Recent Advances and Additives of Bone Cement and Bone Augments for Arthroplastic Surgeries

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A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science in Biological and Agricultural Engineering

in

The Department of Biological and Agricultural Engineering

by
Nicholas P. Totaro
B.S., Louisiana State University, 2011
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ABSTRACT

As life expectancies rise and the average age of our population increases, there has emerged a growing need for joint repair and replacement surgeries due to worn out, torn, or damaged cartilage and bone tissue. This has resulted in an escalating demand for further development of the materials used in joint replacement surgeries and advances in joint repair technology. Researchers in the tissue engineering and regenerative medicine fields have furthered the development of advanced materials for musculoskeletal repair by utilizing growth factors, nanomaterials, and antibiotics within the repair material.

The first aim of this thesis was to provide a summary of the current literature on advances in joint repair materials. While there have been many advances utilizing calcium phosphates to aid in bone regeneration; calcium phosphates now just represent a single ingredient within the state-of-the-art complex biomaterials for joint repair. These combination materials can achieve up-regulation of osteogenesis within the wound site. Furthermore, as the advances in nanofabrication have branched to most fields of science and engineering, the development of complex nanocomposites has become a common strategy for resolving difficult multi-tissue repair problems. The development of this class of bioactive, biomaterial nanocomposites is reviewed within today’s current literature.

The second aim of this thesis was to construct a new biomaterial aiding in joint repair. By utilizing thermally initiated frontal polymerization, a bioactive, degradable bone augment was constructed that would provide orthopedic surgeons a material with an extended working time, good mechanical stability, and potentially osteoconductive and osteoinductive activity. Four ratios of monomers were explored in an effort to optimize the mechanical properties, chemical stability and cytocompatibility. The ratio of 5:1 acrylate monomer to thiol monomer
provided the best overall material characteristics: high cytocompatibility, compressive mechanical strength of 3.65 MPa, and a maximum propagation temperature of 160°C +/- 10°C.
1. INTRODUCTION

1.1 The Need for Joint Replacements

In the 20th century, medicine and healthcare have evolved to increase the average life expectancy from about 48 to 79 years of age.[1] While extending the life expectancy of a population is the goal of healthcare, it has also increased the volume of age-related disorders found in this increasingly aged population.[2] In the early 1900’s, with a shorter life expectancy, there was less demand for joint repair or replacement than is found in today’s society.[3] From a mechanical perspective, the human skeleton contains hundreds of movable and semi-movable joints, and like any metal joint, the material of the human body can withstand a maximum compressive and tensile forces which once reached, the bones reach a failure point. As our bone is loaded and overloaded, microfractures occur regularly. Both microfractures and non-critical sized fractures of bone undergo a self-healing process to repair the damaged bone.[4] However over many years of extensive use our joints are prone to damage through bone deformation, cartilage degradation, and trauma that is effect by the mechanical integrity of bone decreasing with age.[5] Thus there is a substantial driver for improved techniques in the repair of joint damage that provides for better outcomes, in terms of costs, surgical success, service life of the joint repair and patient quality of life. In 2009 alone, there were more than 600,000 total knee replacement (TKA) surgeries costing about 12 billion dollars.[6] While Schroer et al.’s 2010-2011 study showed the large range in time to failure of the TKAs, form 10 days to 31 years, the average time until revision surgery was 5.9 years. Furthermore, they displayed the primary causes for failure in less than 15 years of ware was aseptic loosening, infection, and instability of the prosthetic. After 15 years the next most prominent cause of failure was wear of the polyethylene spacer.[7]
1.2 Overview of a Joint Replacement Surgery

The procedure used today empowers the physician to open the joint being replaced (i.e. hip or knee, etc.), utilize state-of-the-art imaging to map where the head of the joint will be cut, and implant the femoral and tibia prosthetic components. For ease, this overview of the procedure will be done using a knee replacement surgery as an example. By using a bone saw and clamps with set spacing, the femur is shaped to fit a metal prosthetic. Bone cement, a two part polymerizable formulation, is then mixed and added to the shaped femur.[8] The metal – most commonly stainless steel, Co-Cr alloys, or Ti alloys[9] – prosthetic is fit, according to size, over the head of the femur-bone cement layer. The tibia is then shaped, a hole is drilled longitudinally down the center of the tibia, and the prosthetic is anchored to the bone head via this hole.

![Image of post-knee arthroscopy surgery]

Figure 1.1 – Post-knee arthroscopy surgery showing layers of bone, bone cement, and prosthetic.

A high-density polyethylene spacer between the prosthetics of the femur and the tibia minimizes wear on the metal and aids in the movement of the knee.

1.3 Origin of Bone Cement

Replacement joint surgery using polymer anchors, the precedent for today’s procedure, was first performed in 1962 by Dr. John Charnley who developed the use of an acrylic-based polymer to anchor an inert stainless steel prosthetic to the patient’s femur.[10] He also was the
first to utilize the low friction coefficient of high density polyethylene to decrease wear of the metal prosthetic while maximizing mobility of the joint post-surgery. His pioneering effort has led to the extensive use of cemented socket joint replacements and the large market for commercial use of Bone Cement.[10]

1.4 Literature Overview

Many literature reviews of bone cement have been conducted within the past 25 years. These overviews have mainly focused on the polymer chemistry, analyzed the mechanical properties, and drawn comparisons of commercially available bone cements with alternative compositions.[11-13] Even though, the research to better materials and techniques used in joint replacement has been relentless, the U.S. Food and Drug Administration regulations and market forces have retarded evolution of the composition of bone cement over the past 25 years.

Many labs, globally, have done extensive research studying the effects of protein, antibiotic, and synthetic compound additions to improve the biological activity, decrease the inflammatory response and infection rate, and aid in repair of fractures.[14-16] Many of these additives utilize the high surface to volume ratio gained when using nanomaterials. The recent increase in popularity and advances in nanomaterial and composite fabrication has led to a significant increase in the bone augment literature on nanocomposites.

The focus of this section is to provide an extensive and through review of the recent advances pertaining to the effect of additives in Bone Cement and newly developed nanocomposite bone augments.

1.5 Description of the Need for Bone Augment Fabrication

Currently, bone cement and the standard orthopedic surgical technique place a substantial time constraint on a physician. Once the physician has prepared the knee for addition of the
bone cement, he or she is then dependent on the reaction time of the material to continue the procedure. During polymerization, as the cement transitions from liquid to putty like to a solid mass, mainly environmental factors (i.e. room temperature) affect the overall time of the reaction. This makes it difficult for the physician to accelerate or retard the reaction to modify or correct the physical structure of the cement. Once the two parts of bone cement are mixed it is difficult for the physician to advance the procedure. The state-of-the-art bone cement materials tend to be non-degradable and often biologically inert. A preferred advanced bone cement replacement material would provide a long working time, osteoinductive and conductive behavior, a degradation rate matched to new bone growth, bone-like mechanical properties and morphology.

The goal for increased osteogenic properties of a bone construct can be reached through additives within the mixture. These additives typically are not involved in the synthetic mechanism of the construct, but can increase the viscosity and provide additional function. Additives, or fillers, can be synthetic, biologically derived, or a hybrid of the two. The main advantage of using synthetic scaffolds and synthetic fillers is the controlled and reproducible mechanical properties, purity, and bioactivity of the fillers. Despite the benefit of reliability provided by synthetics, their biological activity is typically limited or inert. Biologically-derived additives, due to their innate function within in a biological system, enable more potent biological responses. Unfortunately, obtaining these tissue types, and proving control and reliability have been difficult in the past.[17]
Table 1.1 – A table of the compositions of commercial bone cements [18]:

<table>
<thead>
<tr>
<th>Cement Type</th>
<th>Powder Composition</th>
<th>Monomer Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palacos® R</td>
<td>P(MMA/MA) – 84.25 wt.% +ZrO₂ – 15 wt.% +BPO – 0.75 wt.%</td>
<td>MMA + DMT – 2.0 wt.%</td>
</tr>
<tr>
<td>Simplex® P</td>
<td>P(MMA/styrene) – 75 wt.% +PMMA – 15 wt.% +BaSO₄ – 10 wt.% +BPO – x</td>
<td>MMA + DMT – 2.6 vol.%</td>
</tr>
<tr>
<td>CMW® 1</td>
<td>PMMA – 88.85 wt.% +BaSO₄ – 9.10 wt.% +BPO – 2.05 wt.%</td>
<td>MMA + DMT – 0.816 wt.%</td>
</tr>
<tr>
<td>CMW® 3</td>
<td>PMMA – 88 wt.% +BaSO₄ – 10 wt.% +BPO – 2.00 wt.%</td>
<td>MMA + DMT – 2.487 wt.%</td>
</tr>
<tr>
<td>Endurance</td>
<td>PMMA – 67.05 wt.% +P(MMA/styrene) – 21.10 wt.% +BaSO₄ – 10 wt.% +BPO – 1.85 wt.%</td>
<td>MMA + DMT – 2.00 wt.%</td>
</tr>
<tr>
<td>Zimmer® dough type</td>
<td>PMMA – 89.25 wt.% +BaSO₄ – 10 wt.% +BPO – 0.75 wt.%</td>
<td>MMA + DMT – 2.75 vol.%</td>
</tr>
<tr>
<td>Osteobond™ copolymer</td>
<td>P(MMA/styrene) – 87.5 wt.% +BaSO₄ – 10 wt.% +BPO – 1.2 – 2.5 wt.%</td>
<td>MMA + DMT – 0.75 vol.%</td>
</tr>
<tr>
<td>Sulfix®-60</td>
<td>PMMA – 79.6 wt.% +P(MMA/BMA) – 8.8 wt.% +ZrO₂ – 9.8 wt.% +BPO – 0.84 wt.%</td>
<td>MMA – 84.6 wt.% +BMA – 14.8 wt.% +DMPE – 1.7 wt.%</td>
</tr>
<tr>
<td>Duracem 3</td>
<td>PMMA – 79.7 wt.% +P(MMA/BMA) – 8.8 wt.% +ZrO₂ – 9.8 wt.% +BPO – 0.83 wt.%</td>
<td>MMA – 83.6 wt% +BMA – 14.8 wt% +DMPE – 1.7 wt%</td>
</tr>
<tr>
<td>Boneloc®</td>
<td>P(MMA/BMA) – 90 wt.% +ZrO₂ – 10 wt.% +BPO – 0.1 wt.%</td>
<td>MMA – 50 wt% +DCMA – 30 wt% +IBMA – 20 wt% +DMT – 0.5 wt% +DHPT – 0.9 wt%</td>
</tr>
</tbody>
</table>

Key:
- Polymers:
  - PMMA – poly(methyl methacrylate)
  - P(MMA/MA) – methyl methacrylate/methacrylate copolymer
  - P(MMA/styrene) – methyl methacrylate/styrene copolymer
  - P(MMA/BMA) – methyl methacrylate/butyl methacrylate copolymer
Initiators:
  BPO – benzoyl peroxide
Monomers:
  MMA – methyl methacrylate
  BMA – butyl methacrylate
  DCMA – n-decyl methacrylate
  IBMA – isobornyl methacrylate
Accelerators:
  DMT – N,N-dimethyl-p-toluidine
  DMPE – N,N-dimethyl-amino-phenethanol
  DHPT – dihydroxyl-propyl-p-toluidine

Within the medical field, time management is a major concern for both the patient and the physician. As a patient, increased invasive surgery exposure time can lead to higher infection rates.[19] For the physician, a more time efficient surgery can lead to increased surgical throughput. This has led to the need for time efficient procedures and advanced controllable materials.

By using thermal frontal polymerization (TFP) the bone augment has an extended life span before polymerizing than commercially used bone cement and a shorter polymerization time. This TFP follows a cure-on-demand synthesis of thiol-acrylate monomers based alternative to the previously described PMMA bone cement. This material’s polymerization is heat activated. This empowers the physician to fully shape the highly viscous monomer without the time constraint of the auto-polymerizing MMA. The monomer can be moldable, due to the previously mentioned hydroxyapatite filler; the viscosity is increased with increasing filler. The chemical synthesis follows a free-radical chain growth polymerization with a maximum temperature similar to that of Bone Cement.
1.6 Overview of Thesis

The first chapter introduces the currently used Bone Cement material, its application in arthroplastic surgeries, a review of additive enhancements, the need for construction of a bone augment, and a description of this thesis.

Chapter 2 reviews recent literature for the advances in additives for nano-composites and the up-regulation of osteogenesis.

Chapter 3 details the in vitro fabrication and analysis of a thiol-acrylate bone augment.

Chapter 4 concludes this thesis and provides direction for future research to better the development of bone constructs.
2. STATE-OF-THE-ART BONE CEMENT LITERATURE REVIEW

This literature review extensively examined the most recent advances to the orthopedic thermoset polymer used in knee arthroplastic surgery, known as bone cement, and has laid a foundation for the many types of research and recent discoveries targeted toward increasing the osteogenic properties and/or utilizing nanocomposite materials. Just as the evolution of bone cement has occurred over the past 40 years, it is still occurring today. The demand for consistently strong and increasingly osteogenic and biocompatible scaffolds has driven this body of research. While advances have led to new developments, many are not commercially available, but have potential for use in improved future formulations of bone cement.

The next generation of bone cements has moved toward utilizing ceramics, calcium phosphates, bioglass, antimicrobials, and nanoparticles to increase the biocompatibility and bioactivity of the cement. While non-biodegradable augments have been used more historically for their inert behavior within the body, the next generation of cement development is geared toward regrowth of tissue using bioactive compounds. Following this trend bone cement research is evolving from a development of bioinert to bioactive compounds. Bioactive compounds are beneficial in tissue regeneration, however the paradigm is that these compounds will face regulatory challenges and concern must be given to the fact that growth could lead to incorrect tissue generation, pain and discomfort for the patient, and costly liability.

The advances discussed and reviewed have furthered the research in increasing the biological properties of bone cement. This chapter affords a brief summary of the body of literature that was reviewed along with a discussion of the applicable concepts, models, and literature pertinent to this project is discussed.
2.1 Nano-composites

2.1.1 Additive for self-healing

One addition to the current bone cement composition is to add encapsulated, reactive 2-octyl cyanoacrylate (OCA), then as micro-fractures occur, the OCA will be released, driving polymerization and self-healing at the fracture site.[20, 21] With uses of self-healing, the lifetime of bone cement due to trauma or wear could be greatly extended. The lifespan for the OCA needs further study since in vivo studies testing the lifespan and reactivity of the OCA as a self-healing monomer have not been conducted yet. This concept could be further explored as a fail-safe method for acute fractures to newly installed bone cement.

2.1.2 Bioactive Glass

A recent literature review of the bioactive glass, SiO$_2$ doped with CaO, Na$_2$O, and P$_2$O$_5$, composites was done by Rahaman et al. and addressed the osteogenic properties of bioactive glass with calcium phosphate and doped with antibiotics.[22] Bioactive glass has undergone many formula changes, similar to bone cement, to aid in its’ osteogenic and antimicrobial effects and this technology holds promise as a potential future osteogenic material. Gao et al.’s review of bioactive glass displays the recent use of nano-ceramics with biologically derived materials to form nano-composite materials. In this review it covered a scaffold made of both chitosan and collagen as a combination or hybrid scaffold with bioglass and showed good biocompatibility and bone formation when tested in vitro and in vivo, respectively.[23]

As the research in bioactive glass continues, major limitations and challenges for future studies are balancing the mechanical stability with the degradation rate and tuning the degradation rate to match tissue ingrowth. As new tissue is regenerated, many formulas of bioactive glass do not degrade quickly enough resulting in entrapped non-degraded bioglass
inclusions. The slow rate of degradation leads a release of ions locally and an increase in the pH that can lead to negative cytotoxic effects. Also, for international commercialization, no standard fabrication techniques have been implemented. By using different types of bioglass or sintering at different temperatures the product can have differing degradation rates and change the local pH and ionic environment significantly. And lastly, in the many studies conducted sterilization stability has not been reported, as such, this remains a critical need for commercial adoption.[24] Once these topics have been addressed, bioactive glass may be advanced into the clinical trials.

2.1.3 Calcium Phosphate Cements

Wang et al. provides an up-to-date and thorough review of the proliferation ability for a bone construct out of calcium phosphate. Both the in vitro and in vivo studies reviewed showed an optimistic outlook for use in conjunction with stem cells to produce bone and vasculature.[25] To increase the current effects of calcium phosphates and meet the previously seen mechanical mismatch between bone and the scaffold material a combination of monomers was done. Macroporous scaffolds of tetracalcium phosphate in combination with alginate microbeads, large scale, 322 µm diameter, suture fibers and nano fibers electrospun from poly (D,L-lactide-co-glycolide) (PLGA), and with rhBMP-2, rhTGF-β1, and VEGF were tested in vivo. This study showed the mechanical complexity and synergy of the many material types and growth factors that could be used in bone regeneration. Over 24 weeks, the tetracalcium phosphate scaffold with microbeads, both large and nano-scale fibers and rhBMP-2 had the best bone ingrowth and wound healing in a cranial defect model.[26] Many calcium cements utilize hydroxyapatite as their calcium phosphate agent, however many versions of calcium phosphate have been used. Other calcium phosphate agents, a monetite/phosphorylate chitosan cement, has been developed
to address the resorb-ability of the cement.[27] Other examples of calcium phosphates used for scaffolding are monocalcium phosphate, dicalcium phosphate, β-tricalcium phosphate[28], and octacalcium phosphate[29] and mixtures with hydroxyapatite, i.e., biphasic calcium phosphate.

Table 2.1 – List of Calcium Phosphate monomers. [30]:

<table>
<thead>
<tr>
<th>Ca/P ionic ratio</th>
<th>Compound</th>
<th>Chemical Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>Monocalcium Phosphate Monohydrate (MCPM)</td>
<td>Ca(H₂PO₄)₂·H₂O</td>
</tr>
<tr>
<td>0.5</td>
<td>Monocalcium Phosphate Anhydrous (MCPA)</td>
<td>Ca(H₂PO₄)₂</td>
</tr>
<tr>
<td>1.0</td>
<td>Dicalcium Phosphate Dihydrate (DCPA), mineral monetite</td>
<td>CaHPO₄</td>
</tr>
<tr>
<td>1.33</td>
<td>Octacalcium Phosphate (OCP)</td>
<td>Ca₆(HPO₄)₂(PO₄)₄·5H₂O</td>
</tr>
<tr>
<td>1.5</td>
<td>α-Tricalcium Phosphate (α-TCP)</td>
<td>α-Ca₅(PO₄)₂</td>
</tr>
<tr>
<td>1.5</td>
<td>β-Tricalcium phosphate (β-TCP)</td>
<td>B-Ca₃(PO₄)₂</td>
</tr>
<tr>
<td>1.2-2.2</td>
<td>Amorphous calcium phosphate (ACP)</td>
<td>Ca₉H₅(PO₄)₆nH₂O, n = 3-4.5; 15-20% H₂O</td>
</tr>
<tr>
<td>1.5-1.67</td>
<td>Calcium-deficient hydroxyapatite (CDHA)</td>
<td>Ca₁₀₋ₓ(HPO₄)ₓ(PO₄)₆₋ₙ(OH)₂₋ₓ (0 &lt; x &lt; 1)</td>
</tr>
<tr>
<td>1.67</td>
<td>Hydroxyapatite (HA)</td>
<td>Ca₁₀(PO₄)₆(OH)₂</td>
</tr>
<tr>
<td>1.67</td>
<td>Fluorapatite (FA)</td>
<td>Ca₁₀(PO₄)₆F₂</td>
</tr>
<tr>
<td>2.0</td>
<td>Tetracalcium phosphate (TTCP), mineral hilgenstockite</td>
<td>Ca₄(PO₄)₂O</td>
</tr>
</tbody>
</table>

a Occasionally, CDHA is named as precipitated HA  
b In the case x = 1 (the boundary condition with Ca/P = 1.5), the chemical formula of CDHA looks as follows: Ca₉(HPO₄)(PO₄)₅(OH)₄

The ability to tune bone cement’s mechanical strength compared to native bone has been a topic of interest since the early days of bone graft research. If the mechanical properties of the scaffold and the native tissue are mismatched, a transfer of the loading could be more on the bone cement than the bone, lessening the mechanical stimulus on the bone. This commonly occurs due to error in geometrically positioning the implants on the bone. Unequal static forces are then loaded creating zones of greater or lesser pressure.[31] This lack of a pressure stimulus is similar to being in a lesser gravitational setting and will result in adaptation and reshaping the
native bone to bear less of the load. Ultimately this will lead to bone resorption and potentially resulting in the failure of the implant.[32] To address this concern, many formulations of bone cement have been evaluated.

While calcium phosphate-based cement, silane (3-(trimethoxysilyl) propyl methacrylate (MPS) 1% wt.), has been shown to increase the mechanical strength of the scaffold material.[33] With addition of mesoporous silica nanoparticles (5% wt.) the compressive mechanical strength can be increased.[34] The mechanical mismatch in the elastic modulus of the bone and bone cement can also induce injury or damage to the tissues around the joint. Utilizing linoleic acid-modified bone cement the mechanical properties were better controlled. Linoleic acid addition adjusted the elastic modulus to a similar strength of cancellous bone.[35] A combination of linoleic acid and strontium substituted hydroxyapatite was used to decrease the elastic modulus while maintaining the compressive strength to tune bone cement for vertebroplastic surgeries.[39] By managing the mechanical properties of a cemented construct and tuning them to be similar to native bone, bone resorption may be minimized.

2.2 Addition of Bioactive Ions

The next generations of bone cements will seek to increase the biocompatibility and bioactivity of the augment. By adding ions commonly found in bone such a magnesium or strontium, the local ionic environment can be modulated resulting in increased osteoconduction in the surrounding tissue.[36] The signal of strontium release in vivo from a calcium phosphate based bone cement to bone has shown high biocompatibility and increased osseointegration from the new bone to the bone cement.[37] The ion influx in the local cellular environment was also shown to be synergistic with an apatite/poly-lactide composite scaffold in vivo.[38]
2.3 Addition of Carbon Nanotubes

In order to have a synergistic effect of biocompatibility, high compressive strength, and good fatigue strength, a combination of the calcium phosphate monomer and carbon nanotubes have been explored. The PMMA mixture was adjusted by combining hydroxyapatite with carbon nanotubes or graphene oxide and measured *in vitro*. The cytocompatibility was similar to the control sample and osseointegration was observed.[39] Carbon nanotubes addition to polystyrene has shown to increase the surface roughness of cast grafts and materials. The increase in surface roughness has led to an increase in osteoblast cell proliferation and differentiation.[40] Ormsby et al. have performed one of the few cytocompatibility studies using multi-walled carbon nanotubes (MWCN) with -COOH, -NH2, -unfunctionalized with MG-63 osteoblast cells. Cytocompatibility was tested via an MTT assay after 1, 3, and 7 days of cells seeded on the MWCN-PMMA bone cement. This study showed a significant increase in optical density of the cells cultured on the MWCN-COOH-PMMA compared to the control, while no significant difference was shown between the other MWCN-PMMA and the control. Also, scanning electron microscopy and confocal microscopy displayed similar cellular morphology for MG-63 cells on the MWCN-PMMA cement and the PMMA cement.[41]

Ormsby et al. increased the fatigue properties and minimized the crack propagation by testing different concentrations of multi-walled carbon nanotubes (MWCN) in the bone cement mixture. The end functionality of the MWCN with a carboxyl at 0.1 wt% displayed the highest cyclic loading properties while the unfunctionalized MWCN displayed the highest cytocompatibility.[41]
2.4 Antimicrobial Additives

Infections, which occur in roughly 3.1% of patients in total joint arthroplasties are painful and difficult to resolve for the patient and costly to treat for the healthcare providers. In an attempt to address these issues, antimicrobial agents or antibiotics have been used as additives in the bone cement mixtures.[42] Infection post-primary total knee arthroplasty (TKA) leads to secondary surgeries and further cost the hospital and patient.

Adjustments have been made by Beyth et al. to the composition of bone cement by adding nanoparticles with known antimicrobial properties.[43] These ammonium nanoparticles were bound to the surface of the bone cement to aid in long lasting antimicrobial effects. Other nanoparticle based antimicrobial effects have been explored using silver nanoparticles. Silver nanoparticle antimicrobial properties have been extensively published and have gained attention for applications in the biomedical field.[44] Entrapment of the silver nanoparticles within the bone cement allows for diffusion of silver ions from the surface of the cement into the solution. Bone cement integrity was maintained; silver nanoparticles were synthesized and stabilized with a coating of an organic molecule (tiopronin). The larger nanoparticles (11 nm) showed increased antimicrobial activity resulting in an increased lag phase of the bacterial growth compared to the smaller nanoparticles (5 nm).[45] Bioactive glass with silver has also been studied as a delivery method of silver ions and shown to aid in reducing infection rate.[46] The trade-off with increased antibacterial compounds is the decrease in mechanical integrity and the difficulty in controlling the release rate. There is a need for optimization of these aspects.

Commercially-available bone cement that utilizes an antibiotic is not currently available in the United States. In Europe, some formulas of bone cement do contain antimicrobial reagents. Thus far, antimicrobiotic-laden bone cement has been approved by the FDA to be used
as a secondary application in knee revision surgeries.[47] Hansen et al. addresses the concern for applying antimicrobial reagents to bone cement is the increasing risk of developing antimicrobial resistant bacteria. Currently, the FDA has not approved any antimicrobial-cement combination devices. However physicians are able to combine antibiotics with the bone cement prior to application.[11] Antibiotics, such as vancomycin, have been used to dope bone cement and aid in decreasing infection at the site of implantation.[48] Recently, temocillin was added to a gentamicin-loaded bone cement mixture and tested for drug release and mechanical strength. Significant doses were eluted within an hour, the antimicrobial activity was maintained, with no significant difference in mechanical strength of the cement observed.[49]

Unfortunately, it has been reported that the addition of drugs within a calcium phosphate cement can cause chelation of the calcium, leading to reduced mechanical properties of the cement.[50] The addition of antibiotics remains a controversial topic as reports of insignificant differences between patients with and without antibiotic-loaded bone cement have emerged. Namba et al. followed 22,889 patients post-TKA surgery with 2,030 patients receiving antibiotic-loaded bone cement. Their results showed no reduction in the number of infections with antibiotic added cement versus without the antibiotic added.[51]

2.5 Additive to Increase the Biocompatibility

From Arora et al.'s review of PMMA bone cement in 2013, the suggested future work would be to use bone morphogenic proteins or growth factors to aid in the biocompatibility and osteogenic properties of bone cement.[52] A list of the growth factors suggested to be delivered by drug-bone cement combination to aid in bone generation include: growth hormone, bone morphogenetic protein, transforming growth factor beta, and insulin growth factor.[53]
Table 2.2 – from Verron et al. describes the function of each growth factor[53]:

<table>
<thead>
<tr>
<th>Growth Factors</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Hormone</td>
<td>Bone remodeling</td>
</tr>
<tr>
<td></td>
<td>Proliferation and differentiation of osteoblasts</td>
</tr>
<tr>
<td></td>
<td>Stimulation of osteoclastic resorption activity</td>
</tr>
<tr>
<td>Bone Morphogenic Protein</td>
<td>Proliferation and differentiation of mesenchymal stem cells (MSC) and osteoprogenitor cells</td>
</tr>
<tr>
<td></td>
<td>Ectopic bone formation</td>
</tr>
<tr>
<td>Transforming Growth Factor – Beta</td>
<td>Recruitment, proliferation, and diffusion of MSC’s and osteoprogenitor cells</td>
</tr>
<tr>
<td></td>
<td>Extracellular matrix production</td>
</tr>
<tr>
<td></td>
<td>Angiogenic and inflammation properties</td>
</tr>
<tr>
<td>Insulin Growth Factor</td>
<td>Proliferation and migration of MSC and osteoprogenitor cells</td>
</tr>
<tr>
<td></td>
<td>New bone formation and mineralization</td>
</tr>
</tbody>
</table>

Another common drug delivered is the bisphosphonate synthetic drug family. Bisphosphonate is a structural analog of pyrophosphate that inhibits osteoclast activity and up-regulates osteogenesis in vitro and in vivo.[54] Bose et al.’s review of bisphosphonates in ceramic bone cements displays an increase in bisphosphonate containing combination devices in the literature for bone cements.[55]

To further modify the bone cement formula, originally “0.34 g powder and 173.15 μl liquid, Endurance MV, DePuy Orthopaedics, Warsaw, IN,”[56] N-acetyl cysteine was use to terminate radicals after polymerization and aid in increasing the osteoconductivity of the cement. The modified bone cement formula was able to maintain similar compressive and flexural strength of the control bone cement. The in vitro results with osteoblast linage cells showed higher cytocompatibility and the in vivo study displayed better bone-bone cement adhesion.[56] While maintaining mechanical integrity, addition of phosphorylated 2-hydroxyethylmethacrylate (HEMA-P) was added to powder portion of the PMMA mixture with calcium salts. With the HEMA-P cytocompatibility and calcium deposition was increased when tested in vitro.[57]
When changing the formula of the original bone cement, some physical properties are also modified. With the addition of transforming growth factor – Beta (TGFβ) to the PMMA recipe, the porosity and swelling were increased. Pore sizes of about 150 µm to 200 µm were formed. This \textit{in vivo} study also showed that a combination of increased porosity and release of TGFβ promoted cell in-growth and local inflammation was not detected.[58]

Another biomolecule, chitosan, was added to three PMMA polymers each originating from different sized powder monomers. An \textit{in vivo} study of the temperature of polymerization and bioactivity was conducted. The polymer made with the largest sized monomer powder, 50 µm to 150 µm, and with chitosan showed a decrease in setting temperature from 71.60 ±9.31 °C to 59.04 ±9.59 °C and histology displayed increased osteoid formation compared to the control sample at 4 weeks.[59, 60] Besides chitosan, particles containing collagen from cuttlefish have been used as an additive in PMMA bone cement. The \textit{in vivo} study showed increased adhesion of the construct to the bone and osseointegration compared to the PMMA only control.[61]

\subsection*{2.6 Coloration}
In application, many physicians use a dye to color the bone cement. This will allow a physician to more easily identify the bone cement upon revision surgery. A common dye used is methylene blue, to color the bone cement blue. It has been shown that no mechanical integrity of the bone cement is lost when methylene blue is added.[62]

\subsection*{2.7 Future Work}
From this review many combinations of PMMA bone cement have been reviewed. Throughout this process of analyzing the gains in biocompatibility of bone cement, more research has yet to be done to show reliability and consistency \textit{in vivo} of the bioactive bone cements. More extensive animal trials, which lead to human trials, will need to be done to provide the data for use commercially.
2.8 Conclusion

PMMA bone cement has a significant prevalence in clinical use and has captured majority of the commercial market of bone cements. Due to the consistency, reliability, and historical lifetime there has not been a driving need that outweighs the risk for commercialized bone cement to gain biological activity. As the average person’s lifespan is increased the need for a longer lasting solution to knee replacement rises. This has led to many additives being tested to observe if they are able to improve the biological properties of the cement.

These many additions and adjustments to the original bone cement formulation have led to many breakthroughs in aiding self-healing, improved mechanical strength, improved infection resistance, and increased in osteogenic up-regulation. The additives reviewed here have led to bettering the current understanding of bone cement and substitutions of bone cement for joint arthroplastic procedures. With further research on the consistency of these bioactive materials in vivo some may become a commercialized product.
3. IN VITRO EVALUATION OF THERMAL FRONTALLY POLYMERIZED THIOL-ENE COMPOSITES AS BONE AUGMENTS

3.1 Project Purpose

Due to the large number of total knee replacement (TKR) surgeries conducted per year, and with projections of increased demand to almost a million primary TKR surgeries per year by 2030 in the United States alone, there is a need to discover more efficient working materials as alternatives to current bone cements. There is a need for surgeons and hospitals to become more efficient and better control over the operative environment. One area of inefficiency is the cement steps during TKR. Currently the surgeon has very little control over cement polymerization. This leads to an increase in time, waste, and procedural inefficiencies. There is a clear need to create an extended working time, moldable, osteoconductive, and osteoinductive bone augment as a substitution for the current clinically used bone cement where the surgeon has better control over the polymerization process. This study explored several compositions of pentaerythritol-co-trimethylolpropane tris-(3-mercaptopropionate) hydroxyapatite composite materials prepared via benzoyl peroxide-initiated thermal frontal polymerization. The 4:1 acrylate to thiol ratio containing augment material shows promise with a maximal front temperature of 160 °C +/- 10 °C, with mechanical strength of 3.65 MPa, and 111% cytocompatibility, relative to the positive control. This frontally-polymerized material may have application as an augment with controlled polymerization supporting cemented implants.
3.2 Introduction

During knee replacement surgery, an orthopedic surgeon commonly uses a synthetic material, poly(methyl methacrylate), referred to as bone cement, to aid in stress distribution and adhesion of the metal prosthetic to the patients knee.[11, 13] It is projected that over a million primary total knee replacements (TKR) will occur by 2030 in the United States.[63] While bone cement has been shown to last up to 15 years within a patient, it lacks in osteoconduction and osteoinduction, thus cell ingrowth and bone regeneration does not occur.[64] Many state-of-the-art biomaterials are shifting to a composite augment utilizing calcium phosphate additives to increase the osteogenic properties of the biomaterial.[65] Another major constraint of bone cement is the working time of the material, which greatly constrains clinicians during tissue preparation. According to Lidgren et al., for Palacos® bone cement that was thoroughly mixed by hand at 21°C, it took about 4.27 minutes to harden enough to mold, 5.19 minutes of moldable or handling time, and 10.18 minutes to reach a fully set polymer.[8] Unfortunately, if the physician and operating room staff are unable to prepare the bone cement correctly or if the mold does not fit properly, the entire process must be restarted creating waste, inefficiency, and compromises quality.

After the literature review of the different types of materials recently discovered for support of knee prosthetics, a potential solution to the working lifetime issue might be found by using thermal frontal polymerization (TFP) to form a bone augment. TFP proceeds via a thermally-induced free-radical polymerization mechanism. The polymerization is initiated by the decomposition and radical formation of benzoyl peroxide and follows the mechanism in Scheme 1.
Figure 3.1 – Thiol-ene free-radical chain growth polymerization mechanism.

The thyl radical only forms in the reactions with trimethylolpropane tris-(3-mercaptopropionate) (TMPTMP). The pores within the augment form as a result from the release of CO₂ as a by-product from using the peroxide initiator. This reaction has been demonstrated in water and other organic solvents.[66, 67] It has also been shown that any gases or water dissolved within the monomers can be volatilized during the reaction and lead to bubble formation.[66]

A study by Garber et al. on a novel bone construct displayed high biocompatibility using pentaerythritol-co-trimethylolpropane tris-(3-mercaptopropionate) formed by a copolymerization with an amine-catalyzed Michael addition mechanism.[68] This study utilized a foaming mechanism to generate a porous scaffold and conducted an in vivo model to test the biocompatibility and osteogenic properties.[69] The osteogenic results have led to the potential of utilizing these acrylate and thiol monomers, however due to their lack of cure-on demand these will be used under the free-radical frontal polymerization mechanism, to result in a biocompatible scaffold.
Thermal frontal polymerization utilizes a local free-radical reaction that propagates in a wave like pattern. This front travels due to the coupling of the thermal diffusion and the Arrhenious dependence of the reaction rate of an exothermic polymerization. The front travels at a front velocity dependent on the initiator concentration, temperature, pressure, and the type of monomer.[70]

An extract study was done to test cytotoxicity, and then front velocity measurements and temperature profiles were made. Finally, a pot life study, mechanical property analysis, mass loss analysis, and imaging of the augments were done. Utilizing these monomers via a TFP mechanism instead of the Michael addition mechanism, bone augment synthesis can be conducted with an extended working time and at a rapid reaction rate. This cure-on demand capability empowers the physician to fully shape and mold the monomer form before setting the polymer. This reaction occurs in a short time period and leads to a biologically friendly augment that supports and promotes cell growth. This study uses NIH 3T3 cells for an in vitro cytotoxic evaluation.[71] One application of this material could be as a substitute for currently used bone cement in total knee replacement.

3.3 Materials and Methods

3.3.1 Materials

Materials for augment synthesis were used as obtained from Sigma Aldrich: trimethylolpropane tris(3-mercaptopropionate) (TMPTMP), Luperox A98 - benzoyl peroxide (BPO), hydroxyapatite (HA), and from Alfa Aesar: pentaerythritol triacrylate (PETA).

Augment Synthesis:

Four different composites were synthesized at different ratios of acrylate to thiol. Chen et al.’s procedure to find the lowest concentration of benzoyl peroxide was conducted, and all are
synthesized with 0.5% (w/w) BPO (data not shown).[72] Per Garber et al.’s display of bioactivity, all augments were made with 20% (w/w) HA.[68] The ratio of acrylate to thiol is as follows in Table 3.1:

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Percent PETA by mass</th>
<th>Percent TMPTMP by mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylate only</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>9 to 1</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>4 to 1</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>2 to 1</td>
<td>66</td>
<td>33</td>
</tr>
</tbody>
</table>

Ratios of PETA to TMPTMP with greater amounts of TMPTMP were tested, but were unable to polymerize via thermal frontal polymerization. From henceforth all composites will be referred to by their ratio of PETA to TMPTMP.

Monomers of PETA and BPO were mixed for 24 hours prior to addition of TMPTMP and HA. All augments containing the TMPTMP and HA were mixed in the same order and duration using a rotor for 1 minute for each monomer. Fronts were initiated with a commercial soldering iron or a hot plate depending on the mold (See Supplemental Information).

3.3.2 Cell Culture

NIH 3T3 mouse fibroblast from passages 30 to 55 were cultured in Dulbecco’s Modified Eagle Medium (DMEM) with 10% fetal bovine serum in 25 cm² tissue culture flasks. All cells were incubated at 37°C and 5% CO₂. The media was changed in each flask every 3 days and passaged at 80% confluence.

3.3.3 Extract Cytotoxicity

A 96 well tissue culture plate was seeded with 1 x 10⁵ 3T3 mouse fibroblast cells and incubated at 37°C and 5% CO₂ for 24 hours. Three replicates of 200 mg +/- 2.3 mg augment structures were soaked in 5 mL of DMEM with 10% fetal bovine serum and placed on a rocker
while being incubated at 37°C and 5% CO₂ for 7 days. The extract solution was then filtered through a 0.22 µm pore size filter, and 150 µL per well was placed into a 96 well tissue culture plate that was seeded with 3T3 mouse fibroblast cells. Samples were incubated at 37°C and 5% CO₂ for 24 hours and cellular viability was analyzed using the Alamar Blue assay. The dead control was conducted by adding 100% ethanol to wells with cells for 4 hours prior to staining. This assay called for 10 µL of Alamar Blue reagent to be added to 100 µL of culture medium per well and incubated at 37°C and 5% CO₂ for 4 hours. The fluorescence was measured at an excitation wavelength of 535 nm and an emission wavelength of 595 nm using a plate reader.

3.3.4 **Front Velocity and Temperature Profile**

Monomers of each sample were placed in a metal mold, measuring 0.7 cm x 1.3 cm x 10.2 cm. A commercial soldering iron was used to initiate thermal frontal polymerization. The front velocity was calculated by capturing the reaction via video and determining the time required for the reaction front to travel 1.27 cm in three different portions of the total length of the mold. The temperature profile was captured using a type T thermocouple and collected by a USB TC-08 Thermocouple Data Logger.[67] Polymerization was performed in triplicate and deemed successful if a self-sustaining front propagated.[73]

3.3.5 **Pot Life Study**

Monomers of each augment type were mixed and left at room temperature. The time until bulk polymerization occurred was determined in triplicate.

3.3.6 **Mechanical Testing**

All samples were compressed at 2.5 mm per minute using an Instron (Instron Model 5696, Canton, MA) until a yield point or 10% deformation was reached. The samples were
cylindrical with 6 mm in diameter and 12 mm in height. Sample testing was a modified version of Kim et al.’s protocol.[14]

3.3.7 Mass Retention Test

Three replicates of each augment structure were soaked in DMEM with 10% fetal bovine serum and placed on a rocker while being incubated at 37°C and 5% CO₂ for 7 days. Composite samples were trypsinized (0.25% trypsin, 1X EDTA) for 10 minutes then rinsed three times in phosphate buffered saline (-Ca, -Mg) and freeze dried. Each sample was normalized to its’ initial mass prior to media exposure.[68]

3.3.8 Scanning Electron Microscope Analysis

The cross-sectional view was observed by scanning electron microscopy. The cylindrical augment synthesized for mechanical testing was cut to a 3 mm height. A conductive platinum coating was applied using EMS550X sputter coater for two-2 minute coatings.

3.3.9 Micro-Computerized Tomography Analysis

Each cylindrical structure from mechanical testing was placed into the micro-CT and imaged with a SkyScan Model 1074 microtomography scanner, the x-ray source voltage was 40 kV, the source current was 1000 microamps (uA), and the exposure time ranged from approximately 300 msec to 600 msec. The scanner had a detector with 37 μm pixel size, the x-ray source was 3 μm, and the effective (reconstructed) spatial resolution was about 80 μm full-width-at-half-maximum (FWHM). Scaffold porosity was calculated using the Image Processing toolbox in MATLAB v7.12 (MathWorks, Natick, MA).

3.3.10 Statistical Analysis

All results were expressed as a mean ± percent error. Data were analyzed with a two way t-test. For all comparisons, a p-value < 0.05 was determined significant.
3.4 Results

3.4.1 Effect of Acrylate and Thiol Concentrations

In comparing the reaction properties of the different ratios of acrylate to thiol in Table 3.1, as the amount of thiol was increased, the front velocity decreased from 0.45 cm/sec for the Acrylate only down to 0.12 cm/sec for the 2:1 acrylate to thiol augment, as seen in Figure 3.1. While the front was retarded, the maximum temperature inversely correlated with thiol content with temperatures reducing from 200°C to 124°C.

![Graph showing front position versus time for all acrylate to thiol ratios with 0.5% (w/w) benzoyl peroxide and 20% (w/w) hydroxyapatite.](image)

Figure 3.2 Front position versus time for all acrylate to thiol ratios with 0.5% (w/w) benzoyl peroxide and 20% (w/w) hydroxyapatite.

The temperature for the Acrylate only augment was 200°C +/- 10°C, 9:1 acrylate to thiol augment was 180 +/- 10°C, the 4:1 acrylate to thiol augment was 160°C +/- 10°C, and the 2:1 acrylate to thiol augment was 124°C +/- 4°C. The front velocity of the Acrylate only augment took 6.83 +/- 0.33 minutes to react and cool to 40°C, while the 9:1 acrylate to thiol reached 40°C in 8.10 +/- 0.38 minutes. Similar to the decreasing trend of maximum temperature, the total time decreased from the 9:1 to the 2:1 acrylate to thiol augment with the 4:1 acrylate to thiol augment at 6.38 +/- 0.88 minutes and the 2:1 augment at 5.17 +/- 0.53 minutes, shown in Figure 3.2.
3.4.2 Effect of Acrylate and Thiol Concentrations on Cytocompatibility

Although the reaction properties mostly trended from Acrylate only to the 2:1 acrylate to thiol augment, the biological properties displayed a trend toward the augments with the middle ratios of acrylate to thiol, as shown in Figure 3.3. High biological compatibility from the extracts of the augments was only displayed by the 9:1 and 4:1 acrylate to thiol augments. These augments displayed above 100 percent, normalized to the live control, of cell viability in the extract of these augments.

3.4.3 Pot Life Study

In Figure 3.4, as the amount of thiol was increased there was a decrease in the stability of the system at room temperature before bulk polymerization occurred. The Acrylate only augment lasted greater than 24 hours, while even the 9:1 augment only lasted 2 hours. The 4:1 augment and the 2:1 acrylate to thiol augment had 1 and 0.75 hour pot lives, respectively.
3.4.4 Mechanical Strength

With the addition of thiol, the ultimate compressive strength was observed to increase as shown in Figure 3.5 from as low as 0.87 MPa for the Acrylate only to 3.65 MPa for the 4:1 acrylate to thiol augment. The mechanical strength plateaued at the 4:1 acrylate to thiol augment, thus the 2:1 acrylate to thiol augment withstood the same amount of compressive forces.
The Young’s modulus displays a similar trend as the ultimate compressive strength with the Acrylate only being the stiffest at 6.25 MPa and leveling off at 30.7 MPa for the 4:1 acrylate to thiol augment in Figure 3.6.
3.4.5 Mass Retention Study

Each formulation experienced a small decrease in mass as illustrated in Figure 3.7, except for the 4:1 acrylate to thiol augment. The mass retained was within a range of 1.1% for the Acrylate only, 9:1, and 2:1 acrylate-to-thiol formulation. The 4:1 formulation had a larger range, and lower mass retention at 93.6% retained.

Figure 3.8 – Mass retained after 7 days in tissue culture media at 37°C. *Indicates significance of \( p < 0.05 \).

3.4.6 Porosity and Imaging

Total porosity inversely correlated with increasing thiol concentration, except for the 4:1 acrylate to thiol composition. All samples showed less than 23% porosity with the 2:1 acrylate to thiol formulation having the lowest porosity at 5.9%. The 4:1 acrylate to thiol augment did not hold this trend; with higher porosity than the Acrylate only augment at 23%, as shown in Figure 3.9.

The SEM image in Figure 3.9, of the 4:1 acrylate to thiol augment, shows a cross-sectional image of the morphology. The average pore diameters of the macropores were 120 µm with micropores between 1 µm and 10 µm.
Figure 3.9 – Porosity of augments with varying ratios of acrylate to thiol. All ratios have 0.5% (w/w) benzoyl peroxide and 20% (w/w) hydroxyapatite. **All samples were significant from each other.

Figure 3.10 – Scanning electron microscopy of: a) Acrylate only, b) 9:1 ratio of acrylate to thiol, c) 4:1 ratio of acrylate to thiol, d) 2 to 1 ratio of acrylate to thiol displaying consistent porosity.

The 4:1 acrylate to thiol augment cross-sectional view was also examined via micro-CT imaging as shown in Figure 3.10 a) and reconstructed imaging to display the outer structure in the 3D image shown in Figure 3.11. Figure 3.10 portrays the internal porosity and some slight
elongation of the pores from the bottom left to the top right of image a) and can be compared to the 9:1 augment in image b).

Figure 3.11 - micro-CT cross-sectional slice of: a) Acrylate only, b) 9:1 ratio of acrylate to thiol, c) 4:1 ratio of acrylate to thiol, d) 2 to 1 ratio of acrylate to thiol. Analyzed via MATLAB. Augment cylinders are 6 mm in diameter and 12 mm in height. Scale bar is 6 mm.

Figure 3.12 - micro-CT 3D image of: a) Acrylate only, b) 9:1 ratio of acrylate to thiol, c) 4:1 ratio of acrylate to thiol, d) 2 to 1 ratio of acrylate to thiol. Augment cylinder is 6 mm in diameter and 12 mm in height. Scale bar is 6 mm.
3.5 Discussion

Thermal frontal polymerization works by the feedback between the heat released by the polymerization and the Arrhenius dependence of the polymerization rate on temperature. Most of the temperature dependence is through the decomposition of the initiator. Benzoyl peroxide was chosen as an initiator due to its prevalence in bone cement and other biomaterials.[74, 75] The self-propagating front can be locally initiated and propagate from a heat source to cure the entire polymer. The initiation temperature is dependent on the geometry of the monomer, the materials it is in contact with, and the external environment (i.e. room temperature). These factors also affect the reaction rate, and so the temperature and reaction rate will depend on the application.[70] By using a fine tipped heat source the physician only needs access to a small portion of the monomer and would still be able to cure an area that is covered or not easily reachable.

This study was conducted to determine material’s structure as a function of the thiol concentration. Three ratios of acrylate to thiol were synthesized, 9:1, 4:1, and 2:1 by weight, and one composition without thiol. Ratios of 1:1 and 4:3 by weight of acrylate to thiol did not frontally polymerize due to the high concentration of thiol.[76]

Working with the three ratios of acrylate to thiol and the Acrylate only material, the results display the high extract cell viability of the 9:1 and 4:1 augments. The cell death in the Acrylate only augment is believed to have occurred due to the leeching of excess acrylate monomer that decomposes into acrylic acid.[77] The build-up of excess monomer in the Acrylate only augment is attributed to the low monomer conversion, especially at the surface of the augment. While the excess acrylate monomer caused cell death in the Acrylate only augment, it is hypothesized that hydrolyzed thiol monomers, and/or a change in pH may have
caused the cell death observed in the 2:1 ratio samples.\cite{78} Thiyl radicals are known to onset the mechanism for cell damage by thiols.\cite{79, 80} It is also possible that, due to the lower front temperature, there were unreacted thiols that diffused out of the scaffold and were able to follow a similar pathway to the thiyl radical, disrupting the cell membrane, and lysing the cells. It is presupposed that this release of thiol monomer over the 7 days occurred in all the of the augments with thiol, but the concentration released within the 2:1 acrylate to thiol augment was high enough to lead to cell lysis.

For use as a surgical augment, the temperature change caused by the reaction must be considered. The trends of the augments exhibit the increase in temperature with a decrease in thiol concentration. This is due to lower enthalpy of the copolymerization of a thiol with an acrylate compared to that of the homopolymerization of acrylate. With fewer acrylate-acrylate bonds formed, the temperature decreased in the 9:1, 4:1, and 2:1 augments. When comparing these augments to bone cement’s temperature range, from 70°C to 120°C during polymerization the 4:1 and the 2:1 acrylate to thiol augments are the two that are closest to and within the bone cement range. The bone cement’s duration at an elevated temperature is longer than that of the 4:1 acrylate to thiol augment.\cite{81} The quick heating and rapid cooling could limit the amount of tissue damaged at the surgery site. The time for augments to react and cool to 40 °C for the 4:1 augment was less than that of bone cement, for bone cements reaction time alone is greater than the duration to heat and cool the augments. Thus, this fast reaction is advantageous for the physician’s total working time and the amount of time the tissue is exposed to high, potentially damaging, temperatures.

As shown by Viner et al., as the thiol concentration is increased, bulk polymerization initiated by a redox reaction between the thiol and peroxide becomes favorable. Also, the thiol
acts by decreasing the oxygen inhibition. The greater the concentration of thiol the greater is the decrease in bulk polymerization pot life. This trend of decreased pot life was shown from the 9:1 to the 2:1 acrylate to thiol augments.

The mechanical properties are shown to increase as the thiol concentration is increased, correlating indirectly with the porosity study. As the thiol concentration was increased from the 9:1 to the 2:1 acrylate to thiol formulation, the maximum temperature decreased, likely resulting in less expansion. The decrease in porosity ultimately led to an increase in the mechanical strength of the samples with lower acrylate content. Due to the porosity, the frontally polymerized augment is about one twentieth of the strength of commercially available bone cement.

The large distribution in mass retention and porosity for the 4:1 sample is hypothesized to be a result of the carbon dioxide released from the scaffold. In future studies, adjusting the porosity can be achieved by a change in the concentration of BPO, initiator. This could decrease the amount of gas released, providing a narrower distribution of mass retention and porosity, and result in consistent porosity similar to what is observed within the 9:1 and 2:1 acrylate to thiol augments.

Cordell et al. has shown the importance of micropores for initial cell adhesion and the importance of macropores between 100 µm to 150 µm pores for cell growth and mineral formation. With Cordell et al.’s augments having both micropores and macropores, adequate structure for adhesion, nutrient and waste flow, could lead to cell ingrowth post implantation.
3.6 Conclusion

Utilizing thermal frontal polymerization provides an opportunity for physicians to utilize an alternative to traditional bone cement with an extended working time but a “cure-on demand” capability. This study explored the effect of different ratios of frontally polymerized acrylate to thiol and concluded that the 4:1 ratio of acrylate to thiol augment displayed the optimal cytocompatibility, mechanical strength, and porosity when compared to the Acrylate only, 9:1 and 2:1 acrylate to thiol augments. Based in these results the 4:1 augment may prove useful as a substitution for bone cement in orthopedic repair procedures.

3.7 Acknowledgements

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4. SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

4.1 Summary

Knee arthroplasties were forever changed when Dr. Charnley first used PMMA as a spacer between the metal prosthetic and native bone. PMMA bone cement has been widely characterized in literature, with many modifications, and several unique formulas are used commercially today. Through this study of a triacrylate-co-trithiol by polymerization via free-radical frontal polymerization, a construct was synthesized with potential for use as a substitute for bone cement. This augment was made to address the physician’s concerns of short molding time and to gain control of initiating polymerization. Through use of thermal frontal polymerization, both these concerns were addressed.

This augment provides a similar system to the currently used bone cement where in PMMA bone cement powder and the liquid initiator, are mixed. However the PMMA bone cement commences polymerization once mixed, while the construct developed in this study must be heat catalyzed. These results in increased working time for the physician to shape and mold the monomer mixture. The temperature trigger provides a form of control for the physician and will aid in decreasing the amount of errors, removal of the erroneous cement, and re-molding of the cement. Also, due to the reaction between the thiol and BPO, any monomer not reacted will polymerization in a bulk manner.

To explore the potential of this technique an in vitro study was conducted to test the cytocompatibility, mechanical properties, and the curing time as a function of the ratio of acrylate to thiol monomers by weight percent. The acrylate only, 9 to 1 acrylate to thiol, 4 to 1 acrylate to thiol, and 2 to 1 acrylate to thiol polymers underwent an extract metabolic assay, compression testing, and imaged via micro-computed tomography for porosity analysis.
4.2 Conclusions

The results of this study indicate that the 4 to 1 acrylate to thiol bone augment had a curing temperature most similar to PMMA bone cement while having the highest cytocompatibility, compressive strength, and porosity of the augments tested. This provided a platform for use of frontal polymerization and a “cure-on-demand” system as a synthetic material for a biological application. With innate porosity, due to the production of gas during polymerization, the TFP mechanism is reliable for allotting space for cell ingrowth and nutrient or waste flow for cells. The maximum temperature during polymerization can be tuned with the addition of fillers within the monomers, and the total reaction time is shorter than that of PMMA bone cement.

Through *in vitro* testing, the 9 to 1 and 4 to 1 acrylate to thiol augments has shown positive results from their extract cytotoxicity assays with mouse derived fibroblast cells. This provides more insight to the balance and proper ratio between acrylate and thiol monomers within the polymer to reach a non-toxic level.

The mechanical properties of the augment initially increased with incorporation of thiol monomer then plateaued at higher thiol concentrations. This correlated with the porosity of the each of the monomers and was verified via SEM and micro-CT imaging.

4.3 Recommendations

1. Adjusting and expanding the additions of other bioactive molecules. This may lead to better osseointegration and osteoinduction.

2. By adjusting the hydroxyapatite (filler) weight percentage, the maximum temperature can be decreased further below the curing temperature of state-of-the-art bone cements.
3. While the gas produced induces the necessary porosity needed, measuring expansion of the polymer as a function of porosity could aid in fine-tuning the porosity and in optimizing the clinical application of this technology.

4. Tensile, flexure, and fatigue testing should be done to better understand the constructs mechanical limitations particularly in physiologically relevant conditions.

5. An *in vivo* study within small animals (i.e. murine) could be conducted to better understand the biocompatibility, mechanical strength, and osteogenic potential of the scaffold.
REFERENCES


VITA

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