Development of novel magnesium phosphate bone cement

Niloufar Rostami

University of Toledo
A Thesis

entitled

Development of Novel Magnesium Phosphate Bone Cement

by

Niloufar Rostami

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Master of Science Degree in Bioengineering

________________________________________
Dr. Sarit B. Bhaduri, Committee Chair

________________________________________
Dr. Vijay K. Goel, Committee Member

________________________________________
Dr. Arunan Nadarajah, Committee Member

________________________________________
Dr. Mehdi Pourazady, Committee Member

________________________________________
Dr. Patricia R. Komuniecki, Dean
College of Graduate Studies

The University of Toledo

December 2014
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An Abstract of

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This study aims to develop a novel magnesium phosphate based injectable cement for orthopedic applications. Magnesium phosphate cements (MPC) provide more favorable clinical performances including shorter setting time, and higher resorption rates and strength, as compared to the commonly used calcium phosphate cements. These features make magnesium cements great potential candidates for orthopedic and dental applications. In this study, the magnesium phosphate cement is prepared using a microwave assisted precipitation process. An experimental study is conducted to evaluate developed phases, setting times, injectability, and mechanical strength of the prepared cement. X-ray diffraction and SEM techniques are used to characterize the structure of powders with or without physiological conditions. Physiological conditions are simulated by soaking MPC pellets in Simulated Body Fluid (SBF). To determine the cytotoxicity effects of the as-synthesized magnesium phosphate cements, the mammalian preosteoblast cells are cultured on the MPC samples and the biocompatibility of the materials is confirmed.
Dedicated to my Family
Acknowledgements

I would like to thank my advisor Dr Sarit B. Bhaduri for his support, caring, patience, and providing me with an excellent atmosphere through my master’s thesis research. Moreover, I would also like to thank Dr. Huan Zhou who patiently guided me through the research process. In addition thank to Dr Anand Agarwal for his support and his assistance. I would like to thank Tammy Phares and Dr. Joseph Lawrence in the bioengineering department and in the center for materials and sensor characterization, respectively, for their assistance. Lastly, I would also like to thank Dr. Vijay K. Goel, Dr. Mehdi Pourazady, and Dr. Arunan Nadarajah who were willing to participate in my defense committees.

I am sincerely grateful to my lab mates Maryam Nabiyouni, Elham Babaie, and Sameh Saleh for sharing their truthful and illuminating views on a number of issues related to the thesis.

I would like to thank my husband, Vahid Mortazavian for his aspiring guidance through my master’s thesis. I am thankful for his patience, support and love.

Most importantly, I would like to thank my family for their love and patience. None of this would have been possible without their support.
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SEM and EDS microanalysis for magnesium phosphate cement soaked in SBF. EDS spectra obtained at the point in the sample. The predominant components were found to be oxygen (O), calcium (Ca), and phosphate (P).  

SEM micrograph of MPC with cell growth after 7 days, for two different areas of sample, 2000x magnification.  

SEM micrograph of MPC with cell growth after 7 days, for two different areas of sample, 1200x magnification (above), 1100x magnification (below).  

SEM micrograph of MPC with cell growth after 7 days, 500x magnification.  

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List of Abbreviations

ATP ......................... Adenosine triphosphate
C ............................. Carbon
Ca ............................ Calcium
CaP .......................... Calcium Phosphate
CPC .......................... Calcium Phosphate Cement
CMPC ....................... Calcium Magnesium Phosphate Cement
CT ............................ Computed Tomography
DI ............................ De-Ionized
DNA .......................... Deoxyribonucleic Acid
HA ............................ Hydroxyapatite
HMDS ........................ Hexamethyl Disilazane

Mg ............................ Magnesium
MEM .......................... Minimum Essential Medium
MgO .......................... Magnesium Oxide
MgP .......................... Magnesium Phosphate
MPC .......................... Magnesium Phosphate Cement

Na ............................ Sodium
O ............................. Oxygen

P ............................. Phosphate
PMMA ........................ Polymethyl methacrylate

SBF .......................... Simulated Body Fluid
SEM .......................... Scanning Electron Microscope

$t_i$ ................................ Initial Setting Time
$t_f$ ................................ Final Setting Time

XRD .......................... X-ray Diffraction
List of Symbols

+ .................................. Positive Charge
- .................................. Negative Charge
° .................................. Degrees

α .............................. Alpha
π............................... The value of Pi 3.14159
σ_{max} ........................... Ultimate Compressive Strength

A................................. Area
C................................. Degrees Celsius
D................................. Diameter
F................................. Degrees Fahrenheit
P_{max} ........................... Maximum Load
x................................. Variable of Interest
Chapter 1

Introduction

There are tremendous demands for quick setting fillers such as phosphate cements which can be applied for bone and dental restorations. Over the last several decades, calcium phosphate cement (CPC) as an inorganic phosphate cement has been the main candidate in clinical applications. Recently, there have been some developments toward magnesium phosphate cement (MPC) compositions in biomedical applications. Although both magnesium and calcium belong to the same alkaline earth group, less attention has been given to MPC compositions compared to the CPC compositions. Magnesium based cements are commonly used in civil engineering applications, due to their rapid setting time and high early strength. However, their clinical applications have been very few.

Mg$^{2+}$ is an important intra and extracellular cation and Mg$^{2+}$ deficiency results in multiple physiological problems in the human body such as kidney disfunction. Different magnesium phosphate products are present in various parts of the human body. For example, whitlockite (β-tricalcium magnesium phosphate), struvite and newberyite can be found in salivary gland stones, dental calculi, and kidney stones, respectively [2].

Magnesium phosphate is formed by reacting Mg$^{2+}$ and PO$_4^{3-}$ ions in an aqueous solution. MPC compositions are obtained under acid-base reactions which are often
between magnesia (MgO) and some compounds containing ammonium and phosphate ions. For instance, struvite (MgNH₄PO₄·6H₂O) is a final product after the chemical reaction is completed. MPC compositions relied on ammonium as a phosphoric source have some drawbacks which have made them undesirable in biomedical applications. The presence of ammonium in magnesium phosphate cement can produce odor which is not pleasant. Releasing and storing ammonium during setting can result in toxicity, as another disadvantage of the aforementioned compositions [3]. As compared to struvite, less attention has been given to newberyite as a final product.

This study aims to develop newberyite as the final product via a novel MPC cement composition. The objective is to enhance the mechanical properties as well as other critical features which are important in orthopedic applications, such as setting time, injectability. To achieve an optimum formulation, the reactivity of MPC should be controlled. This is highly affected by Mg/P ratio and water used in the reaction [4]. To design an injectable cement composition, liquid/powder ratio is a controlling factor which should be considered. This experimental effort modifies these parameters to acquire a cement composition with desired requirements.

The heat generated during the setting reaction is an issue for injectable cements. Exothermicity can cause damages to body tissues through injecting cement. This problem can be controlled via a microwave assistant method. The microwave processing controls exothermicity of the reaction by removing all water molecules from the cement paste and preserve the precursor phase in the composition. Moreover, the compressive strength can increase to some degrees by considering microwave treatment. Therefore, it would be desirable to use microwaving treatment and acquire a higher compressive strength [5, 6].
The novel cement obtained in this study is evaluated with regards to setting times, mechanical properties, microstructure, and cell culturing. Cytocompatibility is examined after one day and seven days by measuring cell’s compability.
Chapter 2

Literature Review

2.1 Introduction

Mammalian bones are susceptible to injury or disease. This can be due to various factors including cysts, tumors, implant replacement, and fractures [7, 8]. Therefore, orthopedic research on bone defect repairing is an important topic and has been rapidly growing during the last decades [8, 9]. Over the last 60 years, orthopedic biomaterials have been produced to improve the interaction of materials with biological environments and to produce desirable biological responses [10].

Biomaterials are designed to be embedded in the human body to achieve biological functions. They are typically fixed to different tissues such as bone, cartilage or ligaments and tendons [10].

Bone substitutes can be either allogeneic (i.e. human derivative), xenogeneic (animal derivative) or synthetic. Transmission of diseases is the main difficulty with the non-synthetic substitutes. Metals, polymers, composites, and cements are typical examples of synthetic materials, commonly used in orthopedic applications [7].

Bone cement is one of the most prominent biomaterials increasingly used in orthopedic application. Attempts have been made over the years to develop and design
bone cements. As a result, a number of criteria have been suggested to optimize the bone cement design, as summarized in the Table 2.1 [11].

The rheology of bone cements and their mechanical properties, hardening or setting time, handling and deliverability, as well as biological properties are of main considerations in repairing bone defects. The rheological aspects include injectability, setting time, and viscosity. Biological characteristics consist of biocompatibility, nontoxicity, osteocundoctivity, and resorbability. Ease of preparation, sterilization, and radio opaque are factors which make handling and delivery of bone cements much easier [8, 9].

Table 2.1: Critical requirements for bone cement.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical Properties</td>
<td>To eliminate the stress shielding and carries load, if necessary</td>
</tr>
<tr>
<td>Setting Time (5-15 min)</td>
<td>To assist in the clinical use and after care of patient</td>
</tr>
<tr>
<td>In situ setting at body temperature</td>
<td>To eliminate necrosis of the adjacent tissue</td>
</tr>
<tr>
<td>Bonding to bone and medical grade alloys</td>
<td>To eliminate of fibrous capsule and loosening of the implant</td>
</tr>
<tr>
<td>Bioactive bone in-growth</td>
<td>To transfer stress and chemical attachment of the implants</td>
</tr>
<tr>
<td>Radio opacity</td>
<td>To identify the cement in vivo</td>
</tr>
</tbody>
</table>

Attention to bone cements substantially increased in 1970s such that many studies have been performed on bone replacement with different bone compositions.

The first bone cement developed in the 1960s was made of Polymethyl methacrylate (PMMA) which is an acrylic bone cement [11]. This kind of cement has been used for more than 50 years in plate luting and total arthoplasties. However, due to non-
resorbability and non-bioactivity between the implant and cement, interfaces between cement and both bone and implant will exist. As a result, wear, thermal necrosis from curing process, cracks and fractures within cement layers are drawbacks of PMMA use, which can even lead to the failure of surgical interventions [12-15].

In 1990, Sarkar published a review paper on phosphate based cement materials which have been studied by various researchers [16]. Phosphate bonded materials have been used for concrete repair applications about a century. These materials are classified as cold setting and heat setting compositions. Cold setting types are typically formed by reacting either of oxide and phosphoric acid, or phosphate and phosphoric acid reactions, or by direct adding of liquid phosphate bond [16].

Calcium phosphate gained a huge attention in clinical applications as the first biodegradable phosphate cement. This kind of cement which has desired osteoconductivity, can fill the gap between implant and bone and increase bone-implant interface strength [13]. However, long setting times, low early mechanical strength, and poor degradability are of disadvantages of the calcium phosphate cements [17, 18].

In the late 1990s, magnesium (Mg) based materials and their alloys received much more attention in biomaterial areas. The numbers of studies on MPC have significantly increased during the last 20 years, as shown in Figure 2-1. Recent studies on MPC confirmed that this novel cement has favorable adhesive and osteoconductive properties [13, 19, 20].
To have a better understanding of this unique cement and its exclusive properties, a literature study on the magnesium and Mg-cement and their biological and orthopedic backgrounds is presented. Also, the role of magnesium phosphate cement in the human body with specific reference to orthopedic applications will be explained.

### 2.2 Biological and Orthopedic Background

Mg$^{2+}$ is the 8th and 4th most plentiful element on earth and in vertebrates respectively. This element is also a very crucial cation within cells [21]. The amount of Mg in the human body is about 1 mole or 24g, which has a vital function in cell proliferation, energy metabolism, and apoptosis. In cell proliferation process, the lack of Mg causes growth arrest and the expression levels of proteins are affected. In energy metabolism, Mg modulates critical enzymes and is a required cofactor for activity of over 300 enzymes. Mg participates in both ATP synthesis and apoptosis [21-23].
More than 90% of Mg is located in bone or intracellular spaces and is useful in nerve conduction, muscle contraction, potassium transport, and calcium channels. Kidney has an important role in Mg balance due to its low turnover in bone [22]. One of the main sources of magnesium ions is that, under physiological conditions, the presence of Mg (OH)$_2$ improves osteoblast activity and reduces osteoclast numbers \textit{in vivo}. In biological calcification, magnesium ions concentration is 0.5% in outer tooth enamel layer and 2% in the innermost dentine. Magnesium ions have an important role on the reminilization of teeth [24].

Figure 2-2 demonstrates the total distribution of magnesium in the human body. As shown in this figure, most of concentration of magnesium is in bone.

![Figure 2-2: Total distribution of magnesium in the human body.](image)

As a medical implant, magnesium has strength and modulus as high as cancellous bone. Orthopedic studies [19, 23, 25] indicate the improvement of the bone growth from magnesium based implants and alloy.
Medical implants are biocompatible, but not biodegradable. Magnesium alloys are biodegradable in aqueous physiological situations, due to their high oxidative corrosion rate. As a result, magnesium based alloys are applicable in cardiovascular and orthopedic medical devices. In early 2000, magnesium based materials were introduced. Researchers showed that the presence of Mg increases bone healing and cell adhesion. The corrosion occurring in Mg based implants is often harmless and excretes through urine [23, 26, 27].

2.3 Magnesium Phosphate Cement

Much of the current knowledge about the synthetic bone cement is based on the calcium phosphate (CaP) compositions, due to early regulatory approval at FDA. Recently, magnesium phosphate (MgP) application as bone substitute is seen to increase and several studies have investigated this category of cements [28].

The application of MPC has traditionally been in refractories and rapid setting cement. They have also been used in roads as a binder in the construction and repair of, bridges, decks, potholes, highways, and airfields [29-32]. Recently, MPCs have entered into a few orthopedic applications, due to its high early strength, desired moldability and biocompatibility, self-hardening, and close interaction with boundaries of the defected surface of the bone, which include the advantages of MPC over CPC [12].

In following, a review of the MgP system is provided to get a better understanding of MPC formulation. Moreover, rheological and biological studies which have been performed over recent years are included. Critical factors for bone cement design also explained.
2.3.1 Magnesium Phosphate System Studies

The most significant MgP compositions have Mg/P ratios of 1 or 1.5. A great majority of MgP cement are hydrated at low temperatures. These compositions with increasing basicity are shown in Table 2.2. Brown et al. [31] provided the phase diagram for magnesium and phosphate in an aqueous solutions shown in Figure 2-3. This figure was obtained by applying a representation scheme, because of different degrees of solubility of acid base compositions. The mole fractions of MgO and P$_2$O$_5$ in aqueous phase is plotted as their $10^{th}$ roots [31].

![Figure 2-3: Ternary system MgO-P2O5-H2O at 25 °C [31].](image)
Table 2.2: Hydrated magnesium phosphate (low temperature).

<table>
<thead>
<tr>
<th>Chemical Formula</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg(H₂PO₄)₂</td>
<td>Monomagnesium Phosphate</td>
</tr>
<tr>
<td>Mg(H₂PO₄)₂·2H₂O</td>
<td>Monomagnesium Phosphate dihydrated</td>
</tr>
<tr>
<td>Mg(H₂PO₄)₂·4H₂O</td>
<td>Monomagnesium Phosphate tetrahydrated</td>
</tr>
<tr>
<td>MgHPO₄</td>
<td>Dimagnesium Phosphate</td>
</tr>
<tr>
<td>MgHPO₄·3H₂O</td>
<td>Dimagnesium Phosphate trihydrate (Newberyite)</td>
</tr>
<tr>
<td>MgHPO₄·7H₂O</td>
<td>Dimagnesium Phosphate heptahydrate (Phosphorosslerite)</td>
</tr>
<tr>
<td>Mg₃(PO₄)₂·4H₂O</td>
<td>Trimagnesium Phosphate tetrahydrate</td>
</tr>
<tr>
<td>Mg₃(PO₄)₂·8H₂O</td>
<td>Trimagnesium Phosphate octahydrate (Bobierrite)</td>
</tr>
<tr>
<td>Mg₃(PO₄)₂·22H₂O</td>
<td>Trimagnesium Phosphate docsehydrate (Cattite)</td>
</tr>
</tbody>
</table>

MgP formation is exothermic and results from an acid-base reaction [33, 34]. Magnesium phosphate compositions can take cations such as NH₄⁺, Na⁺ and K⁺ to result in new materials with potentials in orthopedic applications. A MgP system containing NH₄⁺ can produce compounds: such as MgNH₄PO₄₂H₂O (dittmarite), MgNH₄PO₄·4H₂O (schertelite), and MgNH₄PO₄·6H₂O (struvite) [30, 31].

In 1942, the first magnesium phosphate cement for repairing concrete was developed. The composition of this cement consisted of MgO and ammonium phosphate [35]. Most of the magnesium phosphate cements which were used in refractory and concrete repairing included either ammonium dihydrogen phosphate or diammonium hydrogen phosphate. Magnesia (MgO) and phosphate were the compositions considered for road repairs applications [16]. Popovics et al. and Abdelrazig et al. presented the first commercial MPC, based on the ammonium phosphate [33, 36, 37].

Weill et al. and Seehra et al. studied MPC composition based on magnesium oxide (MgO), and Monoammonium phosphate (NH₄H₂PO₄) which are soluble in water [38, 39].
Results from Weill et al. study showed that on the surface of magnesium oxide, basic reactions occur between MgO and NH₄H₂PO₄. To produce Mg (OH)₂ and Mg²⁺, magnesium oxide particles are attached to the surface of hydrogen ions and dissolved in phosphate to produce MgHPO₄. Ammonium ions convert MgHPO₄ to MgNH₄PO₄, by water absorption process [38]. Since setting reactions are rapid, controlling the setting times and ambient temperature the retarders are typically used [16, 38].

The retarder’s role is to make the product insoluble by coating the Mg(OH)₂ and Mg²⁺ surface and limit the MgHPO₄ dissolution and therefore, the reaction rate becomes controllable [38]. Borax and sodium tripoly phosphate compositions are used as retarders to reduce the maximum temperature and the reaction rate. A composition without retarder typically sets in about 3-4 minutes. Retarder’s role is to decrease the rate of consumption of phosphate bonds [16, 38].

Studies on ammonium magnesium phosphate cement show the presence of excessive MgO in all reactions. High strength is exhibited due to presence of products surrounding the center of magnesia particles [16, 29, 33, 36].

Soudee et al. investigated the setting mechanism of magnesium phosphate cement on MgO and monoammonium dihyrogen phosphate with same content. MgO dissociated to MgO²⁺ and reacted with six water molecules and resulted in Mg (H₂O)₆²⁺ complexes. These complexes are large enough to avoid adsorption of water molecules to the MgO surface. Crystallization of struvite structure occurs after PO₄³⁻ and NH₄⁺ and Mg(H₂O)₆²⁺ bonded together by hydrogen bonds [1, 34]. Struvite is one of the highly recommended compositions for clinical application, although struvite is not a very stable product. Figure
2.4 illustrates struvite crystal development process. Based on several conducted studies, struvite (MgNH₄PO₄·6H₂O) is found in kidney stones [2, 40].

Figure 2-4: Struvite crystals development [1].
Cements containing NH₄⁺ and struvite as a primary product have also newberyite as a by-product [40]. Newberyite (MgHPO₄·3H₂O) is found in kidney stones as well as struvite (MgNH₄PO₄·6H₂O) [2].

2.3.2 Rheological and Biological Studies

Several studies have been reported that mechanical, hardening and biological features of magnesium phosphate cement are novel and they have a high potential to be bone substitutes.

Some researchers have shown that by incorporating magnesium in calcium phosphate, cement properties can be improved. Presence of magnesium ions in calcium phosphate compositions forms Whitlockite. Whitlockite (β-tricalcium magnesium phosphate) is found in salivary gland stones, as well as in dental calculi [2]. Investigations on this newly developed cement indicated a shorter setting time and a much better mechanical strength. It was concluded that MPC changes the temperature of the environment and can assist the combination to shorten the setting time, by increasing its amount [18]. Adding magnesium ions to dicalcium phosphate dehydrate can control the rate of reaction by involving in active growth positions. These ions prevent the mineralization of stable hydroxyapatite. Therefore, by stabilization of a precursor reaction they intervene in precipitation process [24, 41]. Most of the magnesium phosphate cements have higher strength, as compared with calcium phosphate cements, which has been attributed to formation of needle-like/plate-like in magnesium phosphate cement. As a result of the microstructure, the crack growth is rendered difficult. Another advantage for this novel cement compared to calcium phosphate cement was higher degradability in SBF [18]. Histological and cell culture studies evaluated bone formation, biocompability and
nontoxicity, respectively. However, there are still some issues with calcium magnesium cements (CMPC), which need to be improved. Hirano et al. reported results on CMPC which composites were designed to use in root canal for filing and repairing teeth. They showed that the setting time was about 40 minutes and the early strength was not high enough. The appropriate setting time for clinical application is about 6-9 minutes [42]. Wu et al. found that CMPC has lower degradation rate than magnesium phosphate, but higher degradation rate than CPC, MPC indicated a better degradability compared to CMPC and CPC. MPCs are degradable, because of their high solubility [18].

Brushite is one of the products of calcium phosphate cement which is resorbable in physiological situations, although very short setting time and low mechanical strength are its drawbacks. Klammert et al. developed brushite cement by adding magnesium phosphate to the composition. The general formula of this cement was Mg x Ca (3-x) (PO₄)₂ with 0 < x < 3. The setting time increased from 2 minutes to 8-11 minutes and compressive strength was improved from 10 MPa to about 40 MPa. Biocompability of the cement was examined with cell line MC3T3-E1 and it was observed that cell proliferation increased. In brushite cements, magnesium ions act as setting retarders and change phase transformation of brushite to hydroxyapatite in vivo. Magnesium ions also form newberyite as the second final product [43].

Liu et al. developed an inorganic bone adhesive agent containing magnesium phosphate, ammonium and apatite like materials. Results indicated that the bone adhesive agent is develops with high early strength with rapid setting time which are favorable in clinical applications. The viscosity was also optimal, which is important in bone fixation. Great biocompatibility was also observed in the final product. It was more degradable and
absorbable compared to PMMA and these features significantly improve both operation and clinical issues [42].

Studies with the aim to compare CPC with MPC indicate that MgP based cements are favorable in equine applications. Waselau et al. compared magnesium phosphate cement with calcium phosphate cement and non-cement situation in eight normal adult horses. Triangular fragments were created in metatarsal bones and replaced with cements. After seven weeks, for all horses, radiographical and CTs results indicated that magnesium protected the Y-shaped area closer to parent bone cement compared to calcium cement or no cement, and the process of bone healing was more significant in magnesium phosphate cement. In MPC situation, a mature woven bone was detected in early stages of bone healing close to or within the cement, and no adverse reaction was observed. The handling property of both cements was good and the hardening time was less than 10 minutes. Magnesium cement brought an immediate attachment of the fragment to the parent bone. Magnesium had a positive effect on periosteal and endosteal bone formation and degradation of osseous callus [19].

Moseke et al. results on magnesium phosphate cement which included trimagnesium phosphate (Mg₃ (PO₄)₂) and highly concentrated ammonium phosphate solution ((NH₄)₂HPO₄) showed a high compressive strength after 24 hours setting and a short setting time [44]. Mestres and Ginebra developed a unique magnesium phosphate cement consisted of an oxide, MgO, and an acidic element with either sodium dihydrogen phosphate, ammonium dihydrogen phosphate, or an equal molar mixture of both [45]. Sodium borate was used as a retarder. Compared to CPC, MPC had a higher early strength. Two major final products were obtained including struvite (MgNH₄PO₄·6H₂O) and
amorphous cement (Na-MPC). Adding sodium borate decreased the maximum temperature and improved the setting time. Excessive MgO in the final product improved the compressive strength and provided antibacterial properties for the cement [45].

Antibacterial and DNA studies of magnesium phosphates demonstrated that MgP cement has great cytocompatibility and antibacterial activity. Mestres et al. examined three kinds of bacteria in the presence of Na- MgP, NH4- MgP and NH4 + Na- MgP cements. They found the antibacterial activity in Na- MgP cement and the bacteriostatic effect in NH4- MgP and NH4 + Na- MgP cements. This was attributed to interactions between of pH and osmolality [46]. Huang et al.’s report on calcium magnesium silicate bioceramics showed that this composition was osteogenic, and bone formation in vivo testing was successful [6]. Magnesium phosphate material results in gene delivery, and in vitro DNA transfection in HeLa cells testing showed that they have high potential in tissue engineering applications [6].

Ewald et al. observed the activity of cells on the surface of three kinds of cements (Hydroxyapatite, brushite and struvite) by using osteoblastic cells (MG 63). Cells grew on struvite well, and their activity was better than other types of cements. Metabolic rate of osteoblasts and protein expression done based on western blotting showed that cytocompatibility of struvite was better than brushite [47].

Liu et al. investigated toxicology of MPC including gene mutation assay (Ames test), chromosome aberration assay (micronucleus test), and DNA damage assay (unscheduled DNA synthesis test). The results showed mutagenicity and potential carcinogenicity of MPC extracts were negative and the cement displayed no toxicity feature [48].
Tamimi et al. determined low cytotoxicity and biocompatibility of newberyite with osteoblast cells assistance. Tamimi et al. showed newberyite’s ability in improving osteogenic activity. They also displayed precipitation condition of magnesium phosphate at concentration of 10-100 times higher than the physiological condition. Based on gene study, magnesium showed similar bone regenerative ability. Osteoblast activity study on newberyite revealed a strong interconnection between crystals, because of collaboration between cell and material and discharging of extracellular matrix [2].

2.3.3 Essential Factors for Magnesium Phosphate Cement

The properties of magnesium phosphate cement are affected by reactivity of magnesia, M/P ratio, retarder, and the amount of water [49]. Chau et al. results showed that mechanical properties of the magnesium based cement are affected by molar ratio of magnesia to phosphate. Choosing an optimum Mg/P ratio is important to have better crystallization and high early strength [4]. Weil et al. found that a high amount of magnesium oxide can cause an accelerating reaction which result in a temperature as high as boiling water and damaging the product. However if the amount of the magnesium oxide is too low, the mass gets harder before completing the reaction of phosphate [38]. Moreover, Qiao et al. believed that altering magnesium oxide content can change the strength which depends on the reactivity of magnesia particles. Moreover, adding retarder delays the setting time and affects strength by reducing it [50]. Hall et al. found that by increasing retarders young modulus can decrease [51].

Wu et al. and Pina et al. found out that P/L (powder/liquid) ratio is an important factor influencing setting time, mechanical strength, and injectability. If the ratio is high, the wettability is poor, and if the ratio is low, injectability and setting time can improve but
strength may decrease. A high amount of liquid causes the structure of the cement to become porous such that particles stay away from each other and their interaction is delayed and setting time increases [12, 52]. Hall et al. observed that a high amount of water is not bounded in a hardened cement and can vaporize during setting, therefore, leave the final product of a porous structure, resulting a poor strength [51, 53].

Based on the previously conducted studies, magnesium has a higher potential over calcium in clinical applications. Most of studies are based on struvite and other compositions for MgP cement, while less attention has been given to newberyite. Struvite is not a very stable composite, while newberyite is one of the compounds which degrade more slowly than struvite.
Chapter 3

Materials and Methods

3.1 Preparation of MgP Powder

The composition of MgP consists of magnesium hydroxide (95% pure Mg (OH)₂, Acros), setting solution, and deionized water (DI). The setting solution was prepared by mixing sodium bicarbonate (NaHCO₃>99.7%, Fisher Scientific, Fair Lawn, NJ), di-ionized water and phosphoric acid (85% H₃PO₄, EMD Chemicals Inc., Gibbstown, NJ). The setting solution composition includes 6 g sodium bicarbonate, 2 ml DI water, and 13 ml phosphoric acid. Sodium bicarbonate increases the reactivity of the initial powder. Deionized water dilutes the setting solution and reduces the combination viscosity. Phosphoric acid acts as a source of phosphorous.

Magnesium hydroxide was mixed with DI water and the setting solution, by using a mortar and a pestle. The MgP powder was made by dissolving 2.47 g of magnesium hydroxide in 1 ml of DI water. Then 3 ml setting solution was added to the dispersed magnesium hydroxide to occur an exothermic reaction by reaching the temperature up to 77 °C (170 °F). The resulting paste is microwaved (Panasonic, max. power 1200 W, 2,450 MHz, NJ, USA) for two minutes with 80% microwave power to dehydrate the cement and
stop the reaction. The resulting hard cement was crushed with a mortar and a pestle. A coffee grinder was used to create a finer MgP powder.

3.2 Fabrication of Cement Samples

Magnesium phosphate cement (MPC) was prepared by mixing MgP powder with MgO and NaH₂(PO₄) using a mortar and a pestle. To make a single test sample which is 2x of the original amount of compositions, 2 g of MgP was combined with 0.6 g of magnesium oxide (98% extra pure powder, MgO, particle size: 99 %< 150 µm (-100 mesh)), 0.6 g sodium phosphate monobasic anhydrous (NaH₂(PO₄), Fisher Scientific, Fair Lawn, NJ) and 0.1 g of boric acid (H₃BO₃, Powder Certified ACS ≥99.5 %, Fisher Scientific, Fair Lawn, NJ) as a retarder. The resultant mixture was then dispersed in 1.1 ml water and placed into a plastic die with circular mold diameter of 12 mm.

The cement was pressed with hand into dies at room temperature condition. The surface of the samples should be smooth without any crack. Cylindrical shaped samples with diameter of 12 mm and height of 3-4 mm were prepared using the compaction die and used for compression testing.

3.3 Working Time and Setting Time Measurement

Determining the working time is one of the essential measurements for cements in biomedical applications. Working time is a period of time from the start of mixing compositions of cement till the cement gains an optimized viscosity for an acceptable usage. In this study, the working time was considered when MgP and other compositions were mixed with water till the paste got ready.
To measure the setting time, the Gillmore needle apparatus (Humboldt Mfg. Co., Schiller Park, IL) was used. The Gillmore needle is an instrument used in penetration tests for measuring the setting time of materials such as Portland cements, pastes, masonry cement, hydraulic hydrated lime, and certain mortars. The Gillmore needle has two stainless steel cylindrical flat-end needles. Initial ($t_i$) and final ($t_f$) setting times of cement samples are determined according to the international standard ISO 9917-1 [54] for dental cement and ASTM C266-89 [55]. Initial setting time is measured by a weight of 0.25 lb (115.12 g) and the diameter of 1/12" (2.12 mm). Final setting time is measured by a needle with diameter of 3/16" (4.8 mm) and weight of 1 lb (453.6g). After preparing the cement, it was immediately pressed using light hand pressure between two glass slides to form a flat sample. Measurements were taken every 30 seconds until the surface of the cement sample was not indented 1 mm.

3.4 Injectability

Injectability is another aspect which should be considered in biomedical applications of cements. Injectability requires good working and setting times. In this study, injectability was examined by using a proper paste which could be injected by a syringe. The optimized created cement was injected for about two minutes after mixing.

For testing the injectability of the cement pastes, the mixed pastes were extruded through a vertical syringe. It is important to monitor the injectability with respect to the L/P ratio. The L/P ratio essentially denotes the ratio of the volume fraction of the liquid to the solid. Injectability changes with respect to the L/P ratio.
3.5 Compression Strength

For mechanical testing of biomedical application cements compressive strength is often evaluated. Compression tests are performed under displacement control with crosshead speed of 0.5 mm/min, according to the American Dental Association [54] using an Instron mechanical testing machine. The plot of compressive stress versus compressive extension was obtained from Bluehill software. Compressive strength was verified from results of 5 duplicate tests.

Compressive test results were obtained from two different procedures, one after 24 hours of immersion in SBF and another one with samples hardened for 10, 30, 45 minutes at 37 °C atmosphere. The last test method was performed to provide information for the clinical applications of the cements. Compressive strength was calculated based on the following equations:

\[ \sigma_{max} = \frac{P_{max}}{A} \]  
\[ A = \frac{\pi D^2}{4} \]

where \( \sigma_{max} \) is the compressive strength, \( P_{max} \) is the peak load, \( A \), and \( D \) are area and diameter of samples, respectively. To prevent the effect of stress concentration, surfaces and/or edges of samples should be smoothened.

3.6 In vitro Evaluation

Mouse preosteoblast cells (MC3T3-E1) were used for in vitro experiment. Before starting cell culture experiment a proper media should be prepared. 100 ml of a complete \( \alpha \)-MEM was used as media, containing 90 ml of \( \alpha \)-MEM (HyClone MEM Alpha
Modification 1X, Thermo Scientific, Logan, UT), 10 ml of fetal bovine serum (HyClone FBS, Thermo Scientific, Logan, UT), and 1000 μl of antibiotic (HyClone Antibiotic, Thermo Scientific, Logan, UT).

MC3T3-E1 cells were grown on 75 cm² culture flasks (BioLite 75 cm² Flask, Thermo Fisher Scientific, Rochester, NY) at 37 °C, 100% relative humidity and 5% CO₂ in complete α-MEM. The complete culture media was changed every two days and cell lines were checked for any contamination. The cells were then detached from the surface of the flask using trypsin (2.5 g/L, EDTA 25 mM solution, Sigma-Aldrich Corp., St. Louis, MO) and the samples were seeded to cement samples in well plates (Tissue Culture Plate, 24 Well, FALCON®, Becton Dickinson Labware, Franklin Lakes, NJ).

Cell culture was conducted with different concentrations of MPC powder and for MPC pellet samples which were soaked for 7 days in SBF. Powders and pellets were autoclaved before cell culture to sterilize from any initial bacterial contaminants. Samples were seeded with 20,000 MC3T3-E1 cells at 37 °C, 100% relative humidity, and 5% CO₂ in complete α-MEM. Hydroxyapatite (HA, Ca₁₀(PO₄)₆(OH)₂) powder was used as control. For comparison, HA powders were prepared similar to the method used for MPC powder.

A chemical drying procedure was conducted in order to perform scanning electron microscope (SEM) investigations on cement samples. Cells underwent a fixation procedure with 4.5% glutaraldehyde in a cacodylate buffer (pH = 7.4). Osteoblasts were dehydrated through sequential washings in 30%, 50%, 70%, 90%, 95%, and 100% ethanol solutions. 2 parts 100% ethyl alcohol and 1 part HMDS (hexamethyl disilazane) was added for 15 minutes. After that 1 part 100% ethyl alcohol and 2 parts HDMS was added for 15 minutes.
Then 2 changes for 15 minutes each with 100% HDMS. Finally the HMDS was removed and allowed the specimen to air-dry in a hood overnight.

Osteoblast morphology was examined on cement samples after 7 days by using a scanning electron microscope (SEM). Additionally, cell numbers on samples were counted after 24 hours and 7 days using CytoTox 96® Non-Radioactive Cytotoxicity Assay kit (Promega).

3.7 Acellular Bioactivity by Soaking in SBF

A body fluid formulation was prepared to mimic the phase transition of MPC in body fluid. The composition of SBF is shown in Table 3.3 [41]. MPC samples were incubated in 50 ml SBF at 37 °C environment for 7 days. The phase of MPC after SBF incubation was characterized using SEM.

![Figure 3-5: MPC samples soaked in SBF for 7 days.](image-url)
Table 3.3: Composition of 1 L test SBF.

<table>
<thead>
<tr>
<th>Order</th>
<th>Reagent</th>
<th>SBF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NaCl</td>
<td>6.5456 g</td>
</tr>
<tr>
<td>2</td>
<td>NaHCO₃</td>
<td>2.2682 g</td>
</tr>
<tr>
<td>3</td>
<td>KCl</td>
<td>0.3727 g</td>
</tr>
<tr>
<td>4</td>
<td>Na₂HPO₄</td>
<td>0.1419 g</td>
</tr>
<tr>
<td>5</td>
<td>MgCl₂·6H₂O</td>
<td>0.3045 g</td>
</tr>
<tr>
<td>6</td>
<td>1 M HCl</td>
<td>10 mL</td>
</tr>
<tr>
<td>7</td>
<td>CaCl₂·2H₂O</td>
<td>0.3881 g</td>
</tr>
<tr>
<td>8</td>
<td>Na₂SO₄</td>
<td>0.072 g</td>
</tr>
<tr>
<td>9</td>
<td>Tris-Base</td>
<td>6.063 g</td>
</tr>
<tr>
<td>10</td>
<td>1 M HCl</td>
<td>33.3 mL</td>
</tr>
</tbody>
</table>

3.8 Characterization of Powders

3.8.1 X-Ray Diffraction Analysis

The crystallographic structures of the magnesium based cement were examined using X-Ray Diffraction (XRD, Rigaku Ultima III) at a speed of one degree per minute in continuous scan mode at 40 kV and 44 mA. The XRD data was collected for a 2θ range between 10° and 60° in order to identify the proper magnesium phosphate cement peaks.

3.8.2 Phase Composition and Morphology of Cement

The surface morphology of magnesium phosphate powder samples was visualized using a Scanning Electron Microscope (SEM, Hitachi S-4800, Hitachi Corp, Tokyo). The samples were crushed into powders and then attached on a carbon tape. An accelerating voltage of 5 kV, a magnification of 1000X, and a distance of 13 mm was used.
In this part of the experiment, samples were soaked in SBF and allowed to set completely for 24 hours in an incubation environment at 37 °C, before being attached on carbon tape. Samples were coated with gold and visualized with accelerating voltage of 5-10 kV, with magnification of 400X and 1000X, and a working distance of about 13 mm.

3.9 Statistical Analysis

Statistical analysis of variance (ANOVA) was performed to evaluate significant differences between mean values. Standard deviation was also evaluated for each a set of samples. In all cases P-value was calculated and results with a P-value of less than 0.05 were statistically considered significant.
Chapter 4

Results

4.1 Phase Composition of Cements Powder

MPC pellet samples were cured at 37 °C in 100% humidity and dried and crushed to fine powders for XRD analysis. The XRD pattern of MPC powder shows the characteristic peaks of magnesium phosphate compounds, as seen in Figure 4-6. XRD was also conducted on samples soaked in alcohol and dried in oven no difference in its pattern was observed, compared with magnesium phosphate cement. Some unreacted MgO was observed in MPC pattern, as can be seen in Figure 4-6. XRD analysis of magnesium phosphate cement indicates presence of newberyite (JCPDS PDF#97-001-5330).
Figure 4-6: XRD scan of MPC powder after 24 hours.
4.2 Cement Morphology

Morphology results for MPC powder and MPC soaked in SBF are shown in Figure 4-7 and 4-8, respectively. Cement powders show clusters of spherical structures. There are crystallographic and structural changes compared to magnesium phosphate cement without soaking in SBF. MPC soaked in SBF indicates an apatite-like feature composed of plate-like amorphous MPC structure. The EDS analysis shows that Ca$^{2+}$ is incorporated in magnesium phosphate cement and main compositions of the cement are magnesium (Mg) and phosphoric (P), as seen in Figure 4-9.

Figure 4-10 and 4-11 show higher levels of phosphorous and magnesium in the structure. Figure 4-12 shows a higher level of calcium.

The in vitro tests indicate pre-osteoblast cells attaching to magnesium phosphate cement after 7 days, as can be seen from Figure 4-13 to 4-15.

Figure 4-7: Scanning characterization of MPC, 10000X magnification.
Figure 4-8: SEM characterization of MPC after 7 days of SBF incubation, 1000X magnification (a, b), 400X magnification (c).
Figure 4-9: SEM-EDS analysis revealed that MPC surface soaked in SBF is composed of Ca, P, Mg, O ions.
Figure 4-10: SEM and EDS microanalysis for magnesium phosphate cement soaked in SBF. EDS spectra obtained at the point in the sample. The predominant components were found to be carbon (C), oxygen (O), sodium (Na), magnesium (Mg), and phosphate (P).
Figure 4-11: SEM and EDS microanalysis for magnesium phosphate cement soaked in SBF. EDS spectra obtained at the point in the sample. The predominant components were found to be carbon (C), oxygen (O), sodium (Na), magnesium (Mg), and phosphate (P).
Figure 4-12: SEM and EDS microanalysis for magnesium phosphate cement soaked in SBF. EDS spectra obtained at the point in the sample. The predominant components were found to be oxygen (O), calcium (Ca), and phosphate (P).
Figure 4-13: SEM micrograph of MPC with cell growth after 7 days, for two different areas of sample, 2000x magnification.
Figure 4-14: SEM micrograph of MPC with cell growth after 7 days, for two different areas of sample, 1200x magnification (above), 1100x magnification (below).
4.3 Setting Time

After mixing MgP with final composition, MPC was set initially in a range of 4-5 minutes and 8-9 minutes for final setting time. For measuring setting time, paste was hard-pressed between two glass slides to form a leveled sample. $t_i$ was measured every 30 seconds till the surface of the cement sample was not indented 1mm. For measuring $t_f$ time was measured till the heavy needle was failed 1 mm indention on the surface of the sample. Initial and final setting time were determined and shown in Table 4.4 for different amounts of retarder.

The liquid to powder ratio (L/P), and amount of retarder are the main factors affecting the setting time measurement. With increasing the L/P ratio, setting time increases and the viscosity of the cement paste decreases. Decreasing this ratio would cause
decreasing setting time and results in non-uniform formation of paste, as seen from Figure 4-16.

Amount of the retarder is important in measuring setting time and strength. Although increasing amount of retarder improves the setting time, the compressive strength can decrease. Decreasing the amount of retarder in mixture may cause reduction in setting time, as seen from Figure 4-17. In this study, adjusting concentration of retarder and L/P ratio allowed to control over the cement setting time. In this study the optimum L/P ratio was found to be 0.33 to get the best setting time and compressive strength.

Figure 4-16: L/P ratio respectively for a, b and c are: 0.21, 0.33, and 0.52.
Table 4.4: Setting times of MPC for boric acid and borax with L/P ratio of 0.33.

<table>
<thead>
<tr>
<th>Mass (g)</th>
<th>Initial Setting Time ($t_i$) (min)</th>
<th>Final Setting Time ($t_f$) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Boric acid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>&lt;30sec</td>
<td>&lt;30sec</td>
</tr>
<tr>
<td>0.05</td>
<td>4.5±0.64</td>
<td>8.5±0.75</td>
</tr>
<tr>
<td>0.085</td>
<td>5.5±0.40</td>
<td>10±0.62</td>
</tr>
<tr>
<td><strong>Borax</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>3±0.47</td>
<td>4.5±0.48</td>
</tr>
</tbody>
</table>

Figure 4-17: Setting time for different amount of boric acid.

4.4 Injectability

The optimized composition was chosen for testing injectability of MPC. Injection test was performed 2 minutes after mixing it. Time was recorded after inserting paste to a syringe. It was found that the paste was workable till 10 minutes. As can be seen in Figure
4-18, after 10 minutes the cement was still injectable and there was consistency after injecting cement with syringe.

![Injectable cement after 6 min (a) and 10 min (b).](image)

Figure 4-18: Injectable after 6 min (a) and 10 min (b).

### 4.5 Compressive Strength

Compressive strength for different amount of retarder is summarized in Table 4.5.

Compression tests showed a typical brittle fracture in the cements. With increasing retarder content, compressive strength decreases, as seen from Table 4.5. The reason for that can be due to the increasing porosity of the cement by adding retarder. A stress-displacement curve is shown in Figure 4-19. After an initial linear elastic compression, compressive failure subsequent to yield point is observed. Strength measurements conducted only on the optimum composition, which was obtained by adding 0.05g of retarder.

Table 4.5: Mechanical strength of MPC for boric acid and borax.

<table>
<thead>
<tr>
<th>Mass (g)</th>
<th>L/P ratio</th>
<th>Compressive Strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Boric acid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td></td>
<td>55.67±2.63</td>
</tr>
<tr>
<td>0.05</td>
<td>0.33</td>
<td>52.48±2.62</td>
</tr>
<tr>
<td>0.085</td>
<td></td>
<td>38.86±4.42</td>
</tr>
<tr>
<td><strong>Borax</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>0.33</td>
<td>25.68±2.9</td>
</tr>
</tbody>
</table>
Figure 4-19: Mechanical strength for different amount of boric acid.

Figure 4-20: Compressive stress behavior on one MPC sample.
4.6 Strength While Setting

Table 4.6 shows the setting strength for different times counted from when the sample is mixed. Result from this mechanical testing show that over a short time after injecting cement, it would gain a strength higher than the human cancellous bone with strength of 2-12 MPa [56].

Table 4.6: Strength while setting for MPC samples.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>26.82</td>
</tr>
<tr>
<td>30</td>
<td>45.37</td>
</tr>
<tr>
<td>45</td>
<td>43.74</td>
</tr>
</tbody>
</table>

Figure 4-21: Load-displacement curve for MPC strength while setting.
4.7 In vitro Evaluation

Cell viability was measured after one day and seven days by considering three duplicate tests. These samples were used for both one day and seven days testing to obtain statistically meaningful results.

Cell culturing results are presented in Tables 4.7 and 4.8 for 1 day and 7 days, respectively. Bar chart representation of results is also shown in Figure 4.22. Day 1 and 7 days results show no statistical cell number variation in all samples (P > 0.05). However cell counting demonstrates that the average cell number increases relative to the MPC concentration in medium, as compared to the HA control. It can also be seen that MPC samples soaked in SBF cell grew well over 7 days cell culturing experiment.

Table 4.7: Unadjusted plate reader results day 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Average</th>
<th>STDEVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>(50mg/ml) MPC Cement</td>
<td>0.306</td>
<td>0.306</td>
<td>0.306</td>
<td>0.487</td>
<td>0.2131</td>
</tr>
<tr>
<td>(100mg/ml) MPC Cement</td>
<td>0.352</td>
<td>0.937</td>
<td>1.407</td>
<td>0.8986</td>
<td>0.5285</td>
</tr>
<tr>
<td>MPC Soaked in SBF</td>
<td>2.163</td>
<td>2.163</td>
<td>2.163</td>
<td>0.8735</td>
<td>0.7848</td>
</tr>
<tr>
<td>Hydroxyapatite (HA)</td>
<td>0.64</td>
<td>0.79</td>
<td>0.625</td>
<td>0.685</td>
<td>0.0912</td>
</tr>
</tbody>
</table>

Table 4.8: Unadjusted plate reader results day 7.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Average</th>
<th>STDEVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>(50mg/ml) MPC Cement</td>
<td>0.63</td>
<td>0.943</td>
<td>0.973</td>
<td>0.8486</td>
<td>0.1899</td>
</tr>
<tr>
<td>(100mg/ml) MPC Cement</td>
<td>2.351</td>
<td>1.997</td>
<td>1.711</td>
<td>2.0196</td>
<td>0.3206</td>
</tr>
<tr>
<td>MPC Soaked in SBF</td>
<td>1.134</td>
<td>1.385</td>
<td>1.777</td>
<td>1.432</td>
<td>0.324</td>
</tr>
<tr>
<td>Hydroxyapatite (HA)</td>
<td>0.962</td>
<td>1.619</td>
<td>1.689</td>
<td>1.4233</td>
<td>0.401</td>
</tr>
</tbody>
</table>
Figure 4-22: Pre-osteoblast cell number for two different concentration of MPC, MPC soaked in SBF and HA (Control).
4.8 Summary and Discussion

Most studies reported in the literature are related to commercially available calcium based cement or PMMA. Formulations of CPC and PMMA have some drawbacks related to their poor mechanical properties and low resorption respectively, which result in limitations to their use in clinical application [57].

The objective of the present study was to provide novel injectable bone cement based on magnesium phosphate with a noticeable mechanical performance. Important factors which are critical for bone cement design were evaluated through conducting an experimental study. Nearly all the studies which have been reported for MPC are based on NH₄-MPC. Although the formulation for MPC has its noticeable features, it presents some problems in clinical applications. The mechanical strength of MPCs has also been shown to be less than 50 MPa.

In this work cement formulation was selected with the aim of obtaining a rapid setting and high early strength of more than 50 MPa. This unique cement consists of two compositions. The first composition is made of Mg (OH)₂ as a basic source and H₃PO₄ as an acidic source, referred to as MgP. The second and final composition is made of a mixture of MgO and NaH₂PO₄ with MgP by adding water. In the second composition, the boric acid was added as a retarder.

Typically a reaction between Mg and phosphate salt occurs at a temperature as high as 100 °C which is not appropriate in medical applications [5]. To solve this problem, microwave treatment was used such that the water content can be quickly and homogenously removed from composition. As a result, the maximum temperature of the reaction reduces which can be beneficial for mechanical properties of the cement. This
method was used only for the first composition, because of crystal nucleation and growth mechanism during MgP preparation which is an exothermic process. This results in no heat generation when mixing materials with MgP powders.

Several factors should be considered to acquire an optimum composition for favored injectable bone cement. Based on conducted studies in literature, an excess of MgO in structure results in higher strength and conversely decreases setting time. Therefore, an ideal ratio for Mg/P is desirable for obtaining a high strength and acceptable setting time. The liquid/powder ratio is another important factor for improving the injectability, which was estimated at 0.33.

A relatively short setting time of this new bone cement can be because of MgO assistance in reaction. However, such rapid reaction can be modified by adding a retarder such as boric acid, sodium borate decahydrate (borax), and citric acid. In this experiment, boric acid was used to control the setting reaction. Although addition of retarder increases the setting time, it may reduce the early strength. Choosing an optimum amount of retarder is another critical factor, which was considered as 0.05g in this study. To obtain cement with a desired setting rate and early high strength, boric acid showed a better result for mechanical performance compared with sodium borate decahydrates.

The acceptable time range for desired injectable bone cement is usually about 6-9 minutes. The novel MPC in this study has a high potential for use in clinical application, because it was injectable after 10 minutes. It was shown that the novel MPC cement can be shaped, injected and set at a 37 °C incubator with 100% humidity.

Compared to other studies [45, 46, 58], this novel magnesium phosphate showed a higher compressive strength attributed to excessive MgO in the cement’s structure.
Another reasoning for a high compressive strength in the novel cement might be formation of newberyite instead of struvite. The MPC sample indicated about 4-8 minutes initial self-setting with a maximum compressive strength of 55 MPa.

In magnesium phosphate reaction mechanism, formation of newberyite occurs with free Mg\(^{2+}\), HPO\(_4\)^{2−}, and H\(_2\)PO\(_4\) ions. Gradual dissolution of Mg (OH)\(_2\) in setting solution results in releasing cations in presence of phosphate anions in acid-base reaction and forming a coordinated network of newberyite. Most studies showed that newberyite is a by-product of struvite decomposition, while the new cement final product is purely based on newberyite.

It was shown that the surface of MPC cement differs after cell culturing and SBF soaking, compared with original MPC. MPC crystal growth was sensitive to the aqueous environment, because of different ionic composition between the cell culturing media and SBF containing Ca\(^{2+}\). Formation of apatite crystals on hardened MPC is obvious in SEM images taken after SBF incubation. However, in other studies based on MPC, this crystal formation was not this much clear. In a study by Klammert et al. [59] on MPC composition based on newberyite, cement implanted into rats resulted in low formation of apatite with whisker like crystals.

Newberyite has been proven to be biocompatible and suitable for clinical applications. Similarly, in our cell culture testing, this novel MPC supports this idea. In vitro study showed that cell proliferation rates grew with increasing the concentration of MPC extract. Moreover, samples soaked in SBF cell grew very well. There is a hypothesis that Mg ions in the MPC structure improve cell growth on MPC samples. It is believed that
this novel magnesium phosphate cement is not cytotoxic and has a high potential for clinical applications as biomaterial cement.

Most studies based on MPC were conducted based on struvite and newberyite as byproduct. This study investigates the high potential magnesium phosphate cement based on newberyite. Although further studies are needed to evaluate the in vivo investigation to prove biological features such as resorption rate at the implantation location and also investigate osteoconductivity to route this bone novel cement in clinical applications. The antibacterial efficiency studies are also needed to evaluate the possibility of using this novel MPC in dental applications. Furthermore, healing process following surgery is very important, such that the cement is radiopaque. Therefore, radiopaque property of the novel MPC can be evaluated and improved to visualize cement flow and placement during an imaging process.
References


