/bone defects in human or animal joints with the help of devices including a bone part, a cartilage layer and a subchondral bone plate or an imitation of such a plate in the transition region between the cartilage layer and the bone part. After implantation, the bone part is resorbed and is replaced by reparative tissue only after being essentially totally resorbed.
6,863,694 July 3, 2000 6,808,585 October 9, 2001	Boyce; Todd M., Kaes; David, Scarborough; Nelson L. Assignee: Osteotech, Inc. (Eatontown, NJ)	An osteogenic osteoimplant in the form of a flexible sheet comprising a coherent mass of bone-derived particles, the osteoimplant having a void volume not greater than about 32% and a method of making an osteogenic osteoimplant having not greater than about 32% void volume, the method comprising: providing a coherent mass of bone-derived particles; and, mechanically shaping the coherent mass of bone-derived particles to form an osteogenic osteoimplant in the form of a flexible sheet.

6,863,900 June 26, 2002	Kadiyala; Sudhakar, Bruder; Scott P. Assignee: Osiris Therapeutics, Inc. (Baltimore, MD)	Disclosed are compositions and methods for augmenting bone formation by administering isolated human mesenchymal stem (hMSCs) cells with a ceramic material or matrix or by administering hMSCs.
6,867,247 May 1, 2002	Williams; Simon F., Martin; David P., Skraly; Frank A. Assignee: Metabolix, Inc. (Cambridge, MA)	Devices formed of or including biocompatible polyhydroxyalkanoates are provided with controlled degradation rates, preferably less than one year under physiological conditions. The polyhydroxyalkanoates can contain additives, be formed of mixtures of monomers or include pendant groups or modifications in their backbones, or can be chemically modified, all to alter the degradation rates. The polyhydroxyalkanoate compositions also provide favorable mechanical properties, biocompatibility, and degradation times within desirable time frames under physiological conditions.
6,884,428 December 16, 2002	Binette; Francois, Bowman; Steven M., Bruker; Izi, Hwang; Julia, Melican; Mora Carolynne, Rezania; Alireza Assignee: DePuy Mitek, Inc. (Norwood, MA)	A biocompatible tissue repair stimulating implant or "scaffold" device is used to repair tissue injuries, particularly injuries to ligaments, tendons, and nerves. Such implants are especially useful in methods that involve surgical procedures to repair injuries to ligament, tendon, and nerve tissue in the hand and foot. The repair procedures may be conducted with implants that contain a biological component that assists in healing or tissue repair.
6,884,518 February 21, 2001	Aho; Allan, Yli-Urpo; Antti	A material suitable for the reconstruction of an individual's tissue, particularly supportive tissue such as bone tissue, insertable into the tissue. The material is characterized by being composed of wood heat-treated within the temperature range of 100-220° C. in the presence of water vapor. The invention also relates the use of the wood.
6,887,488 May 2, 2001	Cui; Fuzhai, Zhang; Shuming, Zhang; Wei, Cai; Qiang, Feng; Qingling Assignee: Tsinghua University (Beijing, CN)	The present invention relates to a nano-calcium phosphates/collagen composite that mimics the natural bone, both in composition and microstructure, as well as porous bone substitute and tissue engineering scaffolds made by a complex of said composite and poly(lactic acid)(PLA) or poly(lactic acid-co-glycolic acid)(PLGA). The invention also relates to the use of said scaffold in treating bone defect and bone fracture.
6,890,333 November 13, 2001	von Hoffmann; Gerard, Cachia; Victor V., Culbert; Brad S Assignee: Triage Medical, Inc. (Irvine, CA)	Disclosed is a fracture fixation device, for reducing and compressing fractures in a bone. The fixation device includes an elongate body comprising a first portion and a second portion that are detachably coupled to each other. The first portion defines a helical cancellous bone anchor and the second portion defines a distal end. An axially moveable proximal anchor is carried by the proximal end of the fixation device and is rotationally locked to the first portion. The device is rotated into position across the femoral neck and into the femoral head, and the proximal anchor is distally advanced to lock the device into place. The second portion is then detached from the first portion.

6,890,354 March 8, 2002	Steiner; Anton J., Gertzman; Arthur A. Assignee: Musculoskeletal Transplant Foundation (Edison, NJ)	The invention is directed toward a bone block, a bone-tendon-bone assembly and method of tendon reconstruction in which at least one tendon replacement is extended between two bone blocks and fixed within each of two bone tunnels in the bones of a joint using interference screws. Each bone block has a central through going bore and at least one substantially parallel channel longitudinally cut in the exterior of the bone block body in which the ligament replacements are seated. One end of each bone block has a rounded recess leading from the central bore to the exterior parallel channel.
6,893,462 August 29, 2001	Buskirk; Dayna, Seid; Chris, Wironen; John F., Gross; James M., Scurti; Gina Assignee: Regeneration Technologies, Inc. (Alachua, FL)	Disclosed herein is processed dermis graft for use in orthopedic surgical procedures. Specifically exemplified herein is a processed dermis graft comprising one or more bone blocks having a groove cut into the surface thereof, wherein said groove is sufficient to accommodate a fixation screw. Also disclosed is a method of processing dermis that results in a dermis derived implant suitable to replace a tendon or ligament in a recipient in need thereof. Other compositions and applications of a dermis derived implant, and methods of manufacture and use, are disclosed.
6,893,666 October 25, 2002	Spievack; Alan R. Assignee: ACell, Inc. (Cambridge, MA)	A matrix, including epithelial basement membrane, for inducing repair of mammalian tissue defects and in vitro cell propagation derived from epithelial tissues of a warm-blooded vertebrate.
6,896,904 June 14, 2004	Spiro; Robert, Liu; Lin Shu Assignee: Depuy Spine, Inc. (Raynham, MA)	Disclosed are bilayer matrices of a polysaccharide such as collagen (COL) and another polysaccharide such as hyaluronic acid (HA) with various COL/HA ratios. Each layers has a porous structure. These materials are useful for tissue regeneration, particularly when used with orthopedic implants and drug delivery.