April 2008

Nanomaterials for Bone Implants

Garrett Devin Cavanaugh
Worcester Polytechnic Institute

Justin Michael Perron
Worcester Polytechnic Institute

Follow this and additional works at: https://digitalcommons.wpi.edu/mqp-all

Repository Citation

This Unrestricted is brought to you for free and open access by the Major Qualifying Projects at Digital WPI. It has been accepted for inclusion in Major Qualifying Projects (All Years) by an authorized administrator of Digital WPI. For more information, please contact digitalwpi@wpi.edu.
Nano-Materials for Bone Implants

A Major Qualifying Project Report:

Submitted to the Faculty

Of the

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirements for the

Degree of Bachelor of Science

By

__________________________________.

Garrett Cavanaugh

And

__________________________________.

Justin Perron

Date:

Approved:

__________________________________

Prof. Jianyu Liang
Abstract

An increase of geriatric patients requires improved orthopedic implant technology. Hydroxyapatite is a material naturally found in bone tissue, but the use of synthesized hydroxyapatite in orthopedic implants is limited because of its poor mechanical properties. The goal of this project is to use nanocrystalline hydroxyapatite and epoxy resin to create composite materials that mimic bone tissue’s nano-structure, test their mechanical properties to determine the influence of nanostructure and characterize the failure mechanisms using electron microscopy.
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>INTRODUCTION</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>BACKGROUND</td>
<td>10</td>
</tr>
<tr>
<td>2.1</td>
<td>Bone Tissue</td>
<td>10</td>
</tr>
<tr>
<td>2.2</td>
<td>Current Orthopedic Implant Technology</td>
<td>15</td>
</tr>
<tr>
<td>2.3</td>
<td>Advantages of Hydroxyapatite as a Bio-material</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>APPROACH</td>
<td>19</td>
</tr>
<tr>
<td>3.1</td>
<td>Synthesizing Hydroxyapatite</td>
<td>19</td>
</tr>
<tr>
<td>3.2</td>
<td>Mechanical Testing</td>
<td>21</td>
</tr>
<tr>
<td>3.3</td>
<td>Characterization</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>METHODOLOGY</td>
<td>24</td>
</tr>
<tr>
<td>4.1</td>
<td>Mold Fabrication</td>
<td>24</td>
</tr>
<tr>
<td>4.2</td>
<td>Sample Fabrication</td>
<td>26</td>
</tr>
<tr>
<td>4.3</td>
<td>Mechanical Testing</td>
<td>31</td>
</tr>
<tr>
<td>4.4</td>
<td>Characterization</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>RESULTS AND ANALYSIS</td>
<td>34</td>
</tr>
<tr>
<td>5.1</td>
<td>Powder Characterization</td>
<td>34</td>
</tr>
<tr>
<td>5.2</td>
<td>Compression Test Results: General Patterns</td>
<td>35</td>
</tr>
<tr>
<td>5.3</td>
<td>Compression Test Results: Composite Properties</td>
<td>38</td>
</tr>
<tr>
<td>5.4</td>
<td>SEM Image Analysis</td>
<td>43</td>
</tr>
<tr>
<td>6</td>
<td>CONCLUSIONS</td>
<td>48</td>
</tr>
<tr>
<td>6.1</td>
<td>Mechanical Properties</td>
<td>48</td>
</tr>
</tbody>
</table>
6.2 Structure and Defects ........................................................................................................51
6.3 Recommendations for future work .................................................................................51

7 REFERENCES ......................................................................................................................54
List of Figures

Figure 2.1 Structure of a collagen fibril. (Fratzl et al. 2007) ................................................. 12
Figure 2.2 Density and modulus graph of human vertebra. (Kopperdahl et al. 1998) ..................... 13
Figure 2.3 Yield Strain and density in bone tissue. (Kopperdahl et al. 1998) ............................... 13
Figure 2.4 A free body diagram of a femur. (Cowin 1989) ....................................................... 14
Figure 3.1 Process flow diagram. ................................................................................................ 19
Figure 4.1 CAD model of the mold and completed HDPE mold. .................................................... 25
Figure 4.2 Picture of purchased hydroxyapatite ......................................................................... 28
Figure 4.3 Picture of samples released from the mold ................................................................. 30
Figure 4.4 MTS Qtest/5 compression tester ................................................................................. 32
Figure 5.1 SEM image of Hydroxyapatite powder .................................................................... 34
Figure 5.2 SEM images of Hydroxyapatite powder .................................................................... 35
Figure 5.3 Example Stress/Strain graphs of compression tests ..................................................... 36
Figure 5.4 Graph representing repeated peaks in a 70% CaP sample ........................................ 37
Figure 5.5 Graphs representing the elastic region slopes in 77%, 70% and 60% CaP samples .......... 38
Figure 5.8 Stress/Strain curve of sample 60% A and 60% B .......................................................... 39
Figure 5.9 Stress/Strain Curve from 70% A, 70% B and 70% C ...................................................... 40
Figure 5.10 Stress Strain Curve from 77%A and 77%B. ................................................................. 41
Figure 5.12 Stress/Strain curve from 50%A, 50%B and 50%C ...................................................... 42
Figure 5.15 SEM images of sample 77A ...................................................................................... 44
Figure 5.17 SEM images of sample 70A ...................................................................................... 45
Figure 5.19 SEM images of sample 60A ...................................................................................... 46
Figure 5.22 SEM images of sample 50C ...................................................................................... 47
Figure 5.24 SEM image of hydroxyapatite powder ....................................................................... 47
Figure 6.1 Ultimate Strength vs. % Epoxy Graph ...................................................................... 48
Figure 6.2 Yield Strength vs. % Epoxy Graph ................................................................. 49
Figure 6.3 Modulus vs. % Epoxy Graph ........................................................................ 50
List of Tables

Table 4.1 Sample compositions ............................................................... 31

Table 5.1 Elastic Modulus and Compressive Strengths .................................. 42
1 Introduction

As a population, standards of living and medicinal science continue to increase and improve, and the life expectancy of our population increase. More and more people will be living longer. The inevitable result of this is an increased geriatric population, and medical science and industry have to address more illnesses and afflictions associated with old age more frequently, including the wear on bodily tissues. One drastic example of this is the degradation of bone tissue, especially in certain joints, such as the knee or the hip, resulting in the need to replace them with orthopedic implants. While the materials used for these have a long history of success, a better understanding of the natural structure, makeup, and formation of bone tissue as well as new fabrication techniques, provide the opportunity for developing new materials and improving current technology.

Bone tissue plays a number of critical roles in the human body. Bones are repositories for calcium, produce both red and white blood cells, and provide structural support for the body. Of particular interest to this project are the mechanical properties of bone tissue, which are significantly superior to those of other materials, both natural and synthetic, that have similar chemical compositions. It is the unique structure of bone tissue that gives rise to these properties. Bone tissue has a nano-scaled hierarchical structure, which consists of hydroxyapatite crystals within a matrix of collagen fibers as well as other proteins (Olszta et al. 2007). Collagen is a common protein in the human body and has a triple helix structure. Hydroxyapatite is a type of calcium phosphate, \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \) (Fratzl et al. 2007). While they are the two main components of bone tissue, neither of these compounds alone are half as strong, stiff, or tough as bone. For instance, hydroxyapatite can be synthesized easily and is ideal for some medical and dental implants because it is bioactive, meaning it stimulates bone growth (Olszta et al. 2007).
However, hydroxyapatite as a bulk solid does not have the necessary mechanical properties, such as strength or stiffness, to be used in load bearing applications. While much has been learned about the structure and growth of bone tissue due to modern microscopy, no reliable method of synthesizing this structure has been developed. Advances in nanotechnology, in particular the fabrication of inorganic crystals on a nano-scale, may provide means for such synthesis.

A material chemically and structurally similar to bone tissue may have a significant impact on current biomaterials technology and the biomaterials market. Orthopedic (bone related) biomaterials dominated biomaterials sales in 2002 with approximately $14 billion in sales and is expected to grow 7% to 9% annually. (Ratner et al. 2004). As their status in the market would indicate, current orthopedic biomaterials are overwhelmingly successful in the long term (>7 years). However, they have been associated with adverse reactions. These reactions are often caused by the degradation of the implant material due to physical wear or electrochemical corrosion (Ratner et al. 2004). The human body may also recognize that the implant is a foreign entity, which would initiate an immune response. Metals are the most common material used in orthopedic implants today, especially in load bearing applications (Ratner et al. 2004). If the natural structure of bone tissue could be reproduced to achieve the necessary mechanical properties in a hydroxyapatite-based biomaterial for orthopedic implants, than perhaps it could be used as an alternative to the metals and other materials currently used. These implants could potentially produce less severe reactions and would also benefit from the qualities that make hydroxyapatite a good candidate for an implant material on its own, such as bioactivity.

Much research has been done on the value of hydroxyapatite-based materials, their synthesis, their mechanical properties, and how both the structure and properties can be improved. Hydroxyapatite crystals can be easily and efficiently synthesized on a nanoscale.
(particle size of 20nm-200nm) (Hernandez et al. 2007). In addition, scaffolds of nano-hydroxyapatite/polymer with high porosities have been created using thermal induced phase separation (TIPS) techniques to mimic the collagen/hydroxyapatite matrix found in natural bone tissue in the hopes of achieving similar mechanical properties (Ma et al. 2004). This technique was successful in both improving the desired mechanical properties and increasing protein absorption. Experiments have also been conducted integrating hydroxyapatite crystals with both various fibers and polymers in an attempt to strengthen the hydroxyapatite. In both cases, the ultimate strengths of the composites were improved to reach roughly 62 MPa, several times the strength of pure hydroxyapatite (Eichmiller et al. 2000, Mayes et al. 2004). However, these experiments did not use nano-sized hydroxyapatite, and did not necessarily mimic the natural collagen/hydroxyapatite.

The goal of this project is to use current technology to design and fabricate a composite material with the best possible approximation of bone tissue’s nano-structure given the constraints of facilities and time, test the mechanical properties of the material to determine how they are affected by said structure, and characterize the failure mechanisms using electron microscopy. In the following chapters we will give more background information on the motivation for the project and available means for synthesis, testing, and characterization. Further on we will discuss the methodology for the experiments as well as their results.

2 Background

2.1 Bone Tissue

Since the purpose of our analysis is to develop a material that may ultimately be used as orthopedic implants, it is essential to have an understanding of the chemical and physical
properties of bone tissue to guide our approach. This is especially true since we intend to mimic the structure of bone tissue. Fortunately the function and structure of bones and bone tissue is well understood. However, the many different aspects of bone tissue structure require a more detailed description than the brief summary provided in the introduction.

Bone tissue falls into two different categories: cortical bone and trabecular or cancellous bone. The shell of every bone consists of cortical bone, but some bones have much thicker shells than others. The thickness of the cortical bone shell is determined by what type of bone it is and what type of loads it is subjected to. Cortical bone is thickest (a few centimeters) in the shafts of long bones found in extremities such as the femur and tibia. These bones are usually subjected to bending and buckling loads. Bones that are subjected to compressive loading have much thinner shells, as thin as few tenths of a millimeter. These bones, such as the head of the femur or a vertebra, are also filled with the “spongy” trabecular bone. (Fratzl et al. 2007).

As mentioned in the introduction, bone is a hierarchical structure. What distinguishes cortical and trabecular bone from each other is differences in the higher levels of this hierarchical structure. Cortical bone has porosity on the order of 6%. These pores are usually blood vessels. The tissue is structured as concentric layers around these pores. These layers are made up of stacked lamellae, or planar layers of bone tissue. Each lamella is about 5 micrometers thick. Trabecular bone has a much higher porosity in comparison, on the order of 80%. This porosity comes from not only blood vessels but osteocytes, or bone cells (Fratzl et al. 2007). Due this high porosity trabecular bone is like a network of interconnected branches, or trabeculae. The thickness of each trabecula is fairly consistent, approximately 2 micrometers (Fratzl et al. 2007).
The nano-sized structures discussed in the introduction are found at the lower levels of the hierarchical structure. The basic building block of bone tissue is mineralized collagen fibrils approximately 100 nm in diameter (Fratzl et al. 2007). These fibrils are made of collagen fibers 300 nm long and 1 – 1.5 nm thick. Within these fibrils are the hydroxyapatite crystals. They usually form as thin flat plates parallel to the long axis of surrounding collagen fibers (Fratzl et al. 2007). They can be 30–50 nm length, 15–30 nm in width, and 2–10 nm in thickness (Ref 2). The crystals appear at regular intervals with a repeating distance of approximately 67 nm. In addition to the crystals within the fibril, the fibril is also coated in hydroxyapatite (Fratzl et al. 2007).

When a collagen fibril is first deposited by osteoblast (bone forming cells), it is mineral free and consists only of collagen. Mineral content increases with time as crystal formation is initiated by collagen or other proteins acting as nucleation sites (Fratzl et al. 2007). The amount of hydroxyapatite in a fibril affects its mechanical properties; the more mineralization has occurred the stronger the fibril becomes (Fratzl et al. 2007). This relationship between strength and content holds for lamellae and trabeculae as well.

A wide range of mineral content in bone tissue results in a wide range of mechanical properties. For instance, the on-axis elastic modulus can vary from .3 MPa to 3000 MPa (Fratzl
et al. 2007). Relationships between bone density and both yield strength and elastic modulus have been found (Kopperdahl et al. 1998).

The graphs below demonstrate such relationships in human trabecular bone. The same study also measured the yield strain, determining that compressive yield strain still varies proportionately with bone density while tensile strain does not. Yield strain does not appear vary to the same extent that strength and modulus does.

Because of the broad range of possibilities, the mechanical properties of bone tissue do not provide an effective standard for comparison. It is difficult to determine if a given material
would be a good substitute for bone if it is both much stronger and much weaker than many samples. However, we can analyze the loading subjected to bone tissue in a typical situation to find the stresses involved, and use that data as a guide in evaluating whether the material or materials studied in this project would be adequate for those situations.

The following free-body diagrams depict the femur of a 1000 N (225 lbs) person standing on one leg and the tibia of the same person as he climbs stairs. This weight is greater than average but makes it easy for scaling and is also closer to a worst-case scenario. This analysis shows compressive stresses ranging from 29.7 to 58.5 MPa for the femur and 72.6 to 90.4 MPa for the tibia. It also shows tensile stresses ranging from 22.2 to 51.6 MPa in the femur 62.6 to 83.4 MPa in the tibia (Cowin, 1989). In addition, for the purposes of this study, the average values found by Cezayirlioglu et al. for femoral bone will serve as a benchmark for our evaluation. These
values are tensile yield strength of 115 MPa, compressive yield strength of 182 MPa, an ultimate tensile strength of 134 MPa and an ultimate compressive strength of 206 MPa.

2.2 Current Orthopedic Implant Technology

The materials used in current orthopedic implants are chosen to withstand cyclic load-bearing applications. Metals, ceramics and polymers are all used in orthopedic biomaterials. Polymers are used in two different ways. The first is as articulating bearing surfaces for joint replacement. The second is as cement between the implant surface and the bone. Polymers or bearing surfaces have low coefficients of friction and low wear rates (Ratner et al. 2004). Ceramics are being used more often as an alternative bearing surface because of their superior wear resistance of ceramics when compared to metal-metal or metal-polymer wear rates. (Ratner et al. 2004) It is the metals that most often provide the strength, ductility, fracture toughness, hardness, corrosion resistance, formability, and biocompatibility needed in load-bearing situations like fracture fixation or total joint arthroplasty. For example, a typical orthopedic implant for a total hip replacement procedure would likely use titanium or a cobalt-chromium alloy the femoral stem, either cemented with PMMA or press fit, combined with a modular cobalt-chromium alloy or ceramic head that “articulates on a ultrahigh-molecular-weight polyethylene (UHMWPE) or ceramic acetabular cup fitted into a titanium or cobalt-chromium cup liner that is cemented, screwed, or press fit into place” (Ratner et al. 2004).

Two types of cobalt-chromium alloys are predominantly used: CoCrMo (ASTM F-75 and F-76) and CoNiCrMo (ASTM F-562). The latter can have increased corrosion resistance when Ni is high enough but this benefit carries the risk of toxicity and immunogenic reactivity from release Ni. CoCrMo is among the strongest, hardest, most fatigue resistant metals used but care must be taken because finishing treatments can reduce these properties. For example, sintering of
porous coatings can reduce fatigue strength from 250-200 MPa to 150 MPa after annealing the material at 1225 deg C (Ratner et al. 2004). Two types of titanium alloys are also used: commercially pure titanium (CPTi) and Ti-6Al-4V. Pure titanium forms an oxide layer and has high corrosion resistance. It is used in porous coatings due to its high ductility. Ti-6Al-4V, on the other hand, has superior mechanical properties and more often used for replacement components (Ratner et al. 2004).

The drawbacks to using the above materials arise from two sources: the degradation of the material due to corrosion and wear, and the human body’s response to the material during the wound healing process. Debris from degradation may be particulate wear, colloidal nanometer-size complexes, free metallic ions, or inorganic metal salts/oxides (Ratner et al. 2004). The clinical issues regarding this debris are how much material is released, where it is transported, what is the chemical form of degraded material, and of course what the consequences of such degradation are (Ratner et al. 2004). One possible outcome of this debris is a risk of metal hypersensitivity, or allergy to metal. This condition is not uncommon, and dermal hypersensitivity affects 10%-15% of the general public. The link between this condition and metal in implants is unclear. Some studies have shown that incidence of metal hypersensitivity is roughly twice as common in patients with metal implants, approximately 25%, regardless of whether the implants function well. Furthermore, when considering only the patients whose implants “failed” (required further surgery) the incidence of hypersensitivity is approximately 50%-60%. However, it must be noted that several published reports have indicated that this condition as lessened after a metal implant was installed, and no correlation has been found between the onset of metal hypersensitivity and implant residence time, infection, reason for removal, or pain. (Ratner et al. 2004).
The degradation of the implant material can also expose the patient to infection. Microbes and pathogens lie dormant in the micro or nano-scale sized cracks and imperfections on the surface of an inadequately sterilized implant. As the implant deteriorates and wears, the cracks can propagate and, bringing the pathogens in direct contact with the surrounding tissue. Infections like these can occur long after the implant was installed, and often contribute to device failure. Infections associated with implant devices are often resistant to anti-bodies and immune responses, and can persist until the device is removed (Ratner et al. 2004).

Even when no infection is present, the implant can often trigger an immune response. The immune system will react to any foreign body regardless of whether or not it is pathological. While an implant will not be “rejected” the same way a transplanted organ sometimes is, the immune response will affect the local tissue, causing inflammation and over long periods of time scarring which can lead to the failure of the device. In a metal hip prosthesis, a prolonged immune response may damage the local bone tissue, causing the implant to loosen (Ratner et al. 2004)

2.3 Advantages of Hydroxyapatite as a Bio-material

Hydroxyapatite has many properties that may reduce or remedy some of the undesired outcomes associated with metallic implants that may occur. Since hydroxyapatite is found in bone tissue and teeth, not only is it non-toxic but it would not be targeted by the immune system as foreign body. Therefore the local tissue inflammation and eventual scarring caused by an immunological response would not be an issue if hydroxyapatite were used. Furthermore, the physiological effects of deterioration may not be as severe since hydroxyapatite is nontoxic and also resorbable.
Hydroxyapatite is also bioactive. The term bioactive refers to materials that have been shown to bond with bone. However, the bonding mechanism of dense hydroxyapatite implants is unique. A cellular bone matrix appears at the surface, producing a narrow amorphous electron-dense band 3-5 micrometers wide. Collagen and mineral crystals forms between this area and the cells, and as the bond matures, it shrinks to .05 to .2 micrometers. TEM images have revealed near perfect crystal alignment between the crystals in the growing bone and the crystals in the implant (Ratner et al. 2004).

Synthetic hydroxyapatite is integrated into bone tissue by the same mechanisms used in the active remodeling of healthy bone, which is the resorption and deposition of the bone mineral. This further develops the bond between the synthetic hydroxyapatite and the bone. Hydroxyapatite is also known to aid the adhesion and proliferation of osteoblasts (Mayes et al. 2002).
3 Approach

The general approach to this project can be seen in Figure 3.1, which is a process flow diagram.

Some of the key steps will be discussed in this section; whereas some of the others will be discussed in the next section Methodology.

![Figure 3.1 Process flow diagram.](image)

3.1 Synthesizing Hydroxyapatite

Synthetic hydroxyapatite is commercially available. The production is usually based on aqueous precipitation or conversion from other calcium rich material. While these methods are common for supplying the material in a bulk form, coating hydroxyapatite onto other material is
also common. For this application sputtering, sol-gel, and plasma spraying are often used, with plasma spraying being a favorite (Ratner et al. 2004).

Sputtering is ejecting atoms of a target material by bombarding the target material with high energy ions. The ejected atoms then collect and form a thin film on a substrate. Plasma spraying consists of spraying the coating material, usually in powder form, into a jet of plasma. The particles are then rapidly heated and sent flying towards the substrate. Once the liquid strikes the substrate it rapidly solidifies to form well structured coatings. The plasma is made with an electric arc, commonly between a tungsten cathode and a copper anode. The gas used for the plasma depends on the coating material, but argon, helium, and hydrogen are commonly used (Lugsheider et al. 1990).

Unfortunately, plasma spraying and sputtering do not produce the nanoscale structures required for this project, whereas the Sol-gel method can. The Sol-gel method is cheap and does not require high temperature or pressure. Another advantage of the Sol-gel method is that it does not require using a vacuum. Also, a sol-gel can be used to make a coating, a powder, or poured into a mold. In a typical sol-gel synthesis, metal or main group element compounds undergo hydrolysis and condensation reactions to form gel materials with extended three dimensional structures (Nalwa 2002). The liquid phase is removed in a drying process to leave a porous material. This may be followed by further thermal treatment, or firing, which promotes further condensation and enhances mechanical properties.

For the purposes of testing and fabrication of usable implants, an ultrafine powder consisting of the hydroxyapatite nanocrystals must be fabricated into a dense bulk material. A significant advantage of sol-gel is that it poured directly into a mold of any shape. The casting
process would be complete after the firing. Since the rate of densification is inversely proportional to particles size, the sintering time required for a given density is less for a nano-sized crystal than for a micron sized crystal. Unfortunately it is still difficult to achieve full densification while retaining the nanoscale microstructure desired (Nalwa 2002). Alternatively, the sol-gel technique could be used to produce an ultrafine hydroxyapatite powder which is then mixed with water into a paste and set, like current calcium-phosphate cements. This process would easily allow for additives to be mixed into the hydroxyapatite to serve as a substitute for the collagen fibers that play such an important role in the structure of natural bone tissue.

3.2 Mechanical Testing

In this project, the hydroxyapatite composites were tested to determine their mechanical properties so that these properties could be compared bone tissue. Mechanical testing has a special significance for ceramic materials because ceramics have high potential for catastrophic brittle failure. The point of failure is of large concern, and careful, focused experiments are required to able to predict when failure will occur. This means determining the ultimate strength and ultimate strain of a material.

Since both yield and ultimate strengths are different depending on whether a material is loading in compression or tension, there are different tests for each. Flexural tests can also be performed to determine the modulus of elasticity in bending. All of these tests determine the strength distributions due to inherent flaws in a large, stressed volume, characterize the stress-strain behaviors for engineering design purposes, and unambiguously qualify the effects of cyclic fatigue loading (Crammer 1998). WPI has several Instron Universal Testing machines in Washburn Laboratories. These machines can perform tensile, compression, flexural and fatigue tests depending on how there are set up. These can be used to find both the ultimate strength and
the yield strength of a sample. The testing machines also record the load and deformation values throughout the entire test. This data can be used to create stress-strain curves for each sample, which can then be used for further analysis. The stress and strain values in the elastic region of a compression of tension test can be analyzed to determine the young’s modulus.

Another useful test to perform on any material is a hardness test. Hardness testing determines a samples resistance to plastic flow. Hardness testing can have different meanings to different fields though; to a mineralogist, it is the resistance to scratching, to a metallurgist it is the resistance to penetration. The hardness test finds very similar characteristics as the tensile stress test. Most hardness tests are performed with a static indentation device. With this device a diamond shaped tip is plunged into the surface of a material at a given pressure. Sometimes a shape other then diamond is used. The tip is then removed and the indentation can be measured for depth and or area. Hardness tests are usually a test to find the relationship between the load and the area or depth of indentation. On larger sized samples the depth of indentation is usually measured; however on the thinner more shallow samples the area of indentation is measured (Chandler 1999). WPI has a variety of hardness testers for indentation and micro hardness testing. A micro hardness tester is a static indentation tester used for the shallower, smaller samples.

3.3 **Characterization**

After the mechanical testing has been completed on each sample, it will be examined to determine its failure mechanisms. The term failure mechanism refers to how cracks and imperfections propagate through a material’s microstructure. Examining the failure mechanisms of each sample could reveal how the micro-structure influenced its mechanical behavior. This is
an incredibly important aspect of the project, as understanding how the microstructure could be manipulated to improve mechanical properties is the ultimate goal of this project.

In order to gain an understanding of the failure mechanisms, each sample will be examined using a scanning electron microscope (SEM). WPI has a JEOL JSM-840 SEM located in Washburn Laboratories. A SEM operates by emitting electrons from an electron source, typically a tungsten or lanthanum hexaboride tip, which acts as a cathode. These electrons are focused by several magnetic lenses as it travels through a tube toward an anode at the other end, much as an optical lens focuses light in optical microscope. In order for the electron beam to travel through the tube without being interfered with, the tube must be in a vacuum.

Images generated from SEMs can be a result of secondary electrons, backscatter electrons, or x-rays. Secondary electrons are electrons that were scattered of the sample by the electron beam. They have a low energy and generally originate only a few nanometers from the surface. Detectors can use these electrons to create a high-resolution image of the sample surface and display its surface morphology in great detail. Backscattered electrons are electrons that were originally from the beam but were scattered back after interacting with the sample. These electrons show a contrast between areas of different composition. X-rays are generated from the interaction of the beam and the sample. This can be used for energy-dispersive X-ray spectroscopy.
4 Methodology

4.1 Mold Fabrication

A crucial element in the fabrication of the composite samples was a suitable mold. How material fills and sets in a mold significantly affects the probability that defects will develop in the sample. To ensure that the mold used would be suitable, a set of design criteria was developed. First, the mold had to be easy to fill completely. This was primarily to avoid malformed samples and samples with defects such as air bubbles or cracks. Second, the sample needed to be easily released once formed. If the material adhered to inside surfaces of the mold too strongly, removing the samples without damaging them could be extremely difficult or impossible. Finally, the mold had to be reusable. There was not enough time nor material to sacrifice a mold for each set of samples and make a new one. Each mold for this project was made in machine shop in Higgins Laboratories at WPI.

The initial mold design was heavily influenced by Mayes’ paper, polymer-calcium phosphate cement composites, with respect to both geometry and materials used. The samples in Mayes’ experiment were cylindrical, 6 mm in diameter 12 mm in depth. These dimensions were used as a guideline for the molds in this project, since both the material tested and the tests conducted in this project are similar to that experiment. Because of the US standard measurements used on manual milling machines, the actual dimensions used for this mold was a diameter of .25 inches with a depth of .5 inches. The material used for the initial mold design was aluminum as opposed to stainless steel. This is due to both availability of aluminum over stainless steel in the Higgins machine shop and the machinability of aluminum, which is much greater than that of stainless steel. This initial mold was created by taking a ¾ inch thick block of
aluminum, then taking a ¼ inch flat end mill and drilling down a 1/2 inch to create a negative of the desired sample geometry within the aluminum block. After this mold was made a simple paste of the hydroxyapatite was made to test its suitability. The paste was made by mixing the hydroxyapatite nanopowder and deionized water using a powder to water ratio of approximately 3:1. This paste was then spatulated into the mold; however, the paste would not go into the holes easily. The thickness of the paste and narrowness of the hole created a problem with airflow. Material at the top of the hole trapped air bubbles in the bottom of the hole, and pushing the paste to the bottom of the hole to ensure complete fullness became a challenge. In addition to the filling problems, the sample could not be released after the material had cured and dried. The surface of the aluminum, especially with the machine marks, was very rough, making it easy for the nanosized particles to adhere to the aluminum. In addition, because the only exposed surface of the sample was the top, it was impossible to apply a force to it that would eject it. Thus the initial mold design failed two of the three design criteria, and a redesign was required.

Figure 4.1 CAD model of the mold and completed HDPE mold.

The second attempt at the mold design and fabrication was much more successful. In order to ensure that such a narrow hole could be easily and completely filled to fulfill the first design criterion, completely filling the mold, it was designed as a hollow tube open at both ends. This allowed air to escape out one end while material was being forced down the other. This
could easily be fabricated by drilling ¼ inch holes in a ½ inch thick piece of material. As for the mold material, plastic replaced aluminum to reduce the degree of which the material adhered to the mold surface. This was done to satisfy with the second design criterion, easy release. A ½ inch stock of HDPE from the Higgins machine shop was used. Furthermore, the mold was split into two parts. Each part consisted of four half cylinders with a diameter of .25 inch and was an exact mirror image of the other part. When the two parts were together the half cylinders became full cylinders .25 inches in diameter and .5 inches in depth. Two 1/4in rods could be placed in two of the holes to ensure the two halves did not slip back and forth, and a standard c-clamp was used to ensure the mold stayed closed. Once the samples were fully formed, the c-clamp could be removed and the two halves pulled apart, thus releasing the sample.

4.2 Sample Fabrication

Fabrication of the composite samples required the nanocrystalline hydroxyapatite on which the composites are based. The original intent of the project was to synthesize the nanocrystals at Worcester Polytechnic Institute (WPI) using the Sol-gel method discussed in the background chapter. Unfortunately, attempts to synthesize the hydroxyapatite nanocrystals this way did not yield satisfactory results. Small amounts of hydroxyapatite were made in the form of a thin film covering a substrate. Unfortunately this thin film was not useful for the purposes of this project. The material needed to have a three-dimensional geometry suitable for undergoing mechanical tests. The thin film did not meet this criterion. Although the research referenced in the background indicated that a sol-gel could in fact be poured into a three-dimensional mold, the sol-gel method has not been used in this fashion at WPI up to this point, and the results of preliminary attempts at synthesizing the nanocrystalline hydroxyapatite with this method produced a number of concerns on whether or not the sol-gel method could indeed create the
necessary sample geometries with the laboratory facilities available at WPI. The first and foremost of these concerns was whether the sol-gel would solidify evenly throughout the entire mold as desired or whether it would only deposit a film on the inside wall of the mold as though the wall of the mold were a substrate, leaving the center of the final sample hollow. Another concern regarding the viability of the sol-gel method for producing a three-dimensional sample was how much the original volume of the sol-gel poured into mold would decrease as the liquid phase was removed during the drying process and the final sample was created. If there was indeed a significant difference between the final volume of the sample and the original volume of the sol-gel, the disparity would affect how the remaining material filled the mold, and the final size and shape of the sample could be difficult to control. Due to these concerns, the sol-gel method was abandoned as a means of synthesizing the nanocrystalline hydroxyapatite. Since other methods of synthesizing nanocrystals are complicating and expensive procedures, such as plasma spraying or sputtering, that require high temperatures, high pressures, and in some cases vacuums, the best option forward was to purchase the hydroxyapatite as opposed to synthesizing it ourselves.

The hydroxyapatite that was used for this project was purchased from Sigma Aldrich, 6177418 Hydroxyapatite Nanopowder. The exact molecular formula for this particular hydroxyapatite sold by Sigma Aldrich is \([\text{Ca}_5(\text{OH})(\text{PO}_4)_3]_x\). The molecular weight is 502.31g/mol. It comes in the form of a dry, solid nanopowder. The particle size is <200nm and the surface area is 9.4m²/g. 100 grams of this hydroxyapatite nanopowder were purchased and came in ten ten gram bottles. Having ten separate bottles was an advantage because it reduced the opportunity for contamination.
Once the hydroxyapatite had been purchased and the final mold had been designed and fabricated, sample fabrication could begin. Due to the small grain size and the expense of the material being used, sample contamination was serious concern. It should be noted that all instruments used to weigh and mix the material were thoroughly cleaned with acetone, isopropyl alcohol, and deionized water in that order. The mold itself was only cleaned with isopropyl alcohol and deionized water to prevent damaging it since it was made from a polymer material and acetone can dissolve some plastics.

The first attempts at sample fabrication were intended to create pure hydroxyapatite nanopowder samples. The purpose of creating those pure samples was to be able to use the results of their mechanical tests as a control to compare the test results of the composite samples. Two sets of pure hydroxyapatite samples were created. For the first set of samples 3.105g of the hydroxyapatite nanopowder were mixed with 1.174g of deionized water for a mixing ratio of 2.64:1g hydroxyapatite to water, or 73% hydroxyapatite by weight. The resultant mixture was a thick and sticky paste that was somewhat difficult to manage. The mixture was then spatulated into the HDPE mold described in the previous section. The mold was then placed in oven at 37°C for one hour so the water in the mixture could evaporate. The temperature 37°C was chosen because this is the natural human body temperature, the cements used in similar
experiments set at this temperature. After this hour the mold was removed and left at room
temperature over night. The second set of pure hydroxyapatite samples were fabricated with
same procedure, the only difference being that 3.077g of hydroxyapatite and 2.06g of deionized
water was used, resulting in a mixing ratio of 1.49:1g hydroxyapatite to water, or 60% hydroxyapatite by weight. The resulting samples of both these sets were extremely delicate and
fragile, indicating extremely poor mechanical properties. This was especially true of the samples
from the second set, 60% hydroxyapatite by weight, which almost fell apart immediately after
being removed from the mold.

After pure, albeit fragile, hydroxyapatite samples were made, composite samples were
fabricated. The apparent fragility of the pure samples suggested that a strong binding agent was
needed. Additionally, the structure of the composite samples was intended to mimic the structure
of natural human bone tissue. This meant that if possible, the binder added to the hydroxyapatite
should function similarly to the collagen fibers in human bone tissue, although this was a
secondary concern compared to increasing the structural integrity of the composite samples.
Based on these criteria, epoxy became the choice for the binding agent for the composite
material.

Epoxies are thermosetting polymers that cure or harden when mixed and reacted with a
catalyzing agent commonly referred to as a hardener. Common epoxies are formed by reacting
epichlorohydrin (C₃H₅ClO) and bisphenol-A (C₁₅H₁₆O₂). This reaction creates the polymer chain
C₃H₅[C₁₀H₂₀O₃]ₙC₁₅H₁₉O₂. Epoxy resins typically perform better than other resins such as polyester
or vinyl ester resins. Another factor that made epoxy resins the best choice for this project was
that medical grade epoxies safe for use with medical implants are available. However, such
epoxy resins are more expensive then the non-medical grade varieties. Although the ultimate
application desired for the composites developed and studied in this project was medical implants, the more immediate focus was on the mechanical properties of the material, therefore a cheap and readily available variety seemed appropriate.

The epoxy resin used in this project was purchased from U.S. Composites, EPOX-635314. The mixing ratio is 3:1 resin to hardener. It has a pot life of 20-25 minutes, a setting time of 3-4 hours, and a drying time of 8-10 hours. It set at the temperature 26.7°C. Four sets of samples were fabricated using both the hydroxyapatite nanopowder and the epoxy. For each set, approximately 3g of hydroxyapatite was weighed out. After the exact amount was recorded, the amount of epoxy needed to attain the desired percentage was calculated. The resin and hardener were mixed with the appropriate 3:1 ratio. The scale was zeroed with the hydroxyapatite still in place, and epoxy was slowly added with a pipette until the scale read the required amount. Then the epoxy and hydroxyapatite were thoroughly mixed together and spatulated into the mold. The material was then left to set and dry in the mold overnight. The four desired percentages were 49.8%  59.5% 69.6% 76.3%. The consistency and texture of the mixtures were all like thick pastes of varying viscosity, except for the 76.3% mixture, which was had a doughy consistency. The samples after drying all released cleanly and appeared firm. Once again it is important to note that all weighing and mixing equipment as well as the mold was thoroughly cleaned.
Table 4.1 Sample compositions

<table>
<thead>
<tr>
<th></th>
<th>Pure HAP Samples</th>
<th>HAP/Epoxy Composites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HAP (g)</td>
<td>Water (g)</td>
</tr>
<tr>
<td>3.105</td>
<td>1.174</td>
<td>2.64</td>
</tr>
<tr>
<td>3.077</td>
<td>2.06</td>
<td>1.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HAP (g)</td>
<td>Epoxy (g)</td>
</tr>
<tr>
<td>3.201</td>
<td>0.995</td>
<td>3.22</td>
</tr>
<tr>
<td>3.065</td>
<td>1.34</td>
<td>2.29</td>
</tr>
<tr>
<td>3.114</td>
<td>2.114</td>
<td>1.47</td>
</tr>
<tr>
<td>3.072</td>
<td>3.095</td>
<td>0.99</td>
</tr>
</tbody>
</table>

4.3 Mechanical Testing

After sample fabrication was complete each sample underwent mechanical testing to gather data on the mechanical properties of the composites so that this data could be compared against the available data on bone tissues mechanical properties. Both compression and hardness tests were performed. However, the samples proved to be too brittle for a hardness test to provide valuable data. The hardness tests were performed on Rockwell hardness testers. Using the C-scale, a brail indenter, and a 150kg mass, the sample shattered into a few pieces. A B-scale test with a 1/16 inch ball indenter and a load of 100kg mass was then tried. The sample once again broke and it was determined that hardness measurements could not be taken at our facilities. Thus the hardness tests were abandoned in order to preserve more samples for compression testing.

The compression tests were performed on a MTS QTest/5 located in Salisbury Laboratories at WPI. The QTest/5 was equipped with a 5kn load cell. The cross-head speed was set at 0.02mm/s. The maximum extension of the compressive head was set at 6cm. The maximum load was set at 2kn. Two samples composed of 77% hydroxyapatite were tested and
labeled samples 77A and 77B. Three samples of each other composition was tested and labeled A, B, & C. While removing a sample after a completed test it was observed that the top compressive head was not completely tight. This was noted as it was a concern that this could affect test results. For testing the samples comprised of 50% hydroxyapatite the 2kn load was not sufficient so the maximum load limit was reset to 4kn and the maximum extension limit was reset to 8mm. Despite these adjustments, one 50% hydroxyapatite sample did not completely fail despite being deformed 8mm. The results of the compression tests will be discussed below in section 5.2.

![MTS Qtest/5 compression tester.](image)

**4.4 Characterization**

After mechanical testing had been completed the samples were studied with a SEM. The images produced by the SEM were gain insight into the structure of the composites that had been developed and to determine how the samples failed during the compression tests. Only one
sample of each composition was imaged due to time constraints. Samples need to be conductive for SEM imaging to work, so all of the specimens were sputter coated with gold and palladium since the specimens themselves were not conductive. Some unused hydroxyapatite nanopowder was also imaged with the SEM, but with mixed results. The powder proved to be too great an insulator and charge buildup made high magnifications blurry despite the use of sputter coating.
5 Results and Analysis

5.1 Powder Characterization
It was important to characterize the hydroxyapatite nanopowder because it was the primary material in the composites. The nanopowder purchased from Sigma Aldrich was advertised to have a particle size of >200nm. A small amount of the powder was placed in a SEM so this could be confirmed and visualized. Unfortunately, the conductive nature of the powder made SEM imaging difficult and limited the level of magnification despite attempts to sputter coat the powder with gold and palladium. The images obtained are shown below. While the resolution on this image is very high, the magnification is not high enough to make a conclusive statement about the nature of the hydroxyapatite powder. However, it does show that the particles do clump together to form large clumps.

![Figure 5.1 SEM image of Hydroxyapatite powder](image)

As shown in Figure 5.2, at higher magnification the build-up of electric charge on the non-conductive material affected the quality of the image and caused bright spots and streaks. This made interpretation of these images very difficult, and prevented imaging at higher
magnification. The first image shows a large clump of particles. The right side of this clump is very bright due to the charge build up. The left side of this clump remains clear. Individual particles that make up the large clump can be discerned on the right side, giving its surface a rough and abrasive appearance. In the following picture individual particles can be discerned that are free standing and not part of any larger group or clump of particles. While the magnification is still not great enough to determine the exact size of the particles in these images, the individual particles do appear to be significantly smaller than 1um when compared to the 10um scale bar. This makes the >200nm particle size at least plausible.

Figure 5.2 SEM images of Hydroxyapatite powder.

5.2 Compression Test Results: General Patterns

Identifying and understanding general trends helps interpret individual results. This section details all the general patterns found in the stress/strain curves of the samples tested. In the next section the test results for each individual sample will be analyzed and compared to others samples of the same composition to determine the mechanical properties of the composites with
relation to their composition. The most immediately recognizable trend in the data was that all the stress/strain curves started low and flat with a negative strain. This was likely due to software compensating for the insecure compression head observed during testing, which would have interfered with the test. The regions of the curves with negative strains were disregarded for all samples and only regions of the curve with a positive strain were discussed.

Figure 5.3 Example Stress/Strain graphs of compression tests

While each of the samples displayed different behaviors, sometimes even with respect to others of the same composition, in response to the compression testing, certain patterns can be found in the stress strain curves of the whole group. The most common of these patterns is the presence of tiny repeated peaks in the stress/strain curve. At least one sample from every set exhibited this behavior, and in some cases all of the samples did. One example, specimen 70C, is shown below to highlight the pattern. The trend regarding these peaks is that samples that did exhibit this pattern in their stress/strain curves were tougher then samples that did not.
Figure 5.4 Graph representing repeated peaks in a 70% CaP sample.

Another pattern is the appearance of linear regions of the curve that end abruptly to give way to another linear region with a similar slope. This phenomenon is particularly evident in the stress/strain curves for sample 77A, 70B, and to a lesser extent 60C, which are depicted in the following figures.
The fact that the MTS QTest/5 was not properly calibrated and the head was not properly secured to perform the tests makes it difficult to appropriately interpret the testing results. However, we did try to calculate an estimate of the Young’s Modulus for the samples by averaging the slopes of linear regions on the stress/strain curves since they can still indicate regions of elastic deformation. This yields a Young’s Modulus of 1301.9 MPa for 77A, 964.12 MPa for 70B, and 919.46 MPa for 60C.

### 5.3 Compression Test Results: Composite Properties

The information about the trends in the data will be used in this section to identify mechanical properties of each composite. The data will be tabulated at the end of this section in Table 5-1 Elastic Modulus and Compressive strengths. Since both phenomena discussed in the previous
section are exhibited by samples in of 60% hydroxyapatite group, this group is a good starting point for this analysis. The abrupt terminations of the linear regions mention in the previous section may indicate imperfections in the sample. For this reason it may not be appropriate to use this data to describe properties of the composite composition the sample belongs to; careful comparison between each sample is required. This is especially true if the stress/strain curves bear little resemblance to others of the same composition, as is the case for sample 60C. In 60B, shown in Figure 5.6, the linear or elastic region at the beginning of the stress/strain curve gradually gives way to a concave downward curve made of the tiny repetitive peaks previously mentioned. This is a stark contrast to 60C, in which the end of the elastic region is the maximum load and failure point for the specimen. Sample 60A, shown in Figure 5.6, seems to be in between each of these, although it appears to resemble 60B more. 60A and 60B also have similar slopes in the elastic region, while 60C is significantly lower than both. The results strongly indicate that better sample preparation is needed to obtain more consistent testing results.

![Stress/Strain curve of sample 60% A and 60% B](image)

**Figure 5.6 Stress/Strain curve of sample 60% A and 60% B**

The point at which the elastic region ends and the plastic region begins was marked on the stress/strain curve and used to estimate interpreted as the yield strength of the material, although it was difficult to determine what that point was on the curve for 60B. The maximum
stress was also marked on the curve and used to estimate the ultimate strength of the material. Only values from 60A and 60B were used to create the estimates in Table 5-1.

All samples of the 70% hydroxyapatite group exhibited the tiny, repeated peaks. 70A and 70B both exhibit the successive linear regions. The curves of all three of the samples were relatively flat in the plastic region; as opposed to the concave downwards shape of the 60A and 60B. The values of the yield and ultimate strengths vary from sample to sample, which again suggest that better sample preparation is needed for more consistent testing results.

![Stress/Strain Curve from 70% A, 70% B and 70% C](image)

The 77% hydroxyapatite samples are difficult to analyze because only two samples could be tested and they are significantly different from each other. 77A fails after the elastic deformation, while there is some plastic deformation in 77B. However the modulus of both
samples is similar. The moduli and yield strengths were averaged in Table 5-1 but the ultimate strength is simply the value of 77B’s ultimate strength.

![Stress Strain Curve from 77% A and 77%B.](image)

Finally, the 50% hydroxyapatite group perhaps the most difficult. One sample, 50A was much weaker than the other two, and another sample, 50B did not really reach its ultimate strength because the load limit was too low. Only 50C can provide estimated data on ultimate strength, so this was the only value used in Table 5 1 Elastic Modulus and Compressive strengths. The yield strengths and moduli of 50B and 50C were averaged.
Figure 5.9 Stress/Strain curve from 50% A, 50% B and 50% C.

Table 5.1 Elastic Modulus and Compressive Strengths

<table>
<thead>
<tr>
<th>Sample</th>
<th>Elastic Modulus (MPa)</th>
<th>Compressive Yield Strength (MPa)</th>
<th>Ultimate Compressive Strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>77A</td>
<td>1301.9</td>
<td>56.31</td>
<td></td>
</tr>
<tr>
<td>77B</td>
<td>1234.1</td>
<td>36.04</td>
<td>41.98</td>
</tr>
<tr>
<td>Avg. 77%</td>
<td>1268</td>
<td>46.175</td>
<td>41.98</td>
</tr>
<tr>
<td>70A</td>
<td>844.79</td>
<td>33.75</td>
<td>40.54</td>
</tr>
<tr>
<td>70B</td>
<td>964.11</td>
<td>46.35</td>
<td>51.64</td>
</tr>
<tr>
<td>70C</td>
<td>1190.9</td>
<td>60.69</td>
<td>75.42</td>
</tr>
<tr>
<td>Avg. 70%</td>
<td>999.933333</td>
<td>46.93</td>
<td>55.866666667</td>
</tr>
<tr>
<td>60A</td>
<td>1387.9</td>
<td>46.47</td>
<td>51.22</td>
</tr>
<tr>
<td>60B</td>
<td>1311.1</td>
<td>56.63</td>
<td>72.37</td>
</tr>
<tr>
<td>Avg. 60%</td>
<td>1349.5</td>
<td>51.55</td>
<td>61.795</td>
</tr>
<tr>
<td>50B</td>
<td>936.69</td>
<td>53.12</td>
<td></td>
</tr>
<tr>
<td>50C</td>
<td>913.66</td>
<td>53.8</td>
<td>100.47</td>
</tr>
<tr>
<td>Avg. 50%</td>
<td>925.175</td>
<td>53.46</td>
<td>100.47</td>
</tr>
</tbody>
</table>
5.4 SEM Image Analysis

Using the SEM to image the samples had two purposes. The first was to study the failure mechanisms in the samples that had been tested. The second was to learn about the structure of the composites that had been developed. The composites were the result of simply mixing two materials together, thus it could certainly not have the highly ordered hierarchical structure natural bone tissue does. However, how well the materials were mixed and how they interacted at the microscopic level also affect the composites’ mechanical properties. These details can be obtained via SEM imaging.

Unfortunately due to time constraints only one specimen from each group could be imaged. The images of 77A in Figure 5.10 show the fracture surface of the material at a relatively low magnification. A large crack starting from the top of the image leads into a large hole deep in the sample. This crack is perpendicular to the direction that force was applied to in the compression test. The fracture surface was formed when the sample split lengthwise down its center. The oddly shaped dark areas in this image are various cross-sections of what were bubbles before the sample split open. The larger the cross-sections, the more misshapen they become. There are smaller cross-sections in the top right corner of the image that look like teardrop shaped craters. While there are spots of hydroxyapatite that appear as white dots or ridges, no concentrated areas of epoxy can be seen and the material looks homogeneous.

The next picture is of the same sample, 77A, at a higher magnification. In this picture the small black spots are epoxy and the small white spots are hydroxyapatite. In this image the hydroxyapatite can be seen on ridges of material that seemed to be chipping off or flaking of the specimen.
The next images are of the sample 70A. This sample was weak compared to others of its composition. At a low magnification this sample looks much like the previous one. There are two holes in the material that are at either the beginning or the end of long this cracks. There are also much more smaller holes. This composition still appears mostly homogenous. At a much higher magnification a difference is clear. The small black circles that are concentrations of epoxy are much more numerous, and while the hydroxyapatite can still be seen in concentrated globes or circles, they are much more prominent on the edges or ridges of the specimen.
With the 60A specimen a difference can be noticed even at a lower magnification. The sample no longer looks homogeneous. Instead, concentrations of epoxy can be seen as two dark round areas in the center of the image, one much larger than the other. Both of these dark areas have a ridge of hydroxapatite leading up to it. The next picture is zoomed in on the same two concentrations of epoxy. In the larger of the two concentrations it appears as though the material is radiating outwards. This indicates that this concentration could be the cross-section of a spherulite of epoxy. The surrounding ridges and globes are shown in great detail as well. The next image is the fracture surface at at even higher magnification. This shows the random organization of the hydroxyapatite crystals very well. In contrast to the spherulite, there is no order in the surrounding hydroxapatite nanopowder.
The next set of pictures is of specimen 50C. The first picture shows the crack in the specimen on a relatively low magnification. One can see where the crack is starting to end towards the right of the picture. The next picture is of the surface of the sample. There are smaller cracks along the outer surface of the specimen that run perpendicular to the main crack. The large white particles of hydroxyapatite are more common where the cracking is more severe. Between two larger cracks the material has bulged outward. The next picture is of the material inside the crack where the two sides are almost joined. None of the other specimens show surface morphology like this.
specimen. Sharp, angular strips of material are being peeled off the specimen in layers. Much of this picture is out of focus because the height differential was too great the SEM’s depth of focus.

Figure 5.13 SEM images of sample 50C

Figure 5.14 SEM image of hydroxyapatite powder
6 Conclusions

6.1 Mechanical Properties

Again, the fact that the MTS QTest/5 was not well calibrated before the tests and the compression head was not secured to perform the tests makes it hard to appropriately interpret the testing results. However, the results of the compression tests led to several conclusions about the estimated mechanical properties of the composites versus their composition. We also attempted a comparison to human bone tissue. The conclusions drawn from the estimated data are:

- Ultimate compressive strength increased with amount of epoxy in the sample

![Ultimate Strength vs. %Epoxy](image)

The ultimate compressive strength values in the chart are the averages for each composition tabulated in Table 5.1. The relationship between the composition and the ultimate strength is clear, with the ultimate strength increasing roughly 20MPa for each ten percent increase in epoxy in the composition.
• Yield compressive strength increased with amount of epoxy in the sample

![Yield Strength vs. %Epoxy](image)

Figure 6.2 Yield Strength vs. % Epoxy Graph

These yield strength values are taken from the averages in table 5.1. Once again the relationship is clear. Compressive yield strength improves roughly 3MPa with every ten percent increase in epoxy.

• Young’s modulus showed no trend associated with the amount of the epoxy in the sample
Once again the values in this graph are taken from Table 5.1 Elastic Modulus and Compressive strengths. There is no correlation to the amount of epoxy and the Young’s Modulus. If data from the samples with 60% hydroxyapatite and 40% epoxy were to be removed, there may be some downward trend. However, there is nothing to indicate that this data should be treated as an outlier.

- **All mechanical properties were far below averages for bone tissue.**

Since the goal of the project was to mimic bone composition and structure it is appropriate to make comparisons of the test results to mechanical properties of natural bone. As discussed in the background section, the data reported by Cezayirlioglu et al would be used for comparison. These data are 206MPa for ultimate compressive strength and 182MPa for compressive yield strength of 182. 206MPa is twice the value the highest recorded ultimate strength, and 182 is three times the value of the highest yield strength.
6.2 Structure and Defects

The analysis of the SEM images resulted in several conclusions about the structure of the composite materials that were developed. These conclusions are listed below.

- The structure of the samples did not mimic that of bone tissue.
  Bone tissue has a nanoscaled hierarchical structure, which consists of hydroxyapatite crystals within a matrix of collagen fibers as well as other proteins. The SEM images of the composite samples showed very little order to the structure of the material. The size and distribution of the epoxy was random. This discussed in greater detail below.

- Large amounts of the epoxy did not integrate with the hydroxyapatite crystals in the samples.
  Certain SEM images showed large spherulites, of epoxy had formed. While these structures probably improved the mechanical properties of the material somewhat they are far different from any structure found in bone tissue, which is mostly lamellar.

- The samples had air bubbles due to resin-hardener reaction
  Large holes were seen in every sample that was examined with the SEM. In some images cracks could clearly be seen emanating from these holes. The bubbles adversely affected the mechanical properties of the samples. These bubbles were the result of using epoxy in the material. Gases often get trapped when mixing the highly viscous resin and hardener, and bubbles can also form during the chemical reaction. It is likely that the presence of bubbles in the samples cannot be divorced from the use of epoxy as a binder.

6.3 Recommendations for future work

From the conclusions it is clear that the goals of this project were not met. The composite materials had mechanical properties significantly inferior to bone tissue. The structure of the
materials was dissimilar from bone tissue as well. However, the conclusion can provide basis for recommendations for future research in this area. These are listed below.

- **Consider using a different binder.**

  The epoxy did not integrate well with hydroxyapatite and was responsible for defects in the samples. It is unlikely that the intricate structure of bone tissue can be mimicked by using an epoxy.

- **Use nanofibers to mimic collagen.**

  Collagen fibers play an important role in bone tissue. It is unlikely that the structure of bone tissue cannot be accurately duplicated without a fibrous material that would play the role that collagen does in natural bone tissue. Carbon nanotubes are a good candidate because of their mechanical properties, but materials should be considered. Biocompatibility should be a chief concern.

- **Revisit the Sol-gel approach.**

  Although Sol-gel did not seem appropriate for this project, the ability to make sol-gel materials in three dimensional molds should be pursued as a possibility in the future. If it can be done with proper facilities and equipment, it may allow for easier fabrication and manipulation of the structure.

- **Apply pressure to the samples in mold during fabrication.**

  The orientation of lamellae in bone tissue is influenced by the direction that stress is applied on them. Perhaps applying stress to the material during fabrication can have this
effect as well. This would result in samples with a more ordered structure. Orientation is an important part of bone structure and therefore is important to mimic.
7 References


