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Development of high strength dicalcium phosphate anhydrous cement with nanosilica sol

Timothy John Luchini

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A Thesis

entitled

Development of High Strength Dicalcium Phosphate Anhydrous Cement with Nanosilica Sol

by

Timothy John Franklin Luchini

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

Master of Science Degree in Mechanical Engineering

Dr. Sarit Bhaduri, Committee Chair

Dr. Ioan Marinescu, Committee Member

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The University of Toledo

December 2012
An Abstract of
Development of High Strength Dicalcium Phosphate Anhydrous Cement with Nanosilica Sol

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Dicalcium Phosphate Anhydrous (DCPA, Monetite) based materials have large potential as bone repair substitutes and drug delivery systems due to high bioactivity and known solubility in body fluid, and they could provide useful scaffolds in tissue engineering and orthopedic applications. Clinicians increasingly need bone scaffolds to replace autogenous tissue in endontic and orthopedic applications as an aging world population is affected by injury and disease. Dicalcium Phosphate Anhydrous is a biologically active material that can act as filler in the absence of bone. However, being a ceramic material, poor mechanical properties and fracture toughness in comparison to bone make DCPA inappropriate for complete load bearing applications. Additionally, the production of DCPA generally utilizes a high temperature exothermic reaction which is difficult to repeat and control as well as having to set before implantation in the human body. A diverse range of materials and fibers have been added to Dicalcium Phosphate Anhydrous Cements to act as reinforcements to increase their mechanical properties. The aim of this study is to produce an improved composite of augmented Dicalcium Phosphate
Anhydrous Cement, through a novel microwave assisted production process, and the addition of nanosilica sol as reinforcement to increase mechanical properties. This is of interest because Silicon is a biocompatible element, which can be formed into biodegradable compounds, shown to enhance the mechanical properties of calcium phosphate cements. This study also investigates the bioactivity of this DCPA based cement infused with silica. The reported properties are designed for use in a clinical setting where a clinician needs 6-12 minutes during a surgical procedure to work with cement after mixing.
Dedicated to Dr. Kenneth Wyckoff, Dr. John Luchini, Dr. Katy Colbry
Acknowledgements

There have been many invaluable sources of guidance for me as I have worked to complete my master’s thesis. I would like to thank my advisor Dr. Sarit B. Bhaduri for his mentorship as I worked through the master’s thesis research process. He deserves a warm thank you for his patience and support. Additionally, Dr. Huan Zhou has made significant time contributions to my success and has helped guide me through the research process, offering instruction in the use of material characterization tools. It was a pleasure to be a student of, and serve as teaching assistant for, Dr. Ioan Marinescu and I particularly enjoyed his teaching style. Lastly, Dr. Mehdi Pourazady was a cheerful and motivating professor and I was thrilled to have him act as a member of my defense committee. My lab mates Nariman Mansouri, Maryam Nabiyouni, and Yufu Ren have taught me instrumental skills and shared their individual expertise with me.

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP</td>
<td>Alkaline Phosphatase</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>CaP</td>
<td>Calcium Phosphate</td>
</tr>
<tr>
<td>CDHA</td>
<td>Calcium Deficient Hydroxyapatite</td>
</tr>
<tr>
<td>CPC</td>
<td>Calcium Phosphate Cement</td>
</tr>
<tr>
<td>CSC</td>
<td>Calcium Silicate Cement</td>
</tr>
<tr>
<td>CSD</td>
<td>Calcium Sulfate Dihydrate</td>
</tr>
<tr>
<td>CSH</td>
<td>Calcium Silicate Hydrate</td>
</tr>
<tr>
<td>DI</td>
<td>De-Ionized</td>
</tr>
<tr>
<td>DCPA</td>
<td>Dicalcium Phosphate Anhydrous (Monetite)</td>
</tr>
<tr>
<td>DCPA+SiO₂</td>
<td>Dicalcium Phosphate Anhydrous Silica</td>
</tr>
<tr>
<td>DCPD</td>
<td>Dicalcium Phosphate Dihydrate (Brushite)</td>
</tr>
<tr>
<td>ESEM</td>
<td>Environmental Scanning Electron Microscope</td>
</tr>
<tr>
<td>FBS</td>
<td>Fetal Bovine Serum</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared Spectroscopy</td>
</tr>
<tr>
<td>HA</td>
<td>Hydroxyapatite</td>
</tr>
<tr>
<td>MCPM</td>
<td>Monocalcium Phosphate Monohydrate</td>
</tr>
<tr>
<td>MEM</td>
<td>Minimum Essential Medium</td>
</tr>
<tr>
<td>OPC</td>
<td>Ordinary Portland Cement</td>
</tr>
<tr>
<td>P</td>
<td>Phosphorous</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
</tr>
<tr>
<td>PMMA</td>
<td>Polymethylmethacrylate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>PSC</td>
<td>Partially Stabilized Cement</td>
</tr>
<tr>
<td>SBF</td>
<td>Simulated Body Fluid</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscope</td>
</tr>
<tr>
<td>Si</td>
<td>Silicon</td>
</tr>
<tr>
<td>Si-α-TCP</td>
<td>Si-doped α-tricalcium phosphate</td>
</tr>
<tr>
<td>Si-CaP</td>
<td>Silicon Substituted Calcium Phosphate</td>
</tr>
<tr>
<td>Si-CDHA</td>
<td>Silicon Substituted CDHA</td>
</tr>
<tr>
<td>SLS</td>
<td>Saliva like Solution</td>
</tr>
<tr>
<td>TCP</td>
<td>Tricalcium Phosphate</td>
</tr>
<tr>
<td>ti</td>
<td>Initial Setting Time</td>
</tr>
<tr>
<td>tf</td>
<td>Final Setting Time</td>
</tr>
<tr>
<td>WPC</td>
<td>White Portland Cement</td>
</tr>
<tr>
<td>XRD</td>
<td>X-Ray Diffraction</td>
</tr>
</tbody>
</table>
List of Symbols

^ ................................................................. Insertion
* ........................................................................... Insertion
† ........................................................................... Positive Charge
- ........................................................................... Negative Charge
° ........................................................................... Degrees
α ........................................................................... Alpha
β ........................................................................... Beta
Δ ........................................................................... Delta, Change in
h ........................................................................... Height
σmax ........................................................................... Ultimate Strength
εmax ........................................................................... Ultimate Strain

A................................................................................. Area
C................................................................................. Degrees Celsius
D................................................................................. Diameter
F................................................................................. Degrees Fahrenheit
K................................................................................. Degrees Kelvin
Pi()............................................................................. The value of Pi 3.14159
Pmax ............................................................................. Maximum Load
x................................................................................. Variable of interest
Chapter 1

Silicon Containing Biocements: A Review

Abstract:

The field of biocements as discussed here is generally a small field of bioceramics, which is a subset of biomaterials: a major area of materials science research focused on generating new materials for use in the human body. Bioceramics of various compositions have been used in bone surgery; biocements are favorable for such applications because of their easy manipulation in filling bone voids. Many of biocements can be combined with growth factors and bone cells, and can be used as a local drug delivery system to aid in bone remineralization. This review focuses on the advantages and disadvantages of various bone cements in clinical settings, exploring the fundamental design of biocements and examining their mechanical properties and cell interactions. More specifically, this work examines two types of biocements: (1) silicon containing, monolithic “self hardening” cements, such as Bioglass®; and (2) composite cements, looking at their inorganic-inorganic and inorganic-organic make ups. Additional discussions focus on silicon substituted materials and silica cement compounds like:
Portland cement, mineral trioxide aggregate, calcium silicate, partially stabilized cement, and resin.

1.1 Introduction

Bone cements are used as a reliable way to anchor joint replacements and pins, to fill voids and defects in orthopedics and endodontics, and as a scaffold for the remineralization of bone. In this context, bone cements are used in surgical procedures, where defects are repaired by the implantation of a calcium phosphate dense material (bone cement) as the mineral phase of bone. Typically, these types of bone grafts are used to mend fractures, fuse vertebrae, correct deformities, provide structural support, and repair defects [1–5]. Ultimately, the surgeon’s objective is the full or partial restoration of the patient’s bone function.

Bone cements are a subset of the class of biomaterials, which include a variety of synthetic and natural materials suitable for use in constructing artificial organs and organ tissues, developing implantable prostheses, and in replacing bone. The application of Si-containing materials as skeletal implants started in the late 1960s [6–9], but attracted little attention until the early 1970s, when Carlisle et al. [10], [11] systematically demonstrated the positive influence of Si for skeleton tissues. In this work, Carlisle et al. analyzed bone using an electron microprobe and showed Si to be highest at the initial stage of bone calcification, with a low Ca/P molar ratio (Ca/P=0.7); the Si content fell below the detection limit at compositions approaching hydroxyapatite (Ca/P=1.67) with
continuous bone mineralization. This research by Carlisle et al. also suggested that Si may be involved with the initiation of mineralizing preosseous tissues. The follow-up study of Si deficiency by Carlisle et al. [12–14] supported this theory and found a lower level of bone growth in young chickens fed a silicon-deficient diet. In the same time period, other groups also reported similar observations in animal studies [15], [16], and it has since become accepted that Si has significant impacts on bone formation and growth.

Silicon (Si) is the second most abundant element on our planet, comprising 26% of the Earth’s crust and found in various concentrations within the body. For example, within the human body silicon is present at approximately 1 ppm in the serum; 2–10 ppm in the liver, kidney, lung and muscle; 200–600 ppm in cartilage and other connective tissues; and crucially 100 ppm in the bone and ligaments [17]. The high concentration of Si in skeletal tissues reflects the important role of Si in bone remodeling (bone metabolism) and covering mineralization [11], [12], cartilage synthesis [18], collagen production [19], [20], bone cell proliferation and differentiation [19], [20]. In controlled conditions, the release of calcium (Ca) and silicon can lead to upregulation and activation of seven families of genes in osteoprogenitor cells, and can induce rapid bone regeneration [21]. Phosphorous is also believed to be an important factor in maintaining bone health; however, the positive function of phosphorous on bone regeneration in implanted materials is still under investigation [22], [23]. Skeletal remodeling and tissue repair in clinical settings is also shown to correlate with silicon; for example, it has been suggested that calcium and silicon ions are critical factors in osteoclast and osteoblast proliferation.
during bone remodeling [24], [25]. The many functions of silicon in the human body justify research interest in Si-containing cements for medical applications.

Within the field of biomaterials, numerous studies of biocements have been completed, including bioactive glass [20], [26] and Si-substituted calcium phosphate (Si-CaP) [27], [28]. In many research cases, these Si-containing biomaterials are combined with each other [29], with a select polymer matrix [30–32], or with functional materials [33–35] to construct graft materials for skeleton tissue repair. It is worth noting that both bioactive glass [36], [37] and Si-CaP [38], [39] are used as bone cement components. The combination of advantageous minerals is also reflected in the Si-containing cements demonstrated in next few sections.

Within the field of biomaterials, special attention is paid to materials that are bioactive, which means that when these materials are placed in a biological environment they form a biologically active, bone-like, apatite layer or carbonate, with a calcium-deficient apatite on the surface [40], [41]. Precursors of apatite are often seen as calcium phosphate spherules [42]. Furthermore, calcium silicates, bioactive glass cement and glass-ceramic cements are all characterized as bioactive materials by the ability to form an apatite layer on their surfaces when in contact with physiological fluids in vivo or with simulated body fluid (SBF) in vitro [40], [43–51].

Biocements generally require a hardening or solidification process, such as sintering, microwave treatments or chemical reactions. For example, the self-setting properties of
calcium silicate cements are due to the progressive hydration reaction of the orthosilicate ions: calcium silicate particles react with water and form a nanoporous amorphous CSH gel on the cement particles, while calcium hydroxide (Ca(OH)$_2$), portlandite) nucleates and grows in the available voids and pore spaces [43]. Additionally, it has been shown that functional groups (like Si-OH) on a wet, freshly exposed cement surface act as nucleation centers for apatite precipitation [51–54].

There is a strong clinical demand for biocompatible bone cements, particularly as needs of an aging world population increases demand for good biocements in medical markets. In general, Si-containing bone cements can be divided into two categories: Si-based and Si-substituted. The structural properties of many silicon-containing bone cements make these materials well-suited to biomedical applications, and to the best of reviewed knowledge there is, no specific review paper linking these needs to Si-containing bone cements. This systematic review examines the broad range of publications available on Si-containing bone cements, and is divided into several sections. This first section has provided a brief introduction of Si-containing biomaterials and biocements. The next several sections discuss Si-containing bone cements, including: hardening mechanisms; biological responses; and the mechanical performance of Si when incorporated in the lattice structure or when bonding with particles of other materials in a composite manner. Another section covers composite cements; specifically examining inorganic-inorganic and inorganic-organic make ups, which respectively include resins and calcium silicate biopolymers. Table 1.1 describes the popularity of various research subjects, while Figure 1-1 demonstrates them in relation to this papers organization.
Table 1.1: Search Results in Science Citation Index Expanded for Key Words

<table>
<thead>
<tr>
<th>Topic</th>
<th>Citations</th>
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<tbody>
<tr>
<td>Composite Cement</td>
<td>7541</td>
</tr>
<tr>
<td>Portland Cement</td>
<td>7370</td>
</tr>
<tr>
<td>Calcium Silicate Cement</td>
<td>1360</td>
</tr>
<tr>
<td>Mineral Trioxide Aggregate</td>
<td>1004</td>
</tr>
<tr>
<td>Silicon Cement</td>
<td>519</td>
</tr>
<tr>
<td>Bio Resin</td>
<td>487</td>
</tr>
<tr>
<td>wTC</td>
<td>459</td>
</tr>
<tr>
<td>Silicon Biomaterial</td>
<td>196</td>
</tr>
<tr>
<td>Partially Stabilized Cement</td>
<td>57</td>
</tr>
<tr>
<td>Bioglass Cement</td>
<td>46</td>
</tr>
<tr>
<td>Silicon Calcium Phosphate Cement</td>
<td>32</td>
</tr>
<tr>
<td>Calcium Silicate-biopolymer</td>
<td>3</td>
</tr>
</tbody>
</table>

Figure 1-1: Organization of the Chapter
1.2 Si-Containing Cements

1.2.1 Bioglass Cements

Bioactive glasses are glass-ceramic biomaterials with extensive applications as bone replacements for diseased or damaged bone. Hench et al. [22], [55–58] are known for pioneering the development of bioactive glasses and significantly influencing the research pathway for Si-containing biomaterials for skeletal tissue applications [56]. More specifically, bioglass became a group of surface reactive glass/ceramic biomaterials with the ability to induce strong bone bonding formation to surrounding skeletal tissues once implanted; conventional bioglass is composed of SiO$_2$, CaO, Na$_2$O and P$_2$O$_5$ in relevant proportions based on Figure 1-2. Once bioactive glasses are implanted, the hydrolysis and polycondensation of SiO$_2$ on the surface is sufficient to induce the mineral phase precipitation of hydroxyapatite (HA) to promote bone growth and form a strong bone bond [55]. If this rate of apatite formation is high enough, soft tissue bonds can also be found. Bioactive glasses composed of SiO$_2$, CaO, Na$_2$O and P$_2$O$_5$ bond most rapidly to bone if SiO$_2$ contents of 45-52% weight are used, providing a strong bond to both soft and hard connective tissue within 5-10 days. Bioactive glasses or glassceramics containing 55-60% SiO$_2$ require a longer time to form a bond with bone, and do not connect to soft tissue at all. After a 60% SiO$_2$ composition is reached, no bonding is found with bone or to soft tissues and the bioglass becomes inert [59]. Figure 1-2 shows where bioactive glasses bond to bone and soft tissues. Region A illustrates the compositional range for bone bonding to bioactive glass and glass-ceramics; the
boundaries are kinetic (not phase equilibrium boundaries), and the glass structure reaction mechanisms responsible for the compositional boundaries have been shown [59]. The glasses shown in region E have the highest level of bioactivity and rapid bone bonding. Increasing the surface area of the glass by making a particulate or a nanoporous sol gel-derived glass extends the bone bonding compositions to higher percentages of SiO₂ in the glass [60], [61].

![Diagram of Na₂O-CaO-SiO₂ Ternary Phase Diagram for Bone-bonding](image)

**Figure 1-2: Na₂O-CaO-SiO₂ Ternary Phase Diagram for Bone-bonding [59].**

Researchers [19], [22] have found that the dissolution products of bioactive glasses promote numerous genes within osteoblast cells, which express growth factors, cytokines, and extracellular matrix components to accelerate bone formation. Hench et al. [22], [57], [62] have outlined a series of material and cellular events occurring on bioactive glass surfaces during bone regeneration. Bioactive glasses have also been applied as particles [63], coating [64], tubes [65], cement [66] and scaffolds [64] for skeletal tissue repairs. Even after four decades, bioactive glass is still a popular topic in
biomaterials research: ongoing work is exploring new directions, including doping of functional elements [33], [67]; preparing composites [32], [68]; and constructing scaffolds with desired architectures [69–71].

Kokubo et al. [66] suggested that a CaO / SiO$_2$ based glass powder could be solidified in a few minutes by mixing with an appropriate solution containing phosphate ions to form hydroxyapatite on its surface. This newly-formed hydroxyapatite could then bond to living bone through the apatite. Based on this concept, the researchers developed a glass powder-based cement that can harden within 4 minutes when mixed with an ammonium phosphate solution. The *in vitro* bioactivity testing confirmed the formation of apatite on the surface of cement and mechanical test observed the cement showed a compressive strength of 80 MPa. The cement formed a tight chemical bond to the living bone in rat tibia within 4 weeks. Subsequent research explored modifications to improve the mechanical and biological performance of this bioglass cement. For example, glass powder composition at the correct weight ratio was shown to provide the highest compressive strength of synthesized cement. Drugs such as cephalexin [72] and indomethacin [73] were loaded to this bioglass cement, and researchers found that the apatite coating interfered with the drug release, slowly releasing the drugs over the course of weeks.

Fu et al. [74] incubated bioglass cement in simulated body fluid (SBF) and reported the compressive strength of cement increased with immersion time, almost doubling after 30 days [74]. The reason for the increased strength is that the bioglass cement structure was
cross-linked with newly formed apatite, which created a uniform, compact structure. In addition, incorporating nano HA in bioglass cement was shown to inhibit bone cancer cells’ proliferation and improve cement strength without negatively influencing the hydration reaction [29], [75]. Instead of regular bioglass powders, a mesoporous bioglass powder was evaluated for making bioglass cement. Once mixed with ammonium phosphate solution, a plastic paste was prepared that had the ability to be molded into different shapes and to be extruded from a syringe. This paste exhibited a 10 minute setting time and, after hardening, showed a porous structure and higher bioactivity as compared to regular bioglass cement [74].

In conclusion, bioglass cements are an extensively studied and relatively well understood area. These materials are biocompatible and bioactive, and altering the design of the system can result in high mechanical strengths. However, above 60% SiO₂ bone bonding decreases unless silica is in a nanosilica sol form. A few commercial forms of bioglass include: Bioglass®, Ceravital®, MEP® a.k.a DOUEK MED®, ERMI ®, NovaBone, PerioGlas, NovaMin and NovaThera [59].

1.2.2 Silicon Substituted Calcium Phosphate Cement

Calcium phosphate (CaP) materials are commonly seen with Si, creating another popular group of inorganic biomaterials for skeletal tissue repair. Both Si-substituted CaP and bioactive glass contain Ca and Si as a main bioactive component. Other names for Si-substituted CaP include “silicate calcium phosphate” and “silicate-substituted calcium
phosphate”. The driving force behind the use of CaP is chemical similarity to the mineral component of hard tissue in the body. Similar to bioactive glasses, Si-substituted calcium phosphate is known for inducing strong bone bonding to skeletal tissues, with the ability to withstand shear and distraction loads. Calcium phosphate cements are of great interest as biomaterials for orthopedic applications requiring a material that (1) becomes a hard mass during the setting process, (2) is biocompatible, (3) is bioactive, and (4) is gradually replaced by the new bone once implanted [37]. The complete list of existing CaP with their detailed information including synthesis, structure, stability, and biomedical applications has been systematically reviewed by Dorozhkin et al. [76–78]. Given the positive impact of Si in the enhancement of bone growth, it is not surprising that incorporating Si into the CaP composition is a popular topic. Si-substituted hydroxyapatite (HA) [79–83] and tricalcium phosphate (TCP) [84–87] are commonly reported CaP materials. The synthesis consists of the sol-gel method [62], [88], hydrothermal method [79], [85], and solid state reactions [89], [90]. Si-substituted CaP materials exhibit improved bone apposition, bone in-growth and cell-mediated degradation in comparison to original CaP [28], [82], [87], [91]. In Si-substituted CaP the biological performance of CaP materials can greatly improve with the addition of Si [28], [92].

Though Si-substituted CaP materials have been widely studied to improve the biological performance of CaP materials, the focus has not necessarily been on Si-substituted calcium phosphate cements. Instead, more attention is put on the combination of calcium phosphate cement and calcium silicate, which will be discussed in the following section.
In general, there are two major groups of calcium phosphate cements (CPC): dicalcium phosphate dihydrate (DCPD), and non-stoichiometric calcium deficient hydroxyapatite (CDHA) [93]. CDHA-based cements are more frequently studied because they set at a pH value closer to the physiological environment. However, under physiological conditions DCPD-based cements are better resorbed than the apatite cements. The concern when using DCPD is the tissue inflammation caused by the material’s low pH, and the release of orthophosphoric acid during the partial transformation from DCPD to CDHA. Komlev et al. [94] reported a DCPD cement that used a sodium polysilicate setting solution to assist the hardening process, resulting in improved bioactivity and an increase cement surface pH to 8.2. On the other hand, several groups investigated the substitution of Si into CDHA-based cement. For example, Su et al. [95], [96] used the mixed powders of tetracalcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA), with 5wt% sodium silicate water solutions as setting solution, to synthesize Si-CDHA. In these experiments, Si-CDHA cement showed faster hardening, higher compressive strength, and better degradability. Both in vitro and in vivo studies indicated Si-CDHA cement has improved biocompatibility and osteogenesis as compared to CDHA cement.

As an alternative to adding Si into the setting solution, Si can be doped into reactant powders. Several reports focus on Si-doped α-tricalcium phosphate (Si-α-TCP) as starting powders [39], [97], [98]. Occasionally, α-TCP hydrolyses into CDHA once in contact with both water and Si-α-TCP. Cement powders of Si-α-TCP can be prepared via solid state reaction of calcium carbonate (CaCO₃), dicalcium phosphate (CaHPO₄) and
CaSiO$_3$ [98], or CaCO$_3$, calcium pyrophosphate (Ca$_2$P$_2$O$_7$ CaHPO$_4$) and CaSiO$_3$ [97], or HA and SiO$_2$ [39] with sintering. In general, this prior research suggests that silicon-substituted calcium phosphates have better mechanical properties than pure calcium phosphates, and that silicon-substituted calcium phosphates have better biological properties than their standard counterparts (without silicon).

1.2.3 Calcium Silicate Cement

Calcium silicate materials are potentially useful for skeleton repairs, since these materials are highly bioactive and induce faster bone mineral deposition than bioactive glasses [99]. Several calcium silicate materials have been investigated, including pseudowollastonite (CaSiO$_3$) [100–103], dicalcium silicate (Ca$_2$SiO$_4$) [53], [104], tricalcium silicate (Ca$_3$SiO$_5$) [43], [104], and calcium silicate gel (CSH) [105]. In addition, other researchers have explored the incorporation of elements such as Sr [106] and Mg [107] into the structure of calcium silicate materials to improve physical, radiopacity, and biological properties.

Compounds like calcium silicate (Ca$_2$SiO$_4$) and tricalcium silicate (Ca$_3$SiO$_5$), which are both components of Portland cement, have the ability to spontaneously develop strength through hydraulic reactions when water is added to the compound [108–110]. This self-setting property results from the progressive hydration of the SiO$_4^{4-}$ (silicate) ions of calcium silicate (Ca$_2$SiO$_4$) and the SiO$_5^{6-}$ (silicate) of tricalcium silicate (Ca$_3$SiO$_5$). In general, when Ca$_2$SiO$_4$ and Ca$_3$SiO$_5$ react with water, a nanoporous amorphous calcium
silicate hydrate (CSH) gel is deposited on the original substrates. Another product of the hydration process, calcium hydroxide (Ca(OH)$_2$), grows on the deposited CSH. As time proceeds, the CSH gels polymerize and harden to provide a solid network to support the cement strength.

Gou et al. [53] describe a self-setting, workable calcium silicate paste that was injectable for minimally invasive surgeries. The hydration yielded a bioactive, biocompatible, and degradable calcium silicate hydrate with strength and cellular structure. Similar reports were also found on the study of tricalcium silicate paste [43], [111]. The self-setting properties can be improved with the substitution of a sodium carbonate (Na$_2$CO$_3$) aqueous solution for regular water [111]. Ammonium phosphate solution has also been used in a liquid phase to replace water and further promote setting and mechanical performance [51].

Various studies have shown that a slower reaction time often results in higher strength. For example, Ca$_2$SiO$_4$ reacts at a slower rate than the Ca$_3$SiO$_5$, but Ca$_2$SiO$_4$ contributes substantially to the strength development shown after 28 days [112],[113]. Chang et al. [104] evaluated cements composed different ratios of Ca$_2$SiO$_4$ and Ca$_3$SiO$_5$, and reported that 20 wt% Ca$_2$SiO$_4$ showed a suitable setting time and best mechanical performance. The resulting cement exhibits excellent apatite-forming ability in physiological solution and biocompatibility in cell culture, as expected. In addition, both Ca$_2$SiO$_4$ and Ca$_3$SiO$_5$ are good candidates for drug loading. For example, loaded gentamicin sulfate exhibited a long-term, sustained release behavior [53], [114] attributed to the interaction of
gentamicin sulfate with the CSH network and the formation of unique nano-to-microporous structure after hydration.

Other calcium silicate cements have been developed as alternatives to calcium silicate and tricalcium silicate cement. For example, tetraethyl orthosilicate \((\text{Si(OC}_2\text{H}_5)_4}\) and calcium nitrate \((\text{Ca(NO}_3)_2\cdot4\text{H}_2\text{O})\) were used as precursors for \(\text{SiO}_2\) and \(\text{CaO}\) separately [115], [116]. The resulting calcium silicate cement achieves the same formation of CSH and the desired bioactivity and biocompatibility.

In bone regeneration, resorbion, and stimulation of biomimetic environments, hydroxyapatite-tricalcium phosphate has been shown to be less effective than calcium silicate cements [115], [117]. Alpha-tricalcium phosphate (alpha-TCP) introduced into wTC-TCP is an active resorbable reactant and phosphate source due to hydrolysis and progressive dissolution [118–120], [121], with calcium ions which are necessary to enhance apatite formation and tissue regeneration [120]. A thicker apatite coating layer forms on wTC-TCP cement and has demonstrated the effectiveness of alpha-TCP to improve apatite formation on calcium silicate materials [122–126] and calcium phosphate ceramics [127]. The biocompatibility of calcium-silicate preparations were studied by Gandolfi et al. [128] with the hypothesis that calcium ions, silicon, and phosphorous released by calcium-silicate cements will provide supportive biomimetic microenvironment for survival and differentiation of cells.
Unhydrated powder of wTC-TCP and wTC are coincident in their Raman spectrum [129] indicating chemical similarity. The amorphous nature of CSH makes it difficult to characterize by methods such as Raman spectroscopy [42]. Observations have shown the presence of monoclinic alite, monoclinic belite, gypsum, anhydrite and calcium carbonate in the spectra [52], [54], [129]. A surface composed of uniform calcium silicate hydrate (CSH) hydrogel is expected as a result of the hydration of belite and alite on wTC and wTC-Bi non-aged cements [54]. The presence of calcium carbonate, anhydrite, and ettringite was also observed due to hydration [54]. Soluble calcium hydroxide has been shown to form during cement hydration and to rapidly release into the ageing media, providing calcium and hydroxyl ions and an alkaline pH [129]. Calcium cations interact with carbon dioxide and phosphate anions, acting as nuclei for the starting of the crystallization process [129].

In conclusion, studies show that higher Si content cement enhances the expression of cell attachment, proliferation and differentiation as compared to lower Si content cement, while higher Ca content can promote the setting reaction [130]. Furthermore, directly using Ca(OH)\(_2\) and nanosilica to produce CSH could be desirable considering the difficulty resulting from the production of large scale quantities of \(\text{Ca}_2\text{SiO}_4\) and \(\text{Ca}_3\text{SiO}_5\) with high purity [105], [131]. The setting process is an exothermic reaction attributed to the breakdown of Si-O-Si bonds. Ca\(^{2+}\) offsets the charge imbalance and bonds to Si-OH and Si-O- giving rise to CSH gel. Though this Ca(OH)\(_2\)-activated nanosilica cement shows shorter setting times and lower heat liberations than pure \(\text{Ca}_3\text{SiO}_5\) cement, no biocompatible results were found.
1.2.3.1 Portland Cement

Portland cement is a common construction material with biomedical potential and chemical similarity to commercially-sold bone cements. According to ASTM C150 [132] there are five official types of Portland cement, which mainly consist of tricalcium silicate (Ca$_3$SiO$_5$) and dicalcium silicate (Ca$_2$SiO$_4$) and which react with water during hydration [133]. Silicate phases of Portland cement undergo hydration followed by a series of physico-chemical reactions resulting in a nanoporous gel of calcium silicate hydrates and a soluble fraction of portlandite (calcium hydroxide, Ca(OH)$_2$) [134].

Two compositions of Portland cement had initial ($t_i$) and final ($t_f$) setting times of 134 $t_i$ and 190 $t_f$ minutes and 130 $t_i$ and 170 $t_f$ minutes respectively [135], which are significantly longer setting times than what is desirable in many clinical applications. Compressive strength after storage in a 100% humidity water bath at 37 °C for 1, 3, and 7 days indicate that Portland cement paste strength increases with longer curing periods [135]. After 7 days of setting, paste specimens of Portland cement have achieved compressive strengths of around 90 MPa [135]. Immersion in SBF has been shown to encourage formation of an apatite layer on the surface Portland cements [136]. An increase in the super saturation of Ca$^+$ is shown not to promote the formation of new crystals of hydroxyapatite on the material surface in SBF [137], [138] and the formation of this homogeneous hydroxyapatite layer is dependent on the content of silicate material [139]. Research has clearly demonstrated apatite precipitates form on calcium silicate
Portland cements when immersed in phosphate solutions [44–46], [140–142]. Depending on the simulated body fluid used carbonated apatite or calcite apatite will preferentially form [129].

1.2.3.2 Mineral Trioxide Aggregate (MTA)

Mineral trioxide aggregate (MTA) is a material similar in chemical structure to both calcium silicate-based cement (CSC) and Portland cement [143–147]. For example, MTA is a cement that generally consists of 80wt% white Portland cement and 20wt% bismuth oxide in a four-to-one ratio [144], and is most commonly used as a root repair material in dentistry [148–154]. The white Portland cement component of MTA is composed of ticalcium silicate, dicalcium silicate, and tricalcium aluminate [112], [155], [156]. [157]. The cements are hydraulic (requiring water) and the cements are able to set through the formation of a nanoporous calcium silicate hydrate gel [158]. Very fine powders cause an increase in water uptake as the surface area is increased, while powders that do no absorb water cause a decrease in the water to powder ratio [159].

As a cement MTA has many attractive qualities including: a source of soluble calcium for remineralization [117], [160]; inorganic phosphates [117], [160]; a hydraulic nature hardening in the presence of moisture [161]; and the release of calcium ions [42], [161]. These qualities can be summarized as attractive chemical [162], physical [163], and biological properties [157]. Both extracellular and simulated body fluids containing a phosphate source allow MTA cements to form calcium phosphate and apatite on its
surface [49], [140], [158], [164]. While MTA has good biocompatibility when compared to traditional dentistry materials for root end filling and repair, MTA is a costly material with poor handling characteristics [149], [165], [166]. Other calcium silicate cements have made additions to the MTA structure with additives like ZnO, MgO, and Fe₂O₃ which can be made to show similar properties [167], and favorably modify the required setting time [168]. Patterns from X-ray diffraction (XRD) data shows similar peaks in hydrated MTA and calcium silicate cements indicating that the MTA and calcium silicate cement chemical structures are similar [143]. Further verifying this, cements of MTA and calcium silicate similarly stimulate bone cells to remodel based on the bone remodeling marker expression patterns found [143].

Sodium fluoride has been introduced into MTA to improve biological properties, showing an increased ability to form apatite, by Gandolifi et al [158]. Other research by Qu et al. [169] demonstrated that fluoride can be added to improve bone integration and bioactivity shown by the positive effect of fluorapatite (Ca₁₀(PO₄)₆OH-F) on osteoblast and bone formation in comparison to hydroxyapatite (Ca₅(PO₄)₃OH) [170]. Furthermore, fluoride can increase the bioactivity of other tricalcium silicates when added as a dopant [171]. Greater solubility has been seen by NaF than CaF₂, particularly in alkaline environments [158], which can be a sign of biocompatibility and is desirable for remodeling. The addition CaF₂ as dopant lowers the pH [171] while NaF has been shown to increase the pH [158], a lower pH correlates to a reduction in the hydration mechanism of cements. The negative impact of NaF comes from slower setting times and increased expansion [163]. This can change the long-term apical sealing in the root canal [172].
The addition of calcium chloride in the composition of calcium-silicate cements reduced the setting time to 30–40 minutes [162], [173–176]. Chin-Lin et al. [143] created a hydrated calcium silicate cement with a similar chemical structure to MTA based on XRD peaks, and a comparison of MTA and their calcium silicate cement showed comparable biocompatibility for both materials. These results showed collagen is expressed on both MTA and calcium silicate cements from the first day, with osteocalcin expression evident on day 7 [143].

One benefit of MTA is the radiopacity provided by the bismuth oxide, which allows clinicians to visually inspect their work and the structure of the implanted area through x-ray. Alternative radiopacifiers should be colorless, nontoxic, and added in minimal amounts to limit material property modifications. The desire to add minimal amounts or radiopacifier requires the addition of elements with high atomic masses [177]. International Standards for dental root canal sealing materials (ISO 6876 Section 7.8 2002) recommend 3mm aluminum for radiopacity values [177], without additives traditional calcium silicate cements have low radiopacity ranging from 0.86 to 2.02mm aluminum [178–180]. Calcium silicate-based cements without additives are not acceptable as root end dental fillers because they are not easily detected on a radiograph [177]. This requires the addition of a radiopaque element such as bismuth oxide [144], barium, or other bismuth compounds [177] to distinguish the cement from the natural tissue [181]. Camilleri et al. [177] created cements with values of 1.62 ± 0.29 mm Al and then showed that this cement could be substituted with Zinc, Barium, Gold, Bismuth, and Silver / Tin in various amounts to reach the 3 mm aluminum standard. Interestingly,
high water-to-cement ratios are shown to reduce the radiopacity of the resulting cement [182].

Besides being an excellent radiopacifier, bismuth oxide has negative effects associated with it including cytotoxicity and decreased mechanical properties. More specifically, increasing concentrations of radiopacifier lowers compressive strength and surface hardness [183]. Bismuth affects the hydration mechanism by forming part of the structure and replacing some silica in the calcium silica hydrate [156], and has been shown reduce the compressive strength of cements [182]. On the other hand, contradictory evidence shows that no significant difference in strength is seen in other calcium silicate cements by varying the amount of bismuth oxide [180]. Camilleri et al. [184] attributed the difference in strength results to nonstandard testing procedures. Exchanging cement for radiopaque materials changes the particle size and distribution therefore changing the water to cement ratio, workability, and strength of final cements [177]. Pure bismuth has been shown to be toxic, inducing cytotoxicity in dental pulp cells [185]. In contrast, other research has shown that MTA is biocompatible and induces cell growth or activity [179], [186]. It is possible that the Bismuth, in conjunction with calcium silicate, has a buffering effect on cell growth but additional research is needed.

In comparison to traditional root-end dental filling materials, MTA is expensive and hard to handle [149], [166] but has been shown to have excellent biocompatibility [145], [175], [187–189]. Unfortunately, the principal limitation of original calcium-silicate MTA cement was related to the slow setting time (more than 170 min) and to difficulty in
hand manipulation for clinical use. Four types of MTA cements sold commercially for applications in endodontics are shown in Table 1.2.

Table 1.2: Four Cements Sold Commercially

<table>
<thead>
<tr>
<th>Product</th>
<th>Manufacturer</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>White ProRoot MTA</td>
<td>Dentsply, Tulsa Dental Products, Tulsa, OK, USA</td>
<td>75% Portland cement (tricalcium silicate, dicalcium silicate, tricalcium aluminate, and tetracalcium aluminoferrite), 20% bismuth oxide, 5% calcium sulfate dehydrate (gypsum) and up to 0.6% trace elements (crystalline silica, calcium oxide, potassium, and sodium sulfate)</td>
</tr>
<tr>
<td>Grey ProRoot MTA</td>
<td>Dentsply, Tulsa Dental Products, Tulsa, OK, USA</td>
<td>75% Portland cement (tricalcium silicate, dicalcium silicate, tricalcium aluminate, and tetracalcium aluminoferrite), 20% bismuth oxide, 5% calcium sulfate dehydrate (gypsum) and up to 0.6% trace elements (crystalline silica, calcium oxide, potassium, and sodium sulfate)</td>
</tr>
<tr>
<td>AH Plus</td>
<td>DeTrey Dentsply, Konstanz, Germany</td>
<td>a mix of epoxide paste (diepoxide, calcium tungstate, zirconium oxide, Aerosil, and pigment) and amine paste (1-adamantane amine, N,N'-dibenzyl-5-oxa-nonandiamine-1,9, TCD-diamine, calcium tungstate, zirconium oxide, aerosol, silicone oil)</td>
</tr>
<tr>
<td>Dycal</td>
<td>De Trey Dentsply, Milford, DE, USA</td>
<td>Base: disalicylate ester of 1,3, butylene glycol; calcium phosphate; calcium tungstate; zinc oxide; iron oxide Catalyst: calcium hydroxide; ethyl toluenesulfonamide; zinc stearate; titanium dioxide; zinc oxide; iron oxide</td>
</tr>
</tbody>
</table>

1.2.3.3 Partially Stabilized Cement (PSC)

Partially stabilized cement (PSC) is a calcium silicate based cement that has traditionally been plagued with low reaction efficiency and low initial strength. This modified silicate cement was developed by Lin et. al [190] and is composed of CaO 69.4%, SiO₂ 21.65,
Fe₂O₃ 3.2%, and Al₂O₃ 5.8%. First synthesized by traditional powder methods, other production methods such as the sol-gel process have since been used. Compositions of PSC are similar to MTA: both are based on calcium silicate Portland cements, but PSC does not include bismuth and substitutes calcium aluminoferrite (Ca₄Al₂Fe₂O₁₀) instead [191]. One benefit of PSC is that it can be formed in one batch and no other mixing is required before use [192]. The resulting cement is either a monoclinic or triclinic crystal structure [190]. Development of the PSC system has shown calcium monoxide (CaO), dicalcium silicate (Ca₂SiO₄), tricalcium silicate (Ca₃SiO₅) tricalcium aluminate (Ca₃Al₂O₆), and calcium aluminoferrite (Ca₄Al₂Fe₂O₁₀) [191][192]. Other research has indicated that transitioning CaO to Ca(OH)₂ during the hydraulic reaction in the liquid phase will raise the environmental pH and can inhibit bacterial growth in the local implant site [146].

The sol-gel method is commonly used in the formation of glass and ceramics [193], where an inorganic polymerization or oxopolymers are formed during hydrolysis and condensation [194]. An oxide network forms during a calcination process creating the morphology of the resulting powders [194].

Clear correlations are drawn between micro-hardness and setting time by Lin et al [190] with improvements after the addition of transition elements and increasing heating temperature during PSC preparation. A sintering temperature around 1400°C in the creation of PSC shortens the setting time and increases the micro-hardness because of the vibration of atoms around their equilibrium positions [190]. Setting time is shortened in
relation to the rate of portlandite formation along with nucleation which increases with defect concentration.

Partially stabilized cement was shown to have setting time improvements with the addition of transition elements like chromium, cobalt, and zinc [190]. Adding zinc and cobalt is more effective at forming monoclinic structures than the addition of chromium after increased crystal defects favoring (CaO)\(_3\)SiO\(_2\) bonds are formed [190]. Also, since cobalt monoxide (CoO) has a smaller molecular weight than zinc monoxide (ZnO), more vacancies and defects can be created by adding CoO to PSC cement in the same ratios. Wang et al. [191] alloyed PSC with zinc to achieve favorable cement mechanical properties; zinc is known to have stimulatory effects on bone formation and a deficiency is known to retard bone growth. Physiological pH limits are shown to be helped at the cellular level with the addition of zinc [195]. This formulation of PSC is the most favorable for root-end filling applications because of the adequate setting time, mechanical properties of PSC with zinc additions, and the promotion of biomineralization [191].

Concerns have been raised about the toxicity of using this cement on patients [191], although good results can be seen on the mechanical properties. Many metal ions are biologically essential but are toxic once biological limits are reached [196]. Wang et al [191] demonstrated that cytotoxicity, cell function, and metabolism are negatively impacted by the presence of cobalt and chromium in the body. These elements have also been shown to lead to cytoplasmic death [197]. In contradiction, cobalt and chromium
can also positively influence cell proliferation, gene expression, and cytokine secretion [198], reinforcing the fact that appropriate levels within biological limits are desired. Wang et al. [191] established that the addition of chromium is more toxic than the addition of cobalt, although neither addition performs satisfactorily during in vitro cell cultures.

1.3 Composite Cements

Research in composite cements seeks to combine the advantages of different components, such as excellent mechanical properties from one material and ideal bioactivity from the second. The specific composites discussed here are engineered from two or more constituents that remain distinct and separate in the final structure – usually one constituent acts as a matrix and the other acts as a filler or reinforcement. In general, they can be classified into two categories based on phases: inorganic-inorganic, and inorganic-organic composite cements.

1.3.1 Inorganic-Inorganic

An inorganic-inorganic composite cement consists of two inorganic constituents added together, where one acts as the matrix and the other acts as the filler. For instance, incorporating calcium silicate into calcium phosphate cements can impact mechanical and biological performance due to the significant role of Si in apatite deposition,
hydration, and cell proliferation [199]. The invention of calcium phosphate cement (CPC) by Brown and Chow et al. [200], [201] sparked a greater interest in skeletal biomaterial research. Calcium phosphate cement is an injectable skeletal substitute material that is biocompatible, bioactive, self-setting, of the proper stiffness, and easily molded into voids [202]. However, calcium silicate has different influences on the mechanical strength, setting time, and degradation of brushite (DCPD) and apatite cements. For brushite cements, calcium silicate was reported to show positive impacts on these properties; specifically, Wang et al. [203] reported a DCPD / amorphous calcium phosphate (ACP) / Ca$_2$SiO$_4$ cement where the addition of 8wt% Ca$_2$SiO$_4$ approximately doubled the compressive strength of the CPC, but did not significantly influence the biodegradability, setting time, injectability, phase evolution, and microstructure of the cement.

Chang el al. [204] presented a composite cement based on β-TCP, Ca$_2$SiO$_4$, and monocalcium phosphate monohydrate (Ca(H$_2$PO$_4$)$_2$*H$_2$O, MCPM). The results indicated that the injectability, setting time, and short-to-long term mechanical strength of the prepared material were higher than those of pure brushite cement. Moreover, the compressive strength of the composite paste increased as aging time increased. Likewise, the specimens showed significantly improved *in vitro* bioactivity in simulated body fluid and similar degradability in phosphate-buffered saline as compared with brushite cement. The reacted paste also possesses the ability to stimulate osteoblast proliferation and promote osteoblastic differentiation of the bone marrow stromal cells. Similar results
were also observed on their DCPD/Ca$_3$SiO$_5$ cement [205] and monocalcium phosphate monohydrate (MCPM)/Ca$_3$SiO$_5$ cement [206].

For regular brushite cement, a low Ca/P ratio and an acidic surrounding environment are necessary for the precipitation of brushite crystals. Otherwise hydroxyapatite (HA) is formed by preference during the hydration of the cement. The incorporation of calcium silicate increased the Ca/P ratio and its hydrated product Ca(OH)$_2$ buffered the acid, resulting in precipitation of CDHA, whose formation rate is much more slow than brushite crystals. Therefore, modified paste showed a delayed setting time and consequently improved the injectability compared with the brushite cement paste. On the other hand, brushite cements are typically weaker than the majority of apatite cements, and a main reason for the comparatively low compressive strength is the extremely rapid setting reaction of the brushite cements, which results in high porosity and consequently weak cement. The retarded setting resulted in a more compact microstructure and the high bioactivity of CSH induced formation of nano-scale CDHA to improve the strength.

On the other hand, for the apatite cements, the incorporation of calcium silicate resulted in different results. Morejon-Alonso et al. [38] tested a cement composed of α-TCP and Ca$_3$SiO$_5$. The results showed that compressive strength decreases drastically in the initial stage and in the pastes with high content of Ca$_3$SiO$_5$. The setting times and degradation rate of composites containing Ca$_3$SiO$_5$ was also observed to change as well. With the addition of Ca$_3$SiO$_5$, the solubility of α-TCP decreases due to the formation of Ca(OH)$_2$ during Ca$_3$SiO$_5$ hydrolysis, which results in alkaline circumstances and delayed
dissolution of α-TCP. Furthermore, the formation of a dense CSH on the surface of α-TCP particles also retards dissolution of α-TCP grains and the precipitation of CDHA. As the CDHA deposition and consumption of Ca₃SiO₅ proceeds, the cement with suitable Ca₃SiO₅ content (5%) finally reaches and surpasses the compressive strength value of the blank α–TCP cement control. However, when excessive Ca₃SiO₅ content (10%) is applied in the cement, the formed C-S-H significantly impeded the CDHA formation, thus resulting in poor compressive strength. On the other hand, the crystallinity of incorporated calcium silicate also affects setting, bioactivity, degradability and mechanical strength of cement. Guo et al. [207] added CaSiO₃ sintered at various temperatures (100-800°C) to commercial available apatite cement. Low-crystalline CaSiO₃ prepared by heat treatment at low temperature was observed to have excellent bioactivity and degradability, but highly crystalline CaSiO₃ resulted in higher compressive strength and shorter setting time, all attributed to the CSH formation ability difference caused by sintering.

Calcium sulfate (CaSO₄) cement has been considered as a potential alternative to CPC. The transformation of plaster of Paris (CaSO₄*1/2H₂O) into calcium sulfate dihydrate (CaSO₄*2H₂O) by a reaction with water has been studied for filling bone cavities [208], [209]. CaSO₄ cement is biocompatible and resorbable, but there are some limitations of this material as compared to CPC: (1) poor mechanical strength; (2) poor bioactivity; and (3) fast resorption. The solution to these drawbacks is to introduce C-S-H formation in CaSO₄ cement. Chang el al. [204] has done work on calcium silicate/CaSO₄ cement. In 2007, Ca₃SiO₅ was added to CaSO₄*1/2H₂O with the purpose of prolonging the setting
time, and improving the strength of the cement. The results indicated that the workability and setting time of the composite pastes are higher than those of pure CaSO$_4$$\cdot$1/2H$_2$O, and the composite pastes showed much better short and long term mechanical properties than those of pure CaSO$_4$$\cdot$1/2H$_2$O. Moreover, the composite specimens showed significantly improved bioactivity and decreased degradability. These results were attributed to the formation of CSH caused by hydration of Ca$_3$SiO$_5$, which constructed a solid network in a shorter time to support the cement structure, showed a significantly lower dissolution rate than that of CaSO$_4$$\cdot$2H$_2$O, and inhibited the contact of CaSO$_4$$\cdot$1/2H$_2$O with water. Following this report, they added CaSO$_4$$\cdot$1/2H$_2$O separately to Ca$_3$SiO$_5$ [210] and Ca$_2$SiO$_4$ [211] with the purpose of decreasing the setting time, and improving the strength of the cement. There are some other reported inorganic-inorganic Si-containing cements, such as Ca$_2$Al$_2$O$_6$/Ca$_3$SiO$_5$ [212], and CaCO$_3$/Ca$_3$SiO$_5$ [213]. Both additives are expected to accelerate the hydration of Ca$_3$SiO$_5$ and improve its mechanical strength.

### 1.3.1.1 Resin

Resin, with a bioactive filler such as HA powder, is the second largest area of biological cement research [214–218]. Charnley et al. [219] introduced polymethylmethacrylate (PMMA) bone cement in 1960 for prosthetic fixation but there was a problem with PMMA bone cement since it was nonadhesive as an anchorage to bone [220] and had poor mechanical properties [221]. This lack of mechanical toughness commonly leads to cement fracture [220], wear debris due to abrasion [221], and prosthetic loosening [222].
The addition of prehardened CPC particles and ceramic whiskers into a resin matrix has been shown to improve the strength and fracture resistance of resin based cements [223]. Xu et al. [223] experimented with two types of whisker additions with surface treatments and silanization: (1) silicon nitride, and (2) silicon carbide. Silicon carbide whiskers are two to three times bigger than silicon nitride and less uniform in diameter, and results suggested that whisker size differences of this magnitude may have only minor effects on the measured composite properties [223]. Incorporating osteoconductive CPC fillers and ceramic whiskers yielded composites with improved mechanical properties compared to composites without whiskers [223].

Silane treatment of fillers on composite properties have also been investigated [224–226]. Dupraz et al. [224] investigated hydroxyapatite particles and whether silanization would suppress their bioactivity, finding that the silanized hydroxyapatite particles maintained their apatite formation ability in a simulated body fluid. Mousa et al. [225] studied the effects of silane treatments on the bioactivity of composites, finding that the cements containing dry silanized bioactive glass-ceramic fillers showed apatite formation in a simulated body fluid. Harper et al. [226] and Labella et al. [218] independently studied the mechanical properties of silanized versus unsilanized hydroxyapatite fillers in bone cements, and found an increase in composite strength when hydroxyapatite fillers were silanized.

The whisker-silica/CPC ratio, increased from 0:1 to 1:0, had significant effects on composite properties, increasing strength approximately three times, work-of-fracture
five times, and modulus two times [223]. Whisker surface treatments and CPC filler silanization also showed effects on composite properties increasing strength with increasing whisker-silica/CPC ratios [223]. Powder used to make the CPC filler consisted of TTCP [Ca$_4$(PO$_4$)$_2$O] and DCPA [CaHPO$_4$] [200], [227–229] in a TTCP/DCPA molar ratio of one [223]. Many bioactive bone cements combine some combination of CaO–SiO$_2$–P$_2$O$_5$–CaF$_2$ (glass), b-tricalcium phosphate(b-TCP), MgO–CaO–SiO$_2$–P$_2$O$_5$–CaF$_2$ (glass), MgO–CaO–SiO$_2$–P$_2$O$_5$–CaF$_2$ (apatite), and wollastonite containing glass-ceramic (AW-GC) powder with a bisphenol-a-glycidyl methacrylate (Bis-GMA) based resin as the organic matrix ((AWC, HAC, and TCPC) [36], [230], [231]. This combination has the ability to bond to bone under nonweight-loading and weight-loading conditions [230], [232]. Uncertainty may remain about whether the uncured resin is carcinogenic [233]. Apatite and wollastonite containing glass-ceramic (AW-GC) powder and bisphenol-a-glycidyl methacrylate (Bis-GMA) based resin have shown to perform when implanted in tibiae, within two weeks of implantation the uncured surface layer of bis-GMA resin was filled with bone-like apatite [233]. Particles of AW-GC were surrounded by bone and were in contact with bone through an apatite layer with no soft tissue intervening. After 4 weeks in tibiae the uncured layer was filled with newly formed bonelike tissue and HA particles were contacting bone through an apatite layer. With a similar TCPC cement in tibiae 8 weeks was necessary to fill with bone like tissue [233]. Neo et al. [234] showed an apatite layer on AW-GC particles 7 days after implantation and on HA particles 10 days after implantation. Yoshifumi et al. [233] indicated that AWC had higher bioactivity and mechanical properties, as well as a more stable bone interface [235], than either HAC or
TCPC. Senaha et al. [236] worked with dog femora and reported that bioactive bone cement consisting of bioactive glass powder and bis-GMA based resin achieved good implant stability under weight-bearing conditions. Matsuda et al. [237] reported that this bio-active bone cement showed higher bonding strength than PMMA bone cement in canine total hip arthroplasty up to 6 months after surgery.

Bisphenol-a-glycidyl methacrylate (Bis-GMA) is an extensively studied [238–243] resin composite that has been used as a dental restorative materials. The effects of filler treatments and cure conditions on composite properties are well understood [223]. Bis-GMA composites with bioactive fillers including hydroxyapatite, bioactive glass, glass ceramics, and calcium phosphates become bioactive and are useful for bone repair applications [36], [217], [235], [244–247]. Ceramic whiskers, with high structural perfection [248], used to reinforce Bis-GMA dental resins have been shown to allow use in large stress-bearing restorations [223], [249–251]. Whisker combination with silica particles as reinforcement create composites with strength and toughness substantially higher than dental resin composites without [249–252].

Oxygen present in blood inhibits the radical polymerization reaction of the resin composite paste implanted in vivo, which means the composite would contain an uncured surface exposing the bioactive fillers at the implant surface and inducing bone bonding [245], [247]. Bis-GMA-based composites can be used as prefabricated implants with abraded surfaces, which has been shown to increase bone-bonding by exposing the bioactive fillers at the implant surfaces [235].
1.3.2 Inorganic-Organic

Polymethylmethacrylate (PMMA) cement is the first well-known cement used for orthopedic applications [219]. PMMA cement is formed by mixing a monomer liquid and a PMMA powder. The monomer liquid is usually a mixture of methylmethacrylate (MMA), accelerator, and a stabilizer, but the PMMA powder usually contains the polymer, copolymers of different molecular weights, an initiator, a radio-opacifier, an accelerator, and sometimes antibiotics and dye [253], [254]. Until now PMMA cement is still considered a major bone cement option in surgery for its years of successful cases, presenting excellent mechanical strength to support implants. However, PMMA has a number of drawbacks, including: lack of bioactivity, poor biocompatibility due to exothermic polymerization and the presence of methacrylate monomer, and possible inflammation caused by debris [254]. One solution to modify the PMMA properties is to add bioactive fillers to PMMA matrix. The advantages of adding bioactive filler to PMMA are the stimulation of bioactivity in PMMA bone cement, better mechanical properties, resulting monomer content decrease to reduce its toxicity to living tissue, and to lower exothermal polymerization reaction. Glass ceramics are widely investigated as filler candidates [255], [256]. Before mixing bioactive fillers to PMMA cement, silane treatment is required. Silane treatment of filler particles was shown to reduce the amount of monomer required to reach sufficient wetting of the filler particles, thus allowing the addition of more filler to the composite to increase its mechanical properties; and allow chemical bonding between the monomer and the covering layer of the coupling agent,
which further improves the mechanical properties and the water resistance of the composite [225], [257], [258]. Makamura et al. [225] mixed silanated apatite-wollastonite glass-ceramics (AW-GC) particles (70 wt%) to PMMA cement, and both *in vitro* and *in vivo* testing confirmed the bioactivity of prepared composite cement. Additionally, it was observed the properties of PMMA itself can play roles in mechanical performance, setting and bioactivity of composite, usually smaller powder diameter and high molecular weight were preferred [225], [259]. As a challenger to AW-GA, bioactive glass exhibited higher bioactivity and mechanical strength. Shinzo et al. [31] used bioactive glass beads, AW-GC particles and HA as fillers (70 wt%) for PMMA matrix. The *in vivo* study showed a significantly higher affinity index value of glass beads filled cement up to 8 weeks after implantation. It was also reported the bending strength of glass beads-filled cement was significantly higher than that of comparisons, attributed to the fact that a spherical shape with a smaller particle of glass beads improved the filling effects of cement, and a smaller spherical shape and a glassy phase resulted in good silane treatment, thereby forming many siloxane bonds (Si-O-Si) on the glass beads to result in good adhesion of glass to PMMA matrix. Their continuous work evaluated the impact of filler size and content of glass beads on PMMA cement, demonstrating smaller filler size [31] and suitable glass bead content (60%) [260] are favored on considering mechanical properties and osteoconductivity.
1.3.2.1 Calcium Silicate with Biodegradable Polymers

Bone and teeth are composed of a collagen organic matrix and a hydroxyapatite mineral phase [261]. Cohesion promoters are often used in an attempt to reduce endontic cements’ susceptibility to brittle fracture and washout from fluid contact [262]. Elastomeric additives like chitosan could help to overcome inherently brittle properties of ceramic materials [263–265], and gelling agents like hydroxypropyl methylcellulose, carboxymethyl cellulose, alginate, chitosan, modified starch could protect from washout of cement [266–268]. It has been clearly shown that the cohesion promoters have a negative effect on the hardening reaction of calcium silicate cements and that mechanical properties do not continually increase with an increase in cohesion promoters [262].

Moldability and syringe injectability are two key aspects contributing to the success of a cement in biomedical applications that require a good working and setting time [269]. Chen et al. [262] showed that more liquid addition was needed for hardening powders with higher calcium (Ca) contents. Their research indicated that calcium was a promoter of crystallinity in the SiO₂-CaO system and in turn shortened setting times. Results showed a hydration mechanism similar to that of Portland cement. Setting time varied by cement and testing conditions but in general Chen et al. [262] showed that SiO₂-CaO cements set in 10-29 minutes, taking less time with increasing calcium content inversely proportional to the Si/Ca ratio [262]. The addition of 5% and 10% gelatin increased the setting time to 25-69 minutes and 108-282 minutes respectively by Gillmore needle (ISO 9917-1) [262]. Others have developed sol-gel-derived tricalcium or dicalcium silicate
cements with initial setting times over 1 hour [43], [53]. In another study calcium silicate cement was shown to set in 22 minutes, while calcium silicate with a chitosan oligosaccharide modifier was shown to set in 27 minutes [269]. Calcium silicate cements with a gelatin addition was shown to set with water in 40 minutes and with water and chitosan oligosaccharide in 55 minutes [269]. Lin et al. [270] concluded that setting times increased with an increase in carboxymethyl chitosan concentration. It was hypothesized that carboxyl groups might bind to calcium ions on the surface of calcium silicate particles, which would improve washout and brittle properties, but could inhibit calcium silicate hydrate formation prolonging setting time [262]. Excessive polyanions destroy the balance of ceramic cements leading to slow or no setting [262].

Diametral tensile stress (DTS) of SiO$_2$-CaO cements were 2.0, 2.6, 2.0, 1.0 MPa with increasing calcium content. Cement composed of 40mol% SiO$_2$ and 60mol% CaO showed DTS values of 2.0, 2.1, and 1.7 MPa for gelatin contents of 0, 5, 10% respectively. The lowest strength was seen from cements with the densest and quickest setting times by Chen et al. [262]. Additionally, macropores can promote biodegradation and bioresorption, which can be critical in craniofacial applications [93]. After immersion in SBF or Tris-HCL cements with cohesion promoters were shown to have significantly higher strength than corresponding cements set outside of SBF or Tris-HCL [269]. The elastic moduli of CSC systems with and without gelatin additions performed in the range of 26.8-43.3 MPa. Ductility can be increased through the uniform dispersion of organic molecules through the ceramic cement[262].
Apatite has been shown to form on the surface of chitosan modified cements after 12 hours, attributed to the rapid dissolution of Ca(OH)$_2$ [270]. Crystalline apatite formation is delayed 1 to 2 days but the amount of apatite on chitosan calcium phosphate cement is the same after soaking in SBF for 3 days [270]. A synergistic combination of chitosan and calcium phosphate cements has shown a better tissue response than pure calcium phosphate cement [264].

Powders with higher silica (SiO$_2$) contents tended to have more dense large particles. With the addition of gelatin a colloidal gel formed densely hydrated cement interwoven with the calcium silicate cement [262]. Gelatin has been shown to partially encapsulate particles and impede hydration of calcium silicate cements when in contact with water [269]. These hybrid gel cements resist washout and were attributed to the adhesive property and negative charge of gelatin prohibiting the penetration of cement paste by liquid [262].
Objectives:

Currently, there are many forms of biocements consisting of calcium, phosphorous, and silicon as their main constituents. As biocements, Monetite cements (DCPA) are traditionally avoided because they are plagued by a rapid exothermic reaction that significantly reduces the strength of resulting ceramics and would kill tissue in a surrounding implantation site. The objectives of the remaining chapters are as follows:

1) Creation of a consistent, microwave assisted, production method of DCPA to be polymerized with nanosilica sol.

2) To show that silicon in the form of a nanosilica sol will have similar positive effects on DCPA Monetite cement strength as shown by the discussions above. An increase in the mechanical strength is expected as a result of the nanosilica sol hydrate gel filling the voids and defects in normal DCPA cement.

3) To demonstrate that polymerization with nanosilica sol will have positive effects on the biocompatibility of DCPA Monetite cements because of the necessity of silicon in the bone remodeling process.

4) To display that DCPA with nanosilica sol will create a resulting cement suitable for trial in clinical settings while retaining the increased strength.
Chapter 2

Development of High Strength Dicalcium Phosphate Anhydrous Cement with Nanosilica Sol

Abstract

Dicalcium Phosphate Anhydrous (DCPA, Monetite) is generally overlooked in biological applications because it has limited load bearing uses: its rapid creation in a high temperature reaction results in a material with low strength and high brittleness. This study implements a method of polymerizing a novel produced, microwave assisted, DCPA powder with nanosilica sol (SiO₂). The result is a material with significantly improved mechanical properties, including a dramatic increase in the compression strength (40-60MPa) under mechanical loads. This study also discusses the formation and effects of a nanosilica sol (SiO₂) addition on the setting time, diametral tensile strength and morphology of the resulting composite cement.
2.1 Introduction

Over the last several decades, there has been a great deal of research in ceramics and their composites used to augment or replace bone; the ceramics used for these purposes are classified as bioceramics. Bone is an intricate, three dimensional structure with a number of organic components, including collagen type I, osteoblasts, and organic acids like citrates and lactates, which give natural bone its flexibility and tensile strength. The inorganic components of bone, like calcium and phosphorous, are organized into an poorly crystallized, carbonated apatitic calcium phosphate structure that gives bone its rigidity and compressive strength [271]. Calcium phosphate based ceramics have a number of desirable characteristics for medical applications, including: biocompatibility; relative inertness to body fluids; high compressive strength; and an aesthetically pleasing appearance [272]. In medicine there is a clear need for an injectable, moldable, self-setting bone filler, such as the material pioneered by Brown and Chow, who developed a self-setting calcium phosphate cement that forms into hydroxyapatite in-situ [201], [273]. Calcium phosphates are relatively easy to synthesize and are used in medical applications because of their similarity with the natural, inorganic component of bone [274], [275]. However, there are several challenges when working with bioactive ceramic cements that are used in injectable paste forms, including susceptibility to brittle fracture and disintegration when in contact with body fluids, such as blood [266].

Various forms of calcium phosphate based bioceramics are used in biomedical applications, such as: tetra-calcium phosphate [TTCP, Ca4(PO4)2O], tri-calcium phosphate [α-TCP, α-Ca3(PO4)2 and β-TCP, β-Ca3(PO4)2], di-calcium phosphate
anhydrous [DCPA, Monetite, CaHPO$_4$], di-calcium phosphate dihydrate [DCPD, Brushite, CaHPO$_4$.2H$_2$O], and octacalcium phosphate [Ca$_8$H$_2$(PO$_4$)$_6$.5H$_2$O, OCP], which is a metastable phase in the CaP family [276]. Additionally, monocalcium phosphate anhydrous [MCPA, Ca(H$_2$PO$_4$)$_2$] and monocalcium phosphate monohydrate [MCPM, Ca(H$_2$PO$_4$)$_2$.H$_2$O] have the highest solubility in and around neutral pH values but are not used as biomaterials due to their high acidity [277]. Although DCPD demonstrates favorable biological qualities, its solubility rate does not make it useful for some medical applications in the same way that DCPA’s greater solubility does [278]. This is partially due to the fact that solubility has a direct relation to the biocompatibility of a substance and the ability of the substance to be replaced (eventually) with living tissue. The presence of CO$_3^{2-}$ is the main source of lattice distortion, creating microstresses and crystalline defects that are the fundamentals of solubility [279]. Small particle sizes and the presence of CO$_3^{2-}$ will alter the solubility, and these characteristics can be designed to mimic biological apatite. In
Table, the Ca/P ratio is critical to the creation, acidity and solubility of the phase. More specifically, the Ca/P ratio of 1.67 is closest to biological bone, and ratios below 1 are highly acidic; as the Ca/P ratio decreases, the biological solubility increases [279].
Table 2.1: Various Phases of Calcium Phosphate Cements [280]

<table>
<thead>
<tr>
<th>Phases</th>
<th>Chemical Formula</th>
<th>Mineral Name</th>
<th>Ca/P ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyapatite (HA)</td>
<td>Ca_{10}(PO_4)_6(OH)_2</td>
<td>Apatite</td>
<td>1.67</td>
</tr>
<tr>
<td>$\alpha$-Tricalcium phosphate ($\alpha$-TCP)</td>
<td>Ca_3(PO_4)_2</td>
<td>Whitlockite</td>
<td>1.5</td>
</tr>
<tr>
<td>$\beta$-Tricalcium phosphate ($\beta$-TCP)</td>
<td>Ca_3(PO_4)_2</td>
<td>Whitlockite</td>
<td>1.5</td>
</tr>
<tr>
<td>Dicalcium phosphate dihydrate (DCPD)</td>
<td>CaHPO_4.2H_2O</td>
<td>Brushite</td>
<td>1.0</td>
</tr>
<tr>
<td>Dicalcium phosphate anhydrous (DCPA)</td>
<td>CaHPO_4</td>
<td>Monetite</td>
<td>1.0</td>
</tr>
</tbody>
</table>

The second component of the cement created in this experiment is a nanosilica sol, which is added to set and strengthen the cement. Colloids consist of solid particles with diameters of 1-100 nm in suspension [193], [281]. A sol is a colloidal suspension (simplest dispersion) of solid particles in a liquid [282], leading to a completely dispersed solution because particles do not adhere to adjacent particles [283]. Coagulation of colloidal particles will result in a color change as agglomerations approach 0.5 µm [283]. A gel of interconnected submicron pores is created where there is adhesion between particles and silica [193], [281], [283]. Distinguishing a gel from a paste can be done by the deformation behavior: hardness and elasticity are characteristic of a gel, while a paste is between plastic and elastic [283].

In cement systems, the critical concentration of calcium ions in solution (which is required to coagulate colloidal silica) increases with decreasing particle size [284]. The effect is most marked at lower pH. Larger particles in a mixture are shown to preferentially coagulate and separate from smaller ones. For coagulation independent of
particle size, a critical number of calcium ions must be absorbed per square nanometer of silica surface. The silica surface contains negative charges, and each adsorbed calcium ion was found to create one additional negative charge on the surface so that each adsorbed calcium ion retains one positive charge. Coagulation is probably due to attraction between surfaces due to positive and negative sites. Allen and Matijevic [285], using Ludox colloidal silica, found that for a given particle size of silica and pH, aggregation occurred at a critical concentration of calcium ions. Depasse et al. [286] suggested alkaline cations are able to coagulate silica between pH of about 7 and 11. It was suggested that the attraction forces are related to acid-base interparticle bonding of salt bonding by acidic cations and silanol-dissociated silanol bonds.

An interesting body of research [287–292] has studied amorphous colloidal silica and aggregate to manufacture self-compacting concretes characterized by low heat development. Results showed less bleeding water, segregation, and improved cohesiveness of fresh cement. Collepardi et al. [287] reported performance of compressive strength in the magnitude of 55 MPa at 28 days, and it was found that amorphous colloidal silica does not negatively affect the durability performance. Aburatani et al. [293] noted that colloidal silica could control the mechanical behavior of organically modified silicate gels with calcium nitrate through sol-gel processing for soft tissue replacements.

Colloidal silica has been shown to act as a bioactive source of silica with the ability to form cements in less traditional methods. The mechanical properties of calcium sulfate
based bone cements can be improved through the addition of colloidal silica: a condensed phase produced from the polymerization process of colloidal silica fills the micropores. The polymerized samples showed improvements in the setting time, compressive strength, and structural properties, versus a control sample of cement created without silica. Additionally, these experiments demonstrated that the presence of silica encouraged the formation of apatite [294].

Bioactive glasses were invented and investigated first by Hench et al. [59] in response to a need for a biomedical implant after talking with an Army colonel who had returned from Vietnam. Since 1969, Hench has been a primary investigator of bone bonding and bioglass ceramic systems, while many researchers have investigated silica systems. For example, Izquierdo-Barba et al. [295] synthesized a bioactive glass with a composition of 80 SiO$_2$–20 CaO (in mol%) by the sol-gel method. The silica sol-gel process traditionally includes three steps: 1) the hydrolysis of an alkoxy silane, 2) the condensation of hydrated silica to form siloxane bonding, and 3) polycondensation by the linkage of an additional silanol group to make cyclic oligomers [296].

DCPA is a material with innate biocompatibility, ease of synthesis, and inexpensive, widely available sources; thus, DCPA should be the material of choice for orthopedic and dental repair applications [297]. However, none of the current DCPA cements are commercially available commercially today, and all require large amounts of heat in their setting processes - which is harmful to surrounding tissue and makes current DCPA cements infeasible for injected human body implants. The advantage of the new,
microwave assisted, DCPA cement synthesis process described in this study is that minimal heat is released in the setting process yet there is a large increase in the strength of the cement. There are minimal changes in the setting time, as compared to a control sample, and this setting time can be controlled by varying the liquid to powder ratio and particle size of the cement powder. Adding nanosilica sol to DCPA allows the cement to increase in mechanical strength while a favorable DCPA Monetite structure for bone tissue applications remains. Much of this can be attributed to the polymerizing or coagulating effect of amorphous nanosilica sol.

2.2 Materials and Methods

2.2.1 Preparation of Powders

The raw DCPA (Dicalcium Phosphate Anhydrous, Monetite) powder is synthesized first, before being polymerized with silica (SiO₂). The DCPA cement powder is synthesized from a calcium hydroxide (Ca(OH)₂=74.10 g/mol, BDH Laboratory Supplies, Poole, UK) reagent, setting solution, and de-ionized water (DI). The setting solution for the cement reaction is prepared by combining sodium bicarbonate (NaHCO₃>99.7%, Fisher Scientific, Fair Lawn, NJ), de-ionized water, citric acid monohydrate (CAM, 100% assay, OCCOOH(CH₂COOH)₂*H₂O, Fisher Scientific, Fair Lawn, NJ), and phosphoric acid (85% H₃PO₄, EMD Chemicals Inc., Gibbstown, NJ).
To yield 15ml of complete setting solution, the setting solution consists of 6g sodium bicarbonate, 0.0032g citric acid monohydrate, 1.95ml DI water, and 13.05ml phosphoric acid respectively. Sodium bicarbonate facilitates the reaction of the initial powder. Citric acid monohydrate helps to control the setting time in some cases, acting as a setting retardant in modifying the injectability of the cement [298–300]. Deionized water acts to dilute the setting solution reducing its viscosity. Phosphoric acid acts as a source of phosphorous and is added by titration over the period of one hour, due to the high reactivity of the solution producing CO₂ gas.

Cement paste is initially prepared by manually mixing calcium hydroxide with DI water and setting solution using a mortar and pestle. A slurry of DCPA is made by dispersing 12.3g of calcium hydroxide in 25ml of DI water, completely dissolving the calcium hydroxide in the water through mixing for one minute. Inspection shows that after one minute mixing, all agglomeration of calcium hydroxide particles is removed. Next, 15 ml setting solution is added to the dispersed calcium hydroxide and an exothermic reaction takes place reaching temperatures up to 72°C (160°F). This is mixed with a mortar and pestle while the reaction is taking place to facilitate the full reaction of components. The resulting paste is then microwaved (Emerson, max. power 100 W, 2450 MHZ, NJ, USA) for ten minutes to dehydrate the cement, stopping the reaction. The resulting hard cement is crushed with a mortar and pestle to create a powder consisting of DCPA.
2.2.2 Comparison of Three Colloidal Silicas

Three silica sources were compared for their effects on strength and setting time: 1) Ludox® SM colloidal silica (GRACE Davidson, Columbia, Maryland); 2) Ludox® HS-30 colloidal silica (GRACE Davidson, Columbia, Maryland); and 3) Ludox® HS-40 colloidal silica (GRACE Davidson, Columbia, Maryland). Each are slightly different in composition and the strength of each is optimized. Further mechanical strength measurements are only conducted on the optimum composition.

Ludox® SM Colloidal Silica is a 30wt. % concentration of SiO$_2$ suspension in H$_2$O. The surface area of the solution is ~345m$^2$/g and the density is 1.22g/ml at 25 °C. The pH of this solution is 10.0, which could also play a role in the hydration of the final cement. The molecular weight is 60.08 g/mol.

Ludox® HS-30 Colloidal Silica is a 30wt. % concentration of SiO$_2$ suspension in H$_2$O. The surface area of the solution is ~220m$^2$/g and the density is 1.21g/ml at 25 °C. The pH of this solution is 9.8, which could also play a role in the hydration of the final cement. The molecular weight is 60.08 g/mol.

Ludox® HS-40 Colloidal Silica is a 40wt. % concentration of SiO$_2$ suspension in H$_2$O. The surface area of the solution is ~220m$^2$/g and the density is 1.3g/ml at 25 °C. The pH of this solution is 9.8, which could also play a role in the hydration of the final cement. The molecular weight is 60.08 g/mol.
2.2.3 Fabrication of Cement Samples

Cement samples are prepared by manually mixing the prepared DCPA powder with colloidal silica (GRACE Davidson, Columbia, Maryland, Ludox® SM colloidal silica) using a mortar and pestle. To create one test sample, 1g of DCPA is combined with 0.5 ml of Ludox® SM Colloidal Silica and placed into a stainless steel powder compaction die (circular mold 12mm in diameter). The cement is compressed with 0.8 MPa of hand pressure at room temperature into powder compaction dies. Samples measuring 12 mm in diameter and 3-4 mm in height are prepared using the stainless steel powder compaction die. This mold is used to make the samples for compression testing and diametral tensile testing.

2.2.4 Setting Time Measurement

Setting time is measured using the Gillmore needle apparatus (Humboldt Mfg. Co., Schiller Park, IL). Initial ($t_i$) and final ($t_f$) setting times of cement samples are determined according to the international standard ISO 9917-1 for dental cement and ASTM C266-89 [301], [302]. According to the American Dental Association, specification No. 61115, complies with ASTM standards C91, C141, C150, C266, C414; AASHTO T154. After preparation of DCPA with silica, the cement is immediately placed in 7.8 mm PVC molds. These PVC mold surfaces are made smooth by pressing on the surface of the mold
with glass slides. The Gillmore needle has two stainless steel cylindrical flat-end needles. Initial setting time is measured by a weight of ¼ lb (115.12 g) and the diameter of 1/12" (2.12mm). Final setting time is measured by a needle of diameter of 3/16" (4.8mm) and weight of 1 lb (453.6 g). Measurements are taken every minute until the surface of the cement sample is not indented 1mm. Two incubation environments are utilized consisting of a room temperature environment at 22 °C and a cold dry fridge environment at 17 °C. Six parallel experiments were carried out for each group. The two temperature settings were chosen and investigated in order to mimic and understand the preparation and operation environments of bone cement in surgery.

2.2.5 Characterization of Powders

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

The surface morphology of DCPA powder samples is visualized using a Scanning Electron Microscope (SEM, Hitachi S-4800, Hitachi Corp, Tokyo). The samples are crushed into powders and then mounted on conducting carbon tape. An accelerating voltage of 10 KV, magnification of 1.00 K and distance of 13 mm is used.
The Fourier Transform Infrared Spectroscopy (FTIR, UMA-600 Microscope, Varian Excalibur Series) is conducted on powders after microwave (Emerson, max. power 100 W, 2450 MHZ, NJ, USA). Each sample was ground using a pestle and mortar then placed to cover the diamond crystal cell. The background was collected before the measurement of each specimen.

2.2.6 Phase Composition and Morphology of Cement

The cements were allowed to set in order to analyze the phase composition, morphology, and mechanical properties. Morphology was monitored using a scanning electron microscope (SEM, Hitachi S-4800, Hitachi Corp, Tokyo). The samples were first allowed to set completely for 24 hours in an incubation environment at 37°C before being mounted on conducting carbon tape. Samples were not coated and were visualized with accelerating voltage of 10 KV. The Fourier Transform Infrared Spectroscopy (FTIR, UMA-600 Microscope, Varian Excalibur Series) was conducted on particles of crushed cement.

2.2.7 Compression Strength

The powder compaction die samples produced a disk specimen then tested in a uniaxial INSTRON Machine. The crosshead rate of loading according to the American Dental Association was set to 0.5 mm/min [301]. A randomized statistical group of 6 samples
was created and tested for each testing variation. Nanosilica sol is added in various concentration to cement samples in solutions of 100%, 80%, 60%, 40%, and 20% GRACE Davidson, Columbia, Maryland, Ludox® SM colloidal silica, diluted with DI water, and evaluated for mechanical properties.

Another test procedure looked at cement samples and mechanically tested for strength as the samples harden for up to 24 hours in a 37°C incubator. These tests were conducted at 30 minutes, 1 hour, 2 hours, 4 hours, 10 hours, and 24 hours after mixing. This was done to provide information for the potential clinical applications of the cements, specifically looking at the time that patients might need to remain immobile before set cement gains strength.

### 2.2.8 Control Sample Comparison

Commercial cement products were chosen and investigated as mechanical strength control samples. The first control sample was osteoconductive bone void filler, CEM-Ostetic™ (Berkeley Advanced Biomaterials, San Leandro, CA). The second control sample was another commercial cement, Calcibon® (Biomet Europe, Synthetic Calcium Phosphate). These samples were mixed per manufacturer’s instructions and placed in the same molds (12 mm diameter, 3-4 mm height), prepared using a stainless steel powder compaction die with 0.8 MPa of hand pressure.
2.2.9 Diametral Tensile Strength and Modulus

The inability to fixture DCPA cements for tensile testing requires the use of the diametral tensile strength testing or Brazilian test. The powder compaction die samples produced a disk specimen then tested in a uniaxial INSTRON Machine. The crosshead rate of loading according to the American Dental Association is set to 0.5 mm/min [301]. A statistical group of 6 samples was created and tested for samples of DCPA + Ludox® SM colloidal silica. The samples were tested after the DCPA powder was mixed and set for 48 hours in a 37°C environment. The diametral tensile strength of each specimen was calculated using the relationship defined as \( \text{DTS} = \frac{2P}{\pi bw} \), where \( P \) is the peak load (Newtons), \( b \) is the diameter (mm), and \( w \) is the thickness (mm) of the specimen. The maximum load at failure was obtained from the recorded load-deflection curve. The elastic modulus is the slope of the linear elastic portion of the load-deflection curve.

2.2.10 Statistical Analysis

Analysis of variance (ANOVA) was used to evaluate significant differences between means in the measured data. Significance of standard deviations in the measured data, from each specimen, under each experimental condition, was evaluated. In all cases an alpha of 0.05 was used and results were considered statistically significant with a P-value of less than 0.05.
2.3 Results

2.3.1 Powder Morphology

Powder morphology results for DCPA (Figure 1, Figure 2) and DCPA+ SiO₂ (Figure 1, Figure 2) cement powders show clusters of plate like structures. Samples of DCPA+ SiO₂ bonding can be seen interlocked in the powders as silica works into the calcium phosphate structure (Figure 1). The plates show no specific orientation but a higher level of porosity can be seen in the DCPA powders (Figure 1).

The particle size of blank powders is a highly referenced characteristic of cements, and so is included in the discussion. Figure 3 and Figure 4 show the ground DCPA particles before mixing with silica. The ground cement samples of DCPA+ SiO₂ are shown in Figure 3, Figure 4, and Figure 5, but both are powders after the microwave dehydration process. DCPA powders show particles sizes from 0.5 to 5 micrometers. DCPA+ SiO₂ powders create particles that are much denser and slightly larger, at 2-20 micrometers.
Figure 2-1: DCPA Powder after Microwave, 7000x Magnification

Figure 2-2: DCPA Powder after Microwave, 5000x Magnification
Figure 2-3: DCPA+ SiO₂ Powder after Setting, 15000x Magnification

Figure 2-4: DCPA+SiO₂ Powder after Setting, 5000x Magnification
2.3.2 Phase Composition of Cements Powder

Cement samples were cured at 37°C in 100% humidity, then were dried and crushed for XRD analysis. The XRD pattern of DCPA and DCPA+ SiO₂ both exhibit the characteristic peaks of DCPA (shown in Figure 2, Figure 3, Figure 4, Figure 5). In addition, patterns belonging to trace amount of unreacted Ca(OH)₂ were also observed. The amount of Ca(OH)₂ was estimated to be around 5.99%, based on the JADE software analysis. The overlaid XRD scan of DCPA against the XRD scan of DCPA+ SiO₂ shows that the additional SiO₂ is amorphous due to the lack of new peaks formed in the XRD scan. This lack of SiO₂ crystallinity is expected. Pure DCPA is identified by four distinct peaks observed on the pattern where 2θ values are 27°, 28°, 31° and 33°.
Figure 2-6: DCPA Characteristic Peaks

Figure 2-7: DCPA with Silica Characteristic Peaks
Figure 2-8: DCPA Characteristic Peaks Showing Ca(OH)$_2$ (Portlandite)
The FTIR data shown in Figure 2-9 are observations of DCPA and DCPA+ SiO₂ showing bands of \( \text{PO}_4^{3-} \) and \( \text{CO}_3^{2-} \) with no significant difference observed. Scans were each corrected for background noise and demonstrate a slight increase in \( \text{PO}_4^{3-} / \text{HPO}_4^{2-} \) bands, which is indicative of a lattice change.
Figure 2-10: FTIR data of DCPA cement and DCPA+ SiO\textsubscript{2} cement powder, P-PO\textsubscript{4}\textsuperscript{3-}/HPO\textsubscript{4}\textsuperscript{2-}, C-CO\textsubscript{3}\textsuperscript{2-}.

### 2.3.3 Cement Morphology

The nanosilica sol concentration in DCPA can be varied to achieve the desired strength and setting time. This study focused on creating a strong paste that could be injected by a syringe. Optimization of this cement created cement which was injectable for 6-12 minutes after mixing. The SEM images presented (Figure, Figure, Figure, Figure) show the microstructure of set DCPA+ SiO\textsubscript{2} cement samples.
DCPA+SiO$_2$ has an irregular combination of two well-defined regions. Region one consists of irregular spheres and trapezoids with higher concentrations of calcium. Their diameters vary from 1 to 10 micrometers, and entangled particles with silica binding the cement together are apparent in the SEM images. In the presence of silica, these DCPA particles show a high degree of symmetry in terms of the order layer structure and distribution. Region number two has a plate-shaped morphology consisting of a higher amount of silica with regular structure. A smooth surface was observed on these plate shapes and some cracking was seen in these surfaces. Figure shows both regions on a sample of set DCPA+ SiO$_2$ cement: spectrum one shows a higher level of silica in the more plate like structures, and spectrum two shows higher levels of phosphorous and calcium.

Small surface cracks were seen in the samples containing higher silica content after the cement disks had been stored in a high temperature oven overnight with 0% humidity. No surface cracks were seen in cement samples that were allowed to set and be preserved in 100% humidity.
Figure 2-11: DCPA+ SiO$_2$ 48hrs Set, Region 1 Left, Region 2 Right

Figure 2-12: DCPA+ SiO$_2$ 48hrs Set, Region 1 Left, Region 2 Right
Figure 2-13: DCPA+ SiO₂ 48hrs Set, Region 1 Left, Region 2 Right

Figure 2-14: DCPA+ SiO₂ 48hrs Set, Two Regions Visible
2.3.4 Setting Time

After mixing, DCPA cements set in the range of 4-14 minutes initially and 6-20 minutes final setting time. With silica, DCPA cements set in the range of 5-10 minutes for initial setting time and 7-28 minutes finally. In this study, adjusting the silica concentration allowed control over the cement setting time. Initial and final setting times are presented in Table and Table for temperature conditions of 7 °C and 22 °C. Each sample is tested five times and averaged. One standard deviation is presented following the average setting time; measurement of the exact setting time is rounded to the nearest minute for sample periods.

Table 2.2: Setting Time at 7 °C

<table>
<thead>
<tr>
<th>Composition</th>
<th>Setting Time Initial (min)</th>
<th>Setting Time Final (min)</th>
<th>Syringe Injectability (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCPA + SiO₂*</td>
<td>9.8 +/- 1.48</td>
<td>27.8 +/- 2.6</td>
<td>7</td>
</tr>
<tr>
<td>DCPA + H₂O*</td>
<td>9.8 +/- 0.89</td>
<td>20 +/- 0.71</td>
<td>6</td>
</tr>
<tr>
<td>DCPA + SiO₂^</td>
<td>6 +/- 0.71</td>
<td>10 +/- 0.71</td>
<td>0</td>
</tr>
<tr>
<td>DCPA + H₂O^</td>
<td>4.4 +/- 0.55</td>
<td>6.2 +/- 0.83</td>
<td>0</td>
</tr>
</tbody>
</table>
* 1g CPC + 0.833ml  Ludox SM Silica or H₂O
^ 1g CPC + 0.5ml  Ludox SM Silica or H₂O

Table 2.3: Setting Time at 22°C

<table>
<thead>
<tr>
<th>Composition</th>
<th>Setting Time Initial (min)</th>
<th>Setting Time Final (min)</th>
<th>Syringe Injectability (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCPA + SiO₂*</td>
<td>9.2 +/- 0.83</td>
<td>28 +/- 0.70</td>
<td>8</td>
</tr>
<tr>
<td>DCPA + H₂O*</td>
<td>13 +/- 0.71</td>
<td>20 +/- 0.71</td>
<td>8</td>
</tr>
<tr>
<td>DCPA + SiO₂^</td>
<td>5.6 +/- 0.55</td>
<td>7.4 +/- 0.55</td>
<td>1</td>
</tr>
<tr>
<td>DCPA + H₂O^</td>
<td>5.8 +/- 0.45</td>
<td>11.4 +/- 0.54</td>
<td>1</td>
</tr>
</tbody>
</table>
* 1g CPC + 0.833ml  Ludox SM Silica or H₂O
^ 1g CPC + 0.5ml  Ludox SM Silica or H₂O
2.3.5 Comparison of Three Silica Sources

Compressive strength of calcium phosphate based cements is the most commonly reported strength results, and from these compressive strength results it is possible to derive the mechanical properties of the cements. Tensile testing is not generally done due to difficulty in fixturing ceramics. The compressive modulus is determined from the slope of the linear region in the stress-strain diagram. Strength and strain are calculated using basic mechanics equations. Three silica sources were compared for their effects on strength and setting time, Ludox® SM, Ludox® HS-30, and Ludox® HS-40 colloidal silica. Each silica source is slightly different and the strength of each was optimized, with further mechanical strength measurements conducted only on the optimum composition.

Table shows compression strength results with two different amounts of unreacted Ca(OH)$_2$. Compression strength (MPa) of DCPA+ SiO$_2$ cements are in Figure. Each bar is the mean value of the compressive strength of six samples with an error bar showing the standard deviation (mean ± standard deviation; n = 6).

Table 2.4: Compression Strength of Varying Ludox Silica Sources with Two Amounts of Unreacted Ca(OH)$_2$, Low Left, High Right

<table>
<thead>
<tr>
<th>Sample DCPA</th>
<th>Strength (MPa)</th>
<th>Sample DCPA</th>
<th>Strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>18.23 +/- 3.13</td>
<td>Water</td>
<td>8.87 +/- 1.45</td>
</tr>
<tr>
<td>SiSM</td>
<td>59.81 +/- 3.95</td>
<td>SiSM</td>
<td>42.97 +/- 3.34</td>
</tr>
<tr>
<td>HS30</td>
<td>44.99 +/- 11.35</td>
<td>HS30</td>
<td>32.64 +/- 2.07</td>
</tr>
<tr>
<td>HS40</td>
<td>55.10 +/- 7.17</td>
<td>HS40</td>
<td>40.89 +/- 3.38</td>
</tr>
</tbody>
</table>
Figure 2-15: Compressive Strength of DCPA+ SiO$_2$ Samples with Two Amounts of Unreacted Ca(OH)$_2$, Low Left, High Right

### 2.3.6 Compression Strength

Compression tests showed typical brittle fracture in the cements, and that increasing silica content also increased strength (up to 100% concentration of nanosilica sol) as shown in Table. For reference, Figure shows a characteristic stress strain curve in an initial linear elastic compression, followed by a yield point and failure soon after.
Table 2.5: DCPA+ SiO$_2$ Mechanical Properties

<table>
<thead>
<tr>
<th>DCPA+ SiO$_2$ Percentage is made 100 with added water</th>
<th>Compressive Strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Ludox SM</td>
<td>42.97 +/- 3.34</td>
</tr>
<tr>
<td>80% Ludox SM</td>
<td>30.80 +/- 3.69</td>
</tr>
<tr>
<td>60% Ludox SM</td>
<td>21.52 +/- 1.39</td>
</tr>
<tr>
<td>40% Ludox SM</td>
<td>18.99 +/- 2.86</td>
</tr>
<tr>
<td>20% Ludox SM</td>
<td>9.79 +/- 0.98</td>
</tr>
<tr>
<td>0% Ludox SM</td>
<td>8.87 +/- 1.45</td>
</tr>
</tbody>
</table>

Figure 2-16: Compressive Strength of DCPA Samples Versus Different Ratios of Nanosilica Sol (Pure DCPA is 0.5ml H$_2$O + 0.0ml Ludox SM)
Many commercial products that can be resorbed and injected are available for testing, and two of particular interests are Calcibon® and CemOstetic™. For this study, these commercial products were prepared in two ways: first, following the manufacturer’s instructions, and second by following the procedure for microwave dehydration outlined in this study. The purpose of this evaluation was to examine commercially available products and the effect of microwaving these powders before creating sample pellets to investigate their strength. Results are shown in Table .
Table 2.6: Comparable Commercial Product Strength Test as Control

<table>
<thead>
<tr>
<th>Commercial Product</th>
<th>Strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcibon</td>
<td>32.997</td>
</tr>
<tr>
<td>Calcibon+Microwave</td>
<td>10.752</td>
</tr>
<tr>
<td>CemOstetic</td>
<td>17.659</td>
</tr>
<tr>
<td>CemOstetic+Microwave</td>
<td>9.256</td>
</tr>
</tbody>
</table>

2.3.8 Strength While Setting

Table shows the setting strength as the sample hardens at room temperature, with the time count starting when the samples are mixed. The data in Figure must be looked at as only a reference, due to the fact that initial setting cements have no true strength.

Table 2.7: Compression Strength vs. Setting Time of DCPA+ SiO₂ (Clinically Interesting Points)

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>Strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 hr</td>
<td>3.21 +/- 0.63</td>
</tr>
<tr>
<td>1 hr</td>
<td>10.33 +/- 1.49</td>
</tr>
<tr>
<td>2 hr</td>
<td>16.12 +/- 6.77</td>
</tr>
<tr>
<td>4 hr</td>
<td>27.96 +/- 1.21</td>
</tr>
<tr>
<td>10 hr</td>
<td>44.96 +/- 4.48</td>
</tr>
<tr>
<td>24 hr</td>
<td>59.77 +/- 5.59</td>
</tr>
</tbody>
</table>
2.3.9 Diametral Tensile Strength

The diametral tensile strength, or Brazilian test, results in good tensile strengths of 3.187 +/- 0.288 MPa. Diametral tensile strength tests showed typical brittle fracture, with strength increasing as silica content increases, up to 100% concentration of nanosilica sol. A characteristic diametral tension test stress strain curve is shown for reference in Figure , an initial linear elastic compression is seen followed by a yield point and failure soon after.
2.4 Discussion

The present study presents a novel production procedure for DCPA cement polymerized with nanosilica sol, which transforms from a pliable and injectable paste like material at operating conditions to a rigid enough material for filling bone defects. Material property results include 5-10 minute initial self-setting cement in a simulated surgical environment at 22°C, while reaching compressive strengths upwards of 60 MPa, under the correct preparation conditions. Traditional DCPA cements harden through a mechanism consuming free Ca$^{2+}$, HPO$_4^{2-}$, H$_2$PO$_4^-$ ions to synthesize DCPA crystals, following the gradual dissolution of Ca(OH)$_2$ in acidic solution and releasing cations, which react with the phosphate anions, forming a coordinated network that consolidates into DCPA around the unreacted Ca(OH)$_2$. Unreacted Ca(OH)$_2$ in the hardened paste is attributed to the DCPA network formed around unreacted Ca(OH)$_2$ working as a barrier to stop further
contact of entrapped Ca(OH)$_2$ with the acidic environment. The availability of water during manufacture will affect the fraction of OH sites occupied by silicon [303], [304]. In this experimental procedure a network can be formed between Ca(OH)$_2$ and silica to stabilize silica during the cement setting.

2.4.1 Phase Composition of Cements

Multiphase calcium phosphate formulations allow researchers and clinicians to fully exploit the desirable properties of a particular phase by increasing or decreasing its presence in the cement to influence overall properties such as compressive strength, biocompatibility, end product phase and degradation rate [305]. When dicalcium phosphate dehydrate (DCPD) is heated above 60-180 °C, it transforms into a more stable DCPA form [306]. Both forms are used in medical calcium phosphate cements [78].

The overlaid XRD scan of DCPA against the XRD scan of DCPA+ SiO$_2$ shows that the additional SiO$_2$ is amorphous due to the lack of new peaks formed in the XRD scan. This lack of SiO$_2$ crystallinity is expected. In addition, patterns belonging to trace amounts of unreacted Ca(OH)$_2$ were observed and associated changes in the setting time and corresponding strength results were noted.
2.4.2 Cement Morphology

A uniform final particle size is important because increasing coagulation is seen with decreasing particle size. In a mixture of different particle sizes, larger particles can preferentially coagulate and separate from smaller ones in the presence of nanosilica sol [285]. A colloidal nanosilica sol forms a dense structure of hydrated cement, which is well indicated based on the contrast between figures of DCPA (Figure , Figure ) and those of DCPA with nanosilica sol (Figure , Figure , Figure ). The amorphous nature of the samples is reasonable because of the low temperature process of polymerization; heat-treating these samples would likely result in a crystalline structure [193], [281]. The gelling process initiated by nanosilica sol creates a particulate disordered structure similar to those seen by other researchers [283], [307] consistent with a hydrogel [308]. In the presence of salts, coagulated colloids become a paste and fluidize under high shear stresses, and the paste consists of both liquid and gel [283], [309], [310]. The suspension is able to flow but is strong enough to gel, so it is a combination of a sol and a gel similar to processes used by Hench et al. [311].

The nanosilica sol concentration in DCPA can be varied based on the required strength and setting time; in this study, concentrations were optimized to create a strong paste that could be injected by a syringe between 6 minutes and 12 minutes after mixing. In this experiment, DCPA+ SiO₂ has an irregular combination of two well-defined regions, with entangled particles with silica binding the cement together. In the presence of silica these DCPA particles show a high degree of symmetry in terms of the order layer structure and
distribution. Region number two has a plate-shaped morphology consisting of a higher amount of silica with regular structure. A smooth surface was observed on these plate shapes and some cracking was seen in these surfaces when incubated at high temperature.

2.4.3 Setting Time

The self-setting properties of calcium silicate cements are due to the progressive hydration reaction of the orthosilicate ions because calcium silicate particles react with water and form an amorphous CSH gel on the cement particles while calcium hydroxide (Ca(OH)$_2$), portlandite) nucleates and grows in the available voids and pore spaces [43]. Functional groups like Si-OH on a wet fresh cement surface have been shown to act as nucleation centers for apatite precipitation. Additionally, varying the particle size has been seen to affect the liquid uptake, changing the setting time. Furthermore, unreacted calcium hydroxide (Ca(OH)$_2$) in the blank powder can be used to increase or decrease the setting time and viscosity of desired cements. These properties can be chosen depending on the desired characteristics of the final cement [312]. Through experimentation the addition of silica into the DCPA matrix could modify the setting time. The addition of silica is uniformly dispersed throughout the DCPA.

Consequently, even though the paste is more workable it requires a longer period of time to cure. In most cases, the L/P ratio is chosen depending on the desired characteristics of the final cement and curing paste properties. In this study, L/P of 0.53 was used. This
ratio was experimentally determined to be optimal for the desired setting time and compressive strength. The L/P ratio is a critical factor determining the setting time. Observations show that the L/P ratio and particle size are directly proportional to the setting time of the cement, and lowering this ratio caused a noticeable decrease in the setting time of the cement while increasing the paste viscosity and decreasing the tendency to flow in a syringe.

2.4.4 Comparison of Three Silica Sources

The most commonly reported strength results for calcium phosphate based cements are compressive strength, from which mechanical properties can be derived. Three silica sources were compared for their effects on strength and setting time, Ludox® SM, Ludox® HS-30, and Ludox® HS-40 colloidal silica. Mechanical strength measurements were made only on the optimized composition of each silica source. Results showed that the same unreacted calcium hydroxide used to vary the setting time had corresponding effects of the strength of the cements. More specifically, a higher level of unreacted Ca(OH)₂ lowered the setting time but also lowered the strength. All silica sources act as a gel in the DCPA structure to increase the strength and polymerize the structure, but Ludox SM was hypothesized to create the largest gains in mechanical strength due to the higher surface area (~345 m²/g vs. ~220 m²/g), allowing for more bonding to take place between the cement and silica.
2.4.5 Compression Strength

The increase in the compressive strengths of DCPA when nanosilica sol is added is attributed to the presence of the intermolecular forces between the silica and DCPA modifying the DCPA’s ability to distribute load. Due to nanosilica sol’s ability to flow and fill voids between DCPA particles, the strength increases accordingly. Furthermore, this avoids the normal vacancies that occur in pure DCPA cements as they set and water is removed from the structure. Under load, these vacancies normally act as initiation points for failure in the cement. Microwave dehydration makes the DCPA cement porous, and thus has a lower density. After crushing and mixing with silica there is a densifying effect. This is in contrast to results from the standard, high temperature creation of DCPA, where the structure remains porous due to the high temperatures. Silicon tends to inhibit grain growth and generate materials with fine microstructures, which may play a role in strength increases [313–315]. The addition of silica sol allows the DCPA powder to become paste like and injectable again for a short window before setting, based on the nanosilica sol mechanism.

The liquid to powder ratio affects the compressive strength of the composite: a too-high liquid to powder ratio will lower the strength of the resulting composite; a too-low liquid to powder ratio will not allow the silica to reach all of the voids between the DCPA particles, and the resulting cement will lack some strength when set. Based on the mechanical testing results, DCPA+ SiO\textsubscript{2} is a good candidate material as a cancellous bone substitute. The DCPA+ SiO\textsubscript{2} is stronger than comparable commercial products used
in bone defect applications. Human cancellous, spongy, or trabecular bone has a maximum stress of 0.70-15.0 (MPa) [316–318] due to its lower density and higher surface area. The other type of human bone is known as cortical or compact bone and has a maximum stress of 131.1-206.0 (MPa) [319–321], designed to support the whole body with its higher density, hardness, and stiffness. This allows DCPA+ SiO$_2$ cements to find applications in bone requirements below 60 MPa.

2.4.6 Control Sample Comparison

Microwaving the commercial products, Calcibon® and CemOstetic™, do not increase their compressive strength. Instead, the commercial products perform better when they are prepared according to manufacturer’s instructions (without microwaving). Neither of these cements incorporates silica or DCPA components by design. During the process of restoration, these cements would cure in place then resorb slowly as they are replaced by newly formed bone. This increase in strength is not seen because the microwave dehydration technique will only work for cements based on an acid-base reaction and not for apatite hydrolysis. The problems with current commercial injectable cements are in their mechanical toughness and final biological properties, although improvements in the technology are being made.
2.4.7 Strength While Setting

DCPA cements are typically weaker than the majority of apatite cements, and the comparatively low compressive strength is due to the extremely rapid setting reaction of the cements, which results in high porosity and consequently weak cement. Using this novel, microwave assisted, production procedure of DCPA cement, the weakness caused by the fast reaction can be bypassed by the microwave dehydration procedure followed by the addition of silica. The data on strength while setting is only useful as a guideline because the cements have not fully set and have a high level of elasticity, which is not present in the final cement. There is difficulty in identifying failure due to the dynamics of a compression test and unset cement derived ceramic materials.

2.4.8 Fracture Analysis

Fracture of the DCPA+ SiO₂ samples generally occurred as a splintering of the diametral surface of the cylindrical cement specimen. This splintering is due to the weak tensile strength of the ceramic and the low level of elasticity seen in it. Following this initial surface splintering, larger pieces would break off of the sample directly before failure. Failed sample surfaces are jagged and irregular as the cements crumble away at failure. Pure DCPA cement samples show a much lower tolerance to loading, beginning to splinter more frequently, and failing sooner that samples with the addition of nanosilica. In samples that were tested before being completely set this same characteristic was not seen and the samples acted in a more elastic manner.
2.5 Conclusion

DCPA has limited use in load bearing locations due to low strength limits caused by a rapid, exothermic, hardening process. The high temperature of the setting process would cause necrosis of the surrounding tissue and limit the useful nature of the cement for medical implant applications. The microwave technique solves the exothermic heat generation problem and improves strength by stopping the reaction and creating a Monetite composition that can be polymerized with silica. The benefits of adding SiO$_2$ and nano-SiO$_2$ have been seen in applications such as construction cements [288], [322], and these benefits can be translated into the same desirable mechanical properties for the creation of a bioactive bone cement.

Significantly improved DCPA cement can be achieved with the polymerizing effect of silica after microwave dehydration. Considerable modifications to setting time and mechanical performance are seen due to bonding between DCPA and silica. This has useful implications for orthopedic and dentistry applications.

Characteristics such as viscosity, yield stress, and injectability impact the use of injectable, self-setting, biomaterials. Compared to a similar self-setting DCPD or DCPA cement, the strength of DCPA+ SiO$_2$ is considerably higher for a minimum cost. It is suggested that the attraction forces are related to acid-base interparticle bonding of salt bonding by acidic cations and silanol-dissociated silanol bonds, resulting in a
homogeneous self-setting paste. The self-setting properties of calcium silicate cements and the progressive hydration reaction of the calcium and silica particles react with water and form a nanoporous amorphous gel on the cement particles, while calcium hydroxide (Ca(OH)$_2$, portlandite) nucleates and grows in the available voids and pore spaces [43]. Functional groups like Si-OH on a wet fresh cement surface have been shown to act as nucleation centers for apatite precipitation.

Finally, the relatively high strength of this cement compared to cancellous bone, the ability to self-harden, pliability while setting, and inject-ability make this novel production procedure for DCPA+ SiO$_2$ a good material for bone repair and regeneration. In the future this material can under in vitro and in vivo trials for application as dental and bone repair materials in the human body and potential commercialization into a product due to its promising features.
Chapter 3

Development of Bioactive Dicalcium Phosphate Anhydrous with Nanosilica Sol for Biomedical Applications

Abstract

Silicon is the second most abundant element in the earth’s crust, apart from oxygen, and is critical in the development of healthy bone and cartilage. Dicalcium Phosphate Anhydrous (DCPA, Monetite, CaHPO₄) is generally overlooked in biological applications because it has limited load bearing uses due to its low strength and brittleness, which is caused by its rapid creation in a high temperature reaction. In this study, a method of polymerizing a novel produced, microwave assisted, DCPA powder with nanosilica sol (SiO₂) is implemented to significantly improve the biological properties. Ultimately, an increase in the cell viability is seen during in vitro experimentation. Moreover, a discussion looks at the biological compatibility of DCPA and nanosilica (SiO₂) sol of the resulting composite cement.
3.1 Introduction

Biocompatibility and bioactivity of any biological cement is the most important point of validation, especially in skeletal or dental applications. Implantable bioceramics should be non-toxic, non-carcinogenic, non-allergic, non-inflammatory, bio compatible, and biofunctional throughout their designed lifespan [323]. A staple characteristic of bioactivity is the ability of the cement to foster growth of new bone in the remodeling process. Hydroxyapatite (HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is the most visibly studied bioceramic material and has been widely used because of its similarity to natural bone mineral. Other synthetic calcium phosphates that are frequently used in biomedical applications include tetra-calcium phosphate (TTCP, $\text{Ca}_4(\text{PO}_4)_2\text{O}$), tri-calcium phosphate ($\alpha$-TCP, $\alpha$-$\text{Ca}_3(\text{PO}_4)_2$ and $\beta$-TCP, $\beta$-$\text{Ca}_3(\text{PO}_4)_2$), di-calcium phosphate anhydrous (DCPA, Monetite, $\text{CaHPO}_4$), di-calcium phosphate dihydrate (DCPD, Brushite, $\text{CaHPO}_4.2\text{H}_2\text{O}$) and octacalcium phosphate ($\text{Ca}_8\text{H}_2(\text{PO}_4)_{6.5}\text{H}_2\text{O}$, OCP).

Bioactively speaking, DCPA is an interesting calcium phosphate based bone regeneration material for rapid healing because it is biocompatible and rapidly resorbed and transformed into bone apatite [324–327]. As a precursor of hydroxyapatite, DCPA is being used for cements with applications in orthopedics [78], [328–330]. Studies have reported that DCPA in combination with $\alpha$-TCP has self-setting properties to form calcium deficient apatite as the end product in vitro [331], [332]. Customarily, researchers have used polymers such as chitosan, PLA, PLGA, PGA, and Alginate.
incorporated into the matrix of calcium phosphate cements to enhance their compressive strength [267], [333]. Alginate is a hygroscopic polymer extracted from seaweed or algae; poly lactic acid (PLA) is a synthetic polymer used to fabricate biodegradable products; and chitosan comes from crustacean shells. The concern with these biocompatible polymers is that they lack sufficient compressive strength and some can lead to inflammation in surrounding tissue during degradation [312].

Producing calcium phosphate based ceramics often involves sintering and high temperatures, leading to a very dense crystalline material (unlike biological apatite) that reduces the bioactivity and corresponding bone growth [62], [334]. Calcium carbonate ($\text{CaCO}_3$) and DCPA have been converted to alpha-tricalcium phosphate through high temperature firing, which resulted in lower biological and hydraulic reactivity [335]. Low temperature production methods, for calcium phosphates, create a less dense implant that can better allow the ingrowth of natural tissue [279].

Silica has been shown to play an important role in biomineralization of organisms such as coral. Carlisle et al. [11], [12], [18], [336] studied the role of silicon in bone calcification and showed higher levels of silica in bone growth regions. Bioactive glasses were invented and investigated first by Hench et al. [59] in response to a need for a biomedical implant after talking with an Army colonel who had returned from Vietnam. Since 1969, Hench has been a primary investigator of bone bonding and bioglass ceramic systems, while many researchers have investigated silica systems. Hench et al. [56] has shown an integral role of silica in bioactivity and osteogenic potential of bioglasses and related
glass ceramics providing surface chemistry and structure for hydroxyapatite bone formation. Silicon-containing bioactive materials, such as bioglass® exhibit excellent carbonated hydroxyapatite forming ability in vitro and in vivo, activating bone related gene expression and cell proliferation [57], [337].

In addition, bioactive glass beads can increase the affinity of bone to and organic matrix of polymethacrylate while keeping the generally desirable handling characteristics and sufficient mechanical strength [260], [38], [39]. Silicon substituted hydroxyapatite and silicon substituted alpha-tricalcium phosphate have been a popular area of research due to the benefits of silicon [79], [85], [87], [88], [90], [97], [314], [315], [340–344]. Calcium silicate bone cements have been shown to promote the precipitation of “bone-like” hydroxyapatite on their surface when exposed to a physiological solution, supporting its ability to integrate with living tissue [51].

Further investigation in silica systems has been done by many investigators. Surface silonal groups (Si-OH) exist on amorphous silica and are effective at inducing hydroxyapatite formation [345]. Li et al. [344] found that hydroxyapatite with silica based composites had much faster bone-like growth than pure hydroxyapatite and the propensity of composites to exhibit better bioactivity with increasing silica content up to 10 wt.%. The formation of bone-like material on the surfaces of these bio-composites was related to the increasing silanol groups associated with the silica content. Hall et al. [346] demonstrated the in vitro bioactivity of silica gels by their ability to nucleate calcium phosphate from a simulated body fluid and the in vitro adhesion and proliferation
of human osteoprogenitor cells. Aburatani et al. [293] noted that colloidal silica (nanosilica sol) containing soft tissue replacements could promote the deposition of apatite within 3 days soaking in simulated body fluids.

In addition, calcium sulfate based bone cements have been created and improved upon through the addition of nanosilica sol for physicochemical and in vitro biological properties. A condensed phase produced from the polymerization process of nanosilica sol filled the micropores and controlled the disintegration of the cement in simulated body fluid. Additionally, the formation of apatite was encouraged by the presence of silica [294]. Various researchers [279] have suggested that it is easier to obtain precursors to biological apatites that will evolve into new compositions in a biological environment than it is to create the final, desired apatite. Like ceramics, compositional and structural characteristics of apatite require a synthetic biological material that can be produced in large, industrial quantities in precise, repeatable batches. When calcium phosphates of DCPA are mixed with nanosilica biological elements mimicking bone, they could form carbonate hydroxyapatite surface crystals; this would meet some of the current biological cement needs while increasing bioactivity and strength.

The objective of this chapter is to evaluate the biological effects of this strong DCPA+SiO$_2$ cement created in chapter 2 and verify the biocompatibility in vitro. Cements such as DCPA and DCPD have been shown to be biocompatible and resorbable bioceramics. It is hypothesized that DCPA+ SiO$_2$ may represent an ideal solution to the problem of long-term implant failure around trabecular bone, as it is resorbed at a rate
similar to cellular metabolism yet stronger than standard injectable cements. It is shown that DCPA+SiO$_2$ cements degrade gradually over a period of time exploiting the body’s natural capacity to repair. The synthesis and setting process of this cement is not a high temperature process and therefore is not harmful to surrounding tissue while being injectable. Adding nanosilica sol to DCPA allows the cement to be as, or more, biocompatible than competing commercial products which do not incorporate amorphous nanosilica sol.

3.2 Materials and Methods

3.2.1 Preparation of Powders

See section 2.2.1

3.2.2 Characterization of Powders

See section 2.2.5

3.2.3 Fabrication of Cement Samples

See section 2.2.3

3.2.4 Evaluation of Anti-washout Properties

Washout resistance evaluation of DCPA formed in the presence and absence of nanosilica sol was tested by visual observation using a saliva like solution (SLS) [267]. It
contains 1.2 mmol/L CaCl₂, 0.72 mmol/L KH₂PO₄, 30 mmol/L KCl, 50 mmol/L HEPES buffer (N-2-hydroxyethyl-piperazine-N’-2’-ethanesulfonic acid) and its pH is adjusted to 7 using 0.1 mol/L NaOH. All the chemicals used are purchased form Fisher Scientific (Fair Lawn, NJ, USA). Pastes of DCPA and DCPA+ SiO₂ are mixed, placed into syringes, and injected into SLS at 22°C and a water bath at 37°C. The sample was considered to pass the washout resistance test if it did not visibly disintegrate in the SLS after an initial time of 5 minutes and final time of 1 day.

3.2.5 Phase Composition and Morphology of Cement

See section 2.2.6

3.2.6 Control Sample Comparison

Commercial cement products were chosen and investigated as biological control samples. The first control was an osteoconductive bone void filler, CEM-Ostetic™ (Berkeley Advanced Biomaterials, San Leandro, CA) and the second control cement was Calcibon® (Biomet Europe, Synthetic Calcium Phosphate). These samples were mixed per manufacturer’s instructions and placed in molds 12 mm in diameter and 3-4 mm in height, prepared using a stainless steel powder compaction die with 0.8 MPa of hand pressure.
3.2.7 Cell Culture

Mouse cells (MC3T3) were grown on 75 cm² culture flasks (BioLite 75 cm² Flask, Thermo Fisher Scientific, Rochester, NY) at 37°C and 5% CO₂ in complete α-MEM. The complete culture media was changed every two days. The cells were then detached from the surface of the flask using trypsin (2.5 g/L, EDTA 25mM solution, Sigma-Aldrich Corp., St. Louis, MO) and the samples were seeded to cement samples in well plates (Tissue Culture Plate, 12 Well, FALCON®, Becton Dickinson Labware, Franklin Lakes, NJ).

Cell Culture was conducted on 12 mm sample disks of DCPA+ SiO₂, DCPA, Calcibon, and CEM-Ostetic. Sample disks were autoclaved before cell culture to sterilize any initial bacterial contaminants. Samples were seeded with 20,000 MC3T3 cells at 37°C with 100% relative humidity and 5% CO₂ in complete α-MEM. The composition of 100 ml complete α-MEM, contains 90 ml of α-MEM (HyClone MEM Alpha Modification 1X, Thermo Scientific, Logan, UT), 10 ml of fetal bovine serum (HyClone FBS, Thermo Scientific, Logan, UT) and 100 μl of antibiotic (HyClone Antibiotic, Thermo Scientific, Logan, UT).

A chemical drying procedure was conducted in order to conduct scanning electron microscope (SEM) investigations on cement samples. Osteoblasts were dehydrated through sequential washings in 30%, 50%, 70%, 90%, 95%, and 100% ethanol solutions. Next, cells underwent a fixation procedure with 4.5% glutaraldehyde in a cacodylate buffer (pH = 7.4). Osteoblast morphology on cement samples after 7 days was examined
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).

3.3 Results

3.3.1 Powder Morphology

See section 2.3.1

3.3.2 Phase Composition of Powders

See section 2.3.2

3.3.3 Cement Morphology

See section 2.3.3

3.3.4 Anti-washout Properties

Under anti-washout investigation, DCPA and DCPA+ SiO₂ maintained their shapes and showed no significant disintegration after 5 min of immersion in SLS (Figure ). This shape was maintained even after 1 day of immersion.
3.3.5 Cell Culture

Many products that can be biologically resorbed and injected are available for testing, and two such commercial products (Calcibon and CemOstetic) were used as controls in this experiment. During the clinical use of these cements, they cure in place then resorb slowly as they are replaced by newly formed bone. The commercial products were prepared following the manufacturer’s instructions. Cell viability was measured after 24 hours and seven days. A total of 12 samples were measured for the 24 hour cell count. A statistical sample size of three was measured for each of four cement samples: DCPA+ SiO₂, DCPA, CEM-Ostetic, and Calcibon. On day seven, three samples were again used for a cell viability analysis on each of four sample types. An additional sample of each group was made and cultured for 7 days to visualize cell viability under the SEM.
Figure 3-2: SEM Micrograph of Calcibon with Collagen Growth after 7 days

Figure 3-3: SEM Micrograph of Calcibon with Collagen Growth after 7 days
Figure 3-4: SEM Micrograph of Cem-Ostetic with Collagen Growth after 7 days

Figure 3-5: SEM Micrograph of Cem-Ostetic with Collagen Growth after 7 days
Figure 3-6: SEM Micrograph of DCPA with Collagen Growth after 7 days

Figure 3-7: SEM Micrograph of DCPA+ SiO$_2$ with Collagen Growth after 7 days
Figure 3-8: SEM Micrograph of DCPA+ SiO\textsubscript{2} with Collagen Growth after 7 days

The *in vitro* tests (Figure , Figure , Figure , Figure , Figure , Figure , Figure ) indicate osteoblast cell proliferation on cement samples. After seven days of cell culture, the number of osteoblast cells were highest on DCPA+ SiO\textsubscript{2}, followed by Calcibon\textsuperscript{®}, DCPA, and lastly Cem-Ostetic\textsuperscript{TM} samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Result 1</th>
<th>Result 2</th>
<th>Result 3</th>
<th>Average</th>
<th>StDev</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCPA+ SiO\textsubscript{2}</td>
<td>0.589</td>
<td>0.610</td>
<td>0.422</td>
<td>0.540</td>
<td>0.103</td>
</tr>
<tr>
<td>DCPA</td>
<td>0.498</td>
<td>0.484</td>
<td>0.441</td>
<td>0.474</td>
<td>0.029</td>
</tr>
<tr>
<td>Calcibon\textsuperscript{®}</td>
<td>0.553</td>
<td>0.509</td>
<td>0.466</td>
<td>0.509</td>
<td>0.043</td>
</tr>
<tr>
<td>CEM Ostetic\textsuperscript{TM}</td>
<td>0.476</td>
<td>0.535</td>
<td>0.492</td>
<td>0.501</td>
<td>0.031</td>
</tr>
</tbody>
</table>
Table 3.2: Unadjusted Plate Reader Results Day 7

<table>
<thead>
<tr>
<th>Sample</th>
<th>Result 1</th>
<th>Result 2</th>
<th>Result 3</th>
<th>Average</th>
<th>StDev</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCPA+ SiO2</td>
<td>1.406</td>
<td>0.588</td>
<td>0.481</td>
<td>0.825</td>
<td>0.506</td>
</tr>
<tr>
<td>DCPA</td>
<td>0.295</td>
<td>0.305</td>
<td>0.786</td>
<td>0.462</td>
<td>0.281</td>
</tr>
<tr>
<td>Calcibon®</td>
<td>0.218</td>
<td>1.185</td>
<td>0.701</td>
<td>0.701</td>
<td>0.484</td>
</tr>
<tr>
<td>CEM Ostetic™</td>
<td>0.074</td>
<td>0.084</td>
<td>0.082</td>
<td>0.080</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Figure 3-9: Osteoblast Cell Numbers for DCPA+ SiO₂, DCPA, Calcibon®, and Cem-Ostetic™
3.4 Discussion

3.4.1 Cement Surface Morphology and Composition

The surface of DCPA and DCPA+ SiO₂ based cements were examined under a scanning electron microscope, and biocompatibility assertions can be made based on these images. Based on the collagen visualized by these micrographs, DCPA+ SiO₂ seems to have the most biocompatible surface. Calcibon® is also highly biocompatible under cell culture conditions. Characteristic DCPA cement performs consistently well after one day and seven days of culturing in cell media. The commercial product CEM-Ostetic has poor cell results after seven days, and it is hypothesized that this is due to its rapid breakdown.

Silica substitution seems to promote biological activity by the transformation of the material surface to a biologically equivalent apatite and increasing the solubility of the material [28]. Morphological surface changes on DCPA+ SiO₂ are identified easily as collagen and cell remains in the SEM images and shows these materials to be bioactive. The mineralization process has been shown to be directly affected by the presence of aqueous silica [347]. The precipitation of hydroxyapatite has been linked to the addition of silica in tests involving inhibitor proteins, which generally restrict its precipitation [348].
3.4.2 Anti-washout Property

Contact with physiological fluids can cause cement washout with some products used in vivo. The results of injection into SLS at room temperature and in a water bath at 37°C does not show that is an issue under the current preparation methods, with some amount of unreacted Ca(OH)$_2$ to allow the cement to react while keeping its low temperature reaction. Additionally, the presence of unreacted calcium hydroxide (Ca(OH)$_2$) can also be used to buffer the pH levels in order to prevent an inflammatory response.

3.4.3 Injectability

One advantage of this DCPA powder production process is the low temperature reaction that takes place. Microwave dehydration does not take place at higher sintering temperatures, which reduces the tendency of the material to harden and coalesce to form large granules reducing reactivity. Pulverization to form powders is not required to increase the surface area and reactivity, but some light grinding does take place.

3.4.4 Biocompatibility of Cement

Biological apatite involves carbonates that occupy positions in the PO$_4^{3-}$ sublattice. Carbonate hydroxyapatite can be obtained at high temperatures when carbonates enter and occupy lattice positions in the OH- sublattice [279]. Low temperature synthesis
routes allow the procurement of carbonate hydroxyapatites with carbonates in phosphate positions, which is more similar to biological apatite [349]. Release of silica complexes to the extracellular media and the presence of silica at the material surface may induce additional stimulatory effects on cells of the bone and cartilage tissue systems [28]. Biological performance increases are seen in synthetic calcium phosphate based materials, which incorporate silicon into their structures far above stoichiometric alternatives [92]. Silicon has been detected in high amounts at grain boundaries leading to a heterogeneous microstructure that may have effects relating to the biological activity of the material [343], [350].

3.4.5 Degradation of DCPA-SiO₂

The investigated DCPA+ SiO₂ materials are bioactive because cells attach and proliferate on day one. Cell numbers on day seven may vary for a number of reasons. For instance, variations may be partially due to the PBS washing process during the cell counting procedure, because cells can be seen under an optical microscope surrounding the cement samples. Another reason may be the cement’s solubility promotes a rapid break down and replacement with natural components. This is a similar phenomenon to those reported by Hench et al. with bioglass® [22], [59], [281], [311] due to the surface degradation of samples. Lai et al. [351] discussed the effects of silicon excretion from bioglass implants in rabbits and saw no adverse effects and the excess silicon was excreted through the animals natural processes. These dissolution products contain high levels of aqueous silica and stimulate ontogenesis and collagen synthesis [337]. Calcium phosphates that
are more soluble have been suggested to induce a higher amount of biomimetic precipitation [352].

Poor stability of this cement composition may be caused by the sterilization autoclave treatment. Based on the results of day one vs. day seven some samples show high cell numbers and some samples show low numbers under the same test conditions. If the cells are alive they cannot be washed away, but the attachment surface could be washed away. As the water vapor and the high temperature in autoclave partially destroy the cement structure the surface for cell attachment may already have begun breaking down. Once the surface is degraded, cells may wash off once the cement matrix breaks away. Ethanol is another sterilization method which may be employed but its results are not ideal and should be a last resort. It is recommended that an ultraviolet or gamma ray radiation technique be used to sterilize cement in future studies. However, based on recent results, it is concluded SiO\textsubscript{2} addition can fight against the instability because it can accelerate apatite deposition to cement to stabilize its structure. Additionally, based on cell numbers after 7 days, the addition of SiO\textsubscript{2} has no clear adverse effects on cell growth.

Day one results show good attachment with some initial cell death and after 7 days two results are seen: some samples show a proliferation of cells, while other samples show the same or less cells than day one results. This is attributed to the surface degradation. This is an advantage of DCPA and DCPA+ SiO\textsubscript{2} cements over stoichiometric hydroxyapatite, because stoichiometric hydroxyapatite does not degrade significantly, which could lead to a permanent fixture that may fail under long term implantation and
require additional treatments [353]. In the case of a silica tri-calcium phosphate sample, it was found that after one year only 10-20% of the scaffold remains and after 2 years the scaffold is replaced completely by laminar bone tissue [354]; this is the ideal result of any bioresorbable bone scaffold because of the natural regeneration seen. Natural bone exhibits deficiencies in Ca, P and OH not seen in stoichiometric compounds [355]. Cement samples may lose calcium from the surface and exchange it for two hydrogens from the medium and have consequences on the biocompatibility of the cement [356].

3.5 Conclusion

The standard exothermic setting process of DCPA cements would generally cause necrosis of the surrounding tissue if used in an injectable form, which limits the useful nature of the cement. The microwave technique developed in this study solves the heat problem and improves strength by stopping the reaction and creating a Monetite composition that can be polymerized with silica. Significantly improved DCPA cement can be achieved with the polymerizing effect of silica after microwave dehydration, and has useful implications for orthopedic and dentistry applications. Adding SiO₂ and nano-SiO₂ to DCPA makes the cement useful as an injectable, self-setting biomaterial. Similar findings have been seen in biological applications before and translate into the same desirable properties for the creation of bioactive DCPA based bone cement.

Biological activity of DCPA+ SiO₂ materials have been increased which can be attributed to a number of different factors, which presumably act synergistically. Silica substitution
in DCPA facilitates precipitation of a biologically equivalent hydroxyapatite on the cement surface as well as direct effects of silica on osteoblasts as silica is released to the extracellular media or present at the material surface. Functional groups like Si-OH on a wet fresh cement surface have been shown to act as nucleation centers for apatite precipitation. Osteoblast cells attach to the DCPA and DCPA+ SiO$_2$ samples as well or better than some commercial products. Cell attachment to sample surfaces shows biocompatibility for in vivo trials. A room temperature preparation process presented here allows the potential incorporation of environmentally sensitive molecules and drugs.

Finally, the biocompatibility of DCPA and DCPA+ SiO$_2$ was supported by the excellent attachment and proliferation of MC3T3 preosteoblast cells under static culture conditions. Based on this finding, DCPA+ SiO$_2$ composite cements are proposed to provide promising features as bone repair materials. Compared to similar self-setting cements the compatibility of DCPA+ SiO$_2$ is improved for a minimum cost. In cancellous bone applications, the ability to self-harden, pliability while setting, and inject-ability make this novel production procedure for DCPA+ SiO$_2$ a good material for bone repair and regeneration. In the future this material can under in vivo trials for application as dental and bone repair materials in the human body.
Chapter 4

Crystallization of Polymerized Dicalcium Phosphate Anhydrous with Nanosilica Sol

Abstract

This study aims to investigate the surface structure and crystallization of cement paste in real time during setting. This requires the utilization of an Environmental Scanning Electron Microscope and Energy Dispersive X-ray analysis (ESEM-EDX). Cement samples are analyzed at 6 minutes after sample preparation and every 1 minute 40 seconds till the estimated final setting time of 30 minutes. The hydration of Dicalcium Phosphate Anhydrous with Nanosilica Sol has been shown to set in wet conditions. During the first few minutes a gel is seen forming as granular precipitates form and a nanosilica sol binds particles together.
4.1 Introduction

4.1.1 Environmental Scanning Electron Microscope

The Environmental Scanning Electron Microscope (ESEM) is an electron optical instrument which enables the examination of the surfaces of soft, hydrated, unfixed, uncoated and electrically insulating specimens under various temperature, pressure, and humidity [357]. Danilatos et al. [358–360] has done extensive work outlining the foundations of the ESEM and its uses, a summary of what these foundations allow for is described. Fresh “wet” sample paste can be directly placed into sample holder because drying and coating treatments are not essential. With the ESEM, information can be gained from samples in various states of interest while bypassing the need for various sample preparation steps like sputter coating, drying, and chemical fixation. The preparation procedures for scanning electron microscopy are time consuming and may change the surface properties of the specimen of interest. With a traditional SEM the continuous in situ changes in chemical compositions and morphology cannot be realized.

4.1.2 Calcium Silicate

Cements are interesting examples of how to use an ESEM to see hydration processes in action. Portland cement is a common construction cement with biomedical applications composted of tricalcium silicates and dicalcium silicates which react with water during
the hydration [133]. Silicate phases of Portland cement undergo hydration followed by a series of physico-chemical reactions resulting in a nanoporous gel of calcium silicate hydrates and a soluble fraction of Portlandite (calcium hydroxide, Ca(OH)$_2$) [134].

Calcium silicate powders with higher silica (SiO$_2$) contents tended to have more dense large particles. With the addition of gelatin a colloidal gel formed densely hydrated cement interwoven with the calcium silicate cement [262]. Gelatin has been shown to partially encapsulate particles and impede hydration of calcium silicate cements when in contact with water [269]. These hybrid gel cements resist washout and were attributed to the adhesive property and negative charge of gelatin prohibiting the penetration of cement paste by liquid [262].

The setting reaction is initiated earlier and hardens faster in wTC and wTC-aTCP calcium-silicate cements releasing noticeably high calcium ions within the first 24 hours of fluid exposure and slowing within 7 days [128]. Calcium chloride is known to accelerate the setting process of calcium silicates and montmorillonite is known to improve dimensional stability [129]. A surface composed of uniform calcium silicate hydrate (CSH) hydrogel is expected as a result of the hydration of belite and alite on wTC and wTC-Bi non-aged cements [54].

Alpha-tricalcium phosphate (alpha-TCP) is a reactive compound which provides phosphate [121] and calcium ions by hydrolysis [119] which is necessary to enhance apatite formation and tissue regeneration [120].
Polymerization of CSH gel hardens over time forming a solid network associated with an increased mechanical strength [43]. Wet calcium silicate cements possess a superficial gel-like structure that envelops the surface particles and calcium phosphate nanoparticles appear as deposits in biological fluids [42]. Nanoparticles form and grow around the cement grains and form a network that fills the spaces between them [43]. This network is established a few minutes after mixing and gains strength as hydration proceeds forming more calcium silica hydrate particles increasing strength due to the forces between calcium silica hydrate particles [361]. The amorphous nature of CSH makes it difficult to characterize by methods such as Raman spectroscopy [42].

In this paper, an ESEM is implemented to visualize the setting process of a DCPA powder polymerized with a nanosilica sol. The same location is investigated over time to see the micrographs and chemical compositions. Methods implement energy dispersive spectroscopy techniques in order to identify regions of the cement as the setting process progresses.
4.2 Materials and Methods

4.2.1 Preparation of Powders

The raw DCPA (Dicalcium Phosphate Anhydrous, Monetite) powder is made first, before being polymerized with silica (SiO₂). The DCPA cement powder is synthesized from a calcium hydroxide (Ca(OH)₂=74.10 g/mol, BDH Laboratory Supplies, Poole, UK) component, setting solution, and de-ionized water (DI). The setting solution for the cement reaction is prepared by combining sodium bicarbonate (NaHCO₃>99.7%, Fisher Scientific, Fair Lawn, NJ), de-ionized water, citric acid monohydrate (CAM, 100% assay, OCCOOH(CH₂COOH)₂*H₂O, Fisher Scientific, Fair Lawn, NJ), and phosphoric acid (85% H₃PO₄, EMD Chemicals Inc., Gibbstown, NJ).

To yield 15ml of complete setting solution, the setting solution consists of 6g sodium bicarbonate, 0.0032g citric acid monohydrate, 1.95ml DI water, and 13.05ml phosphoric acid respectively. Sodium bicarbonate facilitates the reaction of the initial powder. Citric acid monohydrate helps to control the setting time in some cases, acting as a setting retardant in modifying the injectability of the cement [298–300]. Deionized water acts to dilute the setting solution reducing its viscosity. Phosphoric acid acts as a source of phosphorous and is added by titration over the period of one hour, due to the high reactivity of the solution producing CO₂ gas.
Cement paste is initially prepared by manually mixing calcium hydroxide with DI water and setting solution using a mortar and pestle. A slurry of DCPA is made by dispersing 12.3g of calcium hydroxide in 25ml of DI water, completely dissolving the calcium hydroxide in the water through mixing for one minute. Inspection shows that after one minute mixing, all agglomeration of calcium hydroxide particles is removed. Next, 15 ml setting solution is added to the dispersed calcium hydroxide and an exothermic reaction takes place reaching temperatures up to 72°C (160°F). This is mixed with a mortar and pestle while the reaction is taking place to facilitate the full reaction of components. The resulting paste is then microwaved (Emerson, max. power 100 W, 2450 MHZ, NJ, USA) for ten minutes to dehydrate the cement, stopping the reaction. The resulting hard cement is crushed with a mortar and pestle to create a powder consisting of DCPA.

4.2.2 Characterization of Powders

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

The surface morphology of DCPA powder samples is visualized using a Scanning Electron Microscope (SEM, Hitachi S-4800, Hitachi Corp, Tokyo). The samples are crushed into powders and then mounted on conducting carbon tape. An accelerating voltage of 10 KV, magnification of 1.00 K and distance of 13 mm is used.
The Fourier Transform Infrared Spectroscopy (FTIR, UMA-600 Microscope, Varian Excalibur Series) is conducted on powders after microwave (Emerson, max. power 100 W, 2450 MHZ, NJ, USA). Each sample was ground using a pestle and mortar then placed to cover the diamond crystal cell. The background was collected before the measurement of each specimen.

4.2.3 ESEM-EDX

An Environmental Scanning Electron Microscope (ESEM, FEI Quanta 3D FEG Dual Beam Electron Microscope) equipped with a Gaseous Secondary Electron Detector (GSED) was used to image the process. The electron beam resolution is -1.5nm at 30kV in ESEM mode. The samples were ground but were not coated for this analysis and no form of sample preparation took place after starting the reaction of the cement. Samples are placed on 20mm diameter by 6mm thick aluminum sample stubs and initially analyzed at 6.1 Torr, 100% relative humidity, and 4 °C. After initial examination each sample is analyzed at a vacuum of 7.9 Torr, 40% relative humidity, and 22°C. Humidity and temperature are controlled in order to watch the setting process while limiting outside factors.
4.3 Results

4.3.1 Powder Morphology

Powder morphology results for DCPA (Figure , Figure ) and DCPA+ SiO₂ (Figure , Figure ) cement powders show clusters of plate like structures. Samples of DCPA+ SiO₂ bonding can be seen interlocked in the powders as silica works into the calcium phosphate structure (Figure ). The plates show no specific orientation but a higher level of porosity can be seen in the DCPA powders (Figure ).

The particle size of blank powders is a highly referenced characteristic of cements, and so is included in the discussion. Figure and Figure show the ground DCPA particles before mixing with silica. The ground cement samples of DCPA+ SiO₂ are shown in Figure , Figure , and Figure , but both are powders after the microwave dehydration process. DCPA powders show particles sizes from 0.5 to 5 micrometers. DCPA+ SiO₂ powders create particles that are much denser and slightly larger, at 2-20 micrometers.
Figure 4-1: DCPA Powder after Microwave, 7000x Magnification

Figure 4-2: DCPA Powder after Microwave, 5000x Magnification
Figure 4-3: DCPA+ SiO$_2$ Powder after Setting, 15000x Magnification

Figure 4-4: DCPA+SiO$_2$ Powder after Setting, 5000x Magnification
4.3.2 Phase Composition of Cement

Cement samples were cured at 37°C in 100% humidity, then were dried and crushed for XRD analysis. The XRD pattern of DCPA and DCPA+SiO$_2$ both exhibit the characteristic peaks of DCPA (shown in Figure 3, Figure 4, Figure 5, Figure 6). In addition, patterns belonging to trace amount of unreacted Ca(OH)$_2$ were also observed. The amount of Ca(OH)$_2$ was estimated to be around 5.99%, based on the JADE software analysis. The overlaid XRD scan of DCPA against the XRD scan of DCPA+ SiO$_2$ shows that the additional SiO$_2$ is amorphous due to the lack of new peaks formed in the XRD scan. This lack of SiO$_2$ crystallinity is expected. Pure DCPA is identified by four distinct peaks observed on the pattern where 2θ values are 27º, 28º, 31º and 33º.
Figure 3: DCPA Characteristic Peaks

Figure 4: DCPA with Silica Characteristic Peaks
Figure 5: DCPA Characteristic Peaks Showing Ca(OH)$_2$ (Portlandite)

Figure 6: Overlaid XRD Scan of DCPA with DCPA+SiO$_2$
The FTIR data shown in Figure 7 are observations of DCPA and DCPA+ SiO$_2$ showing bands of PO$_4^{3-}$ and CO$_3^{2-}$ with no significant difference observed. Scans were each corrected for background noise and demonstrate a slight increase in PO$_4^{3-}$ / HPO$_4^{2-}$ bands, which is indicative of a lattice change.

Figure 7: FTIR Data of DCPA Cement and DCPA+ SiO$_2$ Cement Powder, P-PO$_4^{3-}$ /HPO$_4^{2-}$, C-CO$_3^{2-}$
4.3.3 ESEM-EDX

Samples initially analyzed at 6.1 Torr, 100% relative humidity, and 4°C show a unique surface. There is a gel-like phase composed of nanosilica sol. After initial examination each sample is analyzed at a vacuum of 7.9 Torr, 40% relative humidity, and 22°C. Increasing the vacuum and temperature causes the environmental dehydration and reabsorption of the gel like phase. The following Figure 8 and Figure 9 show cement after 10 minutes post-mixing with a crack in the surface. Silica is hypothesized to assist in holding the particles together. In Figure 10, a surface is seen after the environment has been taken to 100% relative humidity and water has condensed on the surface. The water film and hydrogel matrix is visible and masks the mineral phase of the surface in Figure 10. Next, Figure 11 shows a set DCPA+ SiO$_2$ cement after 24 hours.
Figure 8: ESEM Micrograph of DCPA+ SiO$_2$ with Silica Polymerization after 10 Minutes

Figure 9: Close-up ESEM Micrograph of DCPA+ SiO$_2$ with Silica Polymerization after 10 Minutes
Figure 10: Setting DCPA+ SiO$_2$ Cement with Nanosilica Sol and Water on the Surface

Figure 11: Set DCPA+ SiO$_2$ Cement
A time lapse study was conducted starting with Figure 12 and results can be seen as a series of micrographs taken every 1 minute 40 seconds. The initial SEM micrograph is taken a 6 minutes due to the time requirements of creating a vacuum in the machine after mixing the cement samples. The final Figure 21 shows the cement after it has been allowed to set for 30 minutes at 47.4% RH and 25.4 °C. The formation of large 20-50µm precipitates can be seen coming out of the cement paste as the setting process takes place. The presence of porosities and aggregates can be seen forming while residual hydrogel fills the space between aggregates.

Figure 12: DCPA+ SiO\textsubscript{2} 6:00 Minutes Setting 47.4%RH 25.4 °C
Figure 13: DCPA+ SiO₂ 7:40 Minutes Setting 47.4%RH 25.4 °C

Figure 14: DCPA+ SiO₂ 9:20 Minutes Setting 47.4%RH 25.4 °C
Figure 15: DCPA+ SiO$_2$ 11:00 Minutes Setting 47.4%RH 25.4 °C

Figure 16: DCPA+ SiO$_2$ 12:40 Minutes Setting 47.4%RH 25.4 °C
Figure 17: DCPA+ SiO$_2$ 14:20 Minutes Setting 47.4%RH 25.4 °C

Figure 18: DCPA+ SiO$_2$ 16:00 Minutes Setting 47.4%RH 25.4 °C
Figure 19: DCPA+ SiO₂ 17:40 Minutes Setting 47.4%RH 25.4 °C

Figure 20: DCPA+ SiO₂ 19:20 Minutes Setting 47.4%RH 25.4 °C
The EDS results shown in Figure 22 and Figure 23 show two spectrums each. The first spectrum is the precipitate and the second spectrum is the gel in each figure. A rough cement surface can be seen forming while the EDS spectrum does not show significant differences between the gel and the aggregate.
Figure 22: DCPA+ SiO₂ – EDS Data 17:40 Minutes Setting 47.4% RH 25.4 °C
4.4 Discussion

The ability to vary pressure, temperature, and humidity is advantageous to visualizing calcium silicate cement under various environments. Hydrated cements, which are still wet or setting can be seen in real time immediately after preparation as the setting reaction takes place. High humidity will prevent the sample from desiccating to the environment and drying out [362]. It has been observed that microcracks can be
observed in concretes and be closed again upon rewetting in some studies [363]. At low vacuum and high humidity the samples are not allowed to desiccate and the wet state can be visualized.

When Portland cement, a hydraulic cement, reacts with water, anhydrous oxides of CaO and SiO\textsubscript{2} react with water to form a solution that contains Ca\textsuperscript{2+}, OH\textsuperscript{-} and silicate ions. Supersaturation with respect to a calcium silicate hydrate follows as the pH goes above 10 and Ca\textsuperscript{2+} concentration exceeds 1mmol/L [364]. Networks of portlandite Ca(OH)\textsubscript{2} nucleate and grows into pores and voids while nanoparticles of calcium silicate hydrate grow around the grains [43].

The present study presents a novel production procedure for DCPA cement polymerized with nanosilica sol, which transforms from a pliable and injectable paste like material at operating conditions to a rigid enough material for filling bone defects. Material property results previously indicated a 5-10 minute initial self-setting cement in a simulated surgical environment at 22°C under the correct preparation conditions. Traditional DCPA cements harden through a mechanism consuming free Ca\textsuperscript{2+}, HPO\textsubscript{4}\textsuperscript{2-}, H\textsubscript{2}PO\textsubscript{4} ions to synthesize DCPA crystals, following the gradual dissolution of Ca(OH)\textsubscript{2} in acidic solution and releasing cations, which react with the phosphate anions, forming a coordinated network that consolidates into DCPA around the unreacted Ca(OH)\textsubscript{2}. Unreacted Ca(OH)\textsubscript{2} in the hardened paste is attributed to the DCPA network formed around unreacted Ca(OH)\textsubscript{2} working as a barrier to stop further contact of entrapped Ca(OH)\textsubscript{2} with the acidic environment. The availability of water during manufacture will
affect the fraction of OH sites occupied by silica [303], [304]. In this experimental procedure a network can be formed between Ca(OH)$_2$ and silica to stabilize silica during the cement setting.

In a mixture of different particle sizes larger particles can preferentially coagulate and separate from smaller ones in the presence of nanosilica sol [285]. A nanosilica sol forms a dense structure of hydrated cement, which is well indicated. The amorphous nature of the samples is reasonable because of the low temperature process of polymerization, if these samples were heat treated a crystalline structure would be expected to result [193], [281]. The gelling process initiated by nanosilica sol creates a particulate disordered structure similar to those seen by other researchers [283], [307] consistent with a hydrogel [308]. Coagulated colloids in the presence of salts become a paste, fluidizing under high shear stresses, consisting of liquid and gel [283], [309], [310]. The suspension is able to flow but is strong enough to gel so it is a combination of a sol and a gel similar to processes used by Hench et al. [311].

4.5 Conclusion

The setting of a microwave assisted DCPA then polymerized with silica has been investigated and outlined for studying the setting morphological evolution. The benefits of adding SiO$_2$ and nano-SiO$_2$ have been seen in many applications such as biological and construction cements. These benefits can be translated into the same desirable
mechanical properties for the creation of a bioactive bone cement. Significantly improved DCPA cement can be achieved with the polymerizing effect of silica after microwave dehydration. Results show that an initial hydrated calcium silicate gel is present.

It is suggested that the attraction forces are related to acid-base interparticle bonding of salt bonding by acidic cations and silanol-dissociated silanol bonds resulting in a homogeneous self-setting paste. The self-setting properties of calcium silicate cements and the progressive hydration reaction of the calcium and silica particles react with water and form a nanoporous amorphous gel on the cement particles while calcium hydroxide (Ca(OH)$_2$, portlandite) nucleates and grows in the available voids and pore spaces [43]. Functional groups like Si-OH on a wet fresh cement surface have been shown to act as nucleation centers for apatite precipitation. Future work on the crystallization process could involve Raman and a time delayed XRD study.
References


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