INFLUENCE OF SILICON DIOXIDE, MAGNESIUM OXIDE AND ZINC OXIDE ON
RESORBABLE TRICALCIUM PHOSPHATE BASED BIOCERAMICS

By

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Chair

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Musculoskeletal disorders and bone deficiencies have been established among the most important human health conditions that exist today, costing an estimated $4.7 billion in the US each year, and afflicting 1 out of 7 Americans. Resorbable calcium phosphate based biomaterials as implant material is important because it will dramatically improve health care by shortening the time necessary for restoration of functional loading of grafted bones. Though calcium phosphates (CaPs) posses exceptional similarities to natural bone they are deficient in one major area, which is, they do not have the mineral content of bone, therefore, the goal of this research is to increase the likeness to bone as much as possible by adding bone based mineral dopants to CaPs which will also affect the resorbable characteristics. This thesis illustrates the analysis of doping tricalcium phosphate ($\beta$-TCP) with magnesia (MgO), silica (SiO$_2$) and zinc oxide (ZnO).

The influence of dopants had a significant effect on the densification of TCP, the binary [TCP-MgO-ZnO, TCP-MgO-SiO$_2$, TCP-SiO$_2$-ZnO] and ternary [TCP-MgO-ZnO-SiO$_2$] compositions increase sample densification over 7% normalized to the theoretical density of TCP. There was apatite growth on the highly porous compositions either on the surface or inside
the samples after biological testing. From SEM analysis it was evident that surface degradation was occurring on all compositions after eight weeks in simulated body fluid (SBF). Compression strength analysis proved that it is possible to tailor TCP compositions for controlled strength loss ceramics. It was found that TCP begins to degrade after 8 weeks in SBF whereas TCP-MgO-ZnO composition began to degrade after 4 weeks; however, TCP-SiO$_2$ 1wt% increased in strength over the 12 week test and the TCP-SiO$_2$-ZnO composition did not show any signs of strength loss over the 3 month test.

Finally, *in-vitro* cell culture was used to determine if the addition of dopants resulted in any cytotoxic effects on the ceramic compacts and it was found that there was no evidence of toxicity within the various compositions. The understanding of calcium phosphates, achieved by this research will assist in the design of a bone graft material that will permit rapid cell growth while providing the initial biomechanical support required for restoration of ambulatory function.
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Dedication

This thesis is dedicated to my mother, Angeleta M. Bernard and father, Stanley A. Bernard who provided both emotional and financial support throughout my time at Washington State University.
CHAPTER 1
INTRODUCTION

1.1 Motivation for Research

Repair or regeneration of skeletal tissues, particularly bone and cartilage, remains an ongoing clinical challenge. Musculoskeletal disorders and bone deficiencies have been established as among the most important health conditions that exist today. Bone defects are frequently caused by trauma, disease and developmental deformity. Repairing such bone sites involves various medical surgical techniques, some of which include the use of autogenous grafts, allogenous grafts, internal and external fixation devices, and replacement implants. A number of materials have been tested for use in the biomedical industry to treat these types of bone defects, which include metals, polymers, ceramics and composites. This research is mainly interested in the use of ceramics which are referred to as bioceramics. Bioceramics are meaningful materials for bone engineering applications because of their good chemical stability, low density, and compositional similarities to that of human bone. Approximately 60% of the bone graft substitutes currently available involve ceramics either alone or in a combination with another material. Calcium phosphates account for most of the ceramic based bone graft substitutes that are currently available. In the United States there are over 650,000 bone graft procedures performed each year. The current standard for bone grafts is the autograft in which tissue is harvested from the patient, this procedure works well because the donor tissue possesses all of the characteristics necessary for new bone growth, such as osteoconductivity and osteoinductivity. Harvesting autograft tissue requires an additional surgery at the donor site which can have negative effects such as infection, inflammation and chronic pain. An alternative
to autografts is allografts. Allografts eliminate donor site morbidity and limited supply issues but also poses a risk of transmissions of donor diseases to the patient, therefore, it is important that the use of calcium phosphate bioceramic be highly considered for bone graft substitute applications.

Tricalcium phosphate (TCP) ceramics is an excellent choice for bone grafting bioceramics because it is biocompatible, bioactive and most important of all bioresorbable. Thus, it has the ability to degrade under physiological conditions of the body to allow bone regeneration. Although there are many reasons for the use of TCP, there are still critical issues concerning the use of these ceramics. Primarily, significant loss of its mechanical properties occurs upon its degradation; therefore, it is not preferable to use in load bearing applications; also, although significant research has been done with this ceramic material, scientists have yet to effectively control the degradation rate in vitro. Another issue is the concern of cell attachment to the ceramic structure once another material is added. It is important to design a material that will be bioactive and allow strong tissue-material interaction first and then slowly dissolve away as tissue grows into the material and transfer mechanical stresses to the newly formed tissue.
1.2 Research Objective

The current research program on bioceramic materials was focused on the design, processing, and characterization of calcium phosphate based bioresorbable ceramics with improved and controlled mechanical degradation, and biological properties for use as bone graft in hard tissue engineering applications. This project attempted to investigate the influence of addition of dopants to tricalcium phosphate (TCP) on mechanical properties and bioactivity. The significance of adding dopants to tricalcium phosphate is to modify the rate of resorption thus, controlling the rate of mechanical degradation, suitable for short-term and long-term bone grafting applications. The primary focus to improve densification was based on the combination and ratio of dopants added to TCP. The other objective of this project was to determine the mechanical degradation rate after compact samples were subjected to a biological study in simulated body fluid. This work involved interdisciplinary research activities between the School of Mechanical and Materials Engineering, the School of Molecular Biosciences, and the Electron Microscopy Center, all at Washington State University, Pullman, Washington.
2.1 Human Skeletal Structure

The human skeletal system provides support and protection for the body as well as sites for muscle attachments and the production of blood cells (Hench, 1993). The skeleton of an adult is made up on average of 206 bones, but this number decreases with age as some bones become fused together; unlike adults the skeleton of an embryo is comprised of about 350 completely cartilaginous bones- over 140 more than adults; as children grow, many of the bones fuse together during the development of the circulatory and nervous system. In an adult skeleton, the cartilage almost completely disappears: it remains in a few places which include certain parts of the ear, the nose, the mouth, the anterior parts of the ribs, and on the surface of the joints. The human skeleton reaches full maturity at about 25 years of age and can be divided into two categories: the axial skeleton, which includes the cranium, the spinal column, and the thorax; the basic function of this section of the skeleton is to protect the internal organs; and the appendicular skeleton, which includes the limbs (upper and lower) and the pelvis; its basic function is to enable movement and support. Of the 206 bones in the human skeletal system, approximately 29 of them are defined as cranial bones; 26 make up the spinal column, roughly 25 can be found in the thorax, also 64 make up both upper limbs including the hands, and 62 make up both lower limbs (Rigutti, 2002).
2.2 Bones

Bone is a porous material that consists of a solid matrix and soft tissue; they also vary in shape and size, and can be considered as long, short, or flat bones. The solid matrix of bones can be classified as (1) cortical bone or as compact with very low porosity and (2) spongy bone or cancellous bone which has very high porosity. The inorganic matrix of the cortical bone consists of a porous structure with an interconnected porosity of approximately 65 volume percent (Hench, 1993). The spongy bone is the porous bone network that is found within the cortical shell as shown in Fig 2.2.1. The spongy bone exists within the ends of long bones, inside the vertebral bones of the spine and sandwiched between layers of compact bone in the skeleton’s plate structure such as the pelvis and skull (Martin, 1999).

![Figure. 2.2.1 Schematic of the interior structure of bones](image_url)
2.3 Bone Growth

Diagrams of the human skeletal system are all practically the same, however, people grow into various shapes and sizes, and so do their bones. Bone development depends strongly on the equilibrium state between bone tissue cells such as osteoblasts, which promote the formation of calcium deposits or bone formation, and osteoclasts, which are responsible for the dissolution and degradation of calcium phosphate by producing enzymes which are able to “melt” salt crystals and “digest” the collagen fibers (TAJ Books Ltd.). The main process through which bone grows is by apposition, which is defined as the process of laying down additional matrix and cells on the free surfaces of hard tissue. It is important that this process is in coordination with the growth of other tissues or the body’s natural proportion can be severely disturbed. The process of new bone formation begins with the secretion of bone matrix by the osteoblast cells that are on the surface of the existing bone matrix, then some osteoblast cells become free at the surface to take place in the formation of new bone cycle, while others are embedded in the bone matrix secretion, this new matrix is mainly type 1 collagen based and is named osteoid. With the addition of calcium phosphate minerals the osteoid turns rapidly into a hard and compact bone matrix. The osteoblast cells that were embedded into the bone matrix cannot form new bone through the apposition process so they become what are called osteocytes which are responsible for maintaining bones. The next step in the bone regeneration process is the erosion or dissolution of old bone matrix, which is done by the osteoclast cells. These cells are released as monocytes into the blood stream and collect at sites of bone resorption. Osteoclasts are capable of tunneling deep into the substance of cortical bone forming cavities that are invaded by other cells. A blood capillary grows down the center of such a tunnel, and
the walls of the tunnel become lined with a layer of osteoblasts, and the formation process begins again.

2.4 Function and Composition of Bone

Within the human, the skeletal system provides four primary functions. First, bones provide a surface for muscle attachment to facilitate movement of the body and they also provide structural support for all organs and tissues in the body. Second, the skeleton system provides protection for all internal organs including the skull that protects the brain. Third, bones act as a storage facility by maintaining and storing over 99% of the body’s total calcium supply. Finally, marrow within bones plays an important role in the development of the body’s immune system (Simske, 1997).

Bones make up about one sixth of the total body mass and have a density of approximately 1.9g/cc. Bone tissue consists of osteoid (90% collagen) which is approximately 50% of bone by volume and 25% by weight. The characteristics that bone is well known for such as its strength and rigidity are derived from the presence of mineral salts that permeate the organic matrix. The mineral phase accounts for 75% of the dry weight and 50% by volume of bone tissue. The principal constituent of bone is calcium phosphates in the form of the ceramic phase hydroxyapatite (HA) \(\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2\) (Mow and Huiskes, 2005). Other major constituents of the mineral phase of bone include calcium (Ca), phosphorous (P), sodium (Na), potassium (K), magnesium (Mg), fluorine (F), chlorine (Cl), carbonate \(\text{CO}_3^{2-}\) and some other trace elements are strontium (Sr), lead (Pb), barium (Ba), iron (Fe), zinc (Zn), copper (Cu) (Shi, 2004). The organic phase of bone is made up of collagen and is a somewhat gelatin like with over 10 different protein types, each type varies by the sequence of amino acids that they are
made of. The bone is mainly composed of Type 1 collagen, this collagen is found in tendons, ligaments and skin.

2.5 Mechanical Properties of Bone

Bone tissue is a calcium phosphate ceramic based material, therefore, bone has very similar mechanical properties such as toughness, compressive strength, low density, lightness, corrosion, and fatigue resistant. However, there is a downside to bone material primarily due to its brittle and viscoelastic nature whose mechanical properties are determined by porosity, degree of mineralization, and other structural factors. The mechanical properties of the human skeletal system have been summarized by many authors from many sources. The following table is an adaptation of these values:

Table 2.5.1 Mechanical properties of bone (Hench, 1993) * (Shi, 2004)

<table>
<thead>
<tr>
<th>Property</th>
<th>Cortical Bone</th>
<th>Cancellous Bone</th>
<th>Articular Cartilage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compressive Strength (MPa)</td>
<td>*137.8</td>
<td>*41.4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>100-230</td>
<td>2-12</td>
<td>-</td>
</tr>
<tr>
<td>Flexural, Tensile Strength (MPa)</td>
<td>50-150</td>
<td>10-20</td>
<td>10-40</td>
</tr>
<tr>
<td></td>
<td>*68.9</td>
<td>*3.5</td>
<td></td>
</tr>
<tr>
<td>Strain to Failure</td>
<td>1-3</td>
<td>5-7</td>
<td>15-50%</td>
</tr>
<tr>
<td>Young’s Modulus (Tensile) (GPa)</td>
<td>7-30</td>
<td>0.5-0.05</td>
<td>0.001-0.01</td>
</tr>
<tr>
<td></td>
<td>*13.8</td>
<td>*3</td>
<td></td>
</tr>
<tr>
<td>Fracture Toughness (K&lt;sub&gt;1c&lt;/sub&gt;)(MPa m&lt;sup&gt;1/2&lt;/sup&gt;)</td>
<td>2-12</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
3.1 Introduction

A biomaterial can be defined as “any substance (other than drug) or combination of part of a system which treats, augments or replaces any tissue, organ or function of the body.” There are many ways to define biomaterials but they all refer to the same idea; materials used in the body to replace parts or functions of living tissues, that are in contact with body fluid. Biomaterials that have been widely used in clinical industry applications include: Sutures (temporary or bioresorbable), Catheters (fluid transport tubes), Blood Bags, Contact Lenses, Intraocular Lenses, Knee and Hip Prostheses, Breast Prostheses (cancer or cosmetic), Dental Implants, Renal Dialyzers (patients), Oxygenators/CPB’s (cardiopulmonary bypass system--facilitates open heart surgery), Vascular Grafts and Pacemakers (pulse generators). Replacement materials for these implants consist of ceramics, polymers, metals, and composites that can be seen in Fig. 3.1.2. Some biomaterials with relatively high mechanical strength such as titanium alloy Ti-6Al-4V, Co-Cr alloy, alumina, or zirconia have been used in joint replacement devices. Others with bioactivity and bone bonding ability such as calcium phosphate ceramics and bioactive glasses have been used as artificial bone grafts. Biomaterials are defined by their applications, and not their chemical compositions. Even though biomaterials have the capability to render excellent physical and chemical likeness to the body they also have some disadvantages that are shown in table 3.1.3.
Biomaterials cover all classes of materials – metals, ceramics, polymers

**Ocular lenses:** acrylates, silicone

**Ear:** HA, Al₂O₃, Ti, silicone

**Cranial:** 316L SS, Ti, acrylic, HA, TCP

**Dental:** acrylic, gold, 316L SS, Co-Cr-Mo, Ti, Ti-Al-V, Al₂O₃, HA, Bioglass

**Maxillofacial reconstruction:** Al₂O₃, HA, TCP, HA/PLA, Bioglass, Ti, Ti-Al-V

**Degradable Sutures:** copolymers of PLA, PGA, PCL, PTMC, PDO

**Heart:** Co-Cr-Mo, Ti-Al-V, pyrolytic C, ePTFE, PET, PUR

**Pacemaker:** 316L SS, Pt, PUR, silicone, PET

**Spinal:** Co-Cr-Mo, Ti, HA, UHMWPE

**Load-bearing Orthopedic:** Al₂O₃, Zirconia, 316L SS, Ti, Ti-Al-V, Co-Cr-Mo, UHMWPE

**Prosthetic joints:** 316L SS, Co-Cr-Mo, Ti, Ti-Al-V, silicone, UHMWPE, acrylic

**Blood vessels:** ePTFE, PET

**Tendon & Ligaments:** PLA/C fiber, ePTFE, PET, UHMWPE

**Bone Fixation:** 316L SS, Co-Cr-Mo, Ti, Ti-Al-V, PLA/HA, PLA, PGA

---

**Figure 3.1.2** Applications of biomaterials in the human body
### Table 3.1.3 Advantages and disadvantage of some biomaterials used in the body (Shi, 2004)

<table>
<thead>
<tr>
<th>Materials</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polymers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nylon</td>
<td>Ductile, Light, Easy to fabricate</td>
<td>Not strong, Prone to creep, Degradable</td>
<td>Suture, Vascular prosthesis, Accetabular cup, Artificial ligament</td>
</tr>
<tr>
<td>Polyester</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silicone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Metals</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ti and its alloys</td>
<td>Ductile, Strong, Tough</td>
<td>Prone to corrosion, Unwanted ion release</td>
<td>Artificial joint, Bone plate and screw, Dental root implant, Pacer, Suture wire</td>
</tr>
<tr>
<td>Co-Cr allos</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stainless steels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Au, Ag, Pt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ceramics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon</td>
<td>Biocompatible, Inert or bioactive, High compression strength, Stiff</td>
<td>Brittle, Weak tension, Sometime fragile</td>
<td>Cardiovascular device, Dental prosthesis, Joint prosthesis, Orthopedic implant</td>
</tr>
<tr>
<td>Aluminum oxide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Composites</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon-carbon</td>
<td>Strong, Stiff, Tailor-made, Distinctive properties</td>
<td>Difficult to make, High production cost</td>
<td>Joint implant, Hart valve bone cement</td>
</tr>
<tr>
<td>Metal- PMMA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA- HDPE</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**3.2 Biocompatibility of Biomaterials in Host Tissue Interfaces**

When a foreign material is present in the human body, there is a specific tissue response elicited by that material; it is a very important that the implant material avoids a toxic response that kills the surrounding tissue. On the other hand, when a material is bioinactive fibroblast cells surround the material and forms a fibrous layer that isolates the implant from the host. Biomaterials that work well in the body can be considered as bioactive. Advantages and disadvantages of bioinert, bioactive and bioresorbable ceramics are represented in table 3.2.1

Bioactivity refers to the inherent properties of a material to participate in specific biological
reactions. Bioactive materials can promote the desired protein adsorption, cell attachment and growth, such as coatings for orthopedic and dental implant applications which consist of CaP ceramics that promote biological fixation by directly binding with bones (Shi, 2004). An important characteristic of a bioactive interface is that it changes with time as do natural tissues which are in a state of dynamic equilibrium.

Table 3.2.1 Advantages and disadvantages of bioinert, bioactive and bioresorbable ceramics (Hench, 1993)

<table>
<thead>
<tr>
<th>Property</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioinert</td>
<td>Minimal biological response, high wear resistance</td>
<td>Limited mechanical properties in tension</td>
</tr>
<tr>
<td>Bioactive</td>
<td>Enhanced bone tissue response, bone bonding</td>
<td>Limited tensile strength and fracture toughness</td>
</tr>
<tr>
<td>Bioresorbable</td>
<td>Material is replaced by normal tissue, thereby excluding possible long term effects</td>
<td>Rate of strength reduction may be too rapid</td>
</tr>
</tbody>
</table>

3.3 Bioceramics

The prerequisite for any synthetic material to be implanted in a body is its biocompatibility, i.e., no inflammatory, or generally adverse, tissue reaction. Additionally, an implant is expected to withstand loads (physiological, mechanical) without substantial deterioration or catastrophic event (reaction, fracture) or altering the environment. The materials must not interact with blood, e.g., produce clotting or denaturing of plasma proteins. The class of ceramics used for repair and reconstruction of diseased and damaged parts of the musculoskeletal system are identified as bioceramics. Different ceramics including calcium phosphate and materials such as Ca aluminates, titanates, zirconates, alumina and silica have
been used for surgical implantations. Many of these ceramics are at their highest energy level and cannot be oxidized any further, they are mostly inert or insoluble in human body fluids, and some are bioactive or surface reactive and bioresorbable or biodegradable (Wise et al., 2000), zirconia, silicon nitrides, and alumina are considered bioinert. Some glass ceramics and dense hydroxyapatite are bioactive whereas tetra calcium phosphate (TTCP) and tricalcium phosphate (TCP) are bioresorbable ceramics (Park, 1992).

3.3.1 Bioactive Ceramics

Bioactive materials such as TCP and HA are a more recent development. They are materials which can react with the local cells to form bonds. Thus, they can be used to transfer loads to and from living cells. Normally cells grow directly adjacent to the material and in some cases may actually digest the material over time. The primary bioactive ceramics are low silica glasses, and various calcium phosphates. These calcium phosphates are very similar to bone material. Bioactive ceramics are often made to be highly porous to increase their active surface. Uniform porosity in the order of 0.3 - 0.4 mm is desirable to facilitate the in-growth of bone into pores in alumina (bioinert material) or hydroxyapatite (bioactive material). The low strength of many bioactive ceramics is their main disadvantage. For this reason they are most often used in composites. Usually a bioactive ceramic is used to coat a bioinert metal, although many different combinations and morphologies have been used successfully. Fiber and particulate reinforcement are commonly used with bioactive materials as well.
3.3.2 Bioresorbable Ceramics

In most cases, with hip replacements, vascular stints, or bone grafts for example, a foreign material is placed inside the body. It is often that this foreign material is only needed for a certain length of time in order to promote natural healing of the surrounding tissues. In those situations the foreign material needs to be removed after it has outlived its usefulness. However, another subsequent surgery is something that should be avoided as it lengthens the total recovery time of the patient and puts them at increased risk of various problems, and drives up the final cost of the procedure. Through the use of bioresorbable materials that degrade in the body over time, the second surgery to remove implanted devices can be avoided. Furthermore, bioresorbable materials can be implanted to support new tissue growth until such a time as the tissue is no longer needed and completely replaced by the newly grown tissue.

Bioresorbable ceramics typically are calcium based materials such as calcium sulfate (plaster of paris), hydroxyapatite, tricalcium phosphate, and other calcium salts. Hydroxyapatite may or may not be resorbable, depending on the particular variety used i.e. HA vs. dense HA or TCP. These materials are designed to degrade in the body over time allowing complete replacement of the implant by new growth of the host tissue. The end result provides the most desirable type of solution in which the implant is only temporary and the remaining tissue is completely biological with the same properties as the surrounding material. This eliminates the problem of limited service life often encountered with implants since the biological tissue that replaced the implant will have the ability to grow and repair itself.

Challenges with resorbable ceramics include: the maintenance of the strength of the implant, the interface during the period of degradation, tissue growth to meet short term performance needs, and the matching of the material resorption rate to the rate of new tissue
growth. Also, larger implants will leave a large quantity of material that must be absorbed by the body; therefore, the selected material must break down into components that are easily metabolized by the body, thus, the predominant use of calcium phosphates.

Calcium phosphate bioceramics may be bioactive or bioresorbable depending on the phase being used. The presence of water and the temperature, both during processing and in service, determine the stable phases of calcium phosphate. At body temperature and in the presence of body fluids, dicalcium phosphate \( \text{CaHPO}_4 \cdot 2\text{H}_2\text{O} \), abbreviated \( \text{C}_2\text{P} \) is the stable phase for use at pH less than 4.2. At a pH of greater than 4.2, hydroxyapatite \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \) is the stable phase at body temperature, in the presence of body fluids. At temperatures above body temperature, other phases occur such as \( \beta \)-tricalcium phosphate (\( \text{Ca}_3(\text{PO}_4)_2 \), abbreviated TCP or \( \text{C}_3\text{P} \)) and tetracalcium phosphate (\( \text{Ca}_4\text{P}_2\text{O}_9 \), abbreviated \( \text{C}_4\text{P} \)). TCP is the amorphous phase of calcium phosphate while HA is the crystalline phase. Amorphous TCP is more resorbable than HA because of the amorphous structure. In other words, the resorbability of calcium phosphate increases with decreasing crystallinity (Chang et al., 1999). At 37°C, the high-temperature phases will react with water (body fluids) to form HA. For example, TCP reacts to form HA on exposed surfaces by the equation:

\[
4\text{Ca}_3(\text{PO}_4)_2 \text{(solid)} + 2\text{H}_2\text{O} \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH}) \text{(surface)} + 2\text{Ca}^{2+} + 2\text{HPO}_4^{2-}
\]

The formation of the HA on the surface decreases the pH of the environment which in turn increases the solubility of TCP and makes for better resorption. Therefore, increasing surface area with the presence of pores in the material will increase resorbability (Ratner et al., 1996).
As with most ceramics, calcium phosphates exhibit low strength compared to metals and a tendency towards brittle fracture. The mechanical properties of calcium phosphates such as compressive strength, tensile strength, and fatigue resistance depend greatly on porosity. The pores in these materials can be of two types: micropores or macropores. Micropores, less than one micrometer in diameter, are the result of incomplete sintering of the material and greatly affect the tensile strength of the material. Macropores, greater than 100 micrometers, are typically created intentionally in order to provide for bone growth through the implant. Compressive strength is affected by the total volume of porosity in the material (Lee et al., 2003). Resorbable calcium phosphates and hydroxyapatites have a multitude of uses throughout the body in skeletal and dental reconstruction that fall into three major categories: guided bone regeneration, coatings or cements, and timed release of medicines. Use of β-TCP rather than hydroxyapatite (HA) in guided bone regeneration is growing because it is more readily resorbable (Zitzman et al.).

3.4 Calcium Phosphates Ceramics

Calcium phosphate (Ca-P) bioceramics have been widely used in medicine and dentistry. Different phases of calcium phosphate ceramics can be used in medicine, depending in whether a bioactive or a resorbable material is desired (Shi, 2004). The stable phases of calcium phosphates ceramics depend on temperature and presence of water, either during materials processing or in service environment. At body temperature (37°C), only two main calcium phosphate phases are stable when in contact with human body fluids. At pH > 4.2, the stable phase is hydroxyapatite (HA, Ca_{10}(PO_4)_6(OH)_2), below pH 4.2, brushite is the stable phase. At higher temperatures, Ca_3(PO_4)_2 (TCP) and Ca_4P_2O_9 (TTCP) are the prominent stable phases. There is a wide variation
of mechanical properties of calcium phosphate as given in table 3.4.1. A list of common calcium phosphate compounds use as biological materials is shown in table 3.4.2.

<table>
<thead>
<tr>
<th>Material</th>
<th>Porosity (%)</th>
<th>Density (mg/m³)</th>
<th>Young's Modulus (GPa)</th>
<th>Microhardness (Gpa)</th>
<th>Compressive strength (MPa)</th>
<th>Tensile Strength (MPa)</th>
<th>Flexural Strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>0.1-3</td>
<td>3.05-3.15</td>
<td>7-13</td>
<td>4.2-4.5</td>
<td>350-450</td>
<td>38-48</td>
<td>100-120</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.7</td>
<td>-</td>
<td>4.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>120-170</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>60-120</td>
<td>-</td>
<td>15-35</td>
</tr>
<tr>
<td></td>
<td>2.8-19.4</td>
<td>2.55-3.07</td>
<td>44-88</td>
<td>-</td>
<td>310-510</td>
<td>-</td>
<td>60-115</td>
</tr>
<tr>
<td></td>
<td>2.5-26.5</td>
<td>-</td>
<td>55-110</td>
<td>-</td>
<td>≤800</td>
<td>-</td>
<td>50-115</td>
</tr>
<tr>
<td>TTCP</td>
<td>Dense</td>
<td>3.1</td>
<td>-</td>
<td>-</td>
<td>120-200</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TCP</td>
<td>Dense</td>
<td>3.14</td>
<td>-</td>
<td>-</td>
<td>120</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7-21</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Other Ca-Ps</td>
<td>Dense</td>
<td>2.8-3.1</td>
<td>-</td>
<td>-</td>
<td>70-170</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 3.4.1** Mechanical properties and strength of calcium phosphate ceramics (Shi, 2004)

<table>
<thead>
<tr>
<th>Usual Symbol</th>
<th>Chemical Formulation</th>
<th>Atomic Ratio Ca/P</th>
<th>Space Group</th>
<th>Solubility Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP</td>
<td>Ca(H₂PO₄)₂.H₂O</td>
<td>0.50</td>
<td>-</td>
<td>1.0 x 10⁻³</td>
</tr>
<tr>
<td>DCPD</td>
<td>CaH₂PO₄.2H₂O</td>
<td>1.00</td>
<td>2/m</td>
<td>1.87 x 10⁻⁷</td>
</tr>
<tr>
<td>DCPA</td>
<td>CaHPO₄</td>
<td>1.00</td>
<td>P1</td>
<td>1.26 x 10⁻⁷</td>
</tr>
<tr>
<td>OCP</td>
<td>Ca₈H₂(PO₄)₅H₂O</td>
<td>1.33</td>
<td>-</td>
<td>5.01 x 10⁻¹⁵</td>
</tr>
<tr>
<td>TCP</td>
<td>Ca₃(PO₄)₂</td>
<td>1.50</td>
<td>R3c</td>
<td>2.83 x 10⁻³⁰</td>
</tr>
<tr>
<td>HAP</td>
<td>Ca₁₀(PO₄)₆(OH)₂</td>
<td>1.67</td>
<td>P6₃/m</td>
<td>2.35 x 10⁻⁵⁹</td>
</tr>
<tr>
<td>TTCP</td>
<td>Ca₄O(PO₄)₂</td>
<td>2.00</td>
<td>P2₁</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 3.4.2** List of main Ca-P compounds that have a biological use as surgical materials (Ravaglioli, 1992)
3.4.1 Hydroxyapatite

Hydroxyapatite (HA) is one of the most biocompatible materials known that has been used as a coating for metal implants, because it enhances bone healing adjacent to implants and establishes high interfacial bone-implant strength (Wise et al., 2000). The term apatite describes a family of compounds having similar structures but not necessarily having identical compositions. Hence, apatite is a description and not a composition. The general chemical formula for this family of compounds is $M_{10}(RO_4)X_2$, where $R$ is most commonly phosphorous, $M$ could be one of several metals although it is usually calcium, and $X$ is commonly hydroxide or a halogen such as fluorine or chlorine. Hydroxyapatite, specifically, calcium hydroxyapatite is the most commonly used calcium phosphate in the medical industry as it possesses excellent biocompatibility and is osteoconductive. The chemical formula for HA is $Ca_{10}(PO_4)_6(OH)_2$ and the $Ca/P$ molar ratio is 1.67:1, its theoretical density is 3.156 g/cc. HA has hexagonal rhombic prisms crystal structure. The principal objective of using HA implants, dense or porous, is to obtain a well osteointegrated bone to implant bridge with sufficient strength to provide normal structure and function. HA has been used as filler for periodontal defects, alveolar ridge augmentation, and maxillofacial reconstruction. Porous HA is mainly used as cancellous bone and substitute or specific filler due to its comparatively low mechanical properties.

3.4.2 Tricalcium Phosphate

The chemical formula for tricalcium phosphate (TCP) is $Ca_3(PO_4)_2$, TCP has four polymorphs, $\alpha$, $\beta$, $\gamma$, and super-$\alpha$. The $\gamma$ polymorph is a high-pressure phase, and the super-$\alpha$ phase is observed at temperatures above 1500°C (Gibson et al., 1996). Hence, the most frequently observed polymorphs of TCP bioceramics are the alpha and beta phases. According
to Elliot, $\alpha$-TCP crystal is in the monoclinic space group $P2_1/a$ with lattice parameters $a=1.2887\text{nm}$, $b=2.7280\text{nm}$, $c=1.5219\text{nm}$, and $\beta=126.20$ degrees. $\beta$-TCP has the rhombohedral space group $R3c$ with unit cell $a=1.0439\text{nm}$, $c=3.7375\text{nm}$ (hexagonal setting) with 21 formula units per hexagonal unit cell.

$\beta$-TCP is stable up to 1125°C, but above this temperature and up to 1430°C, $\alpha$-TCP becomes the stable phase (Elliot, 1994). The dissolution rate of TCP was investigated by various researchers (Black and Hastings, 1998). Jarcho 1977, compared the relative dissolution ratios of dense HA and TCP. The dissolution rate of TCP was 12.3 times higher than that of HA in buffered lactic acid solution (0.4M, pH 5.2) and was 22.3 times higher than that of HA in buffered ethylene diamine tetracetic acid (EDTA) solution (0.05M, pH 8.2). Ducheyne et al. (1980) compared the dissolution rate of six calcium phosphates in calcium and phosphate free solution at pH 7.3, the dissolution rate increased in the following order:

$$HA < \beta\text{-TCP} < \alpha\text{-TCP} < \text{TTCP}$$

Mechanical properties of TCP are rarely available, but it was reported that TCP has slightly higher fracture toughness than HA (Santos et al., 1996). Fully dense and translucent $\beta$-TCP totally free from $\alpha$ modification can attain a maximum strength value of 120 MPa (Tampieri et al., 1997). Most reports have concluded that TCP is biodegradable; however, the exact mechanism for its biodegradation remains unclear. In general, biodegradation of calcium phosphate ceramics is caused by the following factors:

1. Physiological dissolution, which depends on the solubility product of the material and local pH of its environment
2. physical disintegration into small particles as a result of preferential chemical attack on the grain boundaries
3. Biological factors such as phagocytosis which causes a decrease in local pH value.

3.5 Metal Ion Dopants

**Magnesium oxide**

Magnesium is an essential mineral to the human body. It is needed for bone, protein, and fatty acid formation, making new cells, activating B vitamins, relaxing muscles, clotting blood, and forming adenosine triphosphate (ATP; the energy the body runs on). The secretion and action of insulin also require magnesium. Magnesium is the fourth most abundant mineral in the body and is essential to good health. Approximately 60% of total body magnesium is found in bone, where it is thought to form a surface constituent of the hydroxyapatite (calcium phosphate) mineral component. The other half is found predominantly inside cells of body tissues and organs. Only 1% of magnesium is found in blood, but the body works very hard to keep blood levels of magnesium constant (Rude, 1998).

It has been verified that in calcified tissues, the amount of magnesium associated with the apatitic phase is higher at the beginning of the calcification process and decreases with increasing calcification. Also, there is growing evidence that magnesium may be an important factor in the qualitative changes of the bone matrix that determine bone fragility. Magnesium depletion adversely affects all stages of skeletal metabolism, causing ceasing of bone growth, decreased osteoblastic and osteoclastic activities and bone fragility (Percival, 1999).
<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal Structure</td>
<td>Cubic FCC ($a = 4.216 \text{ Å}$)</td>
</tr>
<tr>
<td>Density (g/cm$^3$)</td>
<td>3.58</td>
</tr>
<tr>
<td>Boiling point (°C)</td>
<td>3600</td>
</tr>
<tr>
<td>Melting Point (°C)</td>
<td>2852</td>
</tr>
<tr>
<td>Typical Impurities (ppm)</td>
<td>$\text{Ca} \leq 40, \text{Al} \leq 15, \text{Si} \leq 10, \text{Fe} \leq 50, \text{Cr} \leq 10, \text{B} \leq 5, \text{C} \leq 10$</td>
</tr>
<tr>
<td>Young's modulus (GPa)</td>
<td>250</td>
</tr>
<tr>
<td>Color</td>
<td>White</td>
</tr>
<tr>
<td>Solubility</td>
<td>0.00062 g/100 g water</td>
</tr>
<tr>
<td>Formula weight (g/mol)</td>
<td>40.30</td>
</tr>
</tbody>
</table>

**Table 3.5.1**  General Properties of magnesium oxide

**Figure 3.5.1**  Solid crystal structure diagram of magnesium oxide.

It has been found that the optimum amount of MgO doping into TCP was 1wt%; this percentage has shown good biocompatibility without cytotoxicity (Hyun-Seung et al, 2003). It was also found that $\text{Mg}^{2+}$ substitution into $\beta$-TCP tends to decrease the rate of degradation [L. Hench, 1993]; it is known to stabilize the rhombohedral crystal structure of TCP (Ando, 1958). According to Calderin, Mg is substituted into tri-calcium phosphate in the formula $\text{Mg}_x\text{Ca}_{3-x}(\text{PO}_4)_2$ ($x = 1,2,3$) (Calderin et al, 2002) as shown in Fig. 3.5.2. As expected, because of the smaller ionic radius, the Mg atoms reside closer to the axis of the cluster than the
Ca atoms. When a Mg atom is substituted into the TCP structure the Mg-O bond becomes stronger whereas, the Ca-O bonds are weakened by the increase in bond length compared to the Mg-O interaction, therefore, this may be the reason for Mg stabilizing the structure of TCP (Calderin et al, 2002).

![Figure 3.5.2](image)

**Fig. 3.5.2** Equilibrium interatomic distances (Å) and bond angles (degree) of (a) fully relaxed Ca$_3$(PO$_4$)$_2$ (TCP) fragment (b) fully relaxed MgCa$_{3-1}$(PO$_4$)$_2$ fragment (Calderin et al., 2002).

<table>
<thead>
<tr>
<th>Element</th>
<th>Atomic radius (pm) $10^{-12}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (Ca)</td>
<td>197</td>
</tr>
<tr>
<td>Phosphorous (P)</td>
<td>98</td>
</tr>
<tr>
<td>Oxygen (O)</td>
<td>73</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>160</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>134</td>
</tr>
<tr>
<td>Silicon (Si)</td>
<td>117.6</td>
</tr>
</tbody>
</table>

**Table 3.5.2** Atomic radii of atoms in TCP and the dopants used
**Zinc Oxide**

Zinc has a range of functions in the body. It plays a crucial role in growth and cell division where it is required for protein and DNA synthesis, in insulin activity, in the metabolism of the ovaries and testes, and in liver function. As a component of many enzymes, zinc is involved in the metabolism of proteins, carbohydrates, lipids and energy. The body contains about 2-3g of zinc. There are no specific storage sites known for zinc; it is found in all parts of the body, 60% is found in muscle, 30% in bone and about 5% in skin. The first signs of zinc deficiency are poor immune response and skin problems.

Zinc is an essential trace element with stimulatory effects on bone formation. It was found that zinc doped into beta-tricalcium phosphate to develop zinc-releasing biomaterials can promote bone formation. Also, when the zinc content was higher than 1.20 wt % in TCP, release of zinc from the zinc oxide caused cytotoxicity. Therefore, the zinc content of the composite ceramic must be <1.20 wt % (Ito et al., 2000). The density of zinc is 5.61(g/cm³) and its Melting Point is approximately 1975°C.

TCP based materials doped with some trace elements such as zinc promotes excellent bioactivity which does not exist in the parent materials (Kawamura et al., 1993). As an essential trace metal, Zn inhibits the differentiation of osteoclasts and/or promotes the activity of osteoblasts, affecting the formation of hard tissues, but a high Zn concentration can have serious toxic side effects on cells (Bettger et al., 1993).

*Kawamura et al.* also found that Zn-containing calcium phosphate ceramics stimulated bone formation around the ceramics implanted in rabbit femora, and attributed the effect to the Zn ions released from the ceramics. Therefore, it is important to use Zn-TCP for bone regeneration applications.
Zinc is added into TCP by substitution method and can be seen in Fig. 3.5.3. Zinc replaces the Ca atom causing some distortion in the crystal structure and is believe to be the reason for improved bioactivity in TCP (Yin et al., 2002).

![Equilibrium interatomic distances (Å) and bond angles (degree) of (a) fully relaxed Ca$_3$(PO$_4$)$_2$ (TCP) fragment (b) fully relaxed ZnCa$_{3-1}$(PO$_4$)$_2$ fragment (Calderin et al., 2002).](image)

**Fig. 3.5.3** Equilibrium interatomic distances (Å) and bond angles (degree) of (a) fully relaxed Ca$_3$(PO$_4$)$_2$ (TCP) fragment (b) fully relaxed ZnCa$_{3-1}$(PO$_4$)$_2$ fragment (Calderin et al., 2002).

**Silicon Dioxide (silica)**

Silica is silicon dioxide, a trace mineral found in bones, teeth, skin, eyes, glands and organs. It’s also a major constituent of collagen, which helps keep skin elastic. Silicon molecules in the tissues, such as the nails and connective tissue, give them strength and stability. Silicon is present in bone, blood vessels, cartilage, and tendons, helping to make them strong. Silicon is important to bone formation, as it is found in active areas of calcification. The precise mechanism is uncertain. However, it has been suggested that silicon could facilitate the formation of glycosaminoglycan and collagen components of the bone matrix through its role as a constituent of the enzyme of prolylhydrolase. Alternatively, silicon could have a structural role as a component of glycosaminoglycans and glycosamino-protein complexes, occurring as
silanolate in mucopolysaccharides and linking different polysaccharides in the same polysaccharide chain, or linking acid mucopolysaccharides to protein.

Silicon assists calcium in the maintenance and growth of bones and joints. Retired UCLA Professor, Edith M. Carlisle, Ph.D. found that silicon in the diet of chicks produced denser bone and faster growth compared to chicks deprived of the mineral. Silicon was responsible for a 100% increase in the level of collagen, the protein component of bone, which provides the matrix for calcification and imparts flexibility. In rats, it was found that a silicon deficient diet produced bone deformities and lowered rate of healing of fractures. Silicon played an important role in nutrient interaction: the bones of rats given extra silicon were found to contain 20% more calcium and 10% more phosphorus than those from control rats fed the same diet without the extra silicon.

The 1970 experiments of Carlisle & Schwartz found a correlation between the amount of Si in the diet and the mineralization level of the young bone. Si was found to be located uniquely in the areas where active growth was taking place, declining in relationship to the laying down of hydroxyapatite, or bone crystal, which is the process that transforms young, pliable bone into a hard, calcified structure. This lead the doctors to hypothesize that Silicon acted as a regulating factor for the deposit of bone.

Silicon (density of 2.329 g/cm$^3$) doped calcium phosphate bulk ceramic has been reported to be conducive of both osteoblast deposition and osteoclast resorption, allowing it to fully participate in the bone remodeling processes of the body (Sayer et al., 2003). It is believed that silicon substitutes into the calcium phosphate lattice with a transformation to a saturation composition of $\text{Ca}_3(\text{P}_{0.9}\text{Si}_{0.1}\text{O}_{3.95})_2$ or Si–TCP_{sat} with charge compensation by oxygen vacancy...
formation associated with a proportional decrease of OH ions. There is very little information reported on the requirements of silica in the body so this is a flexible area of research.

### 3.6 Simulated Body Fluid

Simulated body fluid (SBF) is a solution with ion concentration and pH value similar to that of human blood plasma. SBF is known to cause the production of bioactive calcium phosphate precipitation similar to biological mineralization. The purpose for the use of SBF was to simulate human physiological condition. Human body fluid is supersaturated with respect to biological apatite, which constitutes the mineral phase of calcified tissue such as bone, dentine, and enamel in the body and also some pathological calcifications (Shi, 2004). The following table shows the major constituents of simulated body fluid compared to blood plasma.

**Table 3.6.1** Ion concentration in simulated body fluid (SBF) and human blood plasma (Hench, 1993)

<table>
<thead>
<tr>
<th>Ion</th>
<th>Simulated Body Fluid (mM)</th>
<th>Blood Plasma (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>142.0</td>
<td>142.0</td>
</tr>
<tr>
<td>K⁺</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>147.8</td>
<td>103.0</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>4.2</td>
<td>27.0</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>HPO₄²⁻</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

When certain compositions of Ca-P are in SBF, an apatite nuclei can form and spontaneously grow the surface apatite found in normal bone (Hench, 1993). Bone forming cells are expected to
proliferate over fibroblasts on the surface of the apatite layer, therefore, fibrous tissue which usually forms around foreign material is not formed, thus, the surrounding bone can grow directly on the surface of the apatite layer. When this occurs, a tight chemical bond forms between the surface of the apatite and the bone apatite in order to reduce the interfacial energy (Hench, 1993).

**Table 3.6.2** Differences in medium composition and parametric conditions under which the bioactivity reactions at the biomaterial surface take place *in vivo* and *in vitro* (Mow and Huiskes, 2005)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Simulated Body Fluid</th>
<th>Tissue Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity</td>
<td>Acellular</td>
<td>Highly cellular</td>
</tr>
<tr>
<td>Protein content</td>
<td>No Protein</td>
<td>Protein-rich</td>
</tr>
<tr>
<td>Replenishment</td>
<td>None</td>
<td>Fluid turnover (blood circulation)</td>
</tr>
<tr>
<td>Variation in solution composition during contact</td>
<td>Limited to dissolution-precipitation</td>
<td>Unlimited, due to macrophage activity</td>
</tr>
<tr>
<td>pH</td>
<td>7.40</td>
<td>7.0-9.0</td>
</tr>
<tr>
<td>Temperature</td>
<td>37°C</td>
<td>35-40°C</td>
</tr>
</tbody>
</table>
CHAPTER 4
EXPERIMENTAL PROCEDURE

Tricalcium phosphate (TCP) is a bioceramic that exhibits excellent biocompatibility properties; however, it is also known to have poor mechanical characteristics. TCP degrades in the physiological environment, but the rate of strength loss is unpredictable. TCP can be reinforced by the addition of dopant material to improve mechanical properties and modify the rate of degradation under simulated conditions. In this project TCP was doped with various weight percentages of dopant material and processed in two types of samples; compression compacts and disk compacts. These samples were then tested to determine the effect of dopants on: densification, the resorption rate of TCP, the mechanical strength, and if there is any change in biocompatibility or the production of toxic effects in a cellular environment.

Oxide based sintering additives in this research were high purity materials, which included silicon dioxide (99%+ purity), magnesium oxide (96%+ purity) and zinc oxide (99.9%+ purity), all were purchased from Fisher Scientific. Synthetic beta-tricalcium phosphate (BABI-TCP-N100) nano powder was obtained from Berkley Advanced Biomaterials Inc., (CA) with average particle size at 100nm.

4.1 Powder Preparation

Oxide based dopants were used. These dopants were independently added to tricalcium phosphate in multiple weight percentages which included 0.25, 0.5, 1.0, and 5 weight percent. The oxide based dopants used for this work were silicon dioxide, magnesium oxide, and zinc oxide. Powders were weighed and mixed in 250mL translucent polypropylene Nalgene bottles,
and 150g of zirconia milling media balls were added. Batches were made based on 30g of β-TCP. After dopant addition, ball milling was done to minimize the formation of agglomerates and increase the homogeneity of the powders. BET, (Brunauer, Emmett, and Teller) surface area analysis was then done on pure TCP powders that were ball milled at different time intervals of 0, 6, 12, 24 hours to determine which amount of time would be most adequate to use based on the highest surface area after milling. It was noted that after milling for 6 hours there was no noticeable variation in surface area. Therefore, for the remainder of this research ball milling for 6 hours at 70 rpm was used and made it a standard protocol.

4.2 Fabrication of Sample Compacts

After milling, milling media was removed from the powders using a sifter. All powders were appropriately labeled. Extreme care was taken to minimize the exposure of the powders to moisture in the atmosphere because β-TCP is a significantly moisture sensitive product which causes increased agglomeration of the particles. Therefore, when the powders were not in use, they were stored in a pressurized vacuum oven at about 80 degrees centigrade. Powder was measured for each composition and pressed using a uniaxial press. There were two types of samples made for this research, disk compacts for biological analysis and compression compacts for mechanical analysis. The amount of powder weighed to make disk compacts was approximately 0.5g and the amount of powder weighed for compression compacts was approximately 1.15g. There were also two separate mold presses used, to produce the different diameter samples. The disk mold press produced 12mm in diameter by 3mm thick green compacts while the compression mold press made 6.3mm diameter by approximately 20mm tall green compacts. Disk samples were uniaxially pressed at approximately 250 MPa of pressure.
whereas the compression compacts were pressed at around 150 MPa, this was the reason for the significant difference in green densities. Disk sample densities averaged in the mid fifty percentile while the compression samples illustrated densities in the low forty percentile. A total of five compacts for each type of sample made for individual compositions for each type of analysis. Fig. 4.2.1 represents the process flow chart showing the various stages of processing of doped β-TCP compacts.

**Figure 4.2.1** Processing of TCP with metal ion dopants flow chart
Figure 4.3.1 Schematic of the sintering cycle used for TCP ceramics incorporated with oxide based dopants of different compositions

4.3 Sintering

After pressing, all green compacts were placed in a muffle furnace for 2 hours at 1250°C. This temperature and sintering time was based on literature review and densification trends of synthetic calcium phosphates obtained in our laboratory. Porous zirconia setter plates were used to place these samples inside the furnace as shown in Fig. 4.3.2. It is well known that β-TCP has the best densification between 1200°C-1300°C; above 1300°C there is high possibility of β-TCP transforming to α-TCP phase, this also changes the resorption properties and bioactivity characteristics. A slow heating cycle rate was used at 3°C/min; this sintering cycle is shown in Fig. 4.3.1. Good densification is important to improve mechanical and physical properties of β-TCP.
Figure 4.3.2 Images of sintered TCP compact (a) Disk compacts (b) Compression compacts

4.4 Density Measurements

Bulk densities for green and sintered samples were measured for all compositions. Compositions that showed poor densification when the dopant percentage additions were varied, were not used for the remainder of the research, only the compositions that showed the highest densification were used for dopant combinations and mechanical and biological characterization. Based on densification, samples were not subjected to micro hardness testing due to the difficulty in measuring the indent caused by the highly porous surfaces. The following table shows the combination of dopant used for this research based on the highest densifications recorded. Commercial ceramics usually use sintering additives as a measure to increase densification. For this research no additives were used simply because we wanted to identify the specific effects of the dopants being added.
<table>
<thead>
<tr>
<th>Compositions</th>
<th>Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCP</td>
<td>N/A</td>
</tr>
<tr>
<td>TCP-SiO₂</td>
<td>1</td>
</tr>
<tr>
<td>TCP-MgO</td>
<td>1</td>
</tr>
<tr>
<td>TCP-ZnO</td>
<td>0.25</td>
</tr>
<tr>
<td>TCP-SiO₂-ZnO</td>
<td>1 + 0.25</td>
</tr>
<tr>
<td>TCP-SiO₂-MgO</td>
<td>1 + 1</td>
</tr>
<tr>
<td>TCP-ZnO-MgO</td>
<td>0.25 + 1</td>
</tr>
<tr>
<td>TCP-SiO₂-MgO-ZnO</td>
<td>1 + 1 + 0.25</td>
</tr>
<tr>
<td>TCP-SiO₂</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 4.4.1 Weight percent and combination of dopants used for this project

4.5 X-ray Diffraction Analysis

X-ray diffraction was used to test if there were any significant changes to the phases of β-TCP after the introduction of dopants compared to β-TCP processed under the same conditions. Powdered samples were prepared for all compositions by grinding approximately three sintered compacts of each composition separately, in a mortar and a pestle. Each powdered sample was then placed in a specimen holder, individually, and then analyzed using a Phillips expert fully automated diffractometer with Co K-alpha radiation.
4.6  Simulated Body Fluid (SBF)

SBF was used to replicate the human body’s natural physiological environment. There were two stages of this experiment; stage 1 was the use of sintered disk compacts and Stage 2 was the use of sintered cylindrical compression compacts. For the disk compacts, experiments were conducted for durations of 2, 4, 6, 8, and 12 weeks for all compositions except SiO$_2$ 5 weight %, which was only conducted up to 6 weeks. In stage 2, study was conducted for durations of 4, 8, and 12 weeks for all composition except TCP-SiO$_2$ 5 weight % which was conducted only up to 8 weeks.

Preparation

I have followed a standard procedure to make simulated body fluid (Hench, 1993). The procedure instructed for the preparation of 1 liter of solution. First; a solution of 1 normal hydrochloric acid at 100mL was made by adding 8.26mL of concentrated HCL and 91.74mL of de-ionized distilled water and mixing with a magnetic stirring rod. Then the 500mL beaker to be used was washed with approximately 4mL of N HCL and de-ionized water and dried. The beaker was filled with 500 mL of de-ionized water and poured into a 1 L glass Erlenmeyer flask with a magnetic stirring rod. Next, the reagent salts were added in the following order was completely dissolved before adding the next. After all reagents were added, the pH of the solution was measured and adjusted to approximately 7.40 by stirring the solution and adding 1N-HCl solution in. Finally the volume was adjusted by adding de-ionized water to make 1 liter of SBF.
<table>
<thead>
<tr>
<th>Order</th>
<th>Reagent</th>
<th>Purity</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NaCl</td>
<td>For Biological Work</td>
<td>7.996</td>
</tr>
<tr>
<td>2</td>
<td>NaHCO₃</td>
<td>Certified A.C.S</td>
<td>0.350</td>
</tr>
<tr>
<td>3</td>
<td>KCl</td>
<td>Certified A.C.S</td>
<td>0.244</td>
</tr>
<tr>
<td>4</td>
<td>K₂HPO₄.3H₂O</td>
<td>99%+</td>
<td>0.171</td>
</tr>
<tr>
<td>5</td>
<td>MgCl₂.6H₂O</td>
<td>Assay 99.7%</td>
<td>0.305</td>
</tr>
<tr>
<td>6</td>
<td>1N-HCl</td>
<td>-</td>
<td>40 ml</td>
</tr>
<tr>
<td>7</td>
<td>CaCl₂.2H₂O</td>
<td>Assay 99.6%</td>
<td>0.368</td>
</tr>
<tr>
<td>8</td>
<td>Na₂SO₄.10H₂O</td>
<td>Certified A.C.S</td>
<td>0.161</td>
</tr>
<tr>
<td>9</td>
<td>NH₂C(CH₂OH)₃</td>
<td>Assay 100%</td>
<td>6.057</td>
</tr>
</tbody>
</table>

**Table 4.6.1** reagent preparation for simulated body fluid (Hench, 1993)

**Procedure**

In both stage 1 and 2, all sintered compact were placed in 15mL glass vials. As previously mentioned there were nine compositions for each time period, thus a total of 45 samples. The glass vials were filled with 10mL of SBF solution via pipetting from larger flask containing the previously made SBF. This was done until all vials were filled for each time period. This procedure was conducted in a bench hood. All samples were placed in an aluminum tray and placed in an incubator at a regulated temperature of 37°C to simulate the human body’s internal temperature. Then, SBF solution was changed twice per week, typically every Tuesday and Friday. The reason for this was to maintain a somewhat consistent pH.
reading over the duration of the study. The pH changes in SBF are due to the release of ions into the solution from the ceramics compacts. The old SBF solution is pipetted into labeled corresponding glass vials and later measured for any changes in pH. New SBF solution is pipetted into the vial containing the compact samples; this was done for all compositions for all durations.

4.7 Weight Change

Following conclusion of the biological experiment, all compacts were removed from the glass vials and were gently washed by repeated dipping in de-ionized water. All samples were then placed on a zirconia setter plate and placed in a muffle furnace at 200°C where they were left to dry for approximately 72 hours. After the drying process ended, samples were set to cool for 1 hour and then weighed for change in weight measurement due to any apatite formation or dissolution occurrence. Weight measurements were recorded for each composition and each time duration. In general, if there was any Ca-P based apatite such as hydroxycarbonate apatite (HCA) formation, then that would result in weight increase, however, if there is any dissolution of sample occurring then there is a loss in weight.

4.8 Microstructural Analysis

Scanning electron microscopy was used to analyze microstructure of sintered β-TCP structures with various dopant additions and to compare them with that of sintered pure β-TCP compact prior to being subjected to biological experiment. All compositions were observed under SEM to study the effect of dopants on the microstructure of β-TCP. Disk compacts that were subjected to a biological study were also observed under the SEM for all compositions and
all time durations (2, 4, 6, 8, 12 weeks). Both top surface and fracture surface SEM were taken after SBF treatment. Micrographs for β-TCP showed clear grain boundaries and average grain size could be calculated for some compacts, whereas for the compacts containing dopants, their grain boundaries were not always very prominent.

4.9 Mechanical Characterization

Mechanical properties of as-sintered dense β-TCP structures with various dopants were analyzed for failure strength under compressive loading and compared with pure β-TCP samples processed under the same conditions; all compression compacts that were subjected to biological experiments were also evaluated. The ultimate compressive strength of these ceramics compact samples were evaluated by using a screw driven Instron with a constant crosshead speed of 0.33mm/min. Diameter of the cylindrical compacts was approximately 6mm and between 16-20mm in height. The average ratio of height to diameter of the samples tested was more than two to one. Method of testing was based on the compression of two parallel plates with a sample between, the upper plate was attached to a 1000lb. load cell and data was recorded based on crosshead displacement vs. load. Ultimate compression strength was then calculated as the maximum load before the sample broke. Pieces of the broken samples were then placed under the SEM to observe the fracture surfaces. As processed and SBF treated samples were tested to determine the effect of SBF on compression strength.

4.10 Cell Culture

Osteoblast precursor cell line 1 (OPC1) was used for the in vitro analysis. OPC1s were transfected with SV40 oncogene thereby creating a stable line (Winn, 1995). OPC1s were
cultivated with McCoys 5A media (5% FBS, 5% BCS, 100 mg/mL streptomycin sulfate and 100 mg/mL penicillin), and maintained in set conditions of 37°C and humidified 95% air/5% carbon dioxide. Medium was supplemented with 8 mg/mL Fungizone fungicide (Gibco, Rockville, MD). Samples of various compositions of doped TCP were placed in different wells of a 24-well plate and OPC1 were seeded on each individual sample at a density of 2.0 x 144 cells/well. For control, undoped TCP were placed in individual wells of a 24-well plate were seeded at the same cell density. Plates were maintained in standard incubator conditions and media was changed every other day using 1 mL of Mc Coys 5A culture medium. At each experimental time point (days 5 and 11) samples were removed and fixed for scanning electron microscopy.

**SEM Preparation**

Two samples of each material were processed for viewing on the SEM. Scaffolds were fixed in 2%paraformaldehyde/2% glutaraldehyde in 0.1-M cacodylate buffer overnight, post fixed with 2% buffered osmium tetroxide (OsO4) [J. Baker, Phillipsburg, N.J.] Samples were then dehydrated in an ethanol [AARPER Alcohol and Chemical Co., Shelbyville, KY] series; followed by two 5-minute 100% acetone [J. Baker, Phillipsburg, N.J.] rinses. Hexamethyldisilane (HMDS) [Ted Pella, Inc., Redding, CA] was used as a drying procedure. Samples were then placed in 100% HMDS 2x for 1 hour each (Barrias et al., 2004). Scaffolds were air dried; gold coated using a Technics Hummer #V Sputter Coater, and viewed with a Hitachi S570 SEM, images were collected digitally.
CHAPTER 5
RESULTS

5.1 BET Surface Area Analysis

BET surface area analysis was used to determine the appropriate amount of time to allow for ball milling based on the powder surface area. If there is a high surface area count then the amount of agglomerates present will be reduced which is important for good densification.

![BET Surface Area Analysis](chart.png)

**Figure 5.1.1** BET surface area on pure TCP powders ball milled at various times

The plot in Fig. 5.1.1 shows the surface area analysis of powders ball milled from 0 to 24 hours. It became evident that after milling for 6 hours milling the surface area remained within the error range of about 73 m$^2$/g. Therefore, we decided to use 6 hours as the milling time for this project.

After the surface area analysis was concluded, a brief density study was done on the three most significant ball milling times to determine the correlation between surface area and density shown in Fig. 5.1.2. The data indicated that like the surface area, the density for 6, 12 and 24
hour ball-milled samples were all approximately the same, which further justified the use 6 hour ball milling.

Figure 5.1.2 Normalized density measurements of pure TCP sintered compacts after ball milling at various times

5.2 Densification

Good densification is necessary for achieving improved mechanical properties in pressed ceramic compacts. Green ceramic compacts were prepared manually via uniaxial pressing for various compositions of tricalcium phosphate with selected dopant. A silicon based mold releasing agent (CRC Heavy Duty Silicone Multi-use Lubricant) was used to process the green compacts which allowed for applying higher pressing pressure to the compacts and resulted in higher green densities. The use of this releasing agent also prevented samples from sticking to the mold and fracturing during processing. Green and sintered densities were measured and were normalized with respect to the theoretical density of pure TCP (3.07 g/cc). Average green and sintered densities for each of these compositions were calculated and represented separately.
Figure 5.2.1(a) Normalized density measurements of TCP-MgO sintered compacts at various percentage additions

In the preliminary stage of this project the most important task was to determine the ratio of dopant to be added to TCP. Based on literature review, a decision was made to use (0.25, 0.5, 1, and 5 wt%). Fig. 5.2.1(a) shows the effect of MgO on densification based on 0.25 wt% and 1 wt %. It was observed that MgO influenced a slightly higher densification to TCP at 1 wt % addition compared to 0.25 wt%. This ratio for MgO was used for the remainder of the research.

In Fig. 5.2.1(b) the highest density (>70%) for TCP-ZnO compacts were present at 1 wt % addition, however, from literature review, it found that the addition of zinc above 1 wt % may cause some toxic effect, hence, it was decided to use 0.25 wt % zinc. It was determined based on trends in densification that by increasing the amount of SiO₂ (up to 5 wt %) added to TCP it was possible to increase the density which can be seen in Fig. 5.2.1(c). Based on this observation we decided to optimize the density of TCP, by using SiO₂ at 1 and 5 wt % additions.
Figure 5.2.1(b) Normalized density measurements of TCP-ZnO sintered compacts at various percentage additions

Figure 5.2.1(c) Normalized density measurements of TCP-SiO₂ sintered compacts at various percentage additions
Single Dopant System

Density was measured and normalized with respect to theoretical density for both disk and compression compacts. Green density was measured only for the disk compacts. Usually sintered compression compact density was lower than the disk compact density due to the low initial green density.

TCP-MgO (1wt %) had very little effect on densification in terms of disk and compression compacts. The density remained the same at around 73% for the disk compacts as shown in Fig. 5.2.2.

TCP-ZnO (0.25wt%) demonstrated a negative effect on densification for disk compacts by decreasing the density from 73% to 69%. However, there was a positive effect on the compression compacts as shown in Fig. 5.2.3.
The effect of silicon dioxide on sintered density was positive in both disk and compression compacts for TCP doped with SiO$_2$ at 1 wt % and 5 wt %. There was a noticeable increase in densification for the compression compacts of 1wt% addition, from 62% to 72% as shown in Fig. 5.2.4 and Fig. 5.2.5.
Figure 5.2.4 Normalized density calculations of pure TCP versus TCP-SiO$_2$ 1 wt% disk and compression sintered compacts

Figure 5.2.5 Normalized density calculations of pure TCP versus TCP-SiO$_2$ 5 wt% disk and compression sintered compacts
Binary Dopant Systems

Binary composition of TCP-SiO$_2$ (1 wt%)–MgO (1 wt%) had an augmenting effect on the density of TCP. There was over a 9% increase in density for both disk and compression compacts. This occurrence is an indication of the combined effect of the dopants versus the previously observed unary effect as shown in Fig. 5.2.6.

TCP-MgO (1 wt%)–ZnO (0.25 wt%) also improved the density of TCP. There was a significant increase in the compression compact densification from 62% to 78% as shown in Fig. 5.2.7.

**Figure 5.2.6** Normalized density calculations of pure TCP versus TCP-SiO$_2$-MgO disk and compression sintered compacts
Figure 5.2.7 Normalized density calculations of pure TCP versus TCP-MgO-ZnO disk and compression sintered compacts

TCP-SiO$_2$ (1wt%)-ZnO (.25wt%) also increased sample density as shown in Fig. 5.2.8. Based on all three binary systems, we can conclude that the combined effects of these dopants can produce a noticeable increase in the densification of β-TCP sintered compacts.
Figure 5.2.8 Normalized density calculations of pure TCP versus TCP-MgO disk and compression sintered compacts

**Ternary Dopant System** [TCP-SiO$_2$ (1wt%) - MgO (1wt%) –ZnO (0.25 wt%)]

The highest densification was recorded for the ternary system including disk and compression compacts. For the disk compacts there was a 10% increase in density, and a 19% increase for compression compacts as shown in Fig. 5.2.9. The ternary system densification analysis confirms that the combined effect of these dopants on TCP is more pronounced than in the unary or binary compositions.
5.3 pH Change

pH measurements were recorded in this experiment as a baseline guide to determine the effect of various compositions of TCP on the change in pH in a simulated physiological environment. The pH of body fluids is acidic, 7; during wound healing, it becomes slightly alkaline, 7.4, in normal conditions and alkaline >7.4 during bone mineralization (Hench, 1993). The beginning pH of the SBF solution was approximately 7.40, the pH solution was changed twice a week to maintain this constant pH. In Fig. 5.3.1 we observe a change in pH over the 12 week duration of the experiment. All pH changes were positive, i.e., pH always increased; however, except for the 8 week data change, pH did not show any influence of dopant composition.
Figure 5.3.1(a) pH change measurements over the 12 week duration of SBF study of the single component systems
Figure 5.3.1(b) pH change measurements over the 12 week duration of SBF study of binary and ternary component systems
5.4 Weight Change

In this experiment weight change was used to determine apatite formation and/or degradation of TCP. The data collected was the weight change of the post SBF compression compacts. An initial weight was measured before SBF treatment and compared to the final weight after treatment. Weight loss is a sign of occurring dissolution because ions are being released from the ceramic compacts causing a slight decrease in bulk sample weight; however, when these Ca-P ions react with other ions in the SBF solution it is possible to form a calcium phosphate apatite layer on the surface or inside the sample; when this happens the apatite formation has a greater effect on the weight property of the ceramic than does the dissolution effect.

As expected, the samples that showed an increase in weight also showed apatite formation on the surface and inside the samples. Similarly, samples that revealed no significant change in weight also did not show apatite formation. Figures 5.4.1 to 5.4.8 show the effects of weight change on pure TCP versus the various combination of doped TCP.
Figure 5.4.1 Plot illustrating percent weight change for disk and compression compacts of TCP-MgO after removal from simulated body fluid up to 12 weeks.

Figure 5.4.2 Plot illustrating percent weight change for disk and compression compacts of TCP-ZnO after removal from simulated body fluid up to 12 weeks.
Figure 5.4.3 Plot illustrating percent weight change for disk and compression compacts of TCP-SiO$_2$ 1 wt% after removal from simulated body fluid up to 12 weeks.

Figure 5.4.4 Plot illustrating percent weight change for disk and compression compacts of TCP-SiO$_2$ 5 wt% after removal from simulated body fluid up to 12 weeks.
Figure 5.4.5 Plot illustrating percent weight change for disk and compression compacts of TCP-SiO$_2$-ZnO after removal from simulated body fluid up to 12 weeks.

Figure 5.4.6 Plot illustrating percent weight change for disk and compression compacts of TCP-MgO-SiO$_2$ after removal from simulated body fluid up to 12 weeks.
Figure 5.4.7 Plot illustrating percent weight change for disk and compression compacts of TCP-MgO-ZnO after removal from simulated body fluid up to 12 weeks

Figure 5.4.8 Plot illustrating percent weight change for disk and compression compacts of TCP-MgO-ZnO-SiO₂ after removal from simulated body fluid up to 12 weeks
5.5 Microstructural Analysis

The formation of a bone like apatite layer on biomaterials is assumed to be the precondition for their osteoconductivity to induce bone formation on the biomaterials (De Bruijn, et al., 2000). It has been commonly accepted that the bioactivity of bioceramics relies on their ability to induce hydroxyapatite (HA) formation in the physiological environment. Thus, the ability to form apatite in simulated body fluid (SBF) can be regarded as the evidence of bioactivity for bioceramics (Ramila et al., 2001). However, HA is not the only phase of calcium phosphate which may form in the physiological environment (Brown et al., 1987). Other calcium phosphate phases such as octa calcium phosphate (OCP) and dicalcium phosphate dehydrate (DCPD) may be misidentified as HA on bioactive ceramics (Leng et al., 2003). It was found that the stable thermodynamic structure of apatite does not ensure that HA is the most favorable precipitation phase from supersaturated calcium and phosphorous solutions, because the (OCP) and (DCPD) are kinetically more favorable (Biostelle et al, 1990). It has been found that OCP and DCPD can be considered as a precursor phase of HA formation (Elliot, 1994). Theoretical analysis based on nucleation kinetics indicated that the OCP nucleation rates could be much faster than that of HA in the physiological environment (Lu et al., 2005). It is believed the phase of the apatite found in this research is in the hydroxyl carbonate apatite (HCA) phase, however, it was misidentified OCP as HA when using powder X-ray diffraction (XRD) because of the structural similarities between HA and OCP (Leng et al., 2004). For this research, characterization of the apatite formation was not done due to the complications of the process, however, we concluded that the apatite is more favorable to be HCA or OCP, based on what was
found in literature. The apatites found in this experiment had different morphologies; further research is needed to pinpoint what calcium phosphate phase each morphology represents.

All disk samples were subjected to biological study in simulated body fluid up to 12 weeks. Scanning electron micrographs were recorded at the 0, 2, 4, 8, 12 week points of the experiment. After samples were removed for the SBF solution and dried, SEM images were taken of the top surface to observe any changes in microstructure that could be the result of degradation of the calcium phosphate matrix. Some samples revealed formation of an apatite layer on the surface which is good for cell adhesion and provides sites for exceptional implant-tissue interfacial bonding.

Pure TCP as shown in Fig. 5.5.1 was used as control in the SBF solution to visualize the effect of the pure compacts. A well established pattern of degradation was observed over 12 weeks. There was significant increase in porosity from the initial stage to the final stage; this was an indication that some dissolution was taking place. TCP exhibited little ability to induce calcium phosphate apatite formation on its surface; Xin has also reported similar trends with TCP in SBF (Xin et al., 2005). After 12 weeks some of the pores were filled with apatite. The apatite formation on the inside of the sample may have been due to the local release of calcium and phosphorous in the pores which may be more favorable to promote apatite growth in the enclosed environment rather than the free surface. We can conclude that degradation was actively occurring in the TCP ceramics over the 12 week SBF treatment.

In Fig. 5.5.2, showing TCP-1 wt% SiO₂, beginning at the 2 week point there is growth of apatite layer on the surface of the compacts; due to significant apatite growth the grain structure could hardly be seen.
A flake-like apatite layer was formed on the surface of these samples; we believe that the phase of this bone-like apatite is either HCA or OCP. There was enough apatite formation to cover the entire surface of the sample. We concluded that the presence of SiO$_2$ (1wt%) increased the bioactivity of TCP due to the increased amount of apatite on the surface as shown in Fig. 5.5.2. This layer would be ideal for osteoblast cell adhesion, thus, providing good cell-material interfacial bonding.

TCP-5 wt % SiO$_2$ was only studied from 0 to 6 weeks as shown in Fig. 5.5.3. There was no apatite formation visible on the surface and grain structure remained somewhat distinct throughout the 6 week period. There was minimal surface porosity observed which is good for maintaining mechanical strength over the test period.

In Fig. 5.5.4, the surface microstructure of TCP-ZnO composition changed very little throughout the experiment. There was no visible degradation of the grain boundaries. There was also no apatite layer formation and no significant increase in surface porosity. However, some apatite can be seen inside the sample from fracture surface analysis.

In the case of the TCP-MgO composition, as shown in Fig. 5.5.5(a) we observed very distinct grain boundaries. We also observed very unique crystal-like apatite formation on the surface microstructure up to 8 weeks shown in figure 5.5.5 (b) and (d). These crystals are similar to the OCP crystals found by the electrochemical method (Zhang et al., 2005). It is also possible to see the crystals in the pores of the samples in Fig. 5.5.5(d). The pompom-like structures in Fig. 5.5.5(c) were difficult to identify; however, these sharp needle-like balls were only seen on this sample. After 12 weeks all surface apatite formation was no longer visible, revealing an extremely degraded grain structure as shown in Fig. 5.5.5(e).
In Fig. 5.5.6 (a), TCP-MgO-SiO$_2$ compacts, there was a well defined granular structure. At the 2 week point noticeable porosity began to develop as the grain boundaries were slowly degrading as shown in Fig. 5.5.6(b). By the 12th week the grain boundaries were completely eroded as shown in Fig. 5.5.6(e), also not shown in these images but at a lower magnification is evidence of increased porosity on the surface of the sample. There is no apatite formation on the surface of these samples indicating that there is not enough calcium phosphate ion release into the SBF solution to form HCA or OCP crystals on the surface or on the inside of the sample based on fracture surface analysis.

Based on the SEM micrographs of Fig. 5.4.7, the TCP-SiO$_2$-ZnO compacts were very unique on the surface. Initially, there was noticeable porosity in the control compacts as shown in Fig. 5.6.7 (a) and the grain boundaries were to some extent indistinguishable. After two weeks there were signs of apatite formation but in very scarce quantities and the grain structure became even more obscure. The apatites that formed on the surface of these samples were in the form of sponge-like clusters as shown in Fig. 5.5.7 (b) and (c). However, by the eighth week apatite structures were no longer visible on the surface and a significant amount of porosity had developed as shown in Fig. 5.5.7 (d). By the end of the SBF treatment shown in figure 5.5.7(e) the grain boundaries had eroded and the amount of porosity decreased which could have been due to the apatite growth inside the sample.

The micrographs of Fig. 5.5.8 of TCP-MgO-ZnO illustrated the least amount of surface change. Nonetheless, there were signs of dissolution in Fig. 5.5.8(e). It is possible that this composition of TCP-MgO-ZnO reduces the degradation rate of TCP. The grain structure remained intact up to 8 weeks, where there was no sign of apatite formation on the surface, it is possible that the addition of magnesium oxide and zinc oxide together stabilizes the calcium
phosphate structure causing the release of Ca-P ions into the solution for apatite formation more difficult. After 12 weeks of SBF treatment, an excess amount of degradation was visible as shown in Fig. 5.5.8 (e); it is possible that this combination of dopants maintains most of the strength of TCP as it gradually degrades.

The TCP-ternary composition in Fig. 5.5.9 showed very little evidence of change to the surface microstructure. There were no signs of apatite formation, or significant increase in the amount of porosity. There was also no obvious visible change observed from this combination of dopants. It is possible that a longer study may be necessary to identify any significant alterations to samples of this composition.

### Table 5.5.1  Average grain size of sintered compacts before SBF treatment

<table>
<thead>
<tr>
<th>Compositions</th>
<th>Average Grain Size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCP</td>
<td>4.586</td>
</tr>
<tr>
<td>TCP-SiO$_2$ 1 wt%</td>
<td>3.850</td>
</tr>
<tr>
<td>TCP-MgO</td>
<td>6.400</td>
</tr>
<tr>
<td>TCP-ZnO</td>
<td>4.070</td>
</tr>
<tr>
<td>TCP-SiO$_2$-ZnO</td>
<td>4.216</td>
</tr>
<tr>
<td>TCP-SiO$_2$-MgO</td>
<td>4.992</td>
</tr>
<tr>
<td>TCP-ZnO-MgO</td>
<td>4.304</td>
</tr>
<tr>
<td>TCP-SiO$_2$-MgO-ZnO</td>
<td>3.886</td>
</tr>
<tr>
<td>TCP-SiO$_2$ 5 wt%</td>
<td>5.43</td>
</tr>
</tbody>
</table>
Figure. 5.5.1. SEM micrographs illustrating surface microstructures of pure TCP after being subjected to (a) 0 wks in SBF (b) 2 wks in SBF (c) 4 wks in SBF (d) 8 wks in SBF (e) 12 wks in SBF
**Figure. 5.5.2.** SEM micrographs illustrating surface microstructures of TCP doped with 1 wt% SiO₂ after being subjected to (a) 0 wks in SBF (b) 2 wks in SBF (c) 4 wks in SBF (d) 8 wks in SBF (e) 12 wks in SBF
Figure 5.5.3. SEM micrographs illustrating surface microstructures of TCP doped with 5 wt% SiO$_2$ after being subjected to (a) 0 wks in SBF (b) 2 wks in SBF (c) 4 wks in SBF (d) 6 wks in SBF
Figure 5.5.4. SEM micrographs illustrating surface microstructures of TCP doped with 0.25 wt% ZnO after being subjected to (a) 0 wks in SBF (b) 2 wks in SBF (c) 4 wks in SBF (d) 8 wks in SBF (e) 12 wks in SBF
Figure 5.5.5. SEM micrographs illustrating surface microstructures of TCP doped with 1 wt% MgO after being subjected to (a) 0 wks in SBF (b) 2 wks in SBF (c) 4 wks in SBF (d) 8 wks in SBF (e) 12 wks in SBF
Figure 5.5.6. SEM micrographs illustrating surface microstructures of TCP doped with 1 wt% MgO+1 wt% SiO$_2$ after being subjected to (a) 0 wks in SBF (b) 2 wks in SBF (c) 4 wks in SBF (d) 8 wks in SBF (e) 12 wks in SBF
Figure 5.5.7. SEM micrographs illustrating surface microstructures of TCP doped with 0.25 wt% ZnO + 1 wt% SiO$_2$ after being subjected to (a) 0 wks in SBF (b) 2 wks in SBF (c) 4 wks in SBF (d) 8 wks in SBF (e) 12 wks in SBF
Figure. 5.5.8. SEM micrographs illustrating surface microstructures of TCP doped with 0.25 wt% ZnO+1 wt% MgO after being subjected to (a) 0 wks in SBF (b) 2 wks in SBF (c) 4 wks in SBF (d) 8 wks in SBF (e) 12 wks in SBF
Figure 5.5.9. SEM micrographs illustrating surface microstructures of TCP doped with 0.25 wt% ZnO + 1 wt% MgO + 1 wt% SiO2 after being subjected to (a) 0 wks in SBF (b) 2 wks in SBF (c) 4 wks in SBF (d) 8 wks in SBF (e) 12 wks in SBF
5.6 Mechanical Characterization

Mechanical properties of various dopant combinations incorporated into TCP structures were evaluated via uniaxial compression testing. A screw driven Instron machine was used for this purpose; cylindrical samples with average diameter of 6mm and average height of 18mm were used. Structures with 1wt% SiO$_2$, 5wt% SiO$_2$, 1wt% MgO and 0.25wt% ZnO and their combinations were tested for their compression strength and compared with pure TCP structures processed under the same conditions. Five samples of each composition were tested. Results from compression testing were recorded and are illustrated from Fig. 5.6.1 to 5.6.8.

It was found that two mechanisms affected the change in compression strength of the TCP ceramics: (i) the degradation effect which is the loss of strength due to the increase in porosity and weakened grain boundaries, (ii) apatite formation effect which is due to the pores being filled with apatite growth that reinforces the inside to the ceramic causing an increase in strength. Even though apatite growth is occurring it does not mean that degradation is not taking place, it means that the growth of apatite has more of an effect on the compression strength of TCP than degredation.

In Fig. 5.6.1, the TCP compacts gained some strength from 4 to 8 weeks which reinforce the ceramic; however after 12 weeks the degradation effect overcame the growth of apatite causing a decrease in strength that was lower than the initial strength which is evidence of resorption. When doped with zinc the initial strength was lowered, similarly it followed the same trend as in TCP, there was an increase in strength up to 8 weeks then a decrease after the 12th week, however, the final strength after SBF treatment was higher than the initial strength. In the TCP-1wt% SiO$_2$ caused an increase in strength over the 12 week study; therefore, the apatite growth is significantly higher than the amount of degradation occurring so this composition can
be ideal for long term bone regeneration purposes. However, by increasing the amount of SiO$_2$ to 5wt% as shown in Fig. 5.6.3 we are able to reverse the strength characteristics of these samples. This composition provides a high initial strength and quickly degrades after 8 weeks which makes it suitable for shorter term bone regeneration applications. In Fig. 5.6.4 and 5.6.5, there were no positive effects on strength observed, at all times points the strength of these compositions were significantly lower than TCP, also there were no signs of degradation. These compositions would not be suitable for bone regeneration due to the lack of initial mechanical support. The TCP-SiO$_2$-ZnO combination illustrated fairly stable strength measurements throughout the experiment; a longer study may be necessary to see any evidence of degradation as shown in Fig. 5.6.6. The TCP-MgO-ZnO composition shown in Fig. 5.6.7 was most interesting of all. There was over 150% increase in initial strength and a desirable strength loss trend over the 12 week period. This composition can be used in fairly short termed applications where high initial support is required. The ternary composition did not show signs of degradation. The strength remained in the same error range for the duration of the experiment as shown in Fig. 5.6.8.
Figure 5.6.1 Compressive strength of TCP compacts versus TCP+ZnO addition before and after SBF experiments

Figure 5.6.2 Compressive strength of TCP compacts versus TCP+SiO$_2$ 1 wt% addition before and after SBF experiments
Figure 5.6.3 Compressive strength of TCP compacts versus TCP$+\text{SiO}_2$ 5 wt\% addition before and after SBF experiments

Figure 5.6.4 Compressive strength of TCP compacts versus TCP$+\text{MgO}$ addition before and after SBF experiments
Figure 5.6.5 Compressive strength of TCP compacts versus TCP+SiO$_{2}$+MgO addition before and after SBF experiments

Figure 5.6.6 Compressive strength of TCP compacts versus TCP+SiO$_{2}$+ZnO addition before and after SBF experiments
Figure 5.6.7 Compressive strength of TCP compacts versus TCP+MgO+ZnO addition before and after SBF experiments

Figure 5.6.8 Compressive strength of TCP compacts versus TCP+Ternary addition before and after SBF experiments
5.7 Fracture Surface SEM Analysis

SEM images were taken after compression compacts were broken in the mechanical strength tests. The fracture surface was used to observe morphology and apatite formation in the interior of the samples. Images were taken of only the non-SBF compacts and the 12 week compacts. It was observed that all of the fracture surface images were consistent with the weight change analysis. In Fig. 5.7.1 it is evident that after 12 weeks in SBF there was significant flake-like apatite growth on the inside of the pure TCP compact; therefore, SBF penetrated the sample and there was some preferential apatite formation due to the local release of Ca ions in the pore structure. TCP-SiO$_2$ 1wt% samples illustrated a large amount of apatite on the surface and the interior of the compact; there was also a considerable amount of porosity in the non SBF treated compact which was not visible on the surface as shown in Fig. 5.7.2. In Fig. 5.7.3 and 5.7.4, TCP-SiO$_2$ 5wt% and TCP-ZnO there was a significant amount of porosity before SBF treatment, however, after 12 weeks, all the pores were filled with rich flake-like apatite; this apatite growth explained the increase in weight after being in SBF. TCP-MgO composition elicited exceptional apatite growth throughout the inside of the compacts as shown in Fig. 5.7.5, however, these apatite formations were slightly different from the others in that they were petal-like and crystal-like in nature, indicating one of two phases HA or OCP apatite. All the binary and ternary compositions had low porosity and better densification. As expected none of these compositions illustrated apatite growth except TCP-SiO$_2$-ZnO as shown in Fig. 5.7.7; this is consistent with the surface microstructure analysis and weight change data.
Figure 5.7.1. SEM micrographs illustrating fracture surface microstructures of TCP (a) 0 wks in SBF (b) 12 wks in SBF

Figure 5.7.2. SEM micrographs illustrating fracture surface microstructures of TCP doped with 1 wt% SiO₂ (a) 0 wks in SBF (b) 12 wks in SBF
Figure 5.7.3. SEM micrographs illustrating fracture surface microstructures of TCP doped with 5 wt% SiO$_2$ (a) 0 wks in SBF (b) 12 wks in SBF

Figure 5.7.4. SEM micrographs illustrating fracture surface microstructures of TCP doped with 0.25 wt% ZnO (a) 0 wks in SBF (b) 12 wks in SBF
Figure 5.7.5. SEM micrographs illustrating fracture surface microstructures of TCP doped with 1 wt% MgO (a) 0 wks in SBF (b) 12 wks in SBF

Figure 5.7.6. SEM micrographs illustrating fracture surface microstructures of TCP doped with 1 wt% SiO₂+1 wt% MgO (a) 0 wks in SBF (b) 12 wks in SBF
Figure 5.7.7. SEM micrographs illustrating fracture surface microstructures of TCP doped with 1 wt% SiO$_2$+0.25 wt% ZnO (a) 0 wks in SBF (b) 12 wks in SBF

Figure 5.7.8. SEM micrographs illustrating fracture surface microstructures of TCP doped with 0.25 wt% ZnO+1 wt% MgO (a) 0 wks in SBF (b) 12 wks in SBF
Figure 5.7.9. SEM micrographs illustrating fracture surface microstructures of TCP doped with ternary (a) 0 wks in SBF (b) 12 wks in SBF

5.8 Biological Characterization

Cell culture was done using osteoblast precursor cell line 1 (OPC1) to determine if there were any toxic effects caused by the addition of dopants into TCP. It is important that the dopants do not compromise the biocompatibility of TCP by causing any cytotoxicity. If the osteoblast cells are not affected by the dopants then they would tend to spread out and occupy as much surface area as possible, thus, having a more flattened structure. However, if there is some toxicity on the surface; of the material the cells would rather adhere to themselves than the material’s surface therefore; they formed a sort of ball structure that would have the least amount of interaction with the surface. This cell culture experiment was done at two intervals, 5 days
and 11 days. These intervals were chosen because it was found that after day 5, cells on a bioactive surface should begin the proliferation process in which they spread to cover as much surface area as possible; after day 11 some differentiation should be in progress where the osteoblast cells nearest to the material begin to release calcium and phosphorous which are the major constituents of bone material onto the surface. These cells become embedded into the new bone and can no longer take part in the differentiation process, however, more cells grow on the remaining layers of cells and continue the bone regeneration process.

In all samples shown in Fig. 5.8, after 11 days cells began to spread and cover the surface of the ceramic material which is evidence that the dopants did not have any negative or toxic effects on the biocompatibility of TCP. In Fig. 5.8.2 (b), some directional growth and bonding of the cells were observed which was an indication that these cells were joining together to form one structure such as cells joining together to form tissue combining to form organs, and the differentiation process was about to begin. The influence of MgO produced positive effects on the bioactivity of TCP. This same effect was also seen for 5wt% SiO$_2$ in Fig. 5.8.5(b) and for TCP-1wt% SiO$_2$-ZnO in figure 5.8.6(b) and also for the influence of MgO-1wt% SiO$_2$ and ternary shown in Fig. 5.8.8(b) and 5.8.9(b) respectively. In the samples containing ZnO, after 5 days there was development of bud-like apatite on the surface; however, very little cell growth was observed at that time point as shown in Fig. 5.8.3(a), (c). By day 11 there was excellent proliferation of cells and interestingly, they seemed to adhere to the now petal like apatite structures beneath. It has been found that the apatite formation provides a strong cell-material interfacial bond, thus making the use of zinc attractive for further research.
Figure 5.8.1  SEM micrographs illustrating OPC1 cell adhesion and density on the surface of pure TCP ceramic compacts after (a) 5 days at 500X magnification (b) 11 days 500X magnification (c) 5 days 1.5K magnification (d) 11 days 1.5K magnification
Figure 5.8.2  SEM micrographs illustrating OPC1 cell adhesion and density on the surface of TCP doped with 1wt% MgO ceramic compacts after (a) 5 days at 500X magnification (b) 11 days 500X magnification (c) 5 days 1.5K magnification (d) 11 days 1.5K magnification
Figure 5.8.3  SEM micrographs illustrating OPC1 cell adhesion and density on the surface of TCP doped with 0.25 wt% ZnO ceramic compacts after (a) 5 days at 500X magnification (b) 11 days 500X magnification (c) 5 days 1.5K magnification (d) 11 days 1.5K magnification
Figure 5.8.4 SEM micrographs illustrating OPC1 cell adhesion and density on the surface of TCP doped with 1 wt% SiO$_2$ ceramic compacts after (a) 5 days at 500X magnification (b) 11 days 500X magnification (c) 5 days 1.5K magnification (d) 11 days 1.5K magnification
Figure 5.8.5  SEM micrographs illustrating OPC1 cell adhesion and density on the surface of TCP doped with 5wt% SiO₂ ceramic compacts after (a) 5 days at 500X magnification (b) 11 days 500X magnification (c) 5 days 1.5K magnification (d) 11 days 1.5K magnification
Figure 5.8.6  SEM micrographs illustrating OPC1 cell adhesion and density on the surface of TCP doped with 1wt% SiO$_2$+0.25 wt% ZnO ceramic compacts after (a) 5 days at 500X magnification (b) 11 days 500X magnification (c) 5 days 1.5K magnification (d) 11 days 1.5K magnification
Figure 5.8.7  SEM micrographs illustrating OPC1 cell adhesion and density on the surface of TCP doped with 1wt% MgO+0.25 wt% ZnO ceramic compacts after (a) 5 days at 500X magnification (b) 11 days 500X magnification (c) 5 days 1.5K magnification (d) 11 days 1.5K magnification
Figure 5.8.8 SEM micrographs illustrating OPC1 cell adhesion and density on the surface of TCP doped with 1wt% MgO+1 wt% SiO$_2$ ceramic compacts after (a) 5 days at 500X magnification (b) 11 days 500X magnification (c) 5 days 1.5K magnification (d) 11 days 1.5K magnification
Figure 5.8.9 SEM micrographs illustrating OPC1 cell adhesion and density on the surface of TCP doped with Ternary ceramic compacts after (a) 5 days at 500X magnification (b) 11 days 500X magnification (c) 5 days 1.5K magnification (d) 11 days 1.5K magnification
CHAPTER 6
DISCUSSION

6.1 Introduction

The restoration of bone defects by regeneration of natural bone tissue is an important goal in “implantology” and orthopedics. β-TCP is well known as a resorbable material, resorbable meaning the materials’ ability to degrade in the physiological environment and promote active bone regeneration. The primary goal for using TCP as a bone graft substitute (BGS) is that the mechanical strength should degrade at a similar rate to that at which the bone regenerates. Understanding the influence of silicon, zinc, and magnesium on the mechanical and biological properties of TCP was the main objective of this research. One key goal was to evaluate if TCP degradation can be controlled using dopants and compositions can be controlled using dopants and compositions can be tailored for controlled strength loss ceramics, without compromising its biocompatibility. All procedures that were followed in this project were the same for addition of all dopants.

6.2 Influence of Magnesium on Tricalcium Phosphate

It has been found that addition of magnesia into TCP promotes stability of the crystal structure (Ando, 1958); by replacing the larger calcium atoms and creating shorter bond length between Mg-O atoms, therefore, strengthening the interatomic attractions (Claderin et al., 2002). By this increased stability induced by the substitution of Mg into TCP, it has been found to in turn decrease the degradation rate (L. Hench, 1993).
To determine the effects that the addition of magnesia has on TCP, densification analysis was used. It has been found that by improving densification it is possible to improve other mechanical properties. MgO was added into CaP as seen in Fig. 6.2.1, MgO by itself had no significant effect on density which may be due to the small amount of actual magnesium at 0.58wt% being substituted into the crystal structure of TCP. Also, the substitution may only include 1 or 2 atoms and not all 3 calcium atoms, therefore, not having the full effect of increased crystal stability. However, when MgO was combined with SiO$_2$ and ZnO as binary components i.e., TCP-MgO-SiO$_2$ and TCP-MgO-ZnO into TCP, there was a noticeable increase in density which may be due to the additional substitution of the smaller silicon and zinc atoms separately into the TCP crystal structure causing an increase in stability and increasing densification as shown in Fig. 6.2.1. A maximum sintered density of 83% of the theoretical density of TCP (3.07 g/cc) was achieved when MgO, SiO$_2$ and ZnO were all added simultaneously into TCP.

Subsequently all MgO doped ceramic compacts were subjected to a mineralization study in SBF for up to 12 weeks. This procedure was used to identify the influence of magnesium on degradation and mineralization of TCP. SBF solution maintained the same pH by removing old SBF and pipetting new solution into the glass vials twice per week. After the study concluded, samples were dried in a muffle furnace at 200°C for 72 hours. The weight change recorded was used to determine if any apatite formation or degradation had occurred; it was consistent with density of the MgO compositions. Since the TCP-MgO composition was less dense than the other compositions containing magnesia, there was more visible porosity. Porosity permitted apatite formation not only on the surface of the sample but also on the inside of some samples which resulted in an increase in weight. For the binary and ternary compositions, there was a
higher initial density than the single component system and a small decrease in weight was observed suggested more degradation than apatite formation as shown in Fig. 6.2.2. The formation of the apatite layer is very important for bone growth because osteoblast cells have been found to proliferate more when in contact with calcium phosphate apatite layer than fibroblast cells. TCP, when doped with MgO, seemed to promote extensive apatite formation, as shown in 5.5.5, which is a symbol of good bioactivity. However, when combined with other dopants (SiO$_2$ and ZnO), apatite formation decreased rapidly, as shown in Fig. 5.5.6, 5.5.8 and 5.5.9.

Cylindrical compacts of TCP with MgO in various combinations were tested under compressive loading to evaluate their mechanical strength. Results are shown in Fig. 6.2.3. There was no significant increase in initial strength for these compositions except for TCP-MgO-ZnO composition [over 150% increase in initial strength compared to that of pure TCP in the control compacts which was the highest strength recorded in this work]. This composition also illustrated very high strength loss from 0 to 12 weeks, were degradation began from the second week. The pure TCP composition began to degrade after 8 weeks in SBF, whereas, the TCP-MgO composition did not degrade over the entire 12 weeks test; however, these samples had a lower strength that the pure TCP compacts. Also, the TCP-ternary composition showed some strength increase over the duration of the test. It is evident from the collected data that it is possible to tailor compositions for controlled strength loss ceramics.

After compacts were tested for compression strength, fractured samples were collected and mounted for microstructural analysis. In the figures from chapter 5.5 of TCP containing magnesia, there was evidence consistent with the data retrieved from weight change analysis. The TCP-MgO composition showed the only relative weight increase of all the magnesia
compositions due to apatite growth. The other compositions containing magnesia did not show any weight gain and no apatite was seen.

The final method of characterization for the magnesia doped TCP ceramics was based on cell culture analysis. This was done to verify that the addition of dopants to TCP did not compromise its biocompatibility by inducing cytotoxicity. In Fig. 5.8.2, it can be seen that the addition of MgO 1wt% does not influence toxicity to cells; therefore, the TCP ceramics remained bioactive with some improved mechanical properties. In fact, all MgO doped ceramics showed good cell adhesion and biocompatibility test as shown in Figs. 5.8.7, 5.8.8, and 5.8.9.

Figure 6.2.1 Normalized density of TCP doped with MgO in various combinations
Figure 6.2.2 Percent weight change of TCP doped with various combinations of magnesium oxide after compacts have been removed from SBF solution

Figure 6.2.3 Compression strength of TCP doped with various combinations of magnesia after simulated body fluid experiments
6.3 Influence of Silicon Dioxide on Tricalcium Phosphate

To determine the effect of SiO$_2$ on TCP, densification characterization was the initial method used. Based on previous analysis not shown in this thesis of SiO$_2$ doped into commercial TCP, 5wt% SiO$_2$ was chosen for this work because of the interesting data collected. The addition of SiO$_2$ as an individual dopant of TCP resulted in an increase in density. Also, when combined with zinc oxide and magnesia separately as binary composition, TCP-SiO$_2$-MgO and TCP-SiO$_2$-ZnO the density increased to greater than 80% and in the ternary system containing SiO$_2$ the highest density at 83% of the theoretical density of TCP was recorded as shown in Fig. 6.3.1. The exact amount of Si$^{4+}$ that was added into TCP was 0.48wt% and Si was the smallest dopant atom with atomic radius of Ca at 197 pm. This may have had a significant effect on densification.

After samples were removed from SBF solution, they were washed and dried and weight change was recorded. There was a noticeable gradual increase in weight for TCP 1wt% SiO$_2$ which can be attributed to the growth of the flake-like apatite layer as shown in Fig. 5.5.2. In Fig. 6.3.2 TCP-MgO-SiO$_2$ and TCP-ternary were the only compositions that illustrated no considerable weight increase and were consistent with apatite formation. TCP-SiO$_2$-ZnO and TCP-SiO$_2$ 5wt% illustrated an increase in weight even through there was no evidence of surface apatite layer. Most of the apatite growth was inside the samples as can be seen from the fracture surface micrographs. It may have been possible that due to local high concentration of Ca ion inside the sample and the enclosed space, that the apatite has shown a preferential growth. The samples that did not show an apatite layer illustrated signs of surface dissolution and degradation as shown in the figures containing SiO$_2$ in chapter 5.5.
There was a lot of fluctuation in mechanical strength from the different compositions containing SiO$_2$ as shown in figure 6.3.3. 1wt% SiO$_2$, though there was an initial weight loss after four weeks increasing trend in compressive strength may be due to the apatite growth into the pores of the sample causing the strength increase. This composition may be suitable for longer-term applications (beyond 12 weeks). The other significant SiO$_2$ composition was 5wt%. There was an almost uniform loss of strength over the 8 weeks, indicating that this composition can be used for short term applications (six to eight weeks) based on the improved initial compressive strength and that degradation began after the first 4 weeks. The TCP-SiO$_2$-ZnO compacts did not show any signs of degradation after 12 weeks which we can consider for long term applications. All samples containing SiO$_2$ did not influence any toxic reaction to osteoblast (OPCI) cells as shown in the figures in chapter 5.8. However, it was noticed that even though there was apatite growth on some of these silica sample in SBF there was none present along with the cells that were cultured in a similar solution to SBF. Finally, SiO$_2$ retained the bioactivity of TCP while improving other mechanical properties.
Figure 6.3.1 Normalized density of TCP doped with SiO$_2$ in various combinations

Figure 6.3.2 Percent weight change of TCP doped with various combinations of silica after compacts have been removed from SBF solution
Figure 6.3.3 Compression strength of TCP doped with various combinations of silica after simulated body fluid experiments

6.4 Influence of Zinc Oxide on Tricalcium Phosphate

The addition of zinc oxide into TCP improved densification behavior. The zinc oxide addition by itself illustrated contrasting results, the density of the disk compact which was pressed at a higher pressure than the compression compacts i.e. around 250MPa showed a decrease in density whereas the compression compact produced an increase in densification; the exact mechanism for this is unknown. However, despite the variations in the unary compositions, the binary, TCP-ZnO-SiO$_2$ and TCP-ZnO-MgO and ternary compositions demonstrated an increasing trend in density with the ternary composition being the most dense as shown in Fig. 6.4.1. Even though the disk and compression compacts were pressed at different pressures, 250MPa and 150MPa respectively, and there was significant difference in density of the pure TCP compacts, the binary and ternary addition of dopants seemed to promote good densification and the difference between the two compacts became negligible. Therefore, the addition of dopants into TCP promotes improved densification and other mechanical properties.
From the weight change analysis is shown in Fig. 6.4.2. It was noticed that the TCP-ZnO and TCP-ZnO-SiO$_2$ were the only two compositions that produced a weight increase. The other two compositions TCP-ZnO-MgO and TCP-ternary did in fact decrease in weight. This data is consistent with apatite formation on the surface and inside the ceramic compacts. TCP-ZnO composition illustrated the largest weight increase of all the dopants used, however, there was no apatite growth on the surface of these compacts and from the fracture surface analysis it was seen that all the apatite was grown on the inside the sample. This is an indication that this dopant added to TCP promotes preferential apatite growth. From SEM analysis the TCP-ZnO-SiO$_2$ was the only zinc-containing composition that produced some apatite growth on the surface even though it was in such small quantities as shown in Fig. 5.5.7. Other than apatite formation there was very little surface degradation of the ZnO compositions and this is consistent with prior research which used the addition of zinc to inhibit resorption characteristics of TCP (Kishi et al., 1998).

From Fig 6.4.3, it was observed that the TCP-ZnO and TCP-ZnO-MgO were the only ZnO compositions to illustrate some degradation over the 12 week SBF treatment. TCP-ZnO followed the same basic strength degradation pattern of pure TCP, which began degradation after 4 weeks in SBF. The other compositions did not show any considerable strength loss over the 12 week test.

The cell culture analysis for the ZnO compositions was successful. There were no signs of cytotoxicity on any of the compacts. In fact, there was very interesting growth of petal-like apatite on the surface of the TCP-ZnO compacts; cells then attached to this layer and proliferated over the entire sample. The growth of an apatite layer has been known to promote osteoblast cell attachment rather than fibroblast encapsulation of foreign material. This was observed by the
flattened shape of cells, indication that they liked the apatite surface layer as shown in Fig. 5.7.2(b). The cells also began forming multi-layers which is essential for the differentiation process.

Figure 6.4.1 Normalized density of TCP doped with ZnO in various combinations
Figure 6.4.2 Percent weight change of TCP doped with various combinations of zinc oxide after compacts have been removed from SBF solution

![Figure 6.4.2](image)

Figure 6.4.3 Compression strength of TCP doped with various combinations of zinc oxide after simulated body fluid experiments

![Figure 6.4.3](image)
Bulk $\beta$-tricalcium phosphate ceramic compacts were made with the addition of dopants in various combinations. These samples were then tested for physical, mechanical, and biological properties. This research is intended to modify the resorbable properties of TCP by the addition of dopants. Some conclusions that were drawn from this work are as follows:

- The compacts doped with MgO, SiO$_2$ 1wt% and SiO$_2$ 5wt% separately did not indicate any significant increase in densification after being sintered at 1250°C for 2 hours.
- The TCP-ZnO disk compacts decreased in densification by 4%.
- All binary and ternary compositions showed above a 7% increase in densification compared to pure TCP.
- pH analysis demonstrated an increase in pH over the entire duration of the SBF treatment.
- Weight change measurements after SBF treatment indicated that all single component systems and TCP-SiO$_2$-ZnO produced an increase in weight which was an indication of apatite growth, this was supported by SEM data.
- All binary and ternary compositions decreased in weight after SBF treatment, was an indication that there was little or no apatite formed and evidence of some dissolution also supported by SEM.
- Signs of dissolution were evident on the pure TCP compact from SEM analysis.
• TCP-SiO$_2$ 1wt% and TCP-MgO 1wt% illustrated considerable amounts of apatite growth on the surface of the sample. All other compositions did not show any surface apatite growth; however, for some compositions there was growth on the inside of the samples.

• All compositions did show evidence of some surface dissolution after 12 weeks in SBF.

• There was uniform compressive strength increase for the TCP-SiO$_2$ 1wt% compacts indicating that the apatite growth on the inside of the compacts directly produced a strength increase.

• TCP-SiO$_2$ 5wt% and TCP-MgO-ZnO both illustrated a significant increase in initial compressive strength and gradually decreased over the period of the SBF treatment. It was also found that degradation began after 2 weeks for these compositions.

• TCP was found to degrade after 8 weeks in SBF solution.

• The addition of SiO$_2$ 1 wt% was found to increase the compressive strength of TCP upto 12 weeks in SBF.

• TCP-MgO, TCP-SiO$_2$-ZnO, TCP-Ternary all showed no significant signs of degradation over the 12 week biological test.

• SEM images were taken of the fracture surface for all compositions. All single components and TCP-SiO$_2$-ZnO grew apatite on the inside of the compacts, whereas, there was no apatite on any of the other compositions.

• The addition of all dopants proved not to compromise the biocompatibility and bioactivity of TCP. There was no evidence of cytotoxicity from cell culture analysis.
REFERENCES


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Fig 2.1.1 Schematic of the Human Skeletal System
• Pure TCP characteristic peaks have been identified in (A) from XRD analysis
• These peaks are present in all dopant compositions
• There is a slight shift in some peaks
• There are slightly visible new peaks forming
• These shifts and new peaks is insufficient data to indicate that there are new phases being formed in beta TCP