THE STUDY OF INITIATION SYSTEMS AND FORMULATIONS FOR THE DEVELOPMENT OF A NOVEL SILORANE BIOMATERIAL

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ABSTRACT

There are approximately one million hip and knee replacements each year in the United States alone and over 70% are cemented for stabilization. The number of these replacements is expected to rise to 3.5 million per year by 2030 and result in an estimated several fold increase of the current global market of a billion to multi-billion dollars over the next fifteen years. The current commercially available polymethyl methacrylate (PMMA) based bone cements have been used since the 1960's with little change to their composition. They provide strength and longevity for total joint replacements, however they are not without their disadvantages. Issues such as polymerization shrinkage, high curing temperatures, and component toxicity have been reported. In order to address these problems, we replaced the methacrylate-based resin with a silorane-based system, which are novel monomers previously used for dental composites. Our goal is to develop

a new bone cement that would have handling times between 10 – 20 min, curing temperatures under 45 °C, good mechanical strength, and biocompatibility. An important part of this effort centered on the identification and investigation of silorane initiation systems, which can be tailored for specific uses including internal bone cements. The initial screening process utilized the neat resin system followed by differing formulations including modified and unmodified fillers. The tests were based in part on the ISO standard 5833 used for acrylic resin cements and included exothermicity, degree of cure, biocompatibility, and mechanical strength. From these studies, we identified alternative bone cement formulations, which met or exceeded our desired properties as compared to commercially available bone cement.

APPROVAL PAGE

The faculty listed below, appointed by the Dean of the School of Graduate Studies have examined a dissertation titled "The Study of Initiation Systems and Formulations for the Development of a Novel Silorane Biomaterial," presented by Rachel A. Weiler, candidate for the Doctoral of Philosophy degree, and certify that in their opinion is worthy of acceptance.

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CHAPTER 1

INTRODUCTION TO BONE CEMENTS

Current Needs

Each year there are over one million total hip and total knee replacements performed in the United States alone.[1] This number is expected to continue to rise due to the aging population.[2] In addition, the average age of patients is getting younger, especially for knee replacements, due to more wear and tear from sports injuries. By 2030, it is estimated that the number of total hip replacements in the US will grow to over 550,000, which is an increase of 174%. For total knee replacements, the numbers are estimated to climb by 673%, from 450,000 surgeries in 2005 to 3.48 million surgeries per year by 2030. However, these numbers only take into account new surgeries and not revisions.[3] Furthermore, while there are some cementless options, cemented replacements are more common and have better health outcomes such as earlier weight-bearing and less pain.[2,4] Therefore, the development of new and improved bone cements is still of great interest both on the commercial and academic levels.

History of Bone Cements

In general, bone cements are biomaterials that are used for device fixation in knee and hip replacements as well as other total joint replacements (TJR). They have also been used in tumor surgeries, percutaneous vertebroplasty, spacers, and antibiotic beads.[5] Bone cements, which are used for joint replacement, lack adhesive properties. Instead, they hold the implant tightly against the bone and function as a space filler. On an interface level, these cements mechanically interlock a surgically implanted prosthesis with the irregular surface of the bone while improving the prosthesis-cement-bone system's load carrying capacity by the cement's ability to transfer the load from the prosthesis to the bone.[5,6 7]

One of the first total knee prostheses was performed by Themistolke Gluck in 1870, using rosin and plaster to cement an ivory prosthesis.[6] Today, commercially available bone cements are mainly comprised of methyl methacrylate and polymethylmethacrylate (PMMA). PMMA has been available since 1843 and was first used in a biomaterial for dental applications around 1936.[8] The era of modern PMMA bone cements began with Degussa's and Kulzer's patent in 1943, which utilized a tertiary amine to initiate the polymerization of methyl methacrylate at room temperature.[6] In 1958, Sir John Charnley developed a PMMA bone cement capable of anchoring implants.[6,8] He attached an acrylic cup to the femoral head as well as cementing the

metallic implant in the femur using a PMMA bone cement. Since the 1970s, there has been little, if any change in the general composition of bone cements.

Bone Cement Components

Bone cements are comprised of three main components, the monomer, the initiation system, and fillers, all with an appropriate shelf life. The resin is comprised of monomers and the initiator system. Upon polymerization, an optimal resin should have low volumetric shrinkage, low generated heat , and little or no toxicity. The filler components should have compatible chemistry with the resin.[9] The resulting composite material should have all of the properties of the ideal resin plus appropriate mechanical properties.

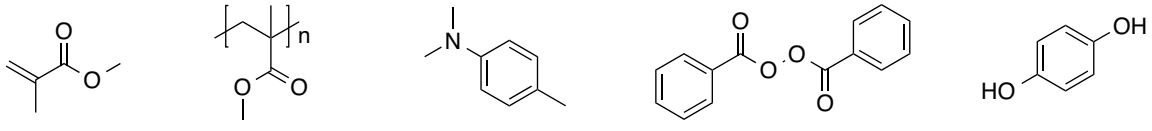
In joint replacement surgeries, there are over 30 commercial PMMA bone cements available, and most of them have similar compositions.[2] They are normally packaged as a two-phase system, a powder and a liquid, both of which are mainly comprised of methacrylates. The powdered portion contains pre-polymerized polymethylmethacrylate (PMMA) beads, a radiopacifier, and an initiator. The liquid portion contains methyl methacrylate monomer, an accelerator, and an inhibitor (Table 1.1).[2] The radiopacifier, typically barium sulfate or zirconium dioxide, is visible in X-rays, which is important for surgical purposes. The accelerator, N,N-dimethyl-*p*-toluidine (DMPT), is used to promote the initiation of the reaction by generating free radicals from decomposition of the initiator, benzoyl peroxide.[8] The inhibitor (hydroquinone) protects the cement against self-polymerization due to light or heat

during storage by acting as a free radical trap. Each component is discussed in more detail in the monomer, initiation, and filler sections of this chapter.

Table 1.1: Typical components of commercial bone cements.[2]

FUNCTION	Component (industry range in total wt%)
RESIN	Methyl methacrylate (**1**, 32.3-33%)
FILLER	Pre-polymerized PMMA beads (**2**, 55.3-66%)
RADIOPACIFIER	Barium sulfate or zirconium dioxide (6-10%)
ACCELERATOR	*N,N*-dimethyl-*p*-toluidine (DMPT, **3**, 0.13-0.93%)
INITIATOR	Benzoyl peroxide (BPO, **4**, 0.5-1.73%)
INHIBITOR	Hydroquinone (**5**, 5-25 ppm)

Figure 1.1: Structures of bone cement components: methyl methacrylate (**1**), PMMA (**2**), DMPT (**3**), BPO (**4**), and hydroquinone (**5**).



1 **2** **3** **4** **5**

Drawbacks of PMMA Bone Cement

PMMA is used in commercial bone cements because of its strength and longevity in total joint replacements.[2] However, there are still many drawbacks with current cements, including toxicity of the monomers, toxicity of the accelerator, high curing temperatures, and polymerization shrinkage. Incomplete curing of the cement leads to unpolymerized monomers, which can leach into the body. These monomers can cause hypotension, inflammation, and tissue irritation, as well as changes in liver function.[10] Due to the volatility of the methacrylate monomer, exposure poses similar health risks in its day-to-day usage in the surgical theater.[11] High concentrations of the residual accelerator, DMPT (**3**), have been found in cement from hip replacements, which were removed after 10 years of surgery. Furthermore, DMPT presents a serious health issue because of its cytotoxicity in combination with possible long-term leaching. Additionally, the high heat of the polymerization may reach temperatures greater than 70 °C.[2,12] The curing heat can cause necrosis of the surrounding bone and tissue. Finally, the resulting polymerization shrinkage is another serious problem. Depending on the method of mixing (e.g., hand or vacuum), the volumetric shrinkage can range between 1-8%,[13,14] which can lead to gaps between the implant and cement, as well as between the cement and bone. Shrinkage, along with poor integration with the bone, leads to loosening of the implant and the need for surgical revision.[13]

Previous Alternatives

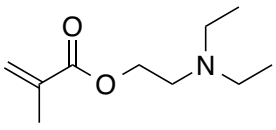
Due to all the previously mentioned problems and the increase in total joint replacements, there is a need for a better or alternative bone cement—but one that would address the drawbacks of current commercially available cement. Previous research focused on the inertness and thus lack of interaction of the cement with the bone, the accelerator, toxicity of the components, and the resulting high curing temperatures. Most of this research centered on making small adjustments to the PMMA formulation, such as substitution or modification of the fillers rather than radically changing the cement's formulation.[2]

In order to address the issue of inertness, the addition of bioactive agents such as hydroxyapatite (HA) was proposed. While there was some improvement with the maximum exotherm, there was no bonding between the HA and the PMMA. Therefore, there was an increase of water absorption, which would result in the initiation of fractures.[2] For the toxicity of the DMPT (**3**) issue, studies were performed with respect to the decrease in the amount of DMPT or alternative accelerators. When less DMPT was used, the resulting exotherm was lower but it resulted in longer setting times. There were no observable advantages when the accelerator was changed from DMPT.[2]

In an attempt to address the drawback of high curing temperatures, additives were added to the liquid monomer (methyl methacrylate, **1**). They included co-monomers such as *N,N*-diethylaminoethyl methacrylate (DEAMA, **6**), methacrylic acid (**7**), and *p*-*N,N*-

diethylaminobenzyl methacrylate (DEABMA, **8**).[2] Resins that incorporated aromatic monomers (e.g., DEABMA) had higher hydrophilicity, shorter setting times, and better mechanical properties compared to those with the aliphatic co-monomers (e.g., DEAMA). However, on average, all of the additive systems, had higher maximum exotherms, lower compressive strength, and consistently higher residual monomer content as compared to commercially available cement.[2] Because of these results, the addition of a co-monomer was not an advantageous alternative.

Figure 1.2: Structures of co-monomer additives: DEAMA (**6**), methacrylic acid (**7**), and DEABMA (**8**).



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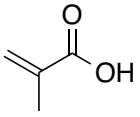

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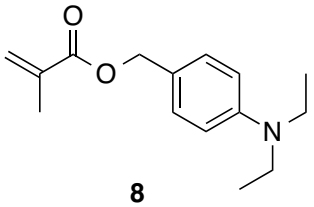

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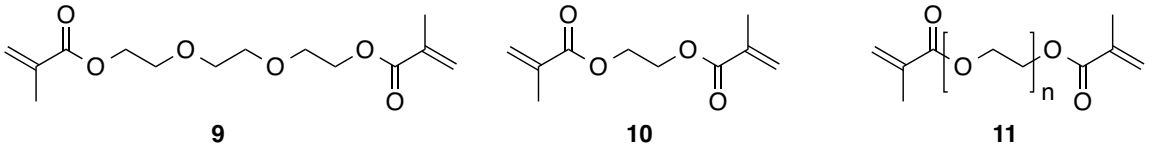
In order to improve the cement's adhesion to the bone and the implant, crosslinking agents were investigated. These compounds were dimethacrylates, which contain more than one "active" unit and would potentially result in a polymer with higher strength. Examples of these additives include triethylene glycol dimethacrylate (TEGDMA, **9**),

ethylene glycol dimethacrylate (EGDMA, **10**), and poly(ethylene glycol) dimethacrylate (PEGDMA, **11**) Unfortunately, there was no increase in mechanical strength upon the addition of the crosslinkers.[2] In both of these additive approaches, methacrylates were used but they did not address the toxicity associated with residual monomer.

Figure 1.3: Structures of crosslinking additives: TEGDMA (**9**), EGDMA (**10**), and PEGDMA (**11**).



9 **10** **11**

Since there was still an issue of toxicity and strength, other non-PMMA alternatives were investigated. Two such examples were the replacement of PMMA with glass polyalkenoate (ionomer) cements (GPCs) and calcium phosphate cements (CPCs).[2] GPCs were first introduced by Kent and Wilson in 1970.[15,16] The GPC-based cements are two phase systems, typically consisting of an acidic polymer solution, poly(acrylic acid), and a basic glass powder, usually calcium fluoroaluminosilicate.[15] When they are combined, neutralization occurs, and the material hardens, during which process fluoride

ions are released.[16] The fluoride release, along with good biocompatibility, has led to the use of GPCs in dentistry as dental fillings.[15,16]

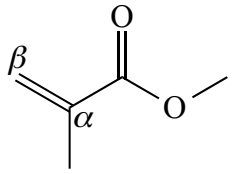
In the 1980s, Brown and Chow developed the first CPCs.[8] Typically, CPCs are also a two-phase system with one or more calcium phosphate compounds as the solid phase, and phosphate-containing solution or water with an additive, such as alginate or succinate, as the liquid portion.[17,18] When the liquid phase becomes saturated, crystals precipitate, and their entanglement leads to the hardening of the cement.[17] CPCs are biocompatible, bioactive, and biodegradable; however, when tested they yielded poor mechanical strength.[18] Because of this drawback, CPCs are mostly used as bone substitutes or grafts in craniofacial applications.[17,18] Both GPCs and CPCs are not ideal cements for joint fixation.

In order to fully understand how to improve a bone cement, each of the three main components, the monomers, initiation system, and fillers, need to be investigated. Since the type of monomers dictates the initiation system used, the monomers will be the first components researched. The monomers that are currently used in commercial bone cement, namely methacrylates, and then possible alternatives, such as alkenes, siloranes and oxiranes, were examined. After the monomers, initiation for the various monomer types was investigated.

Monomers: Methacrylates, Alkenes, Oxiranes, and Siloranes

As mentioned previously, bone cements are mainly comprised of methacrylates. The structure contains contain a methyl group attached to the α carbon ($C_{\beta}=C_{\alpha}(CH_3)$-COOR), and is a methyl ester of the acrylate (Figure 1.4).[19] Methacrylates are not only used in commercial bone cements but also in textiles, paints, plastics, and other biomaterials.[19] In current use within the health care field, there are commercial dental composites, such as Filtek™ Z250, bone cements, such as Simplex® P, contact lenses, microcapsules for drugs, and dialysis membranes.[8]

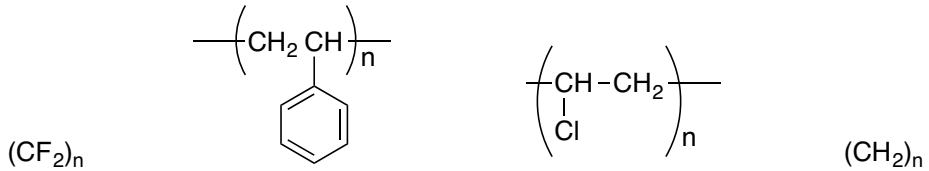
Figure 1.4: Structure of methyl methacrylate (**1**).



Alkenes are another category of monomers that undergo free radical polymerization and contain at least one π bond between two sp^2-hybridized carbons.[20] While they undergo many different reactions, including cationic and free radical reactions, (e.g., allylic halogenation), the polymerization of alkene monomers has

produced many useful polymers. Some of the more familiar materials are poly(tetrafluoroethylene), (Teflon®, **12**), polystyrene (**13**), poly(vinyl chloride) (PVC, **14**), and polyethylene (**15**) (Figure 1.5, where only the simplest repeated unit is depicted).

Figure 1.5 a-d: Common alkene based polymers: Teflon® (**12**), polystyrene (**13**), PVC (**14**), and polyethylene (**15**).

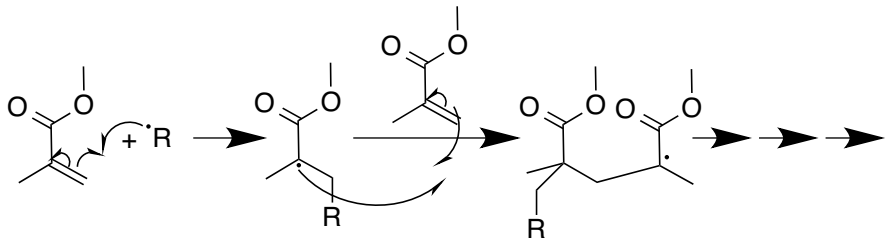


12 **13** **14** **15**

The standard free radical process for monomer units (e.g., alkenes and acrylates) is comprised of three steps: initiation, propagation, and termination.[20] The initiation step is one in which a radical is generated using heat, light, and/or chemical initiation. It does not involve the monomer, but typically an initiator radical. The propagation step is the reaction of at least one radical with a monomer to expand the polymer chain and results in another radical generated. This monomer radical reacts with another monomer to

continue the process (Scheme 1.1). The final step is termination, in which two radicals combine, thus "terminating " the chain reaction.[20]

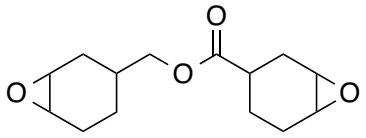
Scheme 1.1: Free radical polymerization propagation step.



Oxiranes

Oxiranes, also known as epoxides, are three-membered rings containing an oxygen and two carbons. They are used in adhesives, paints, coatings, and more recently dental impression materials.[21] Dow's CYRACURE™ UVR-6110 ((7-oxabicyclo[4.1.0]heptan-3-yl)methyl-7-oxabicyclo[4.1.0]heptane-3-carboxylate, **16**), is an example of an epoxy resin that is used as a casting resin and UV resistant decorative coating for metals (Figure 1.6).[22] Oxiranes normally undergo cationic ring opening polymerization, which requires an initiator but is not limited to acids. An example polymerization scheme for cyclohexene oxide is depicted in Scheme 1.2.

Figure 1.6: Structure of UVR-6110 (**16**).



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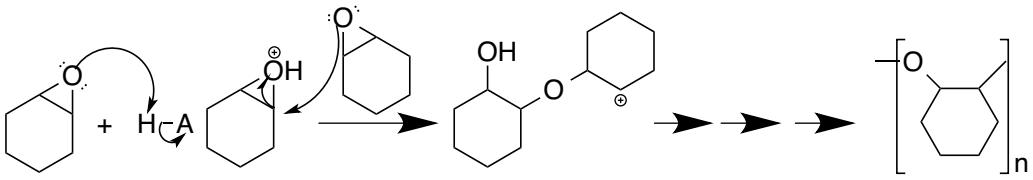
Scheme 1.2: Oxirane cationic polymerization scheme.



Siloranes

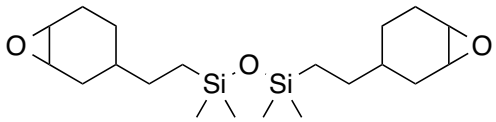
Another class of monomers is one that contains both oxirane and siloxane functional groups, siloranes (disiloxane, **18**). A siloxane compound contains a silicon – oxygen bond (**18**) (Figure 1.7).[23] 1,3-Bis(2-(7-oxabicyclo[4.1.0]heptan-3-yl)ethyl)-1,1,3,3-tetramethyldisiloxane (silorane, **19**) undergoes cationic ring opening

polymerization at the oxirane unit and is one component of a commercially available dental composite distributed by 3M ESPE, and called Filtek™ LS.[24]

Figure 1.7: Structures of oxirane (**17**), a siloxane (disiloxane, **18**), and silorane (**19**).

17

18



19

In an attempt to reduce polymerization shrinkage of dental composites, siloranes were first investigated as an alternative to the current methacrylate-based composites. Polymerization shrinkage is problematic because it can lead to small spaces between the material and the tooth, leading to loosening of the filling and even failure.[23] For example, neat methyl methacrylate resins have been shown to have shrunk up to 22 vol%,[25] whereas the methacrylate-based composite, Filtek™ Z250, resulted in around 2% volumetric shrinkage. On the other hand, the volumetric shrinkage of a silorane composite was less than 1%, which is a significant difference compared to the neat resin.[23] The reduced methacrylate polymerization shrinkage was due to the bonding change from a carbon-carbon double bond into a single bond with a neighboring

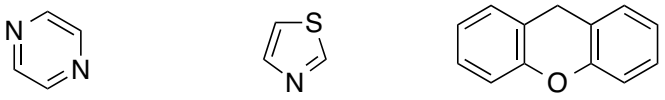
monomer.[26] For siloranes, their large bulky size, along with the oxirane ring opening during polymerization, resulted in less shrinkage.

As mentioned previously, biomaterials are comprised of monomers, an initiation system, and fillers. Methacrylates, alkenes, oxiranes, and siloranes have been discussed and used in biomaterial resin. While they all have shortcomings, the initiation system is important for the determination of a possible commercial biomaterial.

Initiation

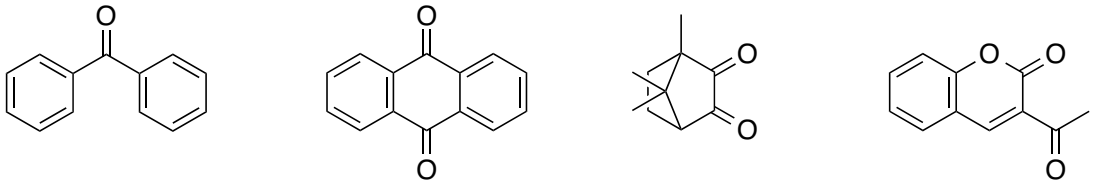
The initiators fall into two categories: "on demand" curing,[32] commonly referred to as photo- or light-induced initiators and "immediate curing," also labeled as chemical curing. For the light-induced curing systems, they are commonly binary or ternary systems. A binary photoinitiation system consists of a light absorbing photosensitizer, which absorbs light to initiate a reaction, and an electron donor, which propagates the reaction. A ternary system includes the addition of an accelerator. A typical photosensitizer is normally a dye or ketone (Figures 1.8 and 1.9). Examples of dyes include substituted azines ($R_2C=N-N=CR_2$, compounds derived from the reaction of hydrazine with a ketones or aldehydes, 1,4-diazine **20**), thiazoles (heteroaromatic compounds containing a sulfur and nitrogen atom, thiazole (**21)**), and xanthenes (xanthene, **22**). However, the preferred photosensitizer is a ketone, such as a monoketone, α-diketone, or ketocoumarin. A few examples of these types of ketones are benzophenone (**23**), anthraquinone (**24**), camphorquinone (**25**), and 3-acetylcoumarin (**26**) (Figure 1.9).[28-30] After the photosensitizer is irradiated, the excited species has a short lifetime in which to react with the monomer to initiate polymerization, leading to slow rates of polymerization when used alone. In the case of camphorquinone, there was only an 18.3% degree of conversion for a methacrylate-base resin after 60 sec of irradiation.[31] Due to the slow reaction time there is need for an additional component, a proton donor.

Figure 1.8: Examples of photosensitizer – dyes: 1,4-diazene (**20**), thiazole (**21**), and xanthene (**22**).



20 **21** **22**

Figure 1.9: Examples of photosensitizer – ketones: benzophenone (**23**), anthraquinone (**24**), camphorquinone (**25**), and 3-acetylcoumarin (**26**).

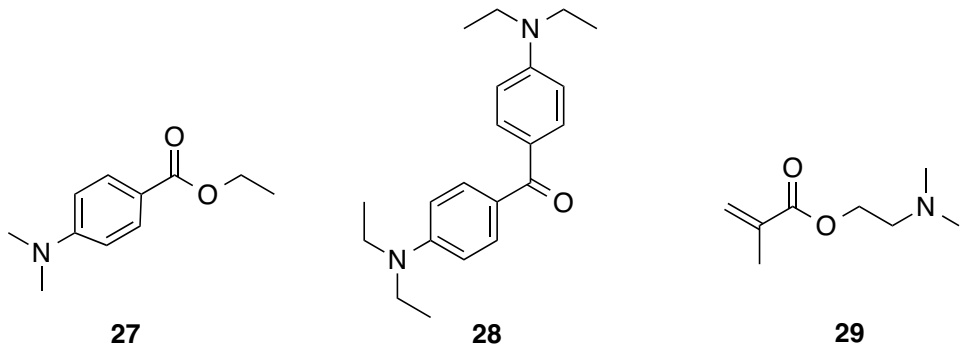


23 **24** **25** **26**

Disubstituted amines are common proton donors in photoinitiation systems (Figure 1.10). A few examples of these reaction promoters are ethyl *p*-dimethylaminobenzoate (EDMAB, **27**), 4,4'-bis(diethylamino)benzophenone (BDEAB, **28**), and 2-dimethylaminoethyl methacrylate (DMAEMA, **29**).[30,32] These compounds react with the excited photosensitizers to generate the radicals needed for polymerization. With the addition of EDMAB to camphorquinone, there was in increase in the degree of

conversion for a methacrylate-base resin to 55% after 60 s of irradiation compared to 18% with camphorquinone alone.[31]

Figure 1.10: Examples of proton donors: EDMAB (**27**), BDEAB (**28**), and DMAEMA (**29**).

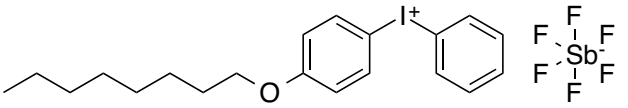


27 **28** **29**

In the case of a ternary photoinitiation system, there is the addition of an accelerator, which is usually a photoacid.[23] Photoacids are compounds that generate an acid after irradiation. In the case of light-induced initiation systems, the photoacids are typically onium salts, such as (4-n-octyloxyphenyl)phenyliodonium hexafluoroantimonate (**30**) and [4-[(2-hydroxytetradecyl)oxy]phenyl]phenyliodonium hexafluoroantimonate (**31**) (Figure 1.11).[30,33] For onium salts, alone they are activated by light below 300 nm, generating a reactive aryliodo radical-cation.[31] This is typically done by using a UV light source; however, that would not be suitable for applications in

health-related fields. When used in conjunction with a visible light photosensitizer and proton donor, the onium salt will decompose in the visible light range, yielding the reactive radical-cation. The addition of an onium salt to the binary system of camphorquinone and EDMAB increased the rate of polymerization for a methacrylate-based resin from 1.65% to 2.73% per s.[31]

Figure 1.11: Onium salts **30** and **31**.



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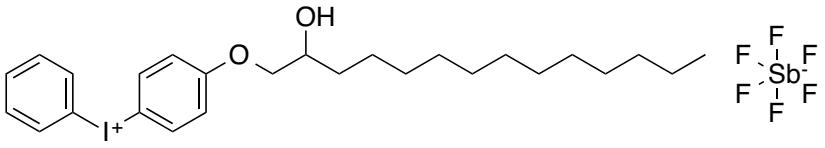
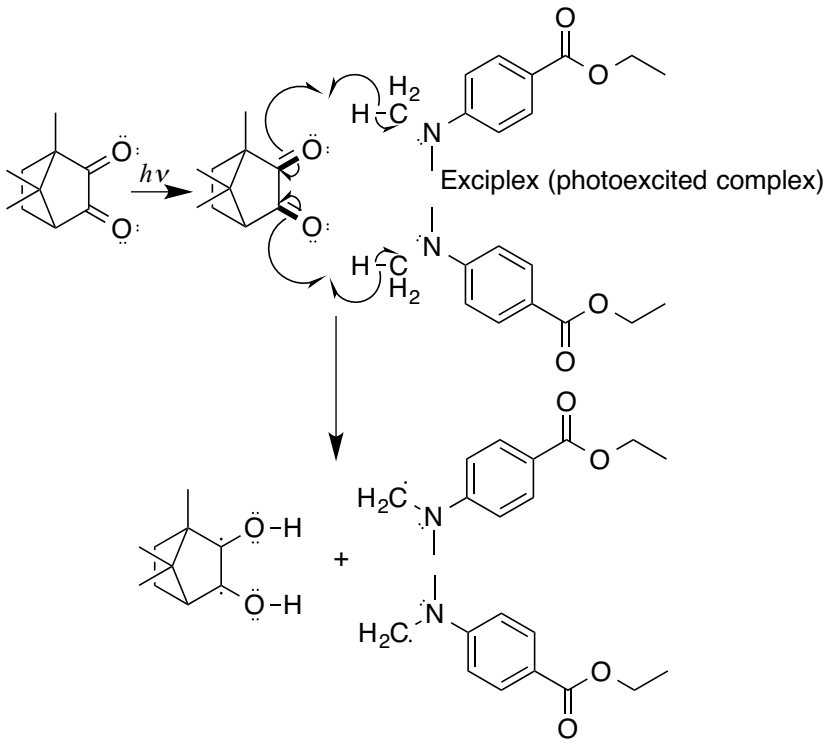


31

These ternary light-cure systems can be used for both free radical and cationic polymerization. For methacrylate dental composites, an excited species is produced from the photosensitizer, camphorquinone, after irradiation with a dental lamp with a wavelength of 470 nm. In this case, the tertiary amine is responsible for the hydrogen

transfer reaction generating the radical.[23,31,34,35] The reaction mechanism for the radical generation is depicted in Scheme 1.3 using camphorquinone and EDMAB. Once the initial radicals have been formed, the polymerization reaction can begin (see Scheme 1.1). In cationic systems, after irradiation, the excited photosensitizer reacts with the onium salt leading to a radical-ion species.[31] When this radical-ion species is reduced, it is the Lewis or Brønsted acid produced from the onium salt that initiates the cationic polymerization.[33,36]

Scheme 1.3: Radical formation during light initiation.

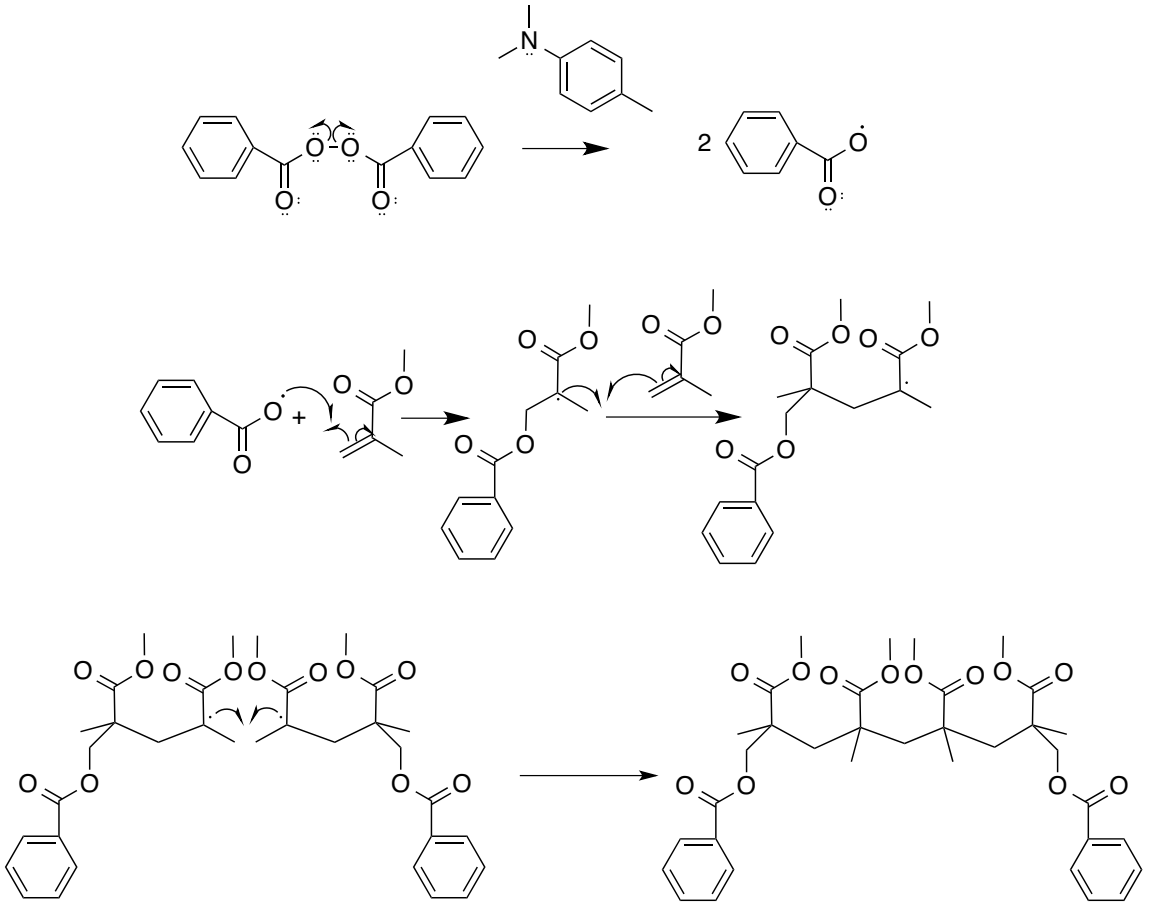


The conjugate base produced in this conversion plays no role in the photochemistry cycle; however, a weak nucleophile is required so as not to prematurely terminate the polymerization reaction.[37]

While light initiation is acceptable for dental composites, light initiation is not feasible for other biomaterials, such as bone cements. Instead, they undergo chemically initiated free radical polymerization, where a chemical, not light, generates the radical.

In a typical bone cement, an amine accelerator, DMPT, decomposes the initiator, benzoyl peroxide (BPO), to generate the radical.[8,38] The mechanism for the free radical polymerization of methyl methacrylate, which is initiated by using this process, is depicted in Scheme 1.4.

Scheme 1.4: Chemically initiated free radical polymerization of methyl methacrylate.

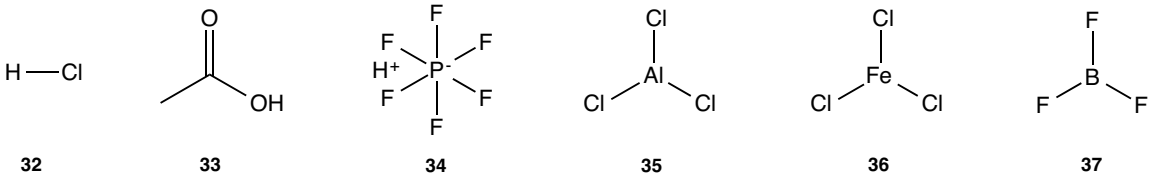


In the case of oxiranes, extreme conditions, such as harsh catalysts and /or high heat applications, are frequently used in the chemical curing process for industrial settings. Typical laboratory conditions include high temperatures (e.g., temperatures greater than 80 °C), prolonged reaction times, and/or a 1:1 ratio of catalyst to monomer for complete conversion to product .[28] These conditions are definitely not suitable for a biomaterial cured internally.

For classical chemical initiators (e.g., Lewis or Brønsted acids), almost immediate curing occurs when the initiation ion is released during the dissociation of the initiator in the monomer system.[39] Acids used for polymerization fall in to the categories of Brønsted, strong, weak , super,[40] and Lewis[41] acids. Brønsted acids are proton donors and the terms "weak" and "strong" acids refer to an acid's ability to dissociate to ions in water. Strong acids completely dissociate in water, while weak acids only partially dissociate. Therefore, stronger acids have larger acid-dissociation equilibrium constants, K_a, than weak acids. An example of a typical strong acid is hydrochloric acid (HCl, **32**), which has a K_a of 1 x 10^3, while acetic acid (CH_3COOH, **33**) is a weak acid with a K_a of 1.8 x 10^{-5} (Figure 1.12 a-b).[20] Acid strength is more commonly referred to as pK_a, the negative logarithm of the acid-dissociation constant. In regards to pK_a, smaller values equate to stronger acids. For HCl, the pK_a is -8, whereas CH_3COOH has a pK_a of 4.8.[20] Acids that are 100 times stronger than sulfuric acid (pKa = -3)[20] belong to the category of "super" acids.[40] An example of a super acid is hexafluorophosphoric acid (HPF_6, **34**).

Rather than limiting an acid to a proton donor, compounds that can accept a pair of electrons are Lewis acids. Examples include aluminum chloride ($AlCl_3$, **35**), iron (III) chloride ($FeCl_3$, **36**), and boron trifluoride (BF_3, **37**) (Figure 1.12). These particular acids are often used in Friedel-Crafts alkylations and acylations, which are important electrophilic aromatic substitution reactions.[20] In the reaction of benzene with acetyl chloride, a Lewis acid catalyst, aluminum chloride, is used, and the halogen becomes a stronger electrophile.

Figure 1.12: Example of acids: HCl (**32**), CH_3CO_2H (**33**), HPF_6 (**34**), $AlCl_3$ (**35**), $FeCl_3$ (**36**), and BF_3 (**37**).



32 **33** **34** **35** **36** **37**

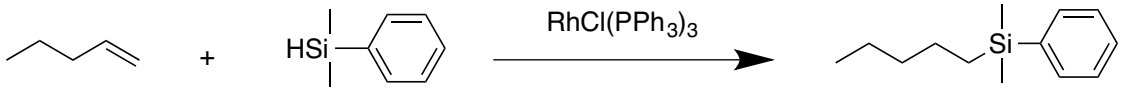
While these acids are quite effective in polymerization[20] and Friedel-Craft reactions, there are several drawbacks. In the case of Friedel-Craft acylation, the Lewis acid is not a true acid catalyst because it complexes with the carbonyl group of the product. In some cases, heat is required for the reaction to occur but it is generally an exothermic

reaction.[20] These are limitations for their use as initiators in biomaterials because stoichiometric requirements, toxicity, sensitivity, and exothermicity can cause cell necrosis.[2,12,42,43]

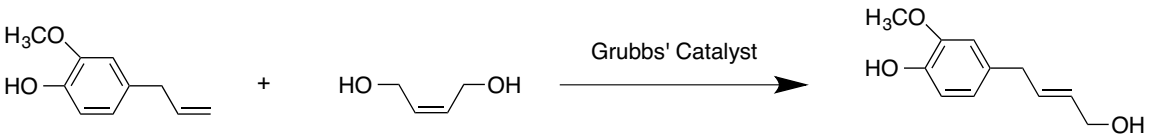
Since the early 1980s, a relatively new subgroup of redox initiators has been investigated.[44,45] This category is comprised of noble metal complexes, such as platinum, palladium, and rhodium catalysts; a reducing agent, such as an organosilane; and an oxidizing agent, such as an onium salt. The original applications for these organometallic complexes were developed for synthetic transformations, which include hydrosilylation (platinum-based Lamoreaux's catalyst, rhodium-based Wilkinson's catalyst) and olefin metathesis (ruthenium-based Grubbs' catalyst) reactions.[44-46] A example of hydrosilylation is the reaction of pent-1-ene and dimethyl(phenyl)silane with Wilkinson's catalyst producing dimethyl(pentyl)(phenyl)silane (Scheme 1.5).[47] Grubbs' catalyst is used in the olefin metathesis reaction of 4-allyl-2-methoxyphenol and (Z)-but-2-ene-1,4-diol with (E)-4-(4-hydroxybut-2-en-1-yl)-2-methoxyphenol as the product (Scheme 1.6).[48] The onium salts have previously been used as photoacids in light initiation systems, and more recently, they have been employed for chemical initiation.[30,33] These redox initiators are used in catalytic amounts, which is advantageous for a biomaterial. Polymerization studies using these redox initiation systems have proceeded rapidly with high reaction exotherms.[49,50] An example of such is the polymerization of 4-vinylcyclohexene-1,2-oxide with Lamoreaux's catalyst and an iodonium salt which

polymerized almost spontaneously with a temperature reaching 258 °C.[49] While this result is not an issue in synthetic methodology, this, along with the possible high toxicity of the organometallic catalysts, would be problematic for its use in health-related applications.[50,51]

Scheme 1.5: Example hydrosilylation reaction.



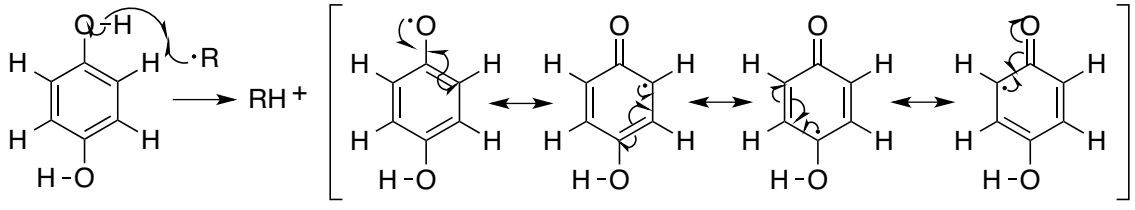
Scheme 1.6: Example of olefin metathesis reaction.



In the development of initiation systems, there are several different factors to consider, including exothermicity, toxicity of acid, and handling times. In order to "tailor" these systems to have the desired properties required for that application,

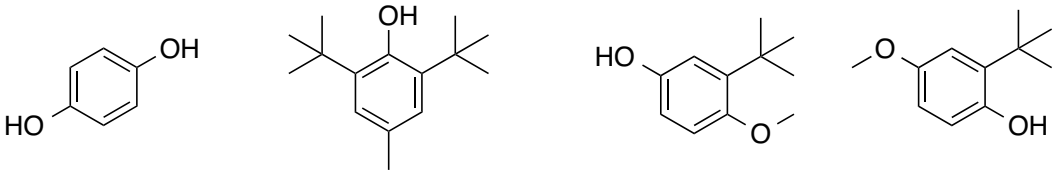
inhibitors can be used in conjunction with the initiators. They can restrict or delay the polymerization reaction by consuming any heat or light generated by the radical and prohibiting it from initiating polymerization.[34] Once the inhibitor is completely consumed, the initiators are free to start the reaction. An example of a free radical inhibitor is hydroquinone (**5**), which is used in commercial bone cements (Figure 1.13). Usually used in ppm quantities, the hydroquinone is first utilized to prevent the methyl methacrylate monomer from self-polymerizing during storage due to light or heat, which results in an increased shelf life. The addition of **5** extends the mixing time of the liquid and powdered portions of the bone cement. Once all of the inhibitor has been consumed, the remaining radicals can then begin the polymerization of the monomers. Hydroquinone is an excellent free radical inhibitor because of its high affinity for free radicals. It is able to donate a proton to a free radical species. The radical becomes trapped in **5** due to its resonance structures (Scheme 1.7).

Scheme 1.7: Hydroquinone as a free radical trap.



There are several other compounds belonging to the hydroquinone class including 2,6-bis(1,1-dimethylethyl)-4-methylphenol (BHT, **38**) and mixture of 2-tert-butyl-4-hydroxyanisole (≤15%) and 3-tert-butyl-4-hydroxyanisole, called BHA (≥85%) (**39**) (Figure 1.13).[52] Both BHT and BHA are commonly used as food additives to extend the shelf life of bread and oils as well as cosmetics.[52,53] In the case of cationic polymerization, strong bases, diols, and crown-ethers, have been also known to inhibit polymerization,[54,55] the same way as the free radical inhibitors, but with ions. Such is the case of 12-crown-4 inhibiting the polymerization of 3,4-epoxy cyclohexyl methyl-3,4-epoxy cyclohexyl carboxylate.[55] The crown ether traps a proton keeping it from the polymerization reaction (Scheme 1.8).

Figure 1.13: Structures of free radical polymerization inhibitors: hydroquinone (**5**), BHT (**38**), and BHA (**39**).



5 **38** **39**

Scheme 1.8: Crown Ether as a proton trap.

Fillers

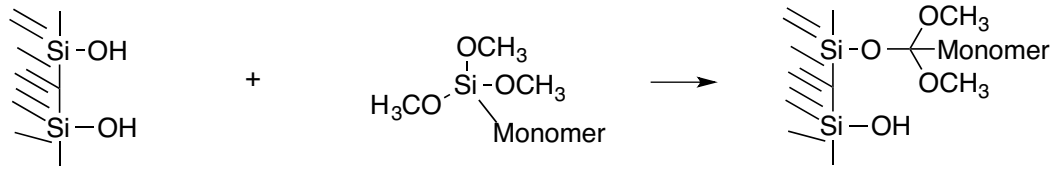
The last of the three main bone cement components is the filler. When choosing a filler, chemical compatibility and a similar refractive index are just two of the characteristics that need to be considered. In order to improve additional properties of the resin, fillers are added to reduce polymerization shrinkage and provide optimal mechanical properties, such as flexural strength and flexural modulus. Usually, an increase in filler equates to an improvement in strength, but this is dependent on a good filler-resin interaction.[35] Certain fillers can also be incorporated as radiopacifiers, which are added to a composition or formulation so as to make the material visible in diagnostic imaging (e.g., x-rays) as well as allowing for differentiation between the cement, bone, and implant. They are typically heavy inorganic metal salts, such as barium sulfate ($BaSO_4$), bismuth oxide (Bi_2O_3), zirconium oxide (ZrO_2), and yttrium (III) oxide (Y_2O_3).[56] These compounds are radiopaque because of their high density, which is due to the high atomic mass of the elements.[56] Typical commercial bone cements use either barium sulfate or zirconium oxide as in the case of Simplex P.

Fillers are essentially comprised of glass and other powders, such as polymer beads or nanofibers. In dental composites, quartz and other silica-based fillers are often used to enhance the mechanical properties, and barium, zirconium, or other heavy metals are added for radiopacity.[35] Bone cements, on the other hand, use pre-polymerized polymer beads to increase the material's strength and either barium or zirconium to

ensure radiopacity.[7] Depending on the application, the formulation is filled to different levels, which changes the viscosity of the cement. For dental composites, the cement is filled from 38 – 85 wt% depending on the particle size, of which 5-15 wt% is the barium sulfate radiopacifier.[21] In the case of typical commercial bone cements, the polymer beads make up the majority of the filler (55 – 60 wt%) with only 6 – 10 wt% of barium sulfate or zirconium dioxide.[2]

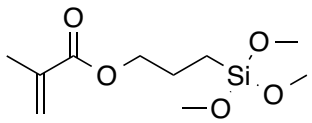
In addition, the filler's surface can be modified in order to improve the resin-filler interaction. Most dental composite fillers are modified so as to reduce hydrophilicity and enhance filler dispersion.[26] Better filler dispersion allows for higher filler loading, which in turn improves the properties of the matrix, resulting in a stronger material with less polymerization shrinkage.[26.35]

Scheme 1.9: Filler modification reaction scheme.



The filler can be modified by a chemical reaction of the Si-O with a compatible monomer, which covalently bonds the monomer to the glass surface. (Scheme 1.9). The rationale is that the functional interface provided by the modification may assist in dispersion, "homogeneity" and improved modulus. The most common monomer used for this purpose in dental glasses is methacryloxypropyltrimethoxysilane (MPS, **40**, Figure 1.14).[26]

Figure 1.14: Structure of methacryloxypropyltrimethoxysilane (MPS, **40**).



40

All three components of a biomaterial formulation, monomer, initiation system, and filler, have now been described. The requirements of a biomaterial, more specifically a bone cement, and the assessments that would be used to evaluate that material, are discussed in the next sections.

Properties of Bone Cement and Standard Testing

There is a set of desired properties that are required for the bone cement formulations, including handling and polymerization times, heat of polymerization, flexural strength, and flexural modulus.

Table 1.2: Desired properties of bone cement.

	ISO 5833 Standard[57]	Desired properties
Exothermicity (°C)	≤90	≤45
Handling time (min)	3-15	≤20
Flexural modulus (GPa)	≥1.8	≥1.8
Flexural strength (MPa)	≥50	≥50
Compressive strength (MPa)	≥70	≥70
Pull out strength – mimic (MPa)	n/a	≥4.5
Pull out strength – *ex vivo* (MPa)	n/a	≥4.5
Pull out strength – *in vivo* (MPa)	n/a	≥4.5
Cytotoxicity (% cell death)	n/a	≤20%

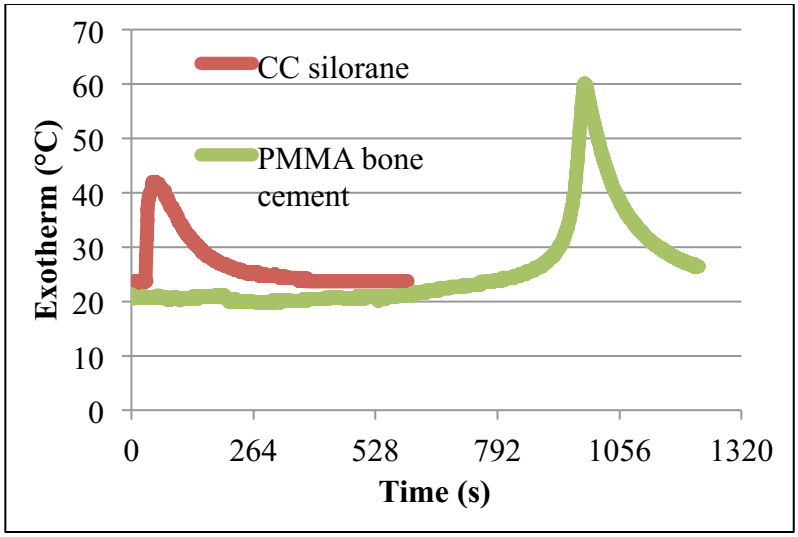
The results of our studies were compared to the standards listed in the International Standard for "Implants for surgery – Acrylic resin cements" ISO 5833 (Table 1.2).[57] In addition to the ISO standard, the other parameters that were investigated include the degree of conversion, biocompatibility, and finally the pull out strength.

For the determination of the polymerization completion or "hardness", the Gillmore Needle Test (GNT) or a penetrometer was used. In both cases, they are used to determine when a material has polymerized to an appropriate hardness. A needle or probe with a specific weight is placed on the top of a sample. Then, observations of the ability of the sample to support the weight of the probe and any visible defects to the surface are made. For the GNT, a common test for concrete and dental materials, two needles with different weights are used to determine the setting time of a material.[35] On the other hand, a penetrometer is an electric device and is used for testing soil compaction. The GNT was chosen because of its ease of use, and because it requires less material than a penetrometer. For this testing, the one-lb. and ¼-lb needles were chosen. The one-lb. needle was placed on the sample and then removed. If no mark was observed, the sample passed. But if a mark or indentation was made, then the sample failed.

Exothermicity is the heat generated during the polymerization of a sample. When a material is used in the body, high polymerization temperatures can cause cell and tissue necrosis.[2,12,42] Exotherms have been measured with something as simple a thermometer.

A thermocouple is more commonly used, and it can record the temperature profile of the reaction. For the testing of exothermicity in this dissertation, a thermocouple was chosen, and the samples were standardized for reproducibility and accuracy. In ISO 5833, there is a defined mold, however a smaller one was developed to limit the amount of wasted resin. The mold was a Delrin® ring (9.9 mm diameter, 0.7 mm thick) taped to a glass slide. The tip of the thermocouple was then placed inside the ring and secured by tape. The sample was mixed and placed in the ring completely surrounding the thermocouple, which measured the temperatures. From the resulting graph, the maximum exotherm (the highest point on the graph) and the rate of polymerization (where the graph flattens out after the peak) were determined. See Figure 1.15 for an example plot.

Figure 1.15: Example exotherm plot.



The handling or working time is the length of time a material can be manipulated and molded. This time is from when the material is mixed until it becomes elastic and can no longer be manipulated.[35] This property is important because it insures that a surgeon can place a material in its desired location before it completely polymerizes. For this test, the amount of time between initiation and when the material began to gel or was too firm to be manipulated by the researcher's hand was recorded in this dissertation. According to the ISO 5833 Standard,[57] for bone cements, the handling time should be between 3 - 15 min. For our material to pass this test, it needs to have a handling time between 5 - 20 min to have the desired properties.

The flexural strength and flexural modulus are measures of the strength and stiffness of a material. The ISO standard is determined using the four-point bend test, which is a standard mechanical test widely used for plastics, concretes, and composites.

With the exception of the specimen size (due to sample conservation), the ISO 5833 guidelines were followed. In this study, the specimen dimensions in ISO 4049 were used.[58] A beam sample (2 x 2 x 25 mm) was held in place at four points, and then a load was applied to the middle of the beam until it fractured (Figure 1.16). From the resulting stress-strain curve, flexural strength was determined by the maximum peak observed on the graph. The flexural modulus was calculated from slope of the curve before fracture (Figure 1.17).

Figure 1.16: Four-point bend test.

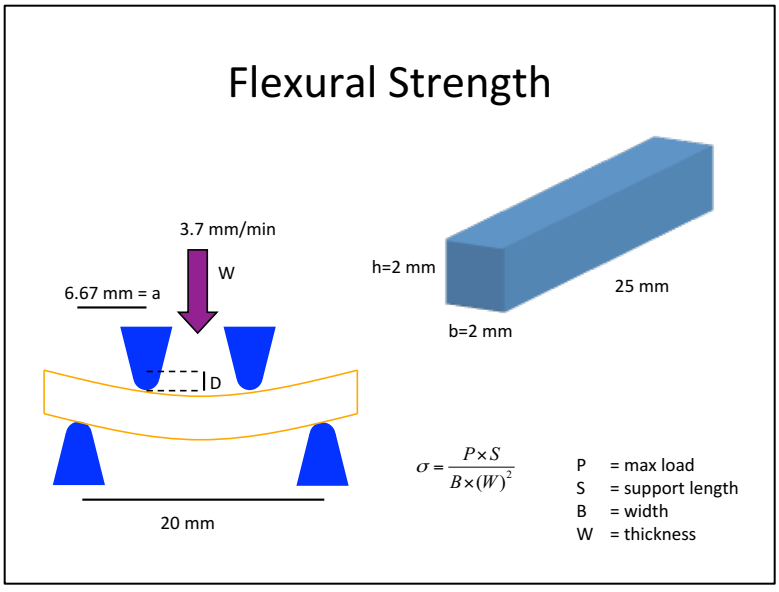
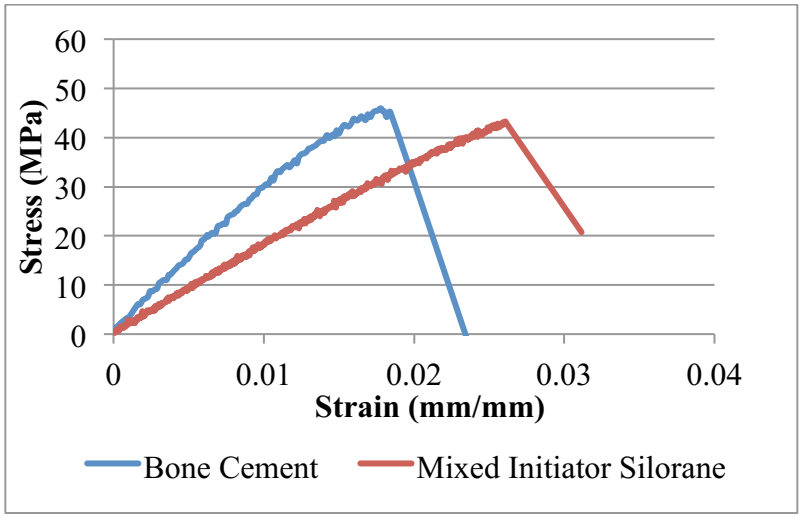


Figure 1.17: Example of stress-strain curve.

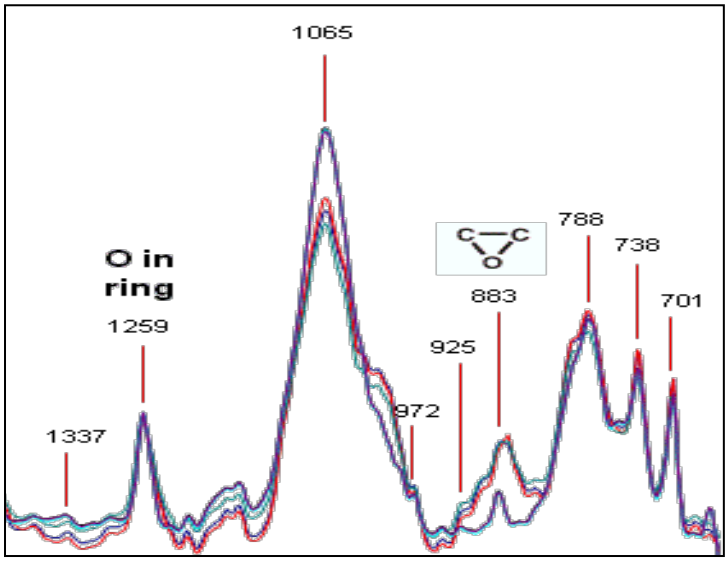


Compressive strength is a measure of "a material's ability to withstand compressive loads without being crushed",[42] and was tested according to guidelines described in ISO 5833. A cylindrical sample (6 x 12 mm) was placed in between plates on the mechanical tester and then a load was applied until the cylinder began to deform or was fractured. The force required for failure was divided by the area of the cylinder to determine the compressive strength (MPa) of the material. The compressive strength for our material should be greater than 70 MPa as according to the ISO 5833.[57]

Fourier Transform Infrared Spectroscopy (FTIR) was used to determine the degree of conversion or degree of cure. These were measured by comparing the change in a peak associated with polymerization (e.g., 883 cm^{-1}, representing epoxide ring opening) to an unchanging peak – an internal standard reference control (e.g., 1257 cm^{-1},

assigned to the Si-O bond in the silorane ring structure). The peak ratios were then calculated (and then reported as a percentage). For this test, a Delrin washer was affixed to the Attenuated Total Reflectance (ATR) accessory with laboratory tape. Approximately 0.1 g of material was placed into the ring that was centered over the ATR crystal. A baseline spectrum was collected before curing. An example spectrum is depicted in Figure 1.18. The peak label "O in ring" is the peak associated with the Si-O bond within the ring in the silorane and remains constant (internal standard). The other labeled peak corresponds to the oxirane units of the silorane, which should decrease upon reaction.

Figure 1.18: Sample FTIR spectrum for degree of conversion determination.



Since a biomaterial has an application for internal use, biocompatibility is an important property. It is a measure of a material's effect, positive or negative, on the cells and surrounding tissues. For our testing, MLO-A5 cells were used, which are post-osteoblast/pre-osteocyte type cells that come from the long bone of mice[59] and were chosen because of our collaboration with Dr. Lynda Bonewald at the UMKC School of Dentistry. With these cells, the Trypan Blue (TB) Exclusion and MTT assays were utilized to determine cell death and cell viability, respectively. Other toxicity assays are possible alternatives, such as other dyes like methyl violet or Evans blue. However, for live cell exclusion, Trypan blue dye is the most common. The TB assay is preferred because a live cell's membrane does not allow the uptake of the dye whereas the dead cell does, leaving the living cells colorless and the dead cells blue.[60] The MTT assay indicates cell viability by measuring the optical density of the purple formazan. Viable cells reduce yellow 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide to formazan, while unviable or dead cells do not. The higher optical density correlates to greater cell viability.[60]

Pull out strength is how strongly an implant is held within the bone by the cement. Depending on the position of the failure during testing, it is a measurement of either the strength of the bone/cement interface or the cement/rod interface. While there is no clear standard sample preparation method, typically a hole is drilled into a bone, and the

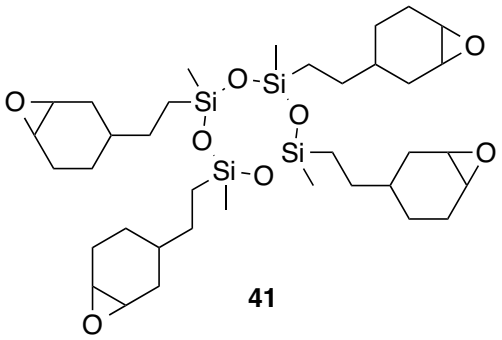
cement and rod are inserted. For testing, the tip of the rod is exposed, and the entire sample placed into a holder and tested on a mechanical tester such as an Instron. The Instron pulls the rod at a constant rate until there is a failure.[61]

More details about each of these test methods can be found in the Appendix A (Materials and Methods). From the collection tests mentioned previously, the proposed biomaterial was evaluated, and its potential use as a new and better bone cement determined.

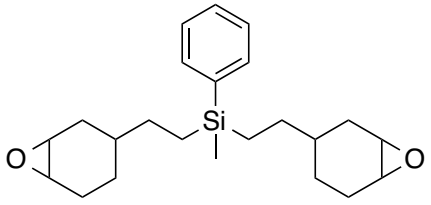
Early Work

A previous project within our collaborative research group was to investigate a new silorane resin, SilMix, as an alternative for dental composites. SilMix is a 50:50 by weight combination of two monomers, 2,4,6,8-tetrakis(2-(7-oxabicyclo[4.1.0]heptan-3-yl)ethyl)-2,4,6,8-tetramethyl-1,3,5,7,2,4,6,8-tetraoxa-tetrasilocane (CYGEP, **41**) and bis[2-(3{7-oxabicyclo[4.1.0]heptyl})-ethyl]methylphenyl silane (PHEPSI, **42**), which were synthesized by Bradley Miller at UMKC. [62] Throughout the research described herein, it was proposed that SilMix is an excellent alternative for bone cements because of its promising physical properties,[63,64] stability in water,[65] and greater biocompatibility.[59,66]

Figure 1.19: CYGEP (**41**) and PHEPSI (**42**).



41



42

Earlier work was centered on the screening of possible fillers for use with light-initiated SilMix. The first level of screening was a pass/fail of polymerization for the individual fillers formulated with SilMix using the GNT. If a filler passed that step, the biocompatibility of the formulation was studied. The final screening was mechanical testing, specifically flexural strength and flexural modulus.[67.68]

Once SilMix had been identified as the base resin system, the next step was the selection of the appropriate filler. The screening test was the one lb. Gillmore needle test (GNT), pass/fail. A variety of fillers were investigated including unmodified glass from MoSci Corporation (Rolla, MO) and alumina nanofibers (ANF) (Dr. Thomas Schuman at Missouri University of Science and Technology, MS&T) (Table 1.3 a-c). SilMix samples containing a ternary light initiation system (3 wt% PIH, 1 wt% CPQ, and 0.15 wt% EDMAB) were filled to 50 wt% with a filler. After irradiation with a dental lamp for two min, the samples underwent the GNT. With the exception of two glasses, all the others passed the GNT. M7 and M8 failed to polymerize and were excluded from further testing. The ANF failed at 50 wt%. It was then tested at 10, 20, and 30 wt%s resulting in a failure at 30 wt%.

Table 1.3 a-c: Glass fillers from MoSci.

a)

Batch Formula Composition Filler	Y_2O_3	Al_2O_3	SiO_2	Na_2O	total
DY5	15	5	80	0	100
DY6	15	15	70	0	100
DY7	14.25	12.25	66.5	5	100
DY8	13.5	13.5	63	10	100
DY9	12.75	12.75	59.5	15	100
DY10	12	12	56	20	100
DY11	11.25	11.25	52.5	25	100

b)

Batch Formula Composition Filler	Li_2O	MgO	BaO	CaO	SrO	Y_2O_3	Yb_2O_3	B_2O_3	Al_2O_3	SiO_2	As_2O_3	AlF_3	total
M-1	3	2	9	0	0	3	0	58	14	8	0	3	100
M-2	3	2	9	0	0	3	0	29	14	37	0	3	100
M-3	3	2	9	0	0	0	3	29	14	37	0	3	100
M-4	3	2	9	0	0	3	0	58	0	22	0	3	100

c)

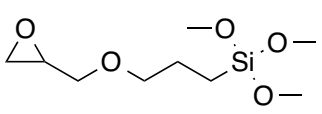
Batch Formula Composition Filler	Na_2O	K_2O	MgO	CaO	BaO	TiO_2	ZrO_2	ZnO	B_2O_3	Al_2O_3	SiO_2	SO_3	Fe_2O_3	Sb_2O_5	total
M-5	0.9	0.4	8.3	0.2	0	0.1	8.8	0	0	20.9	60.4	0	0	0	100
M-6	0.9	0	2.2	22	0	0	4.5	0	7.2	10.6	52.6	0	0	0	100
M-7	9.8	0.4	0.6	5.9	0	0.2	21.8	0	3.1	3.8	54.4	0	0	0	100
M-8	6	8.3	0.1	6	1.9	0	5	4.6	0	1.8	65.2	0.4	0.1	0.6	100
M-9	5.3	6.3	0	0	0	3.6	4.9	7.4	8.1	3.6	60.8	0	0	0	100
M-10	11.6	0.1	3.5	9.6	0	0	1.8	0	0	0.8	72.2	0.3	0.1	0	100
M-12	0	0	0	0	29.1	0	0	0	10.5	5.9	54.5	0	0	0	100

DY5, M1-M4, and M12 glasses along with the ANF were selected to undergo surface modification by Dr. Schuman. The glasses were selected because of their polymerization results as well as having a refractive index similar to that of the resin, SilMix. There were five modifications that were performed with the five fillers. A list of the modifications and structures are given in Table 1.4 and Figure 1.20. All of the modified glasses passed the GNT at 50 wt% (Table 1.5a). The 2-(3,4-epoxycyclohexyl)ethyltrimethoxysilane (ECHE)-modified ANF, on the other hand, failed at 30 and 50 wt%s but passed at 10 and 20 wt%s (Table 1.5b).

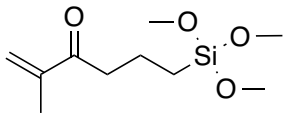
Table 1.4: Filler modifications (**43** – **47**).

Modification	Abbreviation
3-glycidoxypropyltrimethoxysilane	GP-TMS (**43**)
3-methacrylpropyltrimethoxysilane	MAP-TMS (**44**)
2-(3,4-epoxycyclohexyl)ethyltrimethoxysilane	ECHE-TMS (**45**)
[(9,9-diethyl-1,5,7,11-tetraoxaspiro[5.5]undec-3-yl)methyl]trimethoxysilane	1TOSU (**46**)
[3-(9,9-diethyl-1,5,7,11-tetraoxaspiro[5.5]undec-3-yl)propyl]trimethoxysilane	3TOSU (**47**)

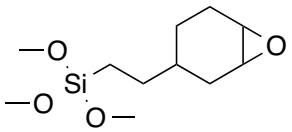
Figure 1.20: Structures of filler modifications (**43** - **47**).



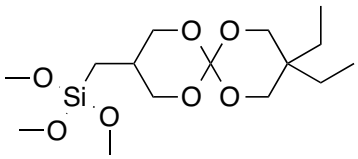
GP-TMS, **43**



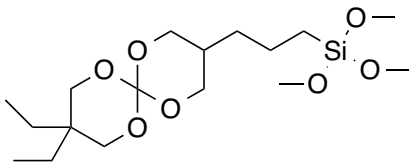
MAP-TMS, **44**



ECHE-TMS, **45**



1TOSU, **46**



3TOSU **47**

Table 1.5 a-b: Modified filler polymerization results.

a)

Filler	Modification	Pass/Fail: 50% filled
M-1	ECHE	Passed
M-2	ECHE	Passed
M-3	ECHE	Passed
M-4	ECHE	Passed
M-12	ECHE	Passed
M-12	1TOSU	Passed
M-12	3TOSU	Passed
DY5	GP-TMS	Passed
DY5	ECHE-TMS	Passed
DY5	MAP-TMS	Passed
DY5	1TOSU	Passed
DY5	3TOSU	Passed

b)

Modified Fillers	Modification	Pass/Fail 50% filled	Pass/Fail 10% filled	Pass/Fail 20% filled	Pass/Fail 30% filled
Alumina Nanofibers	ECHE	Failed	Passed	Passed	Failed

After discussion of these results with our collaborators, it was decided to use DY5, M12, and the ANF for future testing. The cytotoxicity of the fillers were tested as part of the light-cured SilMix system and were found to be biocompatible. More detailed information can be found in Dr. Jennifer Melander's dissertation.[68]

For samples formulated with the DY5, M12, and/or ANF fillers, the flexural strength and flexural modulus were investigated using the four-point bend test. The flexural strength and modulus were determined from the resulting stress-strain curve.. The resins were filled to no more than 50 wt% with various combinations of the fillers. This limit was selected because the majority of filled biomaterials contain 40 – 84 wt% filler. More details can be found in Dr. Jennifer Melander's dissertation[68] and the publication by Melander *et al.*[67] In summary, it was found that incorporation of more than one filler in the resin did not improve either the flexural strength or flexural modulus. It was determined to use either the DY5 or M12 (filled to 50 wt%) as the main fillers for future work.

Summary

As mentioned previously, there is a growing need for an alternative bone cement because of the drawbacks (i.e., toxicity of the monomer and exothermicity of the polymerization) of the currently available commercial bone cements The previous work on dental materials by our collaborators provided us with a possible resin, SilMix, which was investigated as an alternative formulation. Three base fillers (DY5, M12, and ANF) and three surface modifications (ECHE, 1TOSU, and 3TOSU) were identified to move forward in our research. After these two components were identified, the next step was the selection of an appropriate initiation system, which is the third and final component of the bone cement.

CHAPTER 2

CHEMICAL AND MIXED INITIATION SYSTEMS

Introduction

The goal of this research was to develop a silorane-based bone cement alternative. Bone cements are composed of three components, monomer(s), initiation system, and filler. Since SilMix (50:50 by weight ratio of CYGEP and PHEPSI) was selected as the base of a bone cement alternative, our next goal was to choose an appropriate initiation system because a sole light initiation system is not feasible for internal use, which will be discussed below.

There are four main problems with a light initiation system. The first issue is the possibility of cell damage due to the wavelength of the light source required for initiation. Second, the heat radiating from a light source can be a problem for internal use. Some light sources (e.g., 100-watt light bulb) can generate heat greater than 100 °C, which may cause cell and tissue necrosis.[2,12,42] The third issue is the resulting depth of cure, which may lead to incomplete cure. While there is normally ongoing curing or "dark cure", the initial irradiation is at the surface of the material. Therefore, thickness of the material and the refractive index of the filler/formulation affects the completeness and overall time

of cure.[35] This time and depth of cure are issues in joint replacements because patients are required to bear weight on the new joint as soon as possible, sometimes as soon as 24 h post surgery.[3-5] Lastly, there are logistical problems resulting from the use of a light-initiated bone cement. Due to the orientation and placement requirements for implants, it is close to impossible to use a light source internally. A specific example results from the difficulty in the placement of a light source in the femur canal during hip replacement surgeries. Due to these four issues resulting from light initiation requirements, an alternative chemical initiation system was a prerequisite for the development of silorane-based cement. The appropriate initiation system should meet or exceed the following conditions: have an appropriate handling time, generate little heat during the polymerization process, and maintain biocompatibility. The handling time should be between 5 – 15 min to allow the surgeons time to place the material in the surgical site. Ideally, there should be little to no heat produced during polymerization because the heat may cause cell necrosis and damage to surrounding tissue. Finally, it is important that the biocompatibility is similar to light-cured SilMix resins so that it can be considered as a viable alternative to commercially available bone cements.

In order to move forward, there were screening points for each condition before an initiation system would continue to be considered, which were the Gillmore needle test (GNT), pH, exothermicity, biocompatibility, and degree of conversion. The initial screening was a GNT, which was used to determine the time to cure to “hardness”. GNT

and degree of conversion were points used to investigate the handling time. If the formulation passed the GNT by supporting its weight for 30 sec, it was subjected to a pH study to determine the acidity of the material. The acceptable pH difference was within 0.5 units of the light-cured SilMix control. After which, the heat of polymerization was investigated using a thermocouple to measure the exothermicity profile. When the formulations passed the previous three screening parameters, then the biocompatibility and degree of conversion were determined. Ideally, the most desirable system would have a low heat of polymerization, was biocompatible, and had a handling time between five and fifteen min. It was determined that the pure chemical initiation systems with the neat (unfilled) resin would be tested first.

Neat Chemical Cure of SM – GNT

In oxiranes, the ring opening polymerization process is cationic and involves an acid catalyzed protonation of the oxygen.[28,39-41] Using this reasoning, a variety of acids belonging to the categories of weak, strong, super, and Lewis acids were identified (Table 2.1) as possible chemical initiator systems. Initially, SilMix (SM) was combined with one of the fourteen acids at a concentration of less than or equal to 20 wt% (Table 2.2). The "completeness" of polymerization was evaluated using the GNT. If the tested material did not support the one-lb. needle, it resulted in a failure. Hexafluorophosphoric acid (HFPA) was the only acid of all the acids that were tested, which polymerized to hardness (as per the GNT) in less than one h. For sulfuric and triflic acids, polymerization occurred but the resulting materials were too brittle to support the GNT and thus failed the polymerization screening. In the case of acetic acid, no polymerization was observed, even with 20 wt% addition of acid and after 48 h post addition. Some reaction/polymerization was observed in the remaining acids at concentrations as high as 20 wt%. Even so, none of them passed the GNT and were not considered further. The summary of these results are listed in Table 2.2.

Table 2.1: Types of acid catalysts tested.

Acids	Lewis Acid	Weak Acid	Strong Acid	Super Acid
Acetic Acid		X		
Aluminum Chloride	X			
Hexafluorophosphoric Acid				X
Hydrobromic Acid			X	
Hydrochloric Acid			X	
Hydroiodic Acid			X	
Pentafluoropropionic Acid		X		
Phosphoric Acid		X		
Sulfuric Acid			X	
Tin (IV) Chloride	X			
p-Toluenesulfonic Acid		X		
Trichloroacetic Acid		X		
Trifluoroacetic Acid		X		
Triflic Acid (Trifluoromethanesulfonic)				X

It is interesting to note that there was only one acid that passed this initial test, HFPA. It is one of two super acids tested and is an excellent proton donor. The catalytic

process involves homopolymerization – another epoxide adds to the protonated epoxy group. There is no competing reaction because the corresponding counterion, PF_6^-, is non-nucleophilic and does not add to the protonated epoxy group as a nucleophile.[40] On the other hand, HCl is a strong acid and the chloride has the ability to act as a nucleophile and add to the protonated epoxide. The chloride is a smaller, "harder" ion as compared to PF_6^-. It is why the Cl^- is able to compete for the addition to the protonated epoxy group and terminates the polymerization.[40] Since most of remaining acids were more similar to HCl than to HFPA, this reasoning may explain why they did not work. Unfortunately, even though the HFPA system passed, there were drawbacks: short handling time of (less than 30 sec) with rapid polymerization to hardness resulting as brittle crystalline material, which was an unacceptable initiation system for a silorane-based bone cement (Figure 2.1).

Figure 2.1: Pictures of crystalline samples containing a) 4.6 wt% or b) 2.5 wt% HFPA.

a) b)

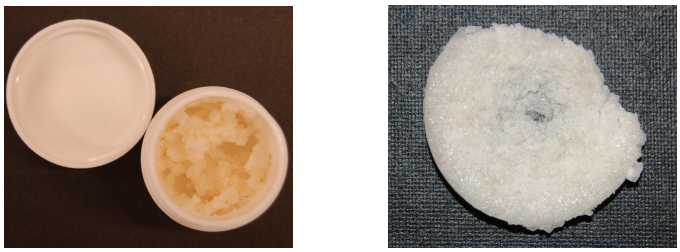


Table 2.2: Summary of polymerization results for initial acids.

Acids	Wt %	GNT Results
Acetic Acid	5%	NR
Aluminum Chloride	13%	NR
Hexafluorophosphoric Acid	**2.5, 4, 4.5, 5%**	**Passed**
Hydrobromic Acid	7.5%	NR
Hydrochloric Acid	2, 5, 10, 14, 18%	Failed – IP
Hydroiodic Acid	6%	NR
Pentafluoropropionic Acid	4, 15, 18%	Failed – HC
Phosphoric Acid	7.8%	Failed – IP
Sulfuric Acid	8%	Failed – Brittle
Tin (IV) Chloride	7.4%	Failed – IP
p-Toluenesulfonic Acid	11.5%	NR
Trichloroacetic Acid	9%	Failed – IP
Trifluoroacetic Acid	7%	NR
Triflic Acid	2, 4%	Failed – Brittle

NR = No Reaction, IP = Incomplete Polymerization, and HC = Some Polymerization at High Concentrations, Bolded samples passed

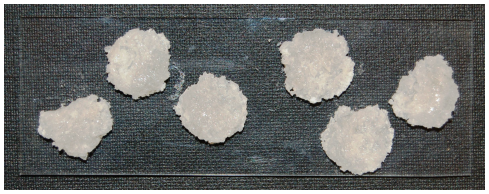
Since there was only one successful monoacid system, HFPA, which had significant drawbacks, combinations of acids and other initiation components were investigated next. Inhibitors (normally amines due to their ability to quench acidic reactions)[54,55] were also included so as to increase the handling time of HFPA. A total of 38 acid combinations with and without inhibitors were studied (Table 2.3). A complete list of all formulations and their polymerization results are listed in Appendix B. Only three of the combination systems were promising, acetic acid/HFPA (1.0/3.2 wt%s), 2-aminopyridine/acetic acid/HFPA (2.7/2.5/8.2 wt%s), and phosphoric acid/PIH (2.2 / 1.6 wt%s). The addition of acetic acid to the HFPA resulted in increased handling times and polymerized material was more amorphous, taffy-like as compared to samples with HFPA alone (Figure 2.2a). The 2-aminopyridine (2.7 wt%), acetic acid (2.5 wt%), and HFPA (8.2 wt%) system produced a smooth material that passed the GNT but in 5 h. The last combination of phosphoric acid (2.2 wt%) and PIH (1.6 wt%), passed the GNT at 6 h time point. The resulting material was a clear, colorless polymer with some intermittent white whiskers (Figure 2.2b). These three formulations, HFPA, 2-aminopyridine/acetic acid/HFPA and the phosphoric acid/PIH, underwent pH testing for the next phase of screening.

Table 2.3: Chemical initiation systems.

Chemical Initiation Systems	
Acetic Acid (AA)	**Phosphoric Acid:PIH (phenyl iodonium salt)**
Aluminum Chloride	**HFPA:Acetic Acid**
Hexafluorophosphoric Acid (HFPA)	HFPA:Butylhydroxytoluene
Hydrobromic Acid	HFPA:Pyridine
Hydrochloric Acid	HFPA:12-crown-4 ether
Hydroiodic Acid	HFPA:Pyridine:Acetic Acid
Pentafluoropropionic Acid	HFPA:Triethylamine
Phosphoric Acid	HFPA:Triethylamine:Acetic Acid
Sulfuric Acid	HFPA:Acetonitrile
Tin (IV) Chloride	HFPA:Acetonitrile:Acetic Acid
p-Toluenesulfonic Acid (*p*-TSA)	HFPA:*p*-Nitroaniline
Trichloroacetic Acid	HFPA:2,6-dinitroaniline
Trifluoroacetic Acid	HFPA:Sulfanilamide
Triflic Acid (Trifluoromethanesulfonic)	HFPA:Phenylenediamine
Triflic Acid:Acetic Acid	HFPA:2-aminopyridine (2AP)
p-TSA:pentafluoropropionic acid	HFPA:Ethanol
Phosphoric Acid:Ethanol	HFPA:Ethanol:Acetic Acid
2-aminopyridine:Potasium-tert-butoxide	**HFPA:2-aminopyridine:Acetic Acid**

Bolded samples passed

Figure 2.2 Pictures of a) HFPA with acetic acid and b) Phosphoric acid with PIH.



Neat Chemical Cure of SM – pH Testing

Since acids were used in the initiation system, it was necessary to determine the pH of the polymerized material as a pre-screening for biocompatibility. If all of the acid from the initiation system was not completely consumed or if there was an acidic by-product produced during the polymerization, the resulting polymer would be acidic, which may cause cell death or necrosis. For bone cells, such as osteoblasts, the optimal environmental pH is 7.4. When the environment becomes more acidic, cell death occurs.[43] Even a decrease as small as 0.4 units can result in a 40% decrease in cell viability.[43] Due to this issue, a test involving pH was developed. Light-cured SilMix (LCSM) was used as the control since it was previously determined as biocompatible [59,66] and must have acceptable pH properties. For samples to move forward onto biocompatibility testing, the change in pH must be within 0.5 units of the pH change produced by the control.

For this study, samples were placed in beakers with deionized or distilled water, and the pH was measured over one h at 15 min increments. The change in pH was determined by taking the difference of the initial pH of the water without the sample and the final pH after one h. The control (LCSM) was compared to HPFA (5.0 wt%), 2-aminopyridine/acetic acid/HFPA (2.7/2.5/8.2 wt%s), and phosphoric acid/PIH (2.2/1.6 wt%s) samples. The control resulted in a 1.11 unit decrease in one h. The sample with the largest change after one h, a 3.55 unit decrease, was the phosphoric acid/PIH (2.2/1.6

wt%s), which also had the longest polymerization time (6 h). The HPFA sample (5.0 wt%) was the next largest, for which in one h the pH decreased by 2.81 units. The 2-aminopyridine/acetic acid/HFPA (2.7/2.5/8.2 wt%s) formulation resulted in a total pH decrease of 1.45 units in one h (Table 2.4), which was within 0.5 units of the control.

Table 2.4: pH change after one h for chemically cured SilMix.

Sample	ΔpH
Light-cure (control)	-1.11
HFPA (5.0 wt%)	-2.81
H_3PO_4 (2.2 wt%)/PIH(1.6 wt%)	-3.55
2-AP(2.7 wt%)/AA(2.5 wt%)/HFPA(8.2wt%)	**-1.43**

Due to the large change in pH and long polymerization time, the phosphoric acid/PIH system was not considered viable moving forward. The 5 wt% HFPA formulation was also not considered for biocompatibility testing, but exothermicity of the polymerization was tested. The 2-aminopyridine/acetic acid/HFPA (2.7/2.5/8.2 wt%s) formulation passed the pH test and was screened for biocompatibility.

Neat Chemical Cure of SM – Exothermicity

As mentioned in the previous chapter, heat generated during the polymerization of PMMA-based bone cement can cause necrosis of surrounding tissue and cells.[2,12,42] A desirable material was required to produce the same, if not less, heat upon polymerization than commercial bone cement. For the exothermicity testing of the HFPA system (5 wt%), commercially available bone cement (Stryker Simplex® P) was used as the control. Both the rate of polymerization and maximum exotherm were found to be significantly different between the control and HFPA system (5 wt%). For the commercial material, a peak exotherm of 62.4 °C was observed approximately 14 min post-initiation and then remained above 45 °C for approximately one min. In contrast, the HFPA initiated sample had a maximum exotherm of 36.1 °C after one min post initiation (Figure 2.3) and was statistically different than the maximum exotherm of commercially available bone cement (Table 2.5). Therefore, HFPA passed the exothermicity screening.

Figure 2.3: HFPA vs. Simplex P exotherms.

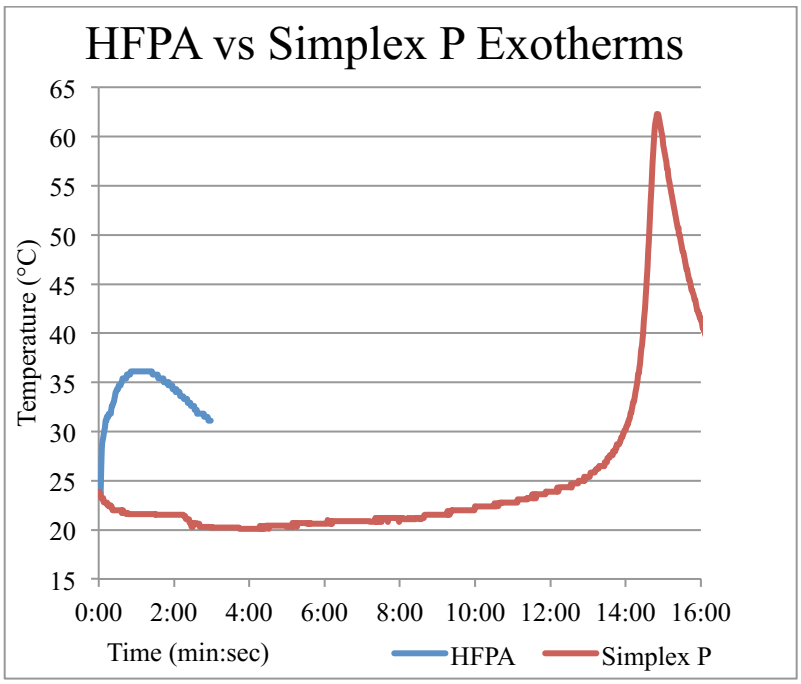


Table 2.5: Exothermicity comparisons for testing initiation systems and control.

Material	Maximum Exotherm	Time of Peak Exotherm
Simplex P	62.4 °C	14 min
HFPA (5 wt%)	36.1 °C	1 min
LCSM	127.2 °C	Immediately after irradiation

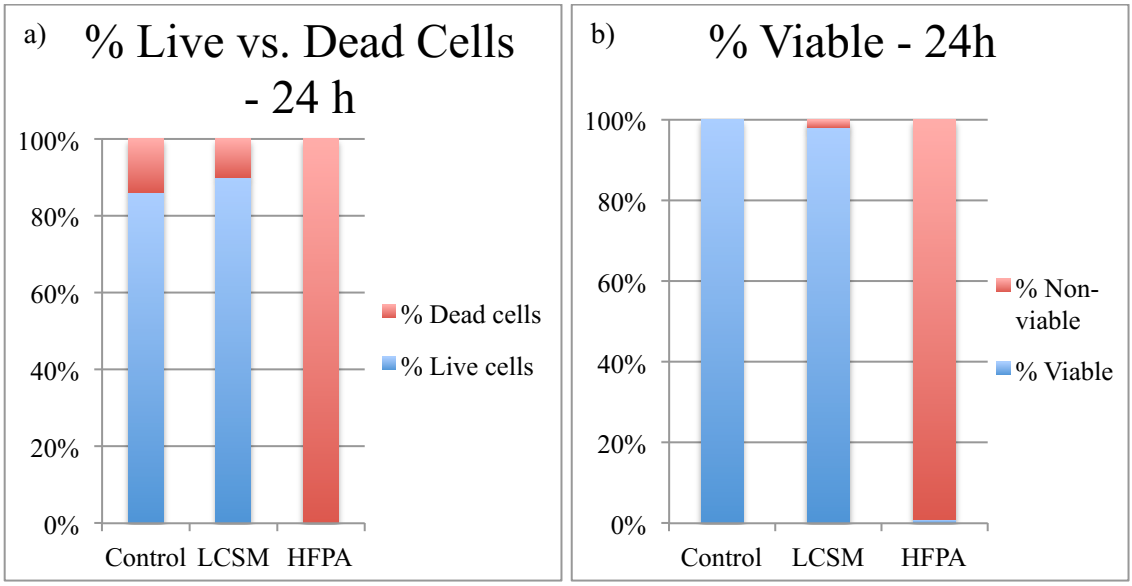
Neat Chemical Cure of SM – Biocompatibility

Biocompatibility was tested using MLO-A5 cells with the Trypan Blue (TB) Exclusion and MTT assays. Cell death was measured using the TB assay while the MTT assay indicated cell viability, both of which were defined in Chapter 1. For these studies, all samples were compared to two controls; empty cell wells and light-cured SilMix (LCSM) samples. The biocompatibility of the chemical initiation system that passed the pH screening (2-aminopyridine (2.7 wt%), acetic acid (2.5 wt%), and HFPA (8.2 wt%)) was tested. Samples (n=4) were prepared in our laboratory and given to our collaborator, Dr. Nalvarte, at the UMKC School of Dentistry for biocompatibility testing. In addition to the TB and MTT assays of the cells in direct contact with the material, the MTT assay of cells in contact with media, which was exposed to polymer samples and contained potential leachable extracts, was also studied.

There was no significant difference between the live/dead cell percentages of LCSM and the empty control wells ($p>0.05$), which was consistent with previous data.[59,66] On the other hand, the SilMix (86.6 wt%), 2-aminopyridine (2.7 wt%), acetic acid (2.5 wt%), and HFPA (8.2 wt%) system resulted in 100% cell death. It was observed as a color change in cells from colorless to blue and a change in the media from blue to yellow, which were attributed to the change in pH observed during that screening test (Figure 2.4 a-b). This result was attributed to residual HFPA, which was not consumed during the initiation reaction. In summary, the HFPA chemically cured samples were not

biocompatible so this initiation system was not appropriate as a possible initiation system for the silorane bone cement.

Figure 2.4: Biocompatibility of chemical-cure SilMix (HFPA) vs. light-cure SilMix (LCSM) a)Trypan Blue and b) MTT of leachables.



Neat Chemical Cure of SM – Summary

A chemical cure system was identified that would polymerize the neat SilMix with a low exotherm. However, it was toxic and had poor handling times. Due to these issues, a new approach toward the initiation system was needed. Investigations began into a dual or mixed initiation system. A "dual" cure system was defined as containing at least one acid and a photosensitizer, which required some type of light source for initiation.

Mixed Initiation of SM – GNT

In an attempt to identify an appropriate initiation system for the silorane material, which did not suffer from any of the previously determined drawbacks, dual cure or mixed initiated systems were also investigated. The mixed initiation systems contained a photosensitizer and at least one acid. They also required direct irradiation with a light source for initiation. As with the chemical initiators, the mixed systems also needed screening tests. As with the previous studies of the chemical systems, the GNT, pH test, degree of conversion, and finally biocompatibility were used for this purpose.

For the GNT test, ten combinations were investigated (Table 2.6) and only two systems showed promise. The first was a combination of SilMix (94.0 wt%), phosphoric acid (2.0 wt%), PIH (3.0 wt%), and camphorquinone (CPQ, 1.0 wt%). The sample polymerized to hardness after five min of irradiation with a 100-watt halogen light bulb (~ 40 cm away). The setup was designed using inexpensive, common materials and is depicted in Figure 2.5. In the second system, the phosphoric acid was replaced with acetic acid to give a less acidic option. This formulation contained SilMix (93.0 wt%), acetic acid (3.0 wt%), PIH (3.0 wt%), and CPQ (1.0 wt%). After five min of irradiation with the halogen light source, the sample polymerized to hardness and resulted in a clear, light yellow polymer (Figure 2.6). Of the eleven combinations tested, only the phosphoric acid/PIH/CPQ and acetic acid/PIH/CPQ systems were moved forward to the

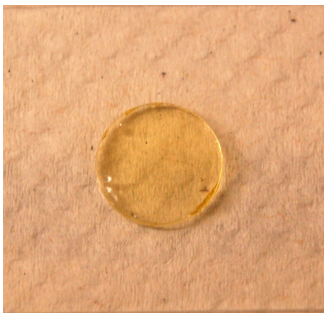
pH screening test. A complete list of the system concentrations investigated and their polymerization results are given in Appendix B.

Table 2.6: Mixed initiated systems.

Mixed Initiation Systems
Acetic Acid:PIH
HFPA:Phosphoric Acid:PIH
Phosphoric Acid:Trifluoroacetic Acid:PIH
Phosphoric Acid:Trichloroactic Acid:PIH
Phosphoric Acid:Triflic Acid:PIH
Phosphoric Acid:*p*-TSA:PIH
Tin (IV) Chloride:PIH
Tin (IV) Chloride:Phosphoric Acid:PIH
Phosphoric Acid:Camphorquinone:PIH
Acetic Acid:Camphorquinone:PIH

Figure 2.5: Halogen lamp set up.

Figure 2.6: SilMix, acetic acid, PIH, and CPQ system.



Mixed Initiation of SM – pH Testing

The mixed initiation systems that passed the GNT underwent pH screening. As was found in the chemical cure investigation, the pH of the material can have an effect on cytotoxicity. The pH test criteria was limited to a pH change within -0.2 to +0.3 units of the control. Those tested formulations were the phosphoric acid/PIH/CPQ (2.0/3.0/1.0 wt%s) and the acetic acid/CPQ/PIH (3.0/3.0/1.0 wt%s) and compared to three of the chemical systems and LCSM as the control. Unfortunately, the pH change for the phosphoric acid system was quite large (2.30 unit decrease after one h) as compared to the control. On the other hand, the pH change of acetic acid system after one h yielded a decrease of 0.81 units, which was less than the control (Table 2.7). Because of these results, only the acetic acid formulation moved on to the additional screening tests, degree of cure and biocompatibility.

Table 2.7: Change in pH for different initiation systems after one h.

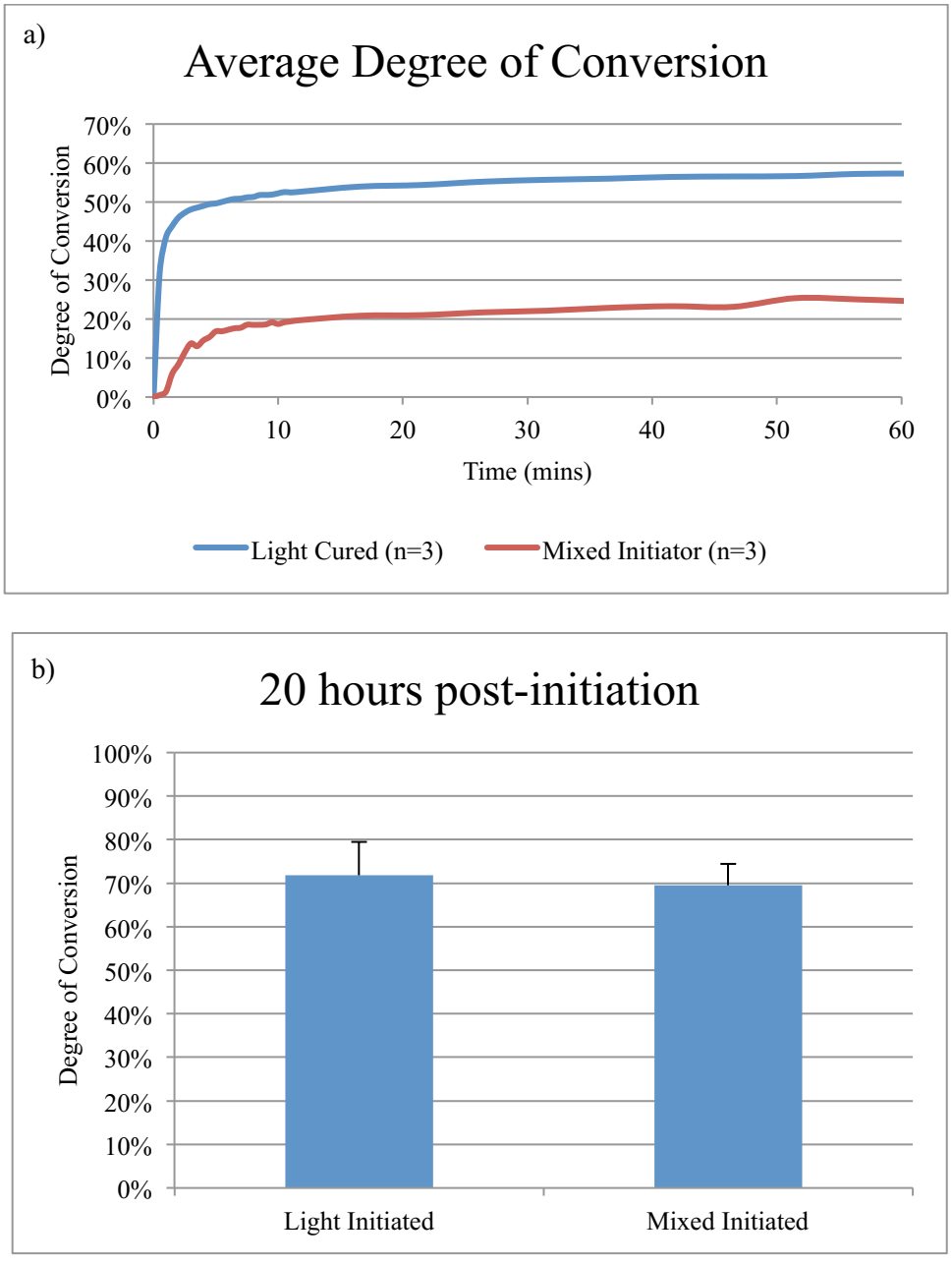
Sample	ΔpH
Light-cure (control)	-1.11
HFPA (5.0 wt%)	-2.81
H_3PO_4 (2.2 wt%)/PIH(1.6 wt%)	-3.55
2-AP(2.7 wt%)/AA(2.5 wt%)/HFPA(8.2wt%)	-1.43
H_3PO_4 (2.0 wt%)/PIH(3.0 wt%)/CPQ(1.0 wt%)	-2.30
CH_3COOH (3.0 wt%)/PIH(3.0 wt%)/CPQ(1.0 wt%)	**-0.81**

Neat Mixed Initiation of SM – Degree of conversion (DC)

There was only one mixed system that had passed the GNT and pH screening tests, the acetic acid formulation (acetic acid (3.0 wt%)/PIH(3.0 wt%)/CPQ(1.0 wt%)). This study was conducted with Dr. Melander at the UMKC School of Dentistry. Fourier Transform Infrared Spectroscopy (FTIR) was used to determine the degree of conversion of SilMix samples by comparing the change in the peak associated with silorane polymerization (883 cm^{-1} representing the oxirane ring opening) to an internal standard peak (1257 cm^{-1} representing the Si-O bond in the CYGEP ring). The peak ratios were then calculated.

The summary of the DC for the acetic acid system and LCSM are found in Figures 2.7a and 2.7b. The DC of the acetic acid system was initially lower than that of the LCSM after 60 min. After one min, the LCSM reached 41% DC; while after 60 min, the mixed system had only achieved 24% DC. However, after 20 h, the mixed system (69%) increased to that of the LCSM (72%). The rate of polymerization of the mixed system was slower, but continued to increase over a longer period of time compared to the LCSM, even when kept in the dark (dark cure). This result allowed the system to move on to biocompatibility testing.

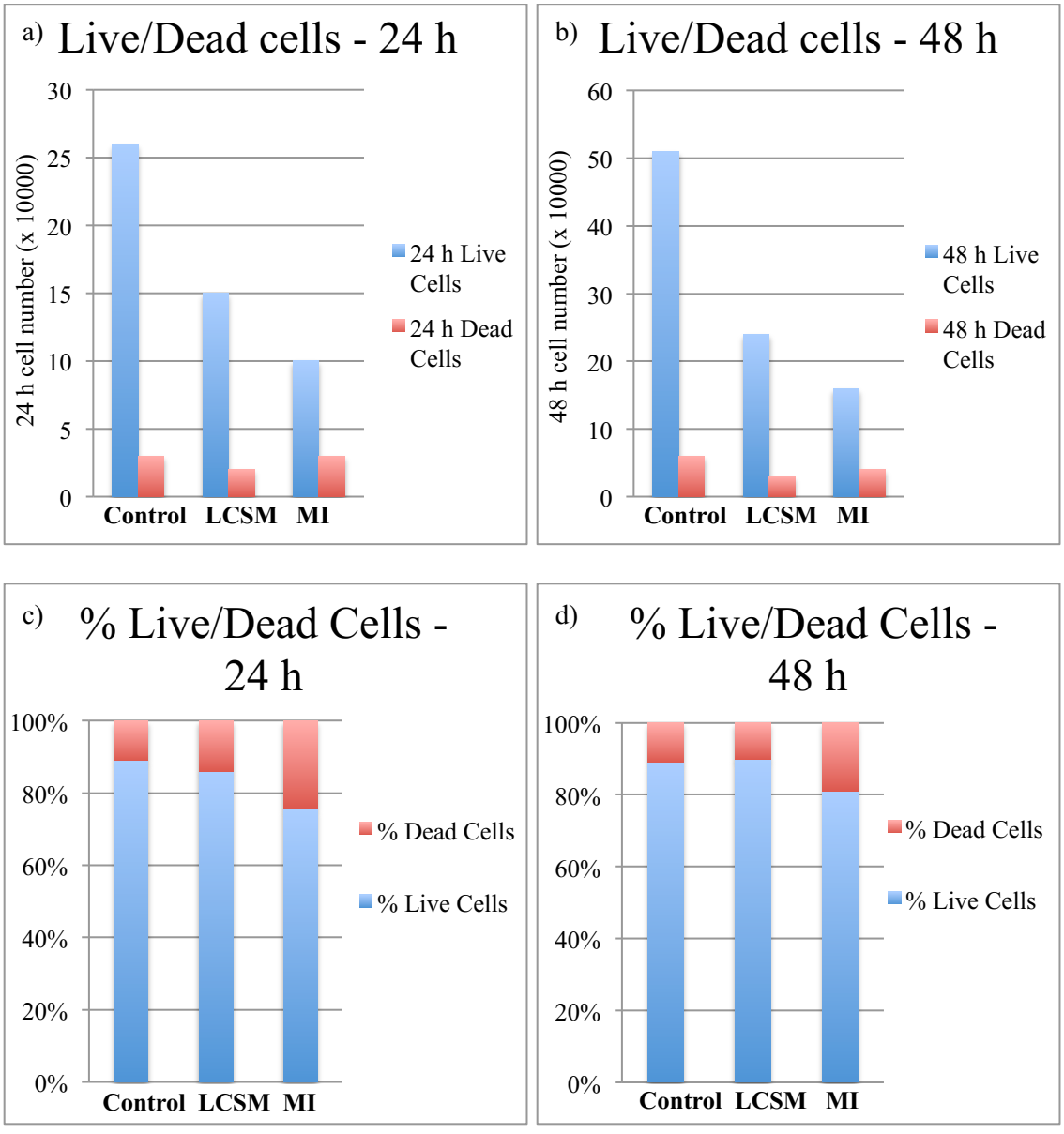
Figure 7: DC of LC and MI SilMix a) from 0 to 60 min and b) after 20 h.



Neat Mixed Initiation of SM – Biocompatibility

The mixed system consisting of SilMix (93.0 wt%), acetic acid (3.0 wt%), PIH (3.0 wt%), and CPQ (1.0 wt%) was tested for biocompatibility using LCSM and an empty cell culture well as controls in the Trypan blue assay. It was the only system to pass all the previous screening tests; GNT, pH, and DC. Samples (n=6) were prepared in our laboratory and given to collaborators at the UMKC School of Dentistry for testing. The MLO-A5 cells cultured with the mixed initiated (MI) system were comparable to the LCSM at 24 and 48 h (Figure 2.8). As with the previous tests, the live cell numbers from both the SilMix samples, at both 24 and 48 h, were lower than the empty wells, but the dead cells numbers for all samples were similar (Figure 2.8 a-b). The percent live/dead cells were comparable for all at both time points (Figure 2.8 c-d). Because of these results, the acetic acid system was found to be biocompatible and thus a viable option for a mixed initiated bone cement.

Figure 2.8: Number of live/dead cells at a) 24 h, b) at 48 h, and percent live/dead cells at c) 24 h, and d) at 48 h.



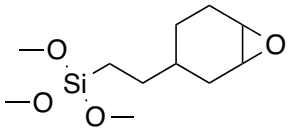
Filled Mixed Initiated SM – Filler Screening

Composites are filled materials and are essentially resins filled with glass or other filler particles. From previous work mentioned in Chapter 1, three fillers and three modifications were identified for use in this bone cement. They were DY5, M12, and alumina nanofibers (ANF). The DY5 and M12 glasses (Table 2.8) were manufactured by collaborators at MoSci Co (Rolla, MO), and the nanofibers were generated by another collaborator, Dr. Thomas Schuman from Missouri University of Science and Technology (Rolla, MO). Dr. Schuman also surface treated all three fillers with modifications synthesized in his laboratory. Of the three modifications identified previously, only one was used for this test, 2-(3,4-epoxycyclohexyl)ethyltrimethoxysilane (ECHE, **45**) (Figure 2.9).

Table 2.8: Filler compositions (in wt%).

Filler	BaO	Ba_2O_3	Y_2O_3	Al_2O_3	SiO_2
DY5	0	0	15%	5%	80%
M12	29.1%	10.5%	0	5.9%	54.5%

Figure 2.9: Structure of ECHE monomer used for filler modification.



ECHE-TMS, **45**

At this point, the best neat mixed system was the acetic acid (AA) system AA:PIH:CPQ (3:3:1 wt%s), which was irradiated for five min with a 100-watt light bulb (~ 40 cm away). The three fillers were added to this system in amounts ranging from 10 – 50 wt%, and the polymerization of the resulting material was tested using the GNT. A sample passed if no mark nor indentation resulted from the placement of the one-lb. needle (Table 2.9).

Table 2.9: Summary of mixed initiation filler screening.

Filler Type	Filler Surface Modification	% Filled (by wt)	Pass/Fail
DY5	None	10%	Pass
DY5	None	20%	Pass
DY5	None	30%	Pass
M12	None	50%	Pass
ANF	None	10%	Pass
ANF	None	20%	Pass
ANF	None	30%	Fail
ANF	None	50%	Fail
ANF	ECHE	10%	Pass
ANF	ECHE	20%	Pass
ANF	ECHE	30%	Pass
ANF	ECHE	50%	Pass

For DY5, all tested samples (10, 20, and 30 wt%s) passed the GNT. M12 glass passed at 50 wt% filled but the disc was slightly wet on top. Unmodified ANF passed at smaller amounts (10 and 20 wts%), but failed at the higher amounts (30 and 50 wt%s).

However, the ECHE-surface modified ANF passed at all tested amounts (10, 20, 30, and 50 wt%). A picture of a mixed initiated sample filled with ECHE-modified ANF is depicted in Figure 2.10. From these results, the DY5, M12, and ECHE-modified ANF passed the polymerization screening and were used for future testing. Next, the optimization of the system was investigated, which included changes in the formulations and alternative light sources.

Figure 2.10: Sample of mixed initiated SilMix.

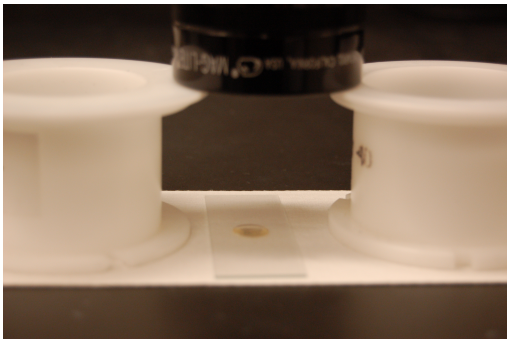
Filled Mixed Initiated SM – Acetic Acid and Temperature Changes

After the fillers were identified, the effect of irradiation time was investigated by increasing the acid component of the initiation system or the temperature during irradiation. It was proposed that the rate of polymerization would increase with an increase in the amount of acetic acid (AA). The tested range was increased from 3 wt% to 6 wt%. No advantage was observed in the filled material (DY5 or modified alumina nanofibers) when the wt% of AA was increased. In addition, there was no change in the polymerization time with the addition of more AA. The samples still required irradiation for the full five min. Next, the effect of heat on a filled mixed system was tested with DY5 (50 wt%). The sample was placed on a hot plate (approximately 35 °C) instead of the bench top (approximately 22 °C) during irradiation. The additional heat did not improve polymerization time. With or without heat, samples filled with DY5 (50 wt%) polymerized at five min under the lamp. Because of these test results, it was determined that the amount of AA (3 wt%) and the heat should remain unchanged.

Filled Mixed Initiated SM – Type of Light Source

The type of light source used to irradiate the filled mixed system AA:PIH:CPQ (3:3:1 wt%s) was investigated. The new light sources were selected by their availability and size, which would eliminate the need to buy large or specialized lights to utilize this bone cement. A typical penlight, flashlight, and 60-watt halogen light bulb were used for this experiment. The more portable light sources, such as a flashlight and a penlight, were investigated first. Five mixed initiation samples, which were filled to 50 wt% with ECHE-modified alumina nanofibers (ANF), were irradiated at one, two, three, four, and five min with a penlight. No change or polymerization was observed in any of the samples. Next, five filled mixed initiated samples containing 50 wt% ECHE modified ANF were irradiated from one to five min with a flashlight (Figure 2.11). The sample that was irradiated for five min with a flashlight (~ 4 cm away) showed some hardening but did not completely polymerize. It barely passed using the ¼ lb. GNT. In order for it to pass the one lb. GNT, the sample required an irradiation time of 8 min. For the next samples, the effect of filler on the rate of the polymerization was carried out by changing the filler to 25 wt% ECHE-modified ANF and 25 wt% DY5. After 10 min of irradiation with the flashlight, the samples polymerized slower than the ECHE-modified ANF alone at 50 wt%.

Figure 2.11: Depiction of flashlight set up.



After the disappointing results with the two portable options, the intensity of the light bulb was changed to 60-watts from the original 100-watts. For samples irradiated with the 60-watt bulb, it took over four times as long (25 min) and at a shorter distance (20 cm) to polymerize the sample. For the 100-watt bulb, it only took five min at 40 cm way. The temperature of the 60-watt light bulb after five min was 77 °C compared to 100 °C with the 100-watt bulb. The cooler temperatures and lower intensity of the 60-watt light source slowed the polymerization of these samples. In summary so far, these portable options are not viable because there was either no or extremely slow polymerization of the samples (Table 2.10).

Table 2.10: Summary of light source trials.

Filler Type and Amount (wt%)	Light Source	Irradiation Time	Pass/Fail
ECHE mod ANF 50%	Pen Light	1 min	Fail
ECHE mod ANF 50%	Pen Light	2 min	Fail
ECHE mod ANF 50%	Pen Light	3 min	Fail
ECHE mod ANF 50%	Pen Light	4 min	Fail
ECHE mod ANF 50%	Pen Light	5 min	Fail
ECHE mod ANF 50%	Flashlight	1 min	Fail
ECHE mod ANF 50%	Flashlight	2 min	Fail
ECHE mod ANF 50%	Flashlight	3 min	Fail
ECHE mod ANF 50%	Flashlight	4 min	Fail
ECHE mod ANF 50%	Flashlight	5 min	Fail
ECHE mod ANF 50%	Flashlight	8 min	Pass
ECHE mod ANF 25% & DY5 25%	Flashlight	10 min	Pass
ECHE mod ANF 50%	60-watt light bulb	25 min	Pass

Summary of Mixed Initiated SM

A biocompatible initiation system that resulted in good handling times, which polymerized a filled formulation in desirable time frame, was identified. However, the samples required direct irradiation with an external light source. Even though the light sources were small, inexpensive, and readily available, they are still inconvenient for use with a bone cement. Another, purely chemical, initiation system was required for this project to move forward.

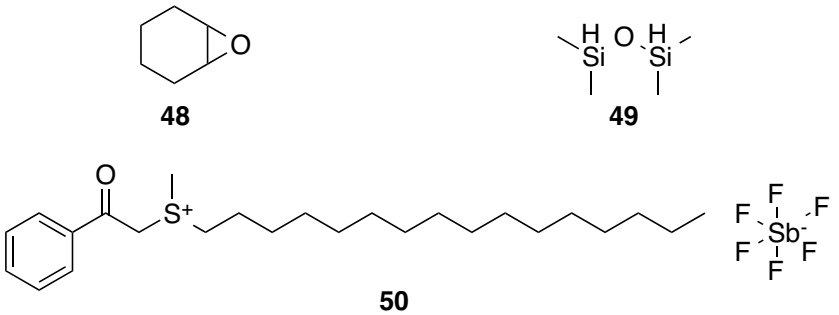
Neat Redox Cure of SM – GNT

With drawbacks of the toxicity of the HFPA option and the requirement of irradiation by a light source for mixed initiated system (AA:PIH:CPQ (3:3:1 wt%s)), there was a need for a paradigm shift for the approach of the initiation system. Therefore, the possibility of organometallic catalyst alternatives was investigated. Redox chemical initiators are a newer class of cationic initiators that were initially used for synthetic reactions.[44-48] There were several considered for the bone cement use, including Pt (e.g., Karstedt and Lamoreaux catalysts), Pd (e.g., Lindlar catalyst), and Rh (e.g., Wilkinson's catalyst) –based catalysts. Lamoreaux catalyst (LMC) was readily available and came to mind because of it is use as the catalyst in the preparation of the CYGEP monomer.[62]

Molleo and Crivello[50] found that a combination of an onium salt, an organometallic catalyst, and a silane would polymerize oxirane monomers. For the polymerization of cyclohexene oxide (**48**), they used 1,1,3,3-tetramethyldisiloxane (TMDS, **49**) as the reducing agent, S-methyl-S-n-hexadecyl-S-phenacylsulfonium hexafluoroantimonate (DPS, **50**) as the oxidizing agent, and Pt-based Karstedt catalyst (Figure 2.12 a-c). They proposed that the addition of catalyst generates a hydride anion from the silane on TMDS. This hydride anion then fragments the DPS, yielding a dialkyl sulfide, an aryl methyl ketone, and a proposed species with "silicenium ion-like"[50] characteristics. This species would then react with any trace amounts of water to give a

strong/super Brønsted acid, which then initiates the polymerization of cyclohexene oxide.[50]

Figure 2.12: Structures of cyclohexene oxide (**48**), TMDS (**49**) and DPS (**50**).



48 **49** **50**

For our system, LMC was added to SilMix at concentrations ranging from 0.1 – 1.0 wt%, and no polymerization was observed even after 48 h. The addition of an organosilane, methylphenylsilane, with LMC to the monomer did not result in polymerization as per the GNT (up to 48 h). Combinations of *p*-(octyloxyphenyl)phenyliodonium hexafluoroantimonate (PIH, **30**) and LMC were studied. This particular combination did polymerize (with or without

methylphenylsilane), but it was found that special care in mixing was needed because the heat generated caused the material to polymerize to hardness in the mixer. As a result, a mixing protocol was established. First, SilMix and PIH were combined in the mixer, and then the LMC was added and mixed in by hand for 30 sec so as to slow the polymerization reaction. The resulting sample was a clear, light brown material that had a handling time of approximately five min before it began to gel. It polymerized to hardness (GNT) between 15 – 30 min depending on the mass of the material. The thicker samples polymerized in 15 min; while the thinner ones took longer (30 min). For these tests, the formulation was 99.89 wt% SilMix, 0.07 wt% LMC, and 0.04 wt% PIH. It was a substantial improvement over the previous systems, which resulted in fast polymerization times with very short handling periods or very slow polymerization times with long working periods. Next, the LMC/PIH system was required to pass the pH and exotherm screenings before it could be tested for biocompatibility.

Neat Redox Cure of SM – pH Testing

For this study, the acidity of a material was measured by the change in pH of deionized or distilled water containing a polymer. As mentioned previously, cell death occurred when the environmental pH became acidic,[43] which was observed in the biocompatibility of the 2-aminopyridine/acetic acid/HFPA initiated material. For a sample to pass the pH test, the pH change must be within -0.2 - +0.3 units of the control. LMC/PIH (0.07/0.04 wt%s) was compared to the control, LCSM, as well as previous chemical cure systems, HPFA (5.0 wt%), phosphoric acid/PIH (2.2/1.6 wt%s), and 2-aminopyridine/acetic acid/HFPA (2.7/2.5/8.2 wt%s). The LMC/PIH (0.07/0.04 wt%s) formulation yielded the smallest decrease in pH of 0.98 units in one h and was the closest to the control (Table 2.11). Due to the small change in pH, the LMC/PIH (0.07/0.04 wt%s) system underwent exothermicity and biocompatibility testing.

Table 2.11: pH change after one h for chemically cured SilMix.

Sample	ΔpH
Light-cure (control)	-1.11
HFPA (5.0 wt%)	-2.81
H_3PO_4 (2.2 wt%)/PIH(1.6 wt%)	-3.55
2-AP(2.7 wt%)/AA(2.5 wt%)/HFPA(8.2wt%)	-1.43
LMC (0.07 wt%)/PIH(0.04 wt%)	**-0.98**

Neat Redox Cure of SM – Exotherm

The ideal material would have a maximum exotherm lower than that of commercial bone cement, specifically 45 °C or below. For this test, the LMC/PIH-initiated SilMix (SilMix 99.89 wt%, LMC 0.07 wt%, and PIH 0.04 wt%) was compared to LCSM (rate and maximum exotherm of polymerization) and a commercial bone cement, Simplex P (maximum exotherm of polymerization). With respect to LCSM, there was a difference in both the rate of polymerization and maximum exotherm. For the LCSM, a peak exotherm of 127.2 °C was achieved immediately after light initiation and remained above 45 °C for approximately one more min. In comparison, the LMC/PIH initiated SilMix reached the maximum exotherm of 34.1 °C at 1-2 min post initiation (Figure 2.13). Simplex P had a maximum exotherm of 62.4 °C. Therefore, the LMC-initiated system resulted in a statistically ($p<0.05$) lower maximum exotherm than bone cement and LCSM (Table 2.12). With an exotherm below the 45 °C threshold, the LMC/PIH system was continued onto biocompatibility testing.

Figure 2.13: LMC/PIH vs. LCSM exotherms.

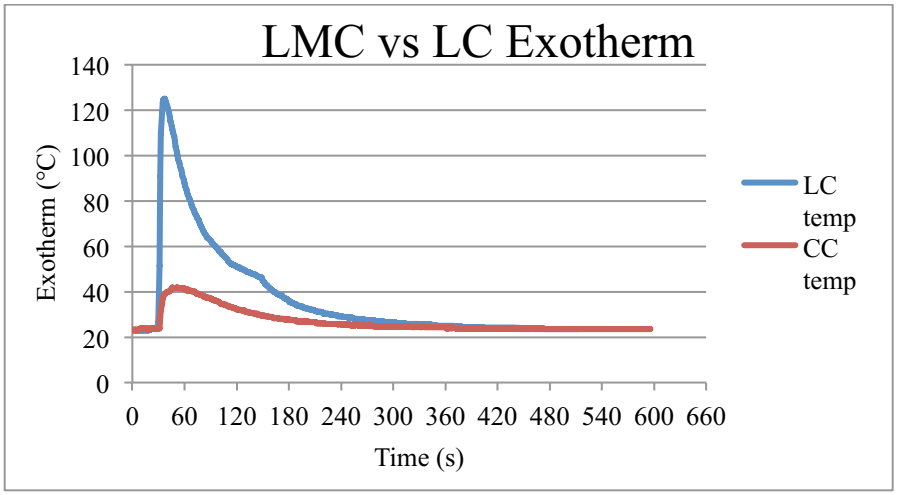


Table 2.12: Exothermicity comparison.

Material	Maximum Exotherm	Time of Peak Exotherm
Simplex P	62.4 °C	14 min
LCSM	127.2 °C	Immediately after irradiation
LMC/PIH (0.07 wt%/0.04 wt%)	34.1 °C	1.5 min

Neat Redox Cure of SM – Biocompatibility

Biocompatibility was tested with MLO-A5 cells using the Trypan Blue (TB) Exclusion and MTT assays for cell death and cell viability, respectively. As with all of previous studies, the LMC/PIH initiated silorane, comprised of SilMix (99.89 wt%), LMC (0.07 wt%), and PIH (0.04 wt%), was compared to two controls: empty cell wells and light-cured SilMix (LCSM). Samples (n=6) were prepared in our laboratory and given to Dr. Bi, a collaborator at the UMKC School of Dentistry, for testing. According to the Trypan blue assay, the number of live cells for the LCSM and the LMC/PIH were significantly less ($p<0.05$) than the empty control wells, however, there was no significant difference in the dead cell count (Figure 2.14). When comparing the live/dead percentages, the LCSM, LMC/PIH, and control wells were the same (Figure 2.15), which indicated that the reduction in the cell number in the SilMix samples was the result of reduced proliferation and not toxicity. This reduced proliferation was likely the result of poor cell adherence to the smooth polymer surface. This phenomenon had been seen with previous SilMix samples.[59] The MTT assay confirmed the biocompatibility of the SilMix samples (Figure 2.16). In the presence of extracts from LCSM (OD=0.95±4) and LMC/PIH (0.96±3), the Formazan products were similar to the controls (0.92±5). With these results, the LMC/PIH biocompatibility studies showed that this particular formulation was comparable to the LCSM and thus a viable initiation system for use in a

bone cement. One last test was undertaken to complete the screening process, degree of conversion.

Figure 2.14: Cell count of live and dead adherent and non-adherent cells after 24 and 48 h incubation with MLO-A5 cells.

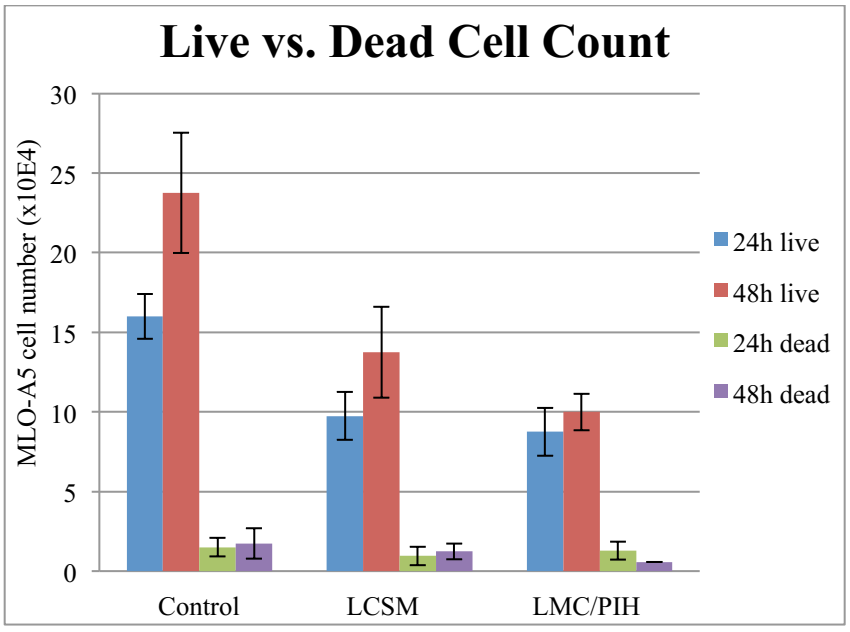


Figure 2.15: Percentage of live and dead cells in the 24 and 48 h cultures.

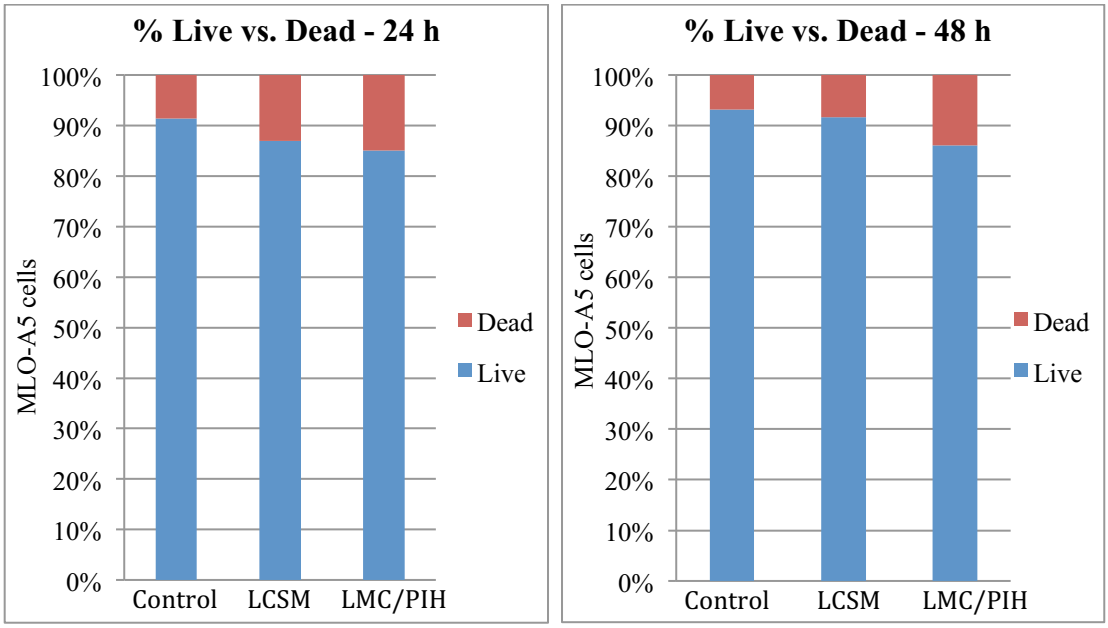
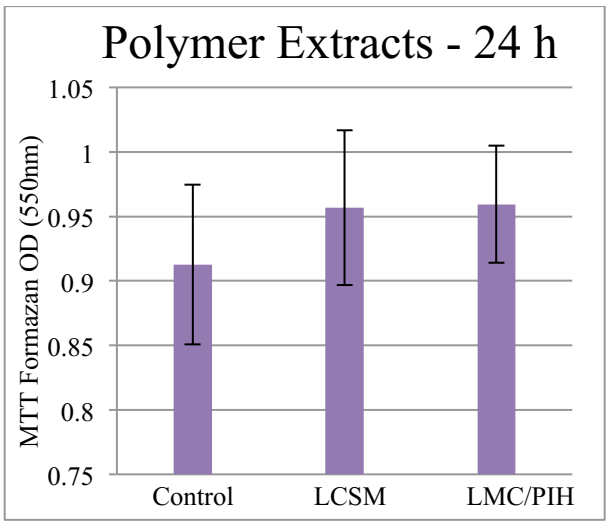


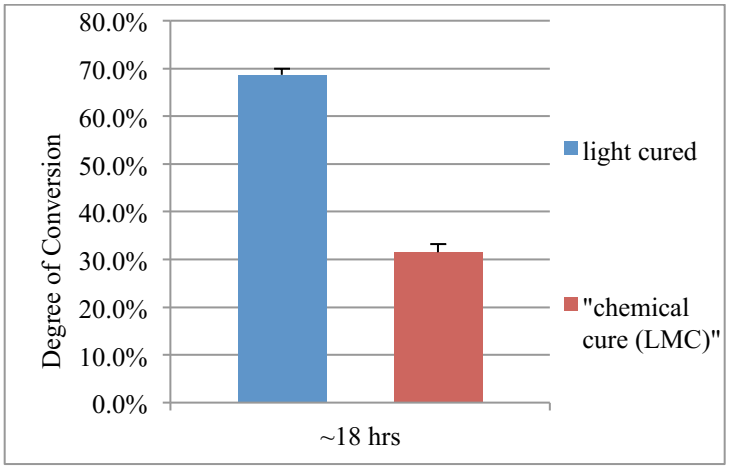
Figure 2.16: MTT formazan product formation: Extracts from 24h incubation of polymers in media with serum.



Neat Redox Cure of SM – Degree of Conversion (DC)

After the LMC/PIH initiation system (LMC (0.07 wt%)/PIH(0.04 wt%)) passed the GNT, pH, exotherm, and biocompatibility testing, it underwent DC testing as compared to LCSM. This study was conducted with Dr. Melander at the UMKC School of Dentistry. Due to the inability to achieve an appropriate baseline for the LMC, the results were analyzed using the LCSM baseline to calculate the degree of conversion. It was found that the LMC system had a lower degree of cure (~30%) as compared to the LCSM (~70%) 18 h post-initiation (Figure 2.17). With this test, it appeared as though the LCSM cured more than the LMC system. However, due to the issue with the baseline, no definitive conclusion could be made.

Figure 2.17: Degree of conversion of LCSM and LMC after 18 h.



Summary

Throughout this work, numerous initiation systems were investigated, but only a few indicated any promise with respect of the parameters required for a bone cement. The mixed system (AA:PIH:CPQ (3:3:1 wt%s) filled to 50 wt% with DY5 and ECHE modified ANF (1:1 by wt) is a possible option for a bone stabilizer with the drawback of the requirement of light initiation. Since one of the main goals for this research was to determine an option whereby direct irradiation with an external light source was not required, the LMC/PIH system consisting of 0.07 wt% LMC and 0.04 wt% of PIH was the best choice with respect of a chemical cure. This formulation had a good handling time (approximately five min) and complete polymerization to hardness in an acceptable timeframe (30 min). The resulting formulation was also biocompatible, which is another key requirement for internal use. The development of a bone cement using the LMC/PIH initiation system is discussed in the next chapter.

CHAPTER 3

SILORANE-BASED BONE CEMENT

Introduction

As discussed in the previous chapters, a viable alternative formulation for a novel silorane bone cement was developed. A neat chemical cure system was identified, as well as, a filled mixed initiated option. The mechanical and handling properties for the formulations were tunable depending on the desired applications. While the biocompatibility and handling times for both systems were acceptable, there were still some drawbacks for each of the options. The chemical cure system was successful but only for the neat silorane resin, however for a composite alternative, filler was required. The filler in the mixed initiated material gave it strength, but it still required some light irradiation for polymerization. This requirement of an external light source limited its use internally and thus not appropriate for a bone cement application. Due to these problems with both alternatives discussed Chapter 2, a new formulation was required and possibly an alternative initiation system.

As a reference, the standard for "Implants for surgery – Acrylic resin cements," ISO 5883 was used to refine and define the viable formulations.[57] Using this standard, emphasis was placed on exothermicity, handling time, flexural strength, and flexural modulus (Table 3.1). Biocompatibility and pull out strength were also investigated for thoroughness. The goal was to identify a material that would be used in live animals, first small (rats), then large (swine).

Table 3.1: Desired properties of bone cement.

	ISO 5833 Standard[57]	Desired properties
Exothermicity (°C)	≤90	≤45
Handling time (min)	3-15	≤20
Flexural modulus (GPa)	≥1.8	≥1.8
Flexural strength (MPa)	≥50	≥50
Compressive strength (MPa)	≥70	≥70
Pull out strength – mimic (MPa)	n/a	≥4.5
Pull out strength – *ex vivo* (MPa)	n/a	≥4.5
Pull out strength – *in vivo* (MPa)	n/a	≥4.5
Cytotoxicity (% cell death)	n/a	≤20%

Prototype 1 (P1) – GNT

After identifying possible initiation systems for SilMix, the next goal was to develop a new bone cement formulation. Currently available bone cements are packaged as two components, a liquid and a powder, both containing methacrylates. Therefore, using this model as a guideline, the initial investigated formulation was comprised of two components. The liquid component was the SilMix comonomer system. For the powdered portion, we used light-cured SilMix (95.85 wt% SilMix (**41**/**42**), 3 wt% PIH (**30**), 1 wt% CPQ (**25**), and 0.15 wt% EDMAB (**27**)), which was crushed into a powder (CSM). The previously identified LMC/PIH system was utilized as the initiation system. The formulations of prototype 1 (P1) were first mimicked after the composition of Simplex P (Table 3.2). The formulation screening was similar to the screening of the initiation systems starting with the Gillmore Needle Test (GNT) to test polymerization time. After a formulation passed the GNT, the exothermicity, mechanical, and possibly degree of cure testing were investigated.

Table 3.2: PMMA Bone cement components vs. P1 components.

PMMA	FUNCTION	P1- Silorane Based Material
Methyl methacrylate (**1**, 32.3-33%)	RESIN	SilMix (**41**/**42**, 35-55%)
Pre-polymerized PMMA beads (**2**, 55.3-66%)	FILLER	Pre-polymerized SilMix (36-56%)
Barium sulfate/zirconium dioxide (6-10%)	RADIOPACIFIER	Yttrium silicate glass (DY5) (9-14%)
N,N-dimethyl-*p*-toluidine (DMPT) (**3**, 0.13-0.93%)	ACCELERATOR	PIH (*p*-(octyloxyphenyl) phenyliodonium hexafluoroantimonate) (**30**, 0.04-0.27%)
Benzoyl peroxide (**4**, 0.5-1.73%)	INITIATOR	Lamoreaux's catalyst (0.10-0.30%)
Hydroquinone (**5**, 5-25 ppm)	INHIBITOR	Not needed

As stated previously, the P1 formulations mimicked the PMMA bone cement components and its ratios. Initially, the crushed SilMix (CSM) was solely light-cured SilMix that was crushed using a coffee grinder and then a mortal and pestle. The first six formulations provided a good starting point (Table 3.3). Changes to the formulations including the LMC/PIH ratio were tested. However, formulation 6 yielded the best handling and polymerization times. The one drawback was the sandy texture. In terms of the eventual use of the material in the body, the graininess could cause irritation and inflammation so was not a possible alternative.

Table 3.3: Initial formulations for P1.

Sample	%SM	%PIH	%LMC	%CSM	%DY5	Consistency	GNT Pass
P1-1	34.35	0.04	0.11	55.87	9.62	damp sand that did not pack together well, rough on top	45min
P1-2	34.31	0.04	0.27	55.78	9.60	damp sand that did not pack together well, rough on top	over 6 h
P1-3	34.34	0.04	0.12	55.88	9.62	wet sand, packed well, top smoothed out	1 h top wet
P1-4	34.33	0.04	0.11	55.89	9.62	wet sand, packed well, top smoothed out (placed on hotplate)	Some parts at 2.5h
P1-5	34.34	0.10	0.11	55.83	9.62	wet sand, packed well, top smoothed out	3.25 h
P1-6	34.24	0.15	0.15	55.84	9.61	wet sand, packed well, top smoothed out	15 min

In order to address this issue, it was decided that the SilMix (CSM) would be milled by Dr. Schuman at MS&T so as to obtain a finer, more consistent powder. The resulting CSM was more like the other glass fillers, smooth and fluffy. There were two different sizes +120 and -120, which were produced as the same time and separated with a 120-sieve. The finer particles passed through the 120-sieve leaving behind larger material

(+120). The first formulations contained the new milled CSM (+120), and their compositions were approximately that of the P1-6 formulation. Unfortunately, it was hard to incorporate all of the new CSM. The resulting material was too thick and took longer to polymerize (over 1 h). Therefore, the formulation was adjusted to obtain polymerization results similar to those of P1-6 (Table 3.4). This result was accomplished by decreasing the amount of the +120 CSM and increasing the DY5, along with toggling the LMC/PIH amounts.

Table 3.4: Prototype 1 with milled crushed SilMix (+120 size).

Sample	%SM	%PIH	%LMC	%CSM	%DY5	Consistency	GNT Pass
P1-m1	34.23	0.15	0.21	55.81	9.60	v. dry, v.hard to mix, dry clay	1.25 h
P1-m2	49.67	0.24	0.30	35.84	13.95	easier to mix, thick paste	30 min
P1-m3	41.91	0.20	0.27	45.84	11.77	hard to mix, v. thick paste, wet clay	15 min

From this study, it was determined that P1-m3 had the most potential due to its good consistency and appropriate handling times. An additional study was used to optimized the amount of LMC. It was found that 0.27 wt% LMC was found to be optimal. The effect of size of the CSM filler was also tested using smaller sized CSM (-

120) and the P1-m3 formulation. It was determined that the resulting material had a thinner consistency and polymerized faster. These studies are summarized in Table 3.5.

Table 3.5: Prototype 1 with milled crushed SilMix (-120 size).

Sample	%SM	%PIH	%LMC	%CSM	%DY5	Consistency	GNT Pass
P1-m3b	41.91	0.20	0.27	45.84	11.77	thick paste	3 min
P1-m4	52.05	0.17	0.17	35.58	11.77	became firmer as mixed	5 min
P1-m5	49.80	0.17	0.17	35.58	14.00	thick paste	15 min
P1-m6	42.18	0.10	0.10	45.58	11.77	thick paste	30 min
P1-m7	49.95	0.10	0.10	35.85	14.00	thick paste	30 min

Four formulations passed the GNT screening test and were subjected to further investigation. The original crushed SilMix formulations, P1-5 and P1-6, along with milled P1-m7, underwent exothermicity testing. The mechanical properties of P1-m6 and P1-m7 and degree of conversion for P1-m7 were determined.

P1 – Exotherm Testing

For the polymerization exotherm, the desired max temperatures was ideally less than 45 °C, which was below the recorded commercial bone cement exotherms (approximately 64 °C) and the ISO standard 5833 of 90 °C. The peak exotherm and the setting times were obtained from this test. The peak exotherm is the highest temperature the sample reaches during polymerization and the highest point on the graph. The setting time is the time it takes the sample to begin hardening. On the graph, it is the point where the exotherm peaks ends and flattens out.

Two formulations using the original crush SilMix were investigated first. Exo 1a-b and Exo 2a-b samples had the same formulation (P1-6). However, there was a difference in the length of time required for the addition of the catalyst; Exo 1a-b took two minutes longer than Exo 2a-b. Exo 3a-b (P1-5) contained less LMC and PIH. All of the samples completely polymerized at 30 min as determined by the GNT. The samples with the highest exotherms were Exo 1a-b, which had setting times that were less than Exo 2 a-b. This result made sense; with the same amount of catalyst, the faster setting time would result in a higher exotherm. As for Exo 3a-b, the lower catalyst amount yielded the lowest exotherm and also the shortest setting time. The formulations, max exotherms, and setting times are listed in Table 3.6 and the graph in Figure 3.1. Besides the differences in exotherms and setting times, there was also a difference in appearance. The Exo 1 samples were very grainy, almost crystalline. Upon observation, Exo 2 and

Exo 3 samples were much smoother, and the Exo 3 samples had a better consistency of the two (Figures 3.2 a-c). All of these samples polymerized well below our 45 °C threshold.

Table 3.6: Formulations, exotherms and setting times of P1-5 and P1-6.

Sample	%SM	%PIH	%LMC	%CSM	%DY5	Max (°C)	Setting Time (s)
Exo 1a (P1-6)	34.25	0.15	0.15	55.84	9.61	34.9 °C	128 s
Exo 1b (P1-6)	34.25	0.15	0.15	55.84	9.61	35.3 °C	156 s
Exo 2a (P1-6)	34.25	0.15	0.15	55.84	9.61	29.5 °C	247 s
Exo 2b (P1-6)	34.25	0.15	0.15	55.84	9.61	32.7 °C	243 s
Exo 3a (P1-5)	34.27	0.10	0.11	55.91	9.61	27.0 °C	88 s
Exo 3b (P1-5)	34.27	0.10	0.11	55.91	9.61	27.0 °C	96 s

Figure 3.1: Plot of exotherms for P1.

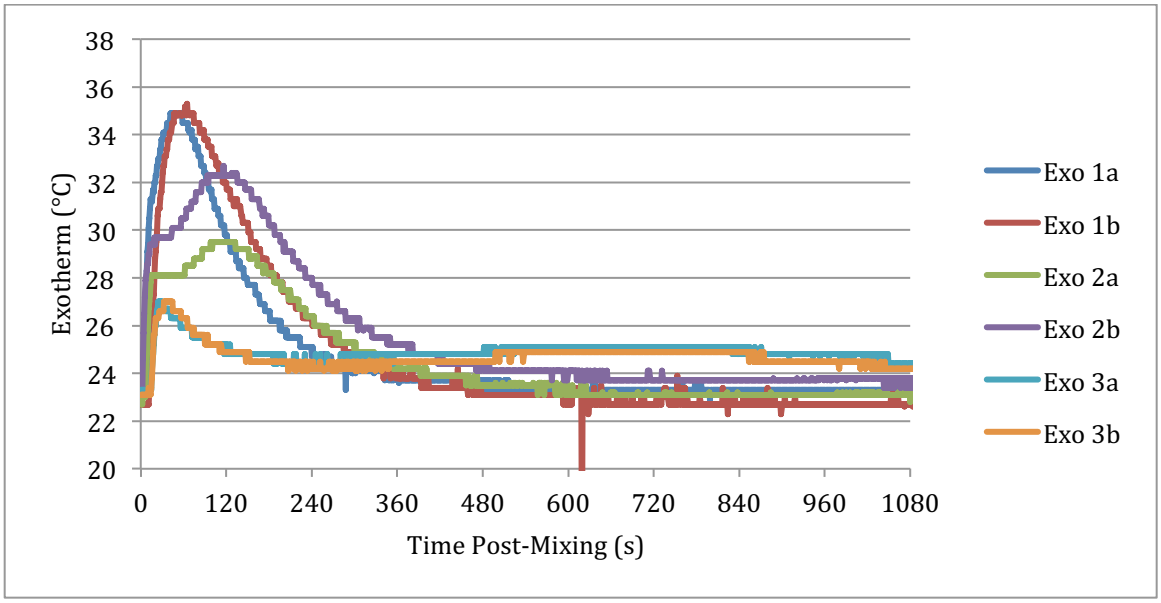
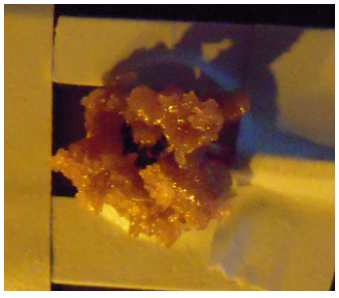
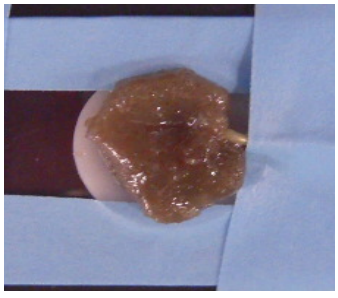


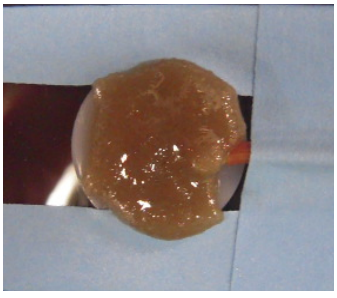
Figure 3.2: Pictures of Exo 1-3



a) Exo 1



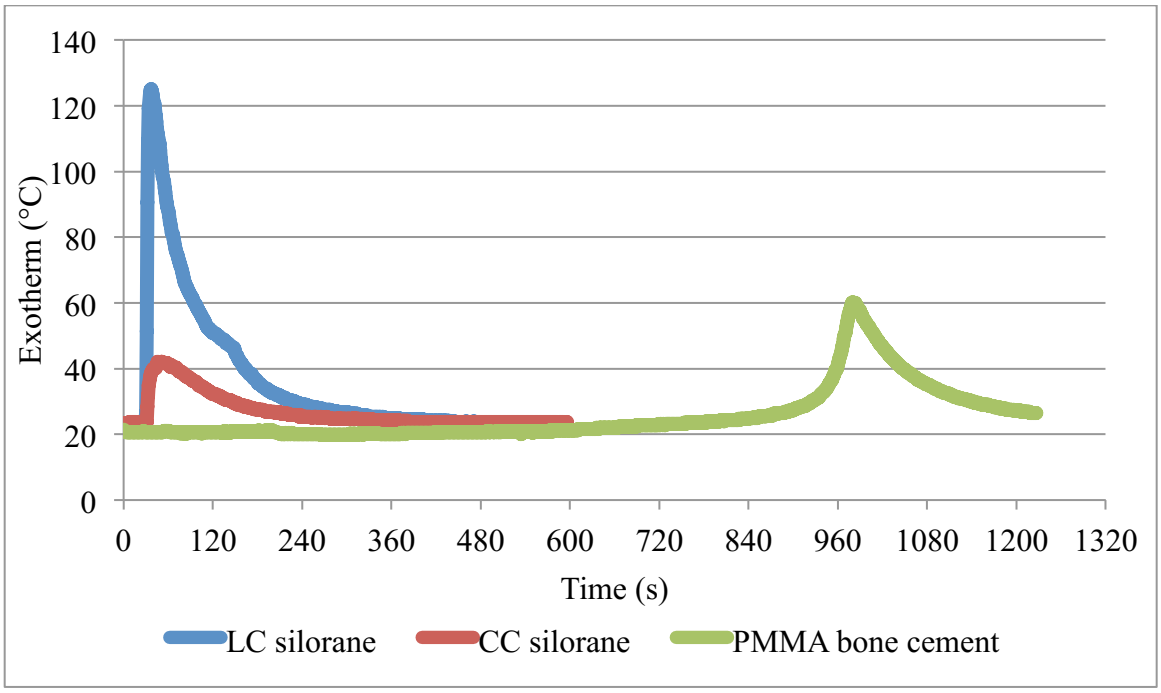
b) Exo 2



c) Exo 3

The exothermicity of the P1-m7 formulation (49.95 wt% SM, 0.10 wt% PIH, 0.10 wt% LMC, 35.85 wt% CSM, and 14.00 wt% DY5) was compared to light-cured SilMix (LCSM) and the PMMA-based bone cement, Simplex P. The P1-m7 exotherm of 40 °C was significantly lower than the others (Figure 3.3). All of the P1-5, P1-6 and P1-m7 formulations passed the exothermicity screening test. Since P1-m7 had a better texture and consistency, it next underwent mechanical testing.

Figure 3.3: Exotherms of P1-m7, LCSM, and commercial bone cement.



P1 – Mechanical Testing

As mentioned previously, flexural strength and flexural modulus are important factors for the development of a bone cement. According to the ISO 5833 standards, a bone cement should have a flexural strength greater than 50 MPa and a flexural modulus greater than 1.8 GPa. After passing the GNT, formulation P1-m6 and P1-m7 were selected for mechanical testing (Table 3.7) with Simplex P as the control.

Table 3.7: Mechanical specimen formulations.

Formulation	%SM	%PIH	%LMC	%CSM	%DY5
P1-m6	42.18	0.10	0.10	45.58	11.77
P1-m7	49.95	0.10	0.10	35.85	14.00
Simplex P	n/a	n/a	n/a	n/a	n/a

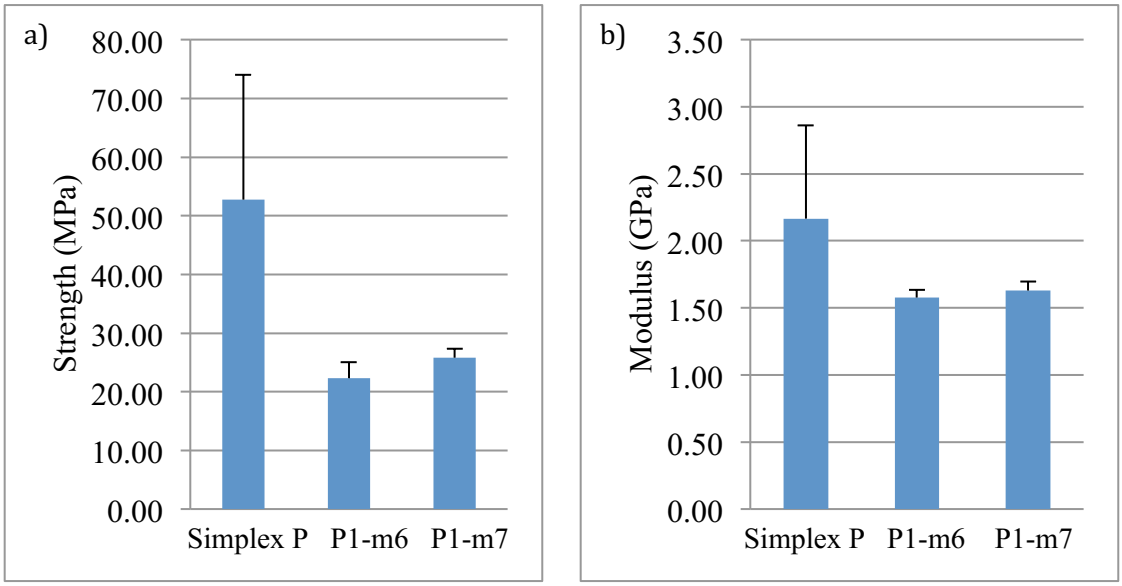
Samples were prepared and given to Dr. Melander at the UMKC School of Dentistry for testing. Neither of the P1 samples performed as well as the Simplex P. Both samples had flexural strengths essentially half of that of Simplex P (ISO 5833 standard > 50 MPa) but close to the desired flexural modulus (1.6 rather than >1.8 GPa according to the ISO 5833 standard). More details are found in Table 3.8 and Figures 3.4 a-b. Due to the poor

strength results, it was decided to investigate the degree of conversion of the better performing P1 formulation, P1-m7

Table 3.8: Flexural strength and modulus of Simplex P, P1-m6, and P1-m7.

Formulation	Flexural Strength (MPa)	Flexural Modulus (GPa)
P1-m6	22.26	1.58
P1-m7	25.77	1.63
Simplex P	52.69	2.17

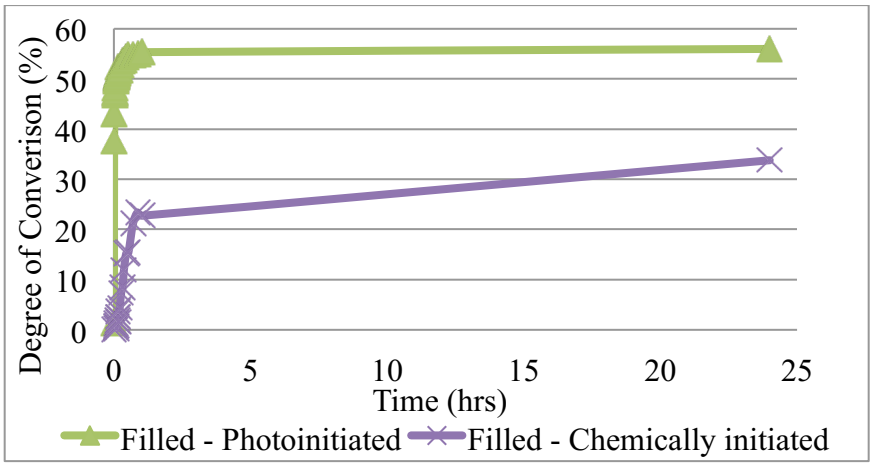
Figure 3.4: a) Flexural strength and b) modulus of Simplex P, P1-m6, and P1-m7.



P1 – Degree of Conversion (DC)

At this point, one P1 formulation performed the best for all of the testing, P1-m7 (49.95 wt% SM, 0.10 wt% PIH, 0.10 wt% LMC, 35.85 wt% CSM, 14.00 wt% DY5). In order to explain the low strength of the formulations, the degree of conversion (DC) of P1 was investigated using a sample of LCSM filled to 50 wt% with DY5 (filled photoinitiated) as the control. This study was performed with Dr. Melander at the UMKC School of Dentistry. The DC of P1-m7 (approximately 33%) was lower than that of the filled LCSM (56%), which was another setback with the P1 formulation (Figure 3.5).

Figure 3.5: Degree of conversion for filled LCMS and P1 (filled chemically initiated).



Disadvantages of P1

There were three main problems with the P1 formulation. The texture of the final material was very grainy due to the consistency of the powdered LCSM. The second issue, low strength of the material (25.77 MPa), was also attributed to LCSM powder. Lastly, the desired handing properties were not achieved using this formulation. When the sample had the desired handling time (5 – 10 min), the material was tacky and took longer than one hour to pass the GNT. When the sample did pass the GNT in less than an hour, the handling time would decrease to less than five minutes at which time the material would begin to gel and could not be manipulated. By increasing the LMC/PIH, it was possible to decrease the polymerization times and also, unfortunately, the handling The textual, strength, and handling issues led to the development of a new formulation, prototype 2 (P2).

Prototype 2 (P2)

In order to improve the texture of the P1 system, the CSM was replaced with modified glass fillers previously tested with the SilMix (see Early Work section in Chapter 1). In addition to the LMC and PIH (**30**), the other two components from ternary the light initiation system, CPQ (**25**) and EDMAB (**27**), were incorporated to help extend the handling time while shortening the total polymerization time. The new initiation system was considered a mixed or dual cured system because it contained both chemical and light sensitive components. However, unlike the previous mixed systems, the P2 samples were not directly irradiated with a light source, instead only ambient light was used. The differences between the components of commercial bone cement, prototype 1 (P1), and prototype 2 (P2) are listed in Table 3.9. The desired properties were the same as with the P1 and are listed in Table 3.10.

Table 3.9: Formulation comparison between PMMA, P1, and P2.

PMMA	FUNCTION	Silorane	P1	P2
Methyl methacrylate (**1**, 32.3-33%)	RESIN	SilMix	35-55%	35-55% (LCSM)
Pre-polymerized PMMA beads (**2**, 55.3-66%)	FILLER	CSM	36-56%	0%
Barium sulfate/zirconium dioxide (6-10%)	RADIOPACIFIER	DY5	9-14%	35-74% (surface-treated
N,N-dimethyl-*p*-toluidine (DMPT) (**3**, 0.13-0.93%)	ACCELERATOR	PIH	0.04-0.27%	(in LCSM)
Benzoyl peroxide (**4**, 0.5-1.73%)	INITIATOR	LMC	0.11-0.30%	0.30-0.80%
Hydroquinone (**5**, 5-25 ppm)	INHIBITOR	N/A	N/A	N/A

Table 3.10: Desired properties of bone cement.

	ISO 5833 Standard[57]	Desired properties
Exothermicity (°C)	≤90	≤45
Handling time (min)	3-15	≤20
Flexural modulus (GPa)	≥1.8	≥1.8
Flexural strength (MPa)	≥50	≥50
Compressive strength (MPa)	≥70	≥70
Pull out strength – mimic (MPa)	n/a	≥4.5
Pull out strength – *ex vivo* (MPa)	n/a	≥4.5
Pull out strength – *in vivo* (MPa)	n/a	≥4.5
Cytotoxicity (% cell death)	n/a	≤20%

P2 – DY5 Formulations

In the beginning of the P2 development, the first formulations investigated were filled with modified DY5 filler. All three modifications identified in Chapter 1 (Early Work section), ECHE (**45**), 1TOSU (**46**), and 3TOSU (**47**), were utilized in this study. The formulations and their polymerization results are listed in Table 3.11. While handling times should ideally be around 15 min, complete polymerization should occur between 30 min and one h as determined by the GNT. Those formulations that passed the GNT between 30 and 45 min underwent mechanical testing for flexural strength and flexural modulus. These formulations are bolded in Table 3.11.

Table 3.11: P2 DY5 formulations and GNT results.

Sample	%SM	%PIH	%CPQ	%EDMAB	%LMC	%DY5	DY5 mod.	GNT Pass
DY5-E-a	47.74	1.49	0.50	0.07	0.39	49.80	ECHE	1.5 h
DY5-E-b	47.73	1.49	0.50	0.07	0.41	49.79	ECHE	1.25 h
DY5-E-c	**47.72**	**1.49**	**0.50**	**0.07**	**0.42**	**49.79**	**ECHE**	**45 min**
DY5-3T-a	47.72	1.49	0.50	0.07	0.42	49.79	3TOSU	15 min
DY5-3T-b	47.74	1.49	0.50	0.07	0.38	49.81	3TOSU	1 h
DY5-3T-c	**47.73**	**1.49**	**0.50**	**0.07**	**0.40**	**49.80**	**3TOSU**	**45 min**
DY5-3T-d	**37.81**	**1.18**	**0.39**	**0.06**	**0.56**	**59.99**	**3TOSU**	**30 min**
DY5-3T-e	**37.76**	**1.18**	**0.39**	**0.06**	**0.62**	**59.99**	**3TOSU**	**45 min**
DY5-3T-f	23.22	0.73	0.24	0.04	0.84	74.93	3TOSU	15 min
DY5-1T-a	37.72	1.18	0.39	0.06	0.65	60.00	1TOSU	15 min
DY5-1T-b	37.77	1.18	0.39	0.06	0.58	60.01	1TOSU	15 min
DY5-1T-c	**38.05**	**1.19**	**0.40**	**0.06**	**0.30**	**60.01**	**1TOSU**	**45 min**

For the formulations that passed the GNT, flexural strength and flexural modulus were investigated using the four-point bend test (Table 3.12). From the ISO 5833 standard, flexural strength is required to be greater than 50 MPa, and a flexural modulus is required to be greater than 1.8 GPa for a bone cement material. Samples were prepared and given to Dr. Melander at the UMKC School of Dentistry for testing.

Table 3.12: Mechanical specimen formulations.

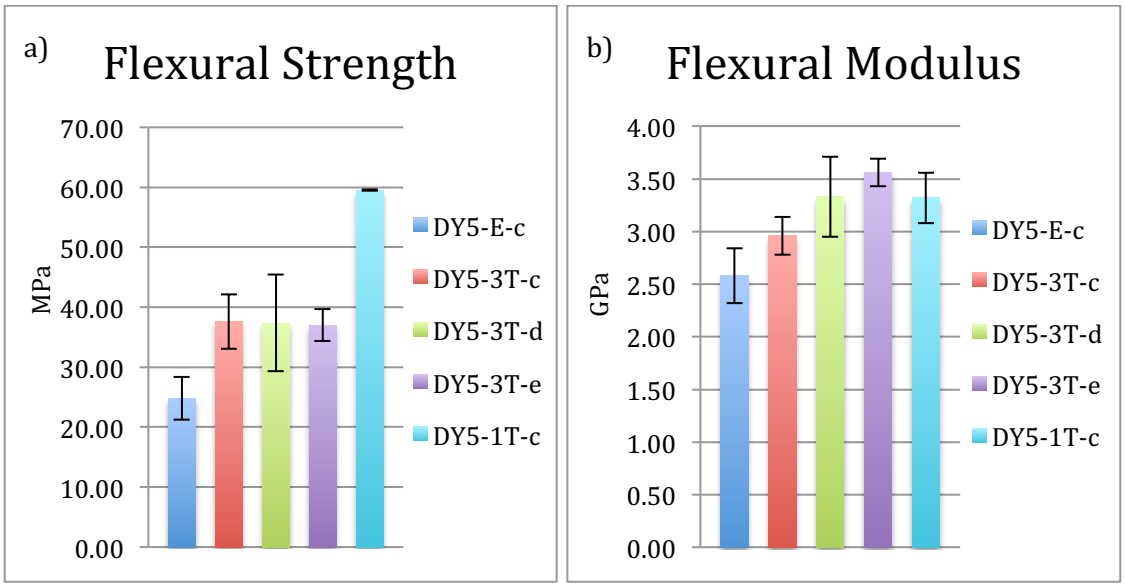
Formulation	%SM	%PIH	%CPQ	%EDMAB	%LMC	%DY5	DY5 mod.
DY5-E-c	47.72	1.49	0.50	0.07	0.42	49.79	ECHE
DY5-3T-c	47.73	1.49	0.50	0.07	0.40	49.80	3TOSU
DY5-3T-d	37.81	1.18	0.39	0.06	0.56	59.99	3TOSU
DY5-3T-e	37.76	1.18	0.39	0.06	0.62	59.99	3TOSU
DY5-1T-c	38.05	1.19	0.40	0.06	0.30	60.01	1TOSU

In contrast to the P1 formulations (~1.6 GPa), all of the formulations exceeded the 1.8 GPa threshold for flexural modulus. For the 3TOSU samples, there was a direct correlation of amount of filler to the modulus. The flexural strength results revealed that there was a difference between the modifications as well as the wt% of filler. The ECHE modification had the lowest strength, while the 1TOSU had the highest. In fact, the DY5-1T-c formulation with a flexural strength of 59.53 ± 0.11 MPa was the best performing prototype of either P1 or P2, which was the first formulation to exceed the minimum strength of 50 MPa. A summary of results can be found in Table 3.13 and Figures 3.6 a-b.

Table 3.13: Mechanical results.

Formulation	Flexural Strength (MPa)	Flexural Modulus (GPa)	n=
DY5-E-c	24.80 ± 3.55	2.58 ± 0.26	9
DY5-3T-c	37.62 ± 4.55	2.96 ± 0.18	6
DY5-3T-d	37.36 ± 8.04	3.33 ± 0.38	7
DY5-3T-e	37.03 ± 2.66	3.56 ± 0.13	3
DY5-1T-c	59.53 ± 0.11	3.32 ± 0.24	2

Figure 3.6: a) Flexural strength and b) modulus of initial DY5 P2 formulations.



Following this strength investigation, it was determined that the ideal composition would contain 60 wt% modified filler, 0.3 wt% LMC, and 39.7 wt% LCSM (SilMix with PIH/CPQ/EDMAB).

P2 – Biocompatibility

The Trypan Blue (TB) Exclusion and MTT assays were used to determine biocompatibility. As mentioned previously in Chapter 1 (Properties of Bone Cement and Standard Testing), TB measures cell death while MTT indicates cell viability. For these studies, all samples were compared to two controls: empty cell wells and light-cured SilMix (LCSM) discs. As with all previous biocompatibility testing, post-osteoblast/pre-osteocyte type cells, MLO-A5, were used. Biocompatibility was performed for all of the components of P2. The highest LMC limit tested before was approximately 0.10 wt%, compared to 0.46 wt% for the P2.

The first P2 formulations were filled to 60 wt% with either 1TOSU or 3TOSU modified M12 (Table 3.14). In addition to the positive controls, a negative control, PMMA commercial bone cement, was also used. Along with TB assay of cells directly on the materials, the toxicity of potential leachables was tested using the MTT assay of cells in contact with media removed from disk wells. Samples were prepared and given to Dr. Bi at the UMKC School of Dentistry for testing.

Table 3.14: Formulations of samples for biocompatibility testing.

Sample ID	%SM	%PIH	%CPQ	%EDMAB	%M12	%LMC	Modification
P2 with 1TOSU (P2-1TOSU)	37.90	1.19	0.39	0.06	60.00	0.46	1TOSU
P2 with 3TOSU (P2-3TOSU)	37.90	1.19	0.39	0.06	60.00	0.46	3TOSU
Neat Light Cured SilMix (LCSM)	95.85	3.00	1.00	0.15	0.00	0.00	N/A
Zimmer Osteobond Copolymer Bone Cement (PMMA)	N/A	N/A	N/A	N/A	N/A	N/A	N/A

As with previous biocompatibility screening for the silorane systems (LCSM and LMC/PIH), the P2 samples were biocompatible. There was no significant difference in the percent live/dead from the empty well control and the silorane formulations (Table 3.15). But there was a difference observed for the PMMA samples, which was expected (Figures 3.7 a-b). As for the leachable study, there was a difference between the P2 samples and the three controls ($p<0.05$), but not significant to be of a concern (Figure 3.8). These results show that this new formulation, P2-M12 with the three different modifications, was biocompatible.

Table 3.15: Percent live vs. dead cells at 24 and 48 h.

Sample ID	%Live – 24 h	%Dead – 24 h	%Live – 48 h	%Dead – 48 h
Control	96.4	3.6	96.8	3.2
PMMA	71.7	28.3	84.0	16.0
LCSM	94.4	5.6	95.6	4.4
P2-1TOSU	94.2	5.8	95.2	4.8
P2-3TOSU	93.1	6.9	93.1	6.9

Figure 3.7: Percent live vs. dead cells at a) 24 h and b) 48 h

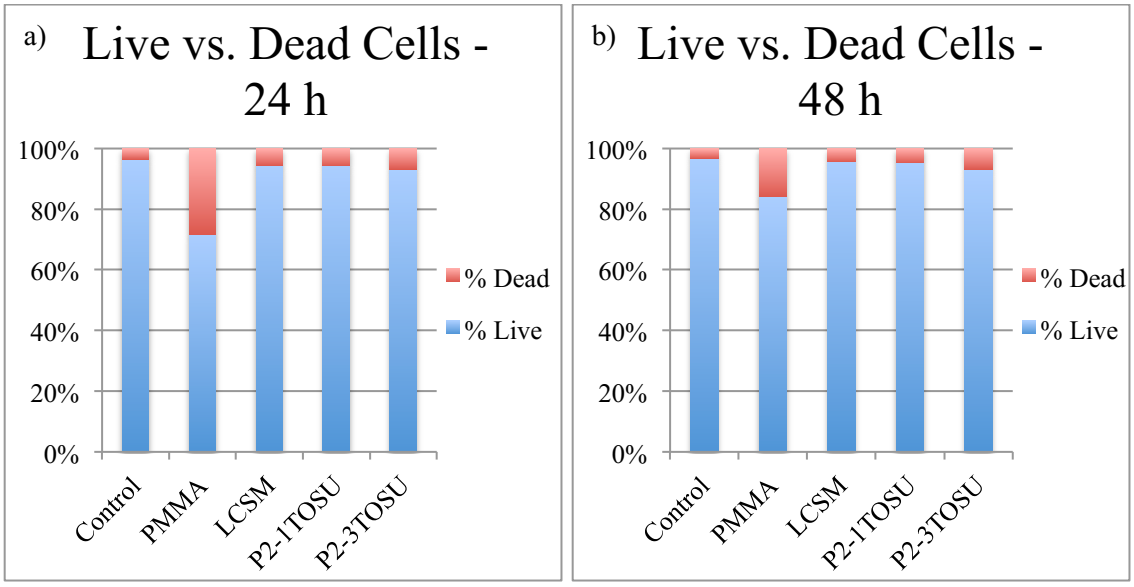
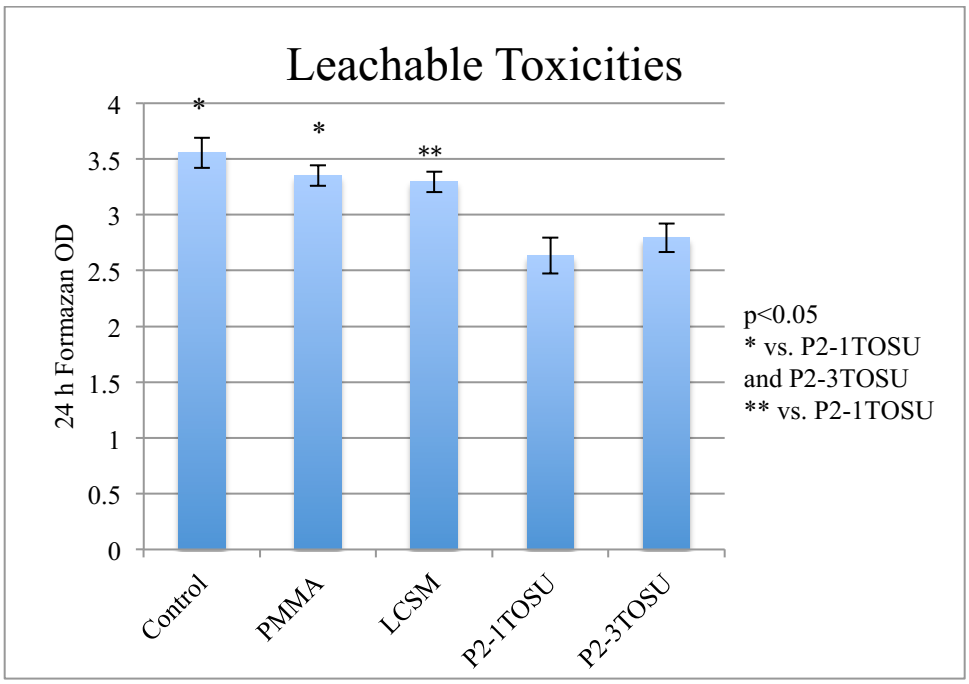


Figure 3.8: MTT assay results for potential leachables.



The next set of biocompatibility testing (TB) was performed using the P2 formulation with 60 wt% 1TOSU-modified DY5 glass. This formulation was compared to empty cell wells, P2 with 60 wt% 1TOSU-modified M12 glass, and a commercial bone cement, Simplex P (Table 3.16). This study was performed by Dr. Bi at the UMKC School of Dentistry.

Table 3.16: Formulations tested.

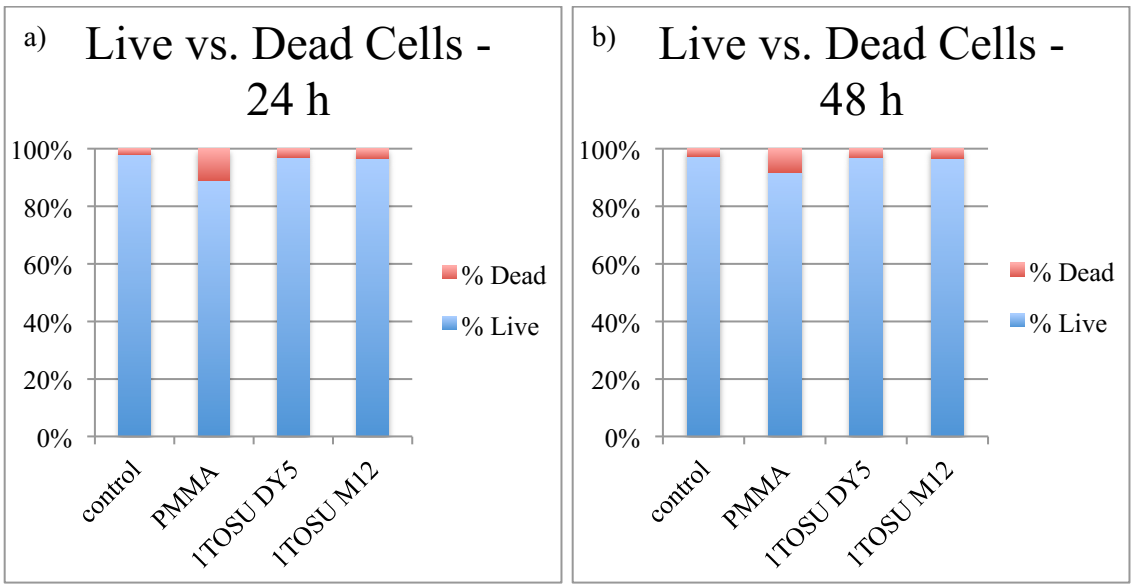
Sample ID	%SM	%PIH	%CPQ	%EDMAB	%Filler	%LMC	Modification
Simplex P (PMMA)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
P2 – 1TOSU mod DY5	37.90	1.19	0.39	0.06	60.00	0.46	1TOSU
P2 – 1TOSU mod M12	37.90	1.19	0.39	0.06	60.00	0.46	1TOSU

As with the previous results for P2 with M12 glass, only one sample the Simplex P samples were significantly different from the empty well control and had higher toxicity than the other samples. The results are given in Table 3.17 and Figures 3.9.

Table 3.17: Percent live/dead cells at 24 and 48 h.

Sample ID	%Live – 24 h	%Dead – 24 h	%Live – 48 h	%Dead – 48 h
Control	97.9	2.1	97.3	2.7
PMMA	88.8	11.2	91.7	8.3
1TOSU DY5	96.9	3.1	96.7	3.3
1TOSU M12	96.5	3.5	96.6	3.4

Figure 3.9: Percent live vs. dead cells a) 24 h and b) 48 h.



From the biocompatibility study, it was determined that the P2 formulation was biocompatible independent of filler (M12 or DY5 glass) or filler modification (1TOSU, 3TOSU, or ECHE). The optimal formulation contains 60 wt% of modified filler, 0.46 wt% LMC, and 39.54 wt% LCSM. With these results in hand, the next step was to determine the strength of this formulation using a pull out test.

P2 – Pull Out Strength

After the biocompatibility of P2 formulation was determined and the amount LMC was optimized, the pull out strength was investigated using a rat model (Table 3.18). It measures the ability of a material to fix a titanium rod in a femur.

Table 3.18: *Ex vivo* pull out sample formulations.

Sample ID	%SM	%PIH	%CPQ	%EDMAB	%LMC	%Filler
Simplex P	N/A	N/A	N/A	N/A	N/A	N/A
P2 – 1TOSU mod M12	37.90	1.19	0.39	0.06	0.46	60.00
P2 – old 1TOSU mod DY5	37.90	1.19	0.39	0.06	0.46	60.00

A hole drilled was drilled down the center of the femur at the distal end. The formulation was injected into the hole, and then an acid etched titanium rod (22 mm x 1.5 mm) was inserted. For this test, the target desired strength was equal to or greater than 4.5 MPa. More information on the pull out procedure can be found in the Materials and Methods section. For *ex vivo* testing, excised rat bones from previously sacrificed animals and etched Ti rods (22 mm x 1.5 mm) were used. P2 formulations (1TOSU M12 and 1TOSU

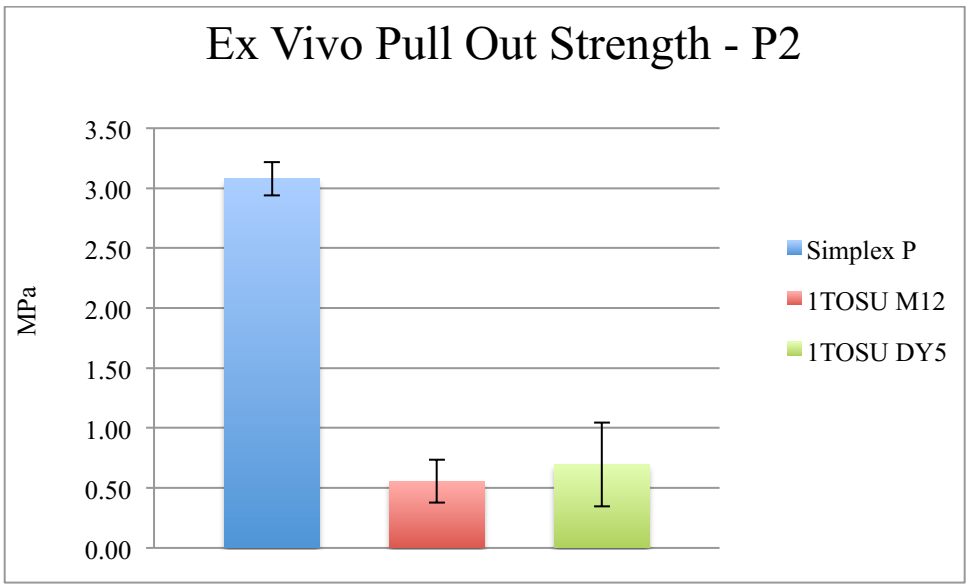
DY5) and Simplex® P (Table 3.18) were compared using this *ex vivo* model. Samples were prepared in collaboration with Dr. Bi, who also conducted the measurements.

The initial results were extremely disappointing because P2 formulations had a fraction of the pull out strength of Simplex P, 0.53 and 0.70 MPa compared to 3.08 MPa (Table 3.19 and Figure 3.10). It is interesting to note that the Simplex P samples did not even meet the minimum criteria of 4.5 MPa. Because of this result, the minimum pull out strength for the P2 formulations was reduced to equal to or greater than 70% of the PMMA control's pull out strength for screening purposes.

Table 3.19: *Ex vivo* pull out strengths of Simplex P and P2 formulations.

Sample ID	Pull Out Strength (MPa)
Simplex P	3.08 ± 0.14
1TOSU M12	0.56 ± 0.18
1TOSU DY5	0.70 ± 0.35

Figure 3.10: *Ex vivo* pull out strengths of Simplex P and P2 formulations.



P2 – Pull Out Mimic

Unfortunately, there were two limitations to the ex vivo rat mode study, the cost and number of rat femurs. Therefore, a mimic rat put out test was developed. It was basically a small tube with an inner diameter of 3 mm that was scored and then imbedded in the specimen holder. The tube was filled with the test formulation, and then the Ti rod was inserted. This mimic setup was used mostly for consistency and product control by comparing batches of catalysts, fillers, and modifications. Additional details of the pull out mimic setup are described in the Material and Methods section.

In order to determine the differences between the glasses, pull out mimic samples were prepared using the ECHE-modified fillers at 60 wt% and 0.32 wt% LMC using Simplex P as the control. These samples were tested by Dr. Bi (Table 3.20).

Table 3.20: Pull out mimic: M12/DY5 comparison formulations.

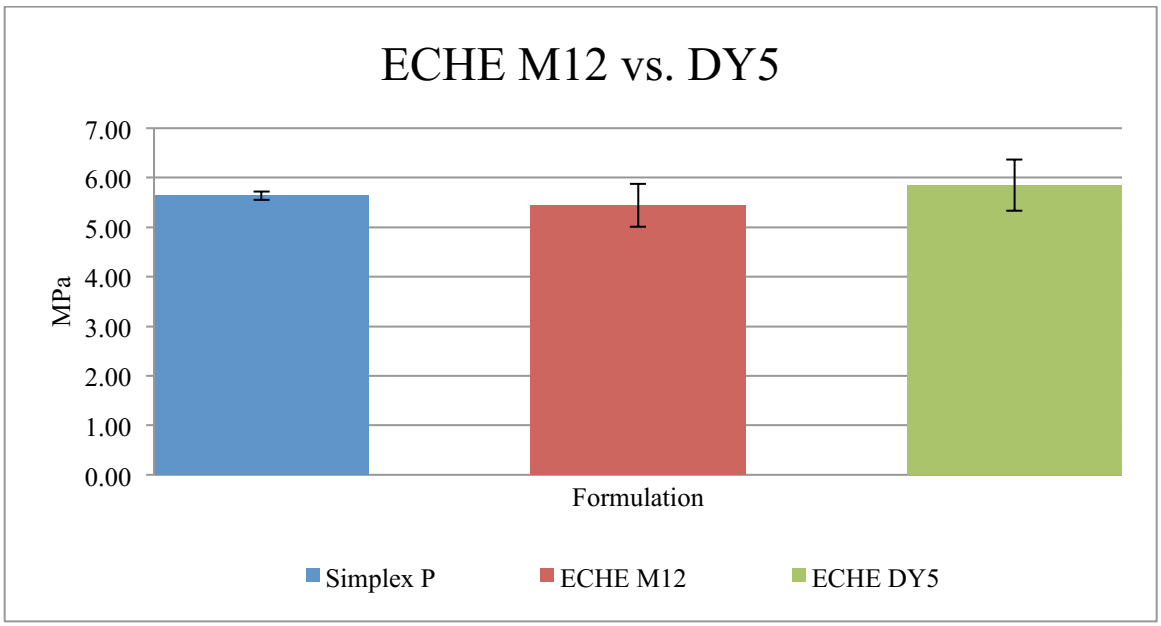
Sample ID	%SM	%PIH	%CPQ	%EDMAB	%LMC	%Filler
Simplex P	N/A	N/A	N/A	N/A	N/A	N/A
P2 with ECHE mod M12 (ECHE M12)	38.03	1.19	0.40	0.06	0.32	60.00
P2 with ECHE mod DY5 (ECHE DY5)	38.03	1.19	0.40	0.06	0.32	60.00

It was found that there was no significant difference between any of the samples. Therefore, there was no difference between the glasses in the same P2 system (Table 3.21 and Figure 3.11). These formulations were investigated further with mechanical and *ex vivo* pull out tests.

Table 3.21: Pull out mimic strength: M12/DY5 comparison.

Sample ID	Pull Out Strength (MPa)
Simplex P	5.64 ± 0.09
ECHE M12	5.44 ± 0.43
ECHE DY5	5.85 ± 0.52

Figure 3.11: Pull out mimic strength: M12/DY5 comparison.



P2 – ECHE Formulations

Since the mimic was successful, the next step was to investigate ECHE 60 wt% filled with 0.32 wt% LMC system. The flexural strength, flexural modulus, compressive strength, and pull out strength were preformed using excised rat bones. The same sample formulations were used for all of these tests, and details can be found in Table 3.22. For the investigation of flexural strength and flexural modulus, the four-point bend test was used. Compressive strength was tested according to ISO 5833. The samples were compared with the commercial bone cement, Simplex P. Specimens were prepared in the laboratory and transferred to Dr. Melander at the School of Dentistry for testing.

Table 3.22: Mechanical specimens – ECHE formulations.

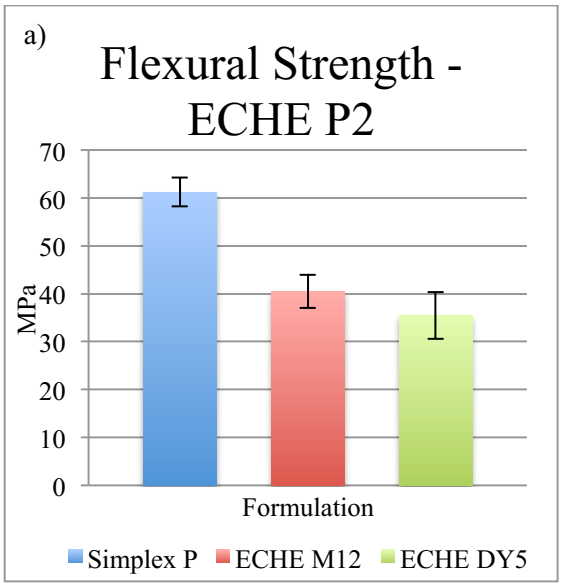
Sample ID	%SM	%PIH	%CPQ	%EDMAB	%LMC	%Filler
Simplex P	N/A	N/A	N/A	N/A	N/A	N/A
P2 with ECHE mod M12 (ECHE M12)	38.03	1.19	0.40	0.06	0.32	60.00
P2 with ECHE mod DY5 (ECHE DY5)	38.03	1.19	0.40	0.06	0.32	60.00

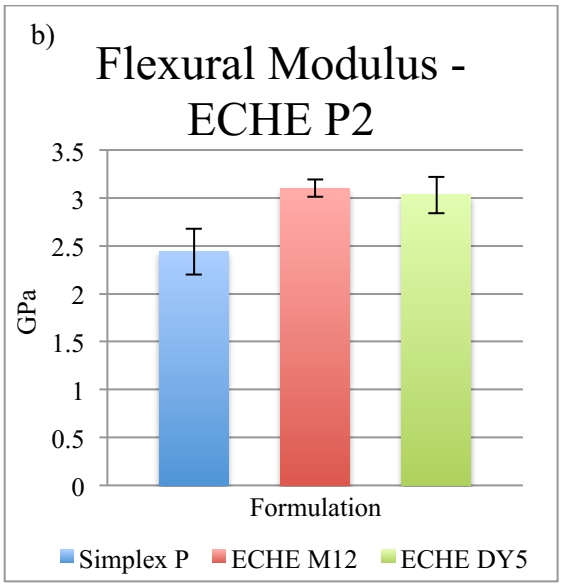
Table 3.23: Flexural strength and modulus – ECHE formulations.

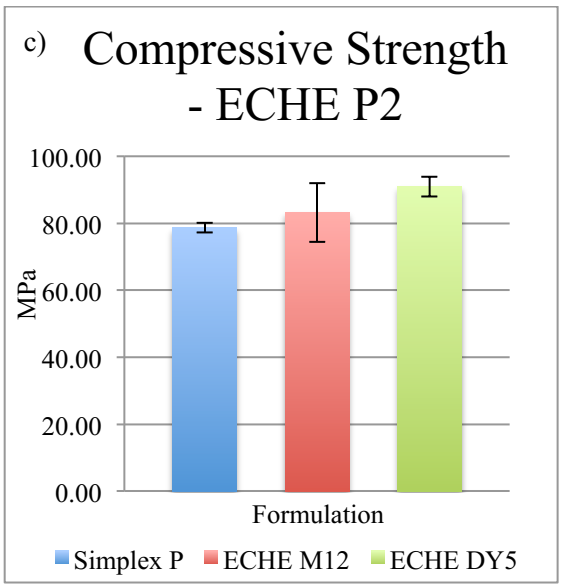
Sample ID	Flexural Strength (MPa)	Flexural Modulus (GPa)	Compressive Strength (MPa)
Simplex P	61.20 ± 2.99	2.44 ± 0.24	78.63 ± 1.41
ECHE M12	40.50 ± 3.46	3.10 ± 0.09	83.17 ± 8.75
ECHE DY5	35.46 ± 4.90	3.03 ± 0.19	90.92 ± 2.95

The flexural strength of this formulation did not meet the ISO 5833 threshold of greater than 50 MPa; however, these samples were closer than the original ECHE DY5 P2 formulation tested (approximately 25 MPa). As for the flexural modulus, all of the samples exceeded the minimum requirement of 1.8 GPa. For compressive strength, all samples were greater than 70 MPa as required by the ISO. The mechanical results can be found in Table 3.23 and Figure 3.12.

Figures 3.12: Results of ECHE formulations for a) flexural strength, b) flexural modulus, and c) compressive strength.





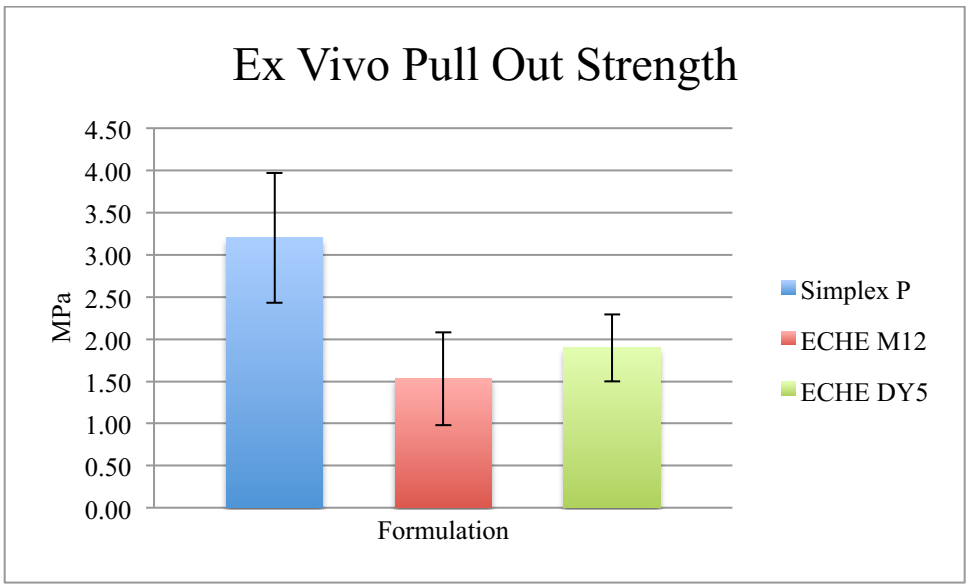


After the mechanical testing of flexural strength, flexural modulus, and compressive strength, the same formulations (Table 3.22) were used for *ex vivo* pull out testing by Dr. Bi. As with the mechanical testing, a PMMA control was used (Simplex P). It was determined that the pull out strengths for our ECHE P2 formulations were significantly lower than the PMMA control (Table 3.44 and Figure 3.13). All the samples including the control were lower in the *ex vivo* test than in the previous pull out mimic. When comparing these results to previous *ex vivo* work, the PMMA controls were similar. On the other hand, these results for the P2 formulations were higher than in the earlier *ex vivo* tests.

Table 3.24: *Ex vivo* pull out strength – ECHE formulations.

Sample ID	Pull Out Strength (MPa)
Simplex P	3.20 ± 0.77
ECHE M12	1.53 ± 0.55
ECHE DY5	1.90 ± 0.40

Figure 3.13: *Ex vivo* pull out strength – ECHE formulations.



While the mimic screening test did result in an improved *ex vivo* result, it was not a realistic mimic for *ex vivo* environment and for *in vivo* systems. With all of the information in hand, it was determined the next step would be to test the formulations in live animal models.

P2 –*In Vivo* Small Animal Model

The next step was to take the best biocompatible formulations to *in vivo* testing in small animals. Mice and rats are the most common small animal model. A rat model was identified due to their larger size, which allows for easier surgeries. From these live animals tests, both inflammation at the surgical site and pull out strengths would be tested 8 weeks post surgery. The surgeries were performed at the UMKC Laboratory Animal Research Core (LARC) with our Dental School collaborator, Dr. Lian Xiang Bi. During surgery, the cement formulations were injected into the femoral canal, and then an acid etched titanium rod was inserted. The rats were sacrificed either one or eight weeks after surgery.

Three formulations were initially studied, and all of then were filled to 60 wt% with either ECHE M12 (n=20), ECHE DY5 (n=15), or 1TOSU DY5 (n=15). A Simplex P control was also tested (n=10). The cement compositions can be found in Table 3.25. Animals were sacrificed at two time points. The animals used for histology and inflammation studies were sacrificed one-week post surgery. Those rats used for the pull out tests were sacrificed 8 weeks post surgery. Once the operated femur was removed, the Ti rod was exposed, and the pull out test was performed.

Table 3.25: Formulations of materials used *in vivo*.

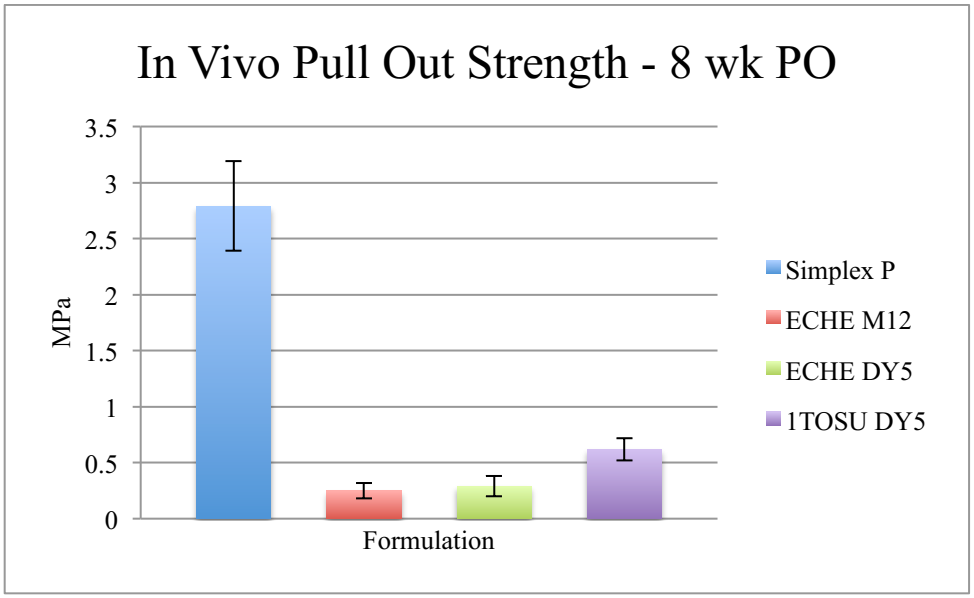
Sample ID	%SM	%PIH	%CPQ	%EDMAB	%LMC	%Filler	Mod.
Simplex P	N/A	N/A	N/A	N/A	N/A	N/A	N/A
P2 with ECHE modified M12 (ECHE M12)	38.03	1.19	0.40	0.06	0.32	60.00	ECHE
P2 with ECHE modified DY5 (ECHE DY5)	38.03	1.19	0.40	0.06	0.32	60.00	ECHE
P2 with 1TOSU modified DY5 (1TOSU DY5)	38.03	1.19	0.40	0.06	0.32	60.00	1TOSU

It was found that the P2 samples only had between 9 and 22% of the strength of the commercial control, Simplex P, which was well below the desired 70%. These results were unexpected. However, there was a decrease in inflammation observed around the surgical site for the P2 formulations. Also, the animals with the P2 formulations lost less weight than the Simplex P animals. However, the low strength was a significant problem that needed to be addressed before moving forward. A summary of the pull out results can be found in Table 3.26 and Figure 3.1.

Table 3.26: *In vivo* pull out strength – ECHE formulations.

Sample ID	Pull Out Strength (MPa)
Simplex P	2.79 ± 0.40
ECHE M12	0.25 ± 0.07
ECHE DY5	0.29 ± 0.09
1TOSU DY5	0.62 ± 0.10

Figure 3.14: *In Vivo* pull out strength – 8 weeks PO.



P2 – Putty Formulation

It is known that an increase in filler is directly proportional to an increase in mechanical strength in dental composites.[35] So in order to improve the mechanical properties of the P2 formulation, a studied was performed, which investigated the optimal amount of modified filler. With an increase in filler, the optimal, amount of LMC was modified to address any changes in handling or polymerization times. The first material was a putty with amounts of 1TOSU DY5 ranging from 65 – 75 wt%s and 0.36 – 0.70 wt%s for LMC. The GNT was used to test for polymerization time. The handling properties were investigated with respect to two points: the time it took for the material to be worked into a ball and the second time point when the ball was unable to be manipulated further. The summary of the putty formulations and handling times can be found in Tables 3.27 and 3.28.

Table 3.27: Putty formulations and polymerization test results.

Formulation	%SM	%PIH	%CPQ	%EDMAB	%Filler	%LMC	GNT Pass
Putty 1 (65% filled)	32.92	1.03	0.34	0.05	65.00	0.65	30 min
Putty 2 (65% filled)	33.07	1.04	0.35	0.05	65.00	0.50	30 min
Putty 3 (65% filled)	33.16	1.04	0.35	0.05	65.00	0.40	30 min
Putty 4 (65% filled)	33.20	1.04	0.37	0.05	65.00	0.36	45 min
Putty 5 (67% filled)	30.96	0.97	0.32	0.05	67.00	0.70	15 min
Putty 6 (67% filled)	30.96	0.97	0.32	0.05	67.00	0.70	15 min
Putty 7 (70% filled)	28.08	0.88	0.29	0.04	70.00	0.70	30 min
Putty 8 (70% filled)	28.13	0.88	0.29	0.04	70.00	0.65	30 min
Putty 9 (75% filled)	23.29	0.73	0.24	0.04	75.00	0.70	45 min
Putty 10 (75% filled)	23.29	0.73	0.24	0.04	75.00	0.70	45 min
Putty 11 (75% filled)	23.29	0.73	0.24	0.04	75.00	0.70	45 min
Putty 12 (75% filled)	23.29	0.73	0.24	0.04	75.00	0.70	45 min
Putty 13 (75% filled)	23.58	0.74	0.25	0.04	75.00	0.40	30 min
Putty 14 (70% filled)	28.42	0.89	0.30	0.04	70.00	0.35	30 min
Putty 15 (72.5% filled)	26.02	0.81	0.27	0.04	72.50	0.35	30 min
Putty 16 (74% filled)	24.58	0.77	0.26	0.04	74.00	0.35	30 min
Putty 17 (74.5% filled)	24.10	0.75	0.25	0.04	74.50	0.35	45 min

Table 3.28: Handling properties of Putty samples.

Putty ID	Time to Form Ball	Time Ball Unworkable
Putty 1	2.5 min	4.5 min
Putty 2	3.5 min	4.5 min
Putty 3	4.5 min	6.5 min
Putty 4	4.5 min	5 min
Putty 5	3.5 min	4.5 min
Putty 6	3.5 min	4.5 min
Putty 7	3.5 min	5 min
Putty 8	3 min	4 min
Putty 9	Immediately	6.5 min
Putty 10	Immediately	6.5 min
Putty 11	Immediately	6.5 min
Putty 12	Immediately	6.5 min
Putty 13	Immediately	5.5 min
Putty 14	4.5 min	6 min
Putty 15	6 min	7.5 min
Putty 16	5 min	10.5 min
Putty 17	3.5 min	7 min

Two formulations were identified as potential candidates, a thick material (74 wt% 1TOSU DY5, 0.35 wt% LMC) and a thinner material (65 wt% 1TOSU DY5, 0.40 wt% LMC). Both of these materials passed the GNT at 30 min. For the handling properties, the thinner material (Putty A) took 4.5 min to form a ball after the LMC was added and a total of 6.5 min when the ball could no longer be manipulated. In the case of the thicker material (Putty B), the times were 5 min and 10.5 min, respectively. It was decided to moved forward with *in vivo* pull out tests using Putty A (thinner material) and Putty B (thicker material).

P2 – Putty *In Vivo* Pull Out

For the comparison of Putty A and B, a rat model utilizing both femurs of six 9-month-old rats was used. In addition to Putty A and B, "regular" P2 and Simplex P were also used (n=3). The formulations and placements of the materials can be found in Table 3.29. To improve the handling and injection times of the Putty, a protocol was developed. The dental syringe was assembled properly, the tip made readily available, and the Ti rod was placed next to the rat before the LMC was weighed. When the LMC was added, the lamp above the surgical table was turned off until the Putty was injected. Once the material was placed in the syringe, it was used immediately. These changes allowed for the Putty samples to be placed without any problems premature polymerization.

Table 3.29: Formulations of samples for *in vivo* testing.

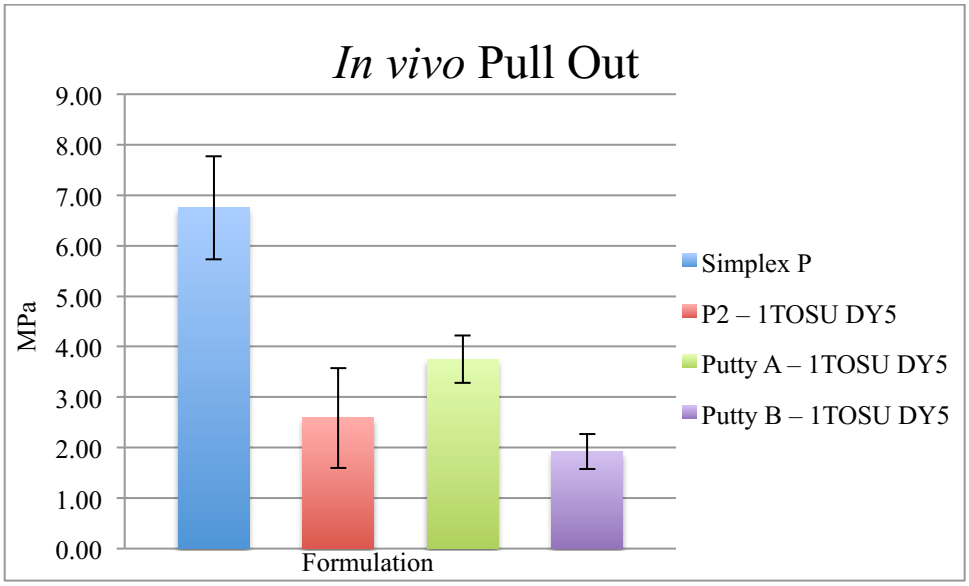
Sample ID	Rat/Leg	%SM	%PIH	%CPQ	%EDMAB	%Filler	%LMC
Simplex P	#1/R	N/A	N/A	N/A	N/A	N/A	N/A
Putty B – 1TOSU DY5	#1/L	24.58	0.77	0.26	0.04	74.00	0.35
Putty A – 1TOSU DY5	#2/R	33.16	1.04	0.35	0.05	65.00	0.40
P2 – 1TOSU DY5	#2/L	38.03	1.19	0.40	0.06	60.00	0.32
Simplex P	#3/R	N/A	N/A	N/A	N/A	N/A	N/A
Putty B – 1TOSU DY5	#4/L	24.58	0.77	0.26	0.04	74.00	0.35
Putty A – 1TOSU DY5	#3/L	33.16	1.04	0.35	0.05	65.00	0.40
P2 – 1TOSU DY5	#4/R	38.03	1.19	0.40	0.06	60.00	0.32
Simplex P	#5/R	N/A	N/A	N/A	N/A	N/A	N/A
Putty B – 1TOSU DY5	#6/R	24.58	0.77	0.26	0.04	74.00	0.35
Putty A – 1TOSU DY5	#6/L	33.16	1.04	0.35	0.05	65.00	0.40
P2 – 1TOSU DY5	#5/L	38.03	1.19	0.40	0.06	60.00	0.32

The animals were sacrificed one week after surgery, and the femurs were harvested. Later that day, the pull out tests were performed by Dr. Bi. While all of the silorane samples were below Simplex P strengths, Putty A had the best silorane *in vivo* strength, 3.75 MPa (Table 3.30 and Figure 3.15).

Table 3.30: *In vivo* P2 vs. Putty A & B pull out strength – 1 week PO

Formulation	Pull Out Strength (MPa)
Simplex P	6.75 ± 1.02
P2 – 1TOSU DY5	2.59 ± 0.99
Putty A – 1TOSU DY5	3.75 ± 0.47
Putty B – 1TOSU DY5	1.92 ± 0.34

Figure 3.15: *In vivo* P2 vs. Putty A & B pull out strength – 1 week PO



Because of these results, it was determined that only the 65 wt% filled Putty A would be used in all future work. However, the strength did not meet the desired criterion. After further reflection and discussion with collaborators, it was decided to focus on the "dryness" of the components of the formulations. It may be a minor change but could have a large impact.

P2 – Moisture Investigation

After the last set *in vivo* results, our collaborator Dr. Schuman, at MS&T, began investigating the role moisture played in the mechanical properties and cure kinetics of our SilMix.[69] Previously, the amount of water was present in SilMix did not appear to interfere with the basic polymerization, exotherm, or mechanical tests. However, when additional water was added to the system, there was a decrease in polymerization and strength. While water did not seem to affect polymerization, too much water appeared to slow down the rate of the reaction.[69] This result would explain the differences observed between the *in vivo*, *ex vivo*, and mimic pull out test results.

The idea was to remove as much water from the different components of the system as possible. The Ti rods were stored in a desiccator after autoclaving, the filler was kept under inert atmosphere once it was modified, and residual water was removed from SilMix. It was assume that the removal of as much moisture from the system prior to its use, any water introduced would not have a significant effect on the polymerization.

Dr. Schuman (MS&T) was able to reduce the water content of the silorane resin using a toluene-water azeotrope and a vacuum pump. The water content was determined using Karl Fischer titration. Before drying, the average water content was 0.18 wt%, while after drying, it decreased to approximately 0.1 wt% for dry and 0.03 wt% for ultra-dry SilMix.[69] For future testing, the dry SilMix (0.1 wt%) was chosen due to the better degree of conversion [69] The dry components were tested *in vivo* next.

P2 – *In Vivo* Pull Out Dried Putty

After the moisture study performed by Dr. Schuman, the dried material was investigated in a wet environment. Samples were prepared to compare the original 60 wt% filled material to the same formulation but using dried materials. Putty A was also tested using the dry materials. Fifteen 10-month-old rats underwent surgery on their right femurs. As with all rat surgeries, they were performed at the LARC with Dr. Bi. Formulations containing Simplex P, Original P2 (60 wt%), Dry Original P2 (60 wt%), and Dry Putty A (65 wt%) were tested (n=3). "Dry" meant that the filler and SilMix were dried, and a desiccated Ti rod was used. For more information on the formulations and placements of these samples see Table 3.31.

Table 3.31: Sample information for *in vivo* testing of "dried" material.

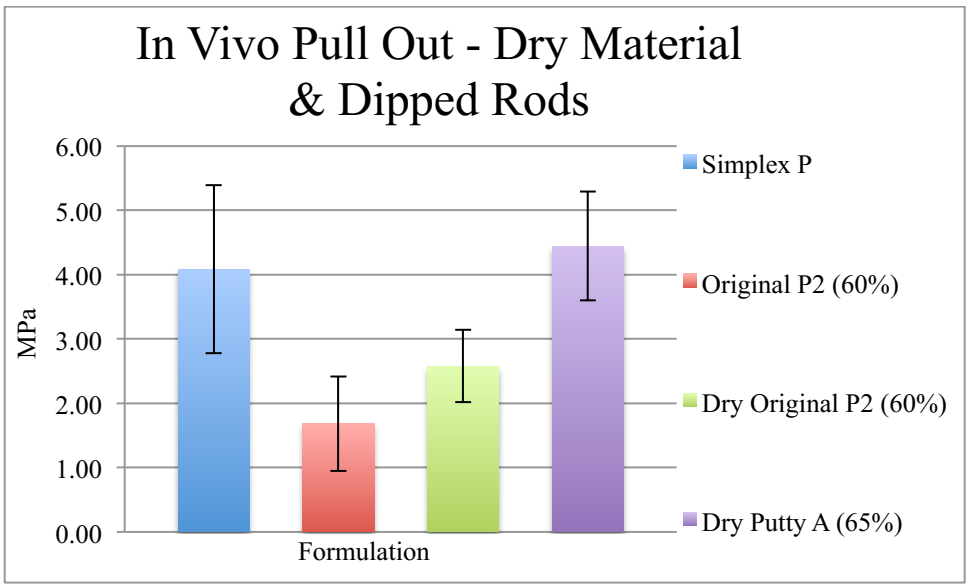
Sample ID	%SM	%PIH	%CPQ	%EDMAB	%Filler	%LMC
Simplex P	N/A	N/A	N/A	N/A	N/A	N/A
Original P2 (60%)	38.03	1.19	0.40	0.06	60.00	0.32
Dry Original P2 (60%)	38.03	1.19	0.40	0.06	60.00	0.32
Dry Putty A (65%)	33.16	1.04	0.35	0.05	65.00	0.40

One week after surgery, the animals were sacrificed, and the femurs were harvested. The pull out tests were performed that same day by Dr. Bi. It was found that there was no significant difference between Simplex P and Dry Putty A formulations. The dry P2 samples were stronger than the regular P2 samples (Table 3.32 and Figure 3.16). From these results, the Dry Putty A formulation was chosen for future testing.

Table 3.32: *In vivo* pull out strength of dry material investigation – 1 week PO.

Formulation	Pull Out Strength (MPa)
Simplex P	4.08 ± 1.31
Original P2 (60%)	1.68 ± 0.73
Dry Original P2 (60%)	2.58 ± 0.56
Dry Putty A (65%)	4.44 ± 0.85

Figure 3.16: *In vivo* pull out strength of dry material investigation – 1 week PO.



From this study and all the previous testing, it was determined that the ideal material was a putty filled to 65 wt% with 1TOSU DY5 using dried SilMix and filler. The Ti rods would be kept free of excess water by storage in a desiccator prior to use. For the majority of the previous *in vivo* tests, these sample replicates were limited to no more than four. Due to a small sample size, it was determined to run a larger scale investigation comparing Simplex P with our dry Putty A.

P2 – *In Vivo* 8 weeks Pull Out Dried Putty

Surgery was performed on the right femurs of seventeen 13-month-old rats at the LARC with Dr. Bi. Dry Putty A (n = 9) was compared to Simplex P (n = 8). The sample information can be found in Table 3.33.

Table 3.33: Sample information for *in vivo* testing.

Sample ID	%SM	%PIH	%CPQ	%EDMAB	%Filler	%LMC
Simplex P	N/A	N/A	N/A	N/A	N/A	N/A
Dry Putty A (65%)	33.16	1.04	0.35	0.05	65.00	0.40

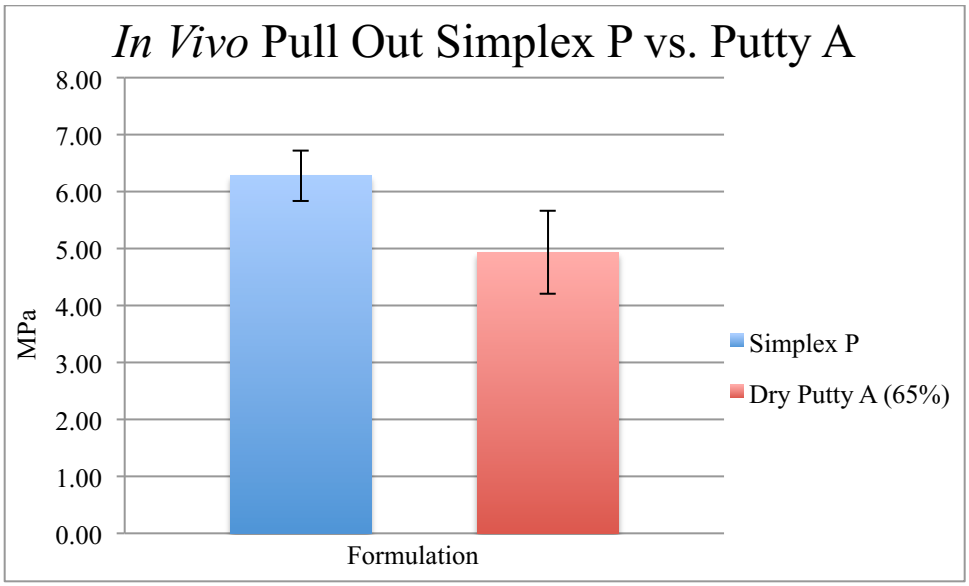
Over the course of the eight weeks, four rats died or were sacrificed (one Simplex P and three Putty A). This was the first time that there was a post-operative death of an animal from the SilMix group. Necropsies were done, and any issues were attributed to the age of the animals. With the previous *in vivo* testing, younger 9-10 month old rat were used. After the deaths, there were only five Putty A and seven Simplex P animals. At the end of 8 weeks, the animals were sacrificed, and the femurs were harvested. The pull out test was done later the same day. While pull out strengths for Putty A were

lower than for Simplex P, the two groups were not statistically different (Table 3.34 and Figure 3.17).

Table 3.34: *In vivo* Simplex P vs. Putty A pull out strength – 8 weeks PO.

Formulation	Pull Out Strength (MPa)
Simplex P	6.28 ± 0.44
Dry Putty A (65%)	4.94 ± 0.73

Figure 3.17: *In vivo* Simplex P vs. Putty A pull out strength – 8 weeks PO.



In summary from all of the *in vivo* data for dry Putty A (33.16 wt% SM, 1.04 wt% PIH, 0.35 wt% CPQ, 0.05 wt% EDMAB, 65 wt% 1TOSU DY5, and 0.40 wt% LMC), a viable bone cement with comparable strength to Simplex P was found. This formulation met or exceeded the desired requirements for the *in vivo* studies. With the exception of Flexural Strength, Putty A met or exceeded the ISO standard 5833. With this, the next step for this material was large animal testing.

P2 – *In Vivo* Large Animal Model

Swine were chosen for their similarity to humans in regards to orthopedics. Surgery was performed by Dr. Donna Pacicca on 16 pigs (n=8 each Simplex P and Dry Putty A) at the National Swine Research and Resource Center, Columbia MO. The surgery protocol was similar in nature to the rat studies with the exception of the use of a larger implant (100 mm x 6.35 mm vs. 22 mm x 1.5 mm for the rat) and larger cement delivery device. The animals were sacrificed at 8 weeks after surgery. The histology and pull out studies were ongoing during the preparation of this dissertation.

Summary

At the start of this research, numerous initiation systems were investigated for the formulation of a bone cement alternative. However, only two exhibited potential for this purpose. The first candidate was a filled mixed system of AA:PIH:CPQ (3:3:1 wt%s) filled to 50 wt% with DY5 and ECHE modified ANF (1:1 by wt). Unfortunately, the requirement of direct irradiation with an external light source for polymerization limited its applicability for internal use.. The other alternative was a neat pure chemical option of LMC/PIH (0.07 wt% LMC and 0.04 wt% of PIH), which did not require irradiation for polymerization. This formulation was biocompatible, and the handling and polymerization times were within the acceptable range, approximately five and 30 min, respectively. It was utilized for the generation of prototype 1 (P1), which contained SilMix (CSM) and glass filler in addition to the LMC/PIH initiation system. There were drawbacks with P1, which included the grainy consistency of the material, low flexural strength of (25.8 MPa), and poor handling properties. Due to these issues, prototype 2 (P2) was developed, which was comprised of a mixed initiation system, LMC/LIS (PIH, CPQ, EDMAB) and polymerized without the use of direct irradiation from an external light source. With respect to consistency, it was improved with P2 due to the removal of the CSM and an increase in glass filler. Further investigations with P2 with respect to the filler were performed with either M12 or DY5 glass with one of three modifications (ECHE, 1TOSU, and 3TOSU). All of the components in P2 formulations had good

biocompatibility and low polymerization exothermicity, however the *in vivo* pull out strengths were well below the desired threshold of ≥ 70% of the PMMA control's pull out strength. Due to results of the moisture study, a bone cement alternative, Dry Putty A formulation (33.16 wt% SM, 1.04 wt% PIH, 0.35 wt% CPQ, 0.05 wt% EDMAB, 65 wt% 1TOSU DY5, and 0.40 wt% LMC), was identified. This formulation met all of the desired properties of biocompatibility, *in vivo* pull out strength, low exothermicity upon polymerization, and lack of inflammation response at surgical site. There were no complications resulting from the use of this material in 8 swine and over 50 rat subjects. The summary of the P2 bone cement alternative properties is found in Table 3.35.

The future work on this project will be in the areas of therapeutic beads and spacers, as well as, studies of the incorporation of antibiotics and antifungals into this material. Due high polymerization temperature of commercial PMMA bone cement, the number of antibiotics that can be incorporated for the treatment of infection is limited to those that are heat stable. With a low polymerization exotherm, a wider variety of antibiotics may be incorporated into the P2 alternative. From initial elution studies of vancomycin into P2, it provided proof of concept that antibiotics can be incorporated into Putty A and with similar elution profiles to that of Simplex P.

Table 3.35: Summary of P2 properties.

	ISO 5833 Standard[57]	Desired properties	P2 (60 & 65% filled)
Exothermicity (°C)	≤90	≤45	26 ± 0.5
Handling time (min)	3-15	≤20	8-10
Flexural modulus (GPa)	≥1.8	≥1.8	3.1
Flexural strength (MPa)	≥50	≥50	40.5
Compressive strength (MPa)	≥70	≥70	90.9
Pull out strength – mimic (MPa)	n/a	≥4.5	4.1
Pull out strength – *ex vivo* (MPa)	n/a	≥4.5	2.0
Pull out strength – *in vivo* (MPa)	n/a	≥4.5	4.9
Cytotoxicity (% cell death)	n/a	≤20%	<5%

APPENDIX A

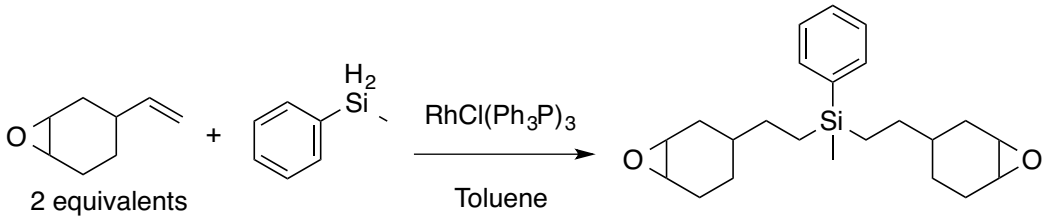
MATERIALS AND METHODS

Materials

SilMix is a 1:1 combination by wt ratio of PHEPSI (bis[2-(3{7-oxabicyclo[4.1.0]heptyl})-ethyl]methylphenyl silane) and CYGEP (2,4,6,8-tetrakis(2-(7-oxabicyclo[4.1.0]heptan-3-yl)ethyl)-2,4,6,8-tetramethyl-1,3,5,7,2,4,6,8-tetraoxa-tetrasilocane). The general reaction schemes for the synthesis of the two monomers are given below and described in Dr. Bradley Miller's dissertation.[62] ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA 400 MHz nuclear magnetic resonance spectrometer operating at 399.8 MHz and referenced to $CDCl_3$ (Cambridge Isotopes). Unless otherwise noted, commercial chemicals were used as supplied without further purification. Starting materials were obtained from the following sources: methylphenylsilane (Gelest); 4-vinyl-1-cyclohexene-1,2-epoxide (Aldrich); and 2,4,6,8-tetramethylcyclotetrasiloxane and Wilkinson's Catalyst (Alfa Aesar). Lamoreaux's catalyst was synthesized from an adapted procedure by Lamoreaux.[62,70]

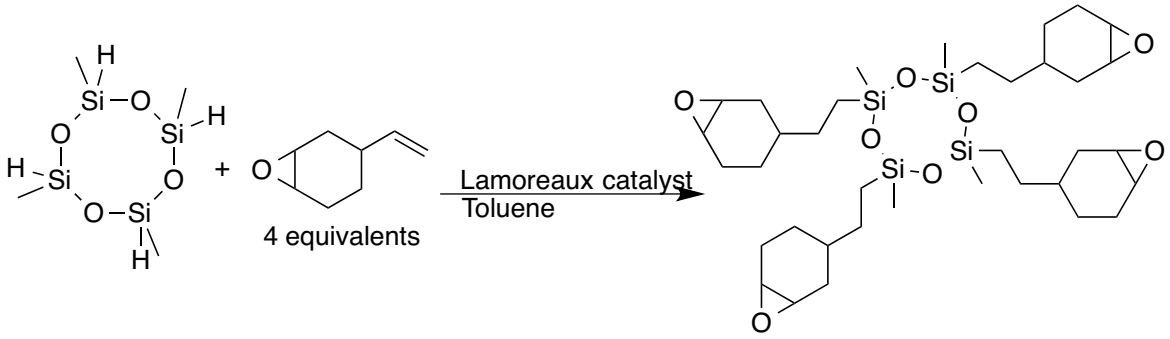
Bis[2-(3{7-oxabicyclo[4.1.0]heptyl})-ethyl]methylphenyl silane (PHEPSI)[62,71] – PHEPSI was prepared according to an adapted procedure from Crivello. ^{1}H NMR ($CDCl_3$, 399.8 MHz) 0.21 (s, 3H), 0.71 (m, 4H), 0.78-2.20 (m, 18H), 3.11 (m, 4H), 7.34 (m, 3H), 7.45 (m, 2H) ppm; ^{13}C NMR ($CDCl_3$, 100.5 MHz) 10.6, 10.8, 23.5, 23.9, 25.1, 26.7, 29.9, 30.1, 30.5, 31.5, 32.4, 35.2, 51.8, 52.6, 53.1, 127.6, 128.7, 133.6, 138.0 ppm.

PHEPSI reaction scheme



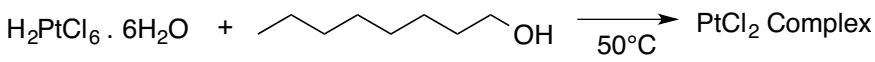
2,4,6,8-tetrakis(2-(7-oxabicyclo[4.1.0]heptan-3-yl)ethyl)-2,4,6,8-tetramethyl-1,3,5,7,2,4,6,8-tetraoxa-tetrasilocane (CYGEP)[62,72] – CYGEP was prepared according to an adapted procedure from Aoki. ^{1}H NMR ($CDCl_3$, 399.8 MHz) 0.02 (s, 12H), 0.44 (s, 8H), 0.79-2.18 (m, 36H), 3.12 (m, 8H) ppm; ^{13}C NMR ($CDCl_3$, 100.5 MHz) 13.9, 23.5, 24.0, 25.2, 26.8, 29.2, 29.8, 30.3, 31.5, 32.0, 35.0, 51.8, 52.2, 53.5 ppm.

CYGEP reaction scheme:



Lamoreaux's catalyst (LMC)[62,70] – LMC was prepared according to an adapted procedure from Lamoreaux and not characterized.

LMC reaction scheme



Sample Preparation Methods

For neat light-cured SilMix (LCSM): SilMix (SM) was combined with the *p*-(octyloxyphenyl)phenyliodonium hexafluoroantimonate) (PIH), camphorquinone (CPQ), and ethyl *p*-dimethylaminobenzoate (EDMAB) in a high-speed mixer until no particles were visible (between 30 min to an hour depending on the amount of material). The final composition of the LCSM was 95.85 % SM, 3.0 % PIH, 1.0 %, CPQ, and 0.15 % EDMAB (by total weight of sample).

For filled Light Cured samples: LCSM was combined with the filler at the wanted amount and mixed in a high-speed mixer between 15 and 30 minutes depending on the amount of material.

Neat Chemical Cure of SM: SilMix (1 g) and the acid catalyst were combined using a FlackTek Speed Mixer and mixed for 5 min.

Neat Dual Cure samples: SilMix (1.5 g) and the photosensitive compound(s) of the initiation system were combined using a FlackTek Speed Mixer and mixed for periods of 5 – 30 min depending on the amount of material. Then, the acid catalyst component was added, and the sample was mixed for another 5 – 10 min depending on the amount of catalyst.

For filled Dual Cure samples: Samples were prepared by combining SilMix (1.5 g) and the photosensitive compound(s) of the initiation system using a FlackTek Speed Mixer and mixed for periods of 5 – 30 min depending on the amount of material. Then,

filler (1:1 by wt) was added and mixed in a high-speed mixer between 15 and 30 minutes depending on the amount of material. Finally, the acid catalyst component was added, and the sample was mixed for another 5 – 10 min depending on the amount of catalyst.

Crushed SilMix (CSM) for prototype 1 (P1) samples: LCSM was polymerized on glass slide. At first the polymer was ground using a coffee grinder to start and then a mortar and pestle. In order to attain more uniform particles, the samples were milled (MS&T collaborators).

For Prototype 1 (P1) samples: In one cup, the CSM was combined with the filler and mixed in the mixer on high for 1 min. In another cup, SM and PIH were mixed for 15 min. The dry components were added to the wet components and mixed by hand. Finally the LMC was added using a needle and syringe (by weight on a balance) and mixed by hand for approximately 30 sec.

For Prototype 2 samples (silorane bone cement): Light-cured SilMix (LCSM) was prepared as stated previously. A portion of the LCSM was combined with the glass filler and mixed in the high-speed mixer (approximately 25 min). The material was allowed to cool to room temperature, and then the Lamoreaux Catalyst (LMC) was added using a needle and syringe (by weight on a balance) and mixed by hand for approximately 30 sec.

Sample Test Methods

Gilmore Needle test: The one – lb. needle was placed on the sample (~ 0.1 g) and then removed. If a needle indented or marked the sample,, then the sample "failed"; if no mark or indentation was observed, then the sample "passed".

pH test: The pH of the deionized or distilled water (~60 mL) was taken initially by placing a pH probe in the beaker, waiting five min, and then taking the reading. Then, a polymerized sample (~60 mg) was placed in the water. The resulting pH of the water containing the sample was then measured at 15-min increments for the first h.

Exotherm Test: Exothermic temperature testing was conducted with a K-type thermocouple (Omega, Stamford, CT). A Delrin® washer (McMaster-Carr, Aurora, OH) was first affixed to a glass slide with lab tape. Then, the thermocouple was slightly bent to place the tip within the Delrin® washer without touching the glass slide and secured with lab tape such that the tip was in the center of the washer. The silorane material was mixed with initiators and then mounded onto the thermocouple tip using a glass rod to ensure that the tip was completely covered with composite (~ 0.125 g). A data logger (OM-PLTC, Omega Engineering Inc., Stamford, CT) was used to collect temperature readings from the thermocouple at 1 Hz for 30 min post-light initiation. The maximum temperature was determined from this test.

Flexural Strength and Flexural Modulus: The resins were injected into borosilicate glass tubes (VitroCom, Mountain Lakes, NJ) coated with a silicone spray mold release (Mark V Laboratory, East Granby, CT). The specimens were irradiated with a dental lamp for two min on the top of the samples (three consecutive regions at 40 s apiece) and for 40 s on the bottom in a scanning motion. The samples were removed from the glass and yielded beams that were 25 mm x 2 mm x 2 mm, as per ISO specification 4049.[55] The beams were stored at room temperature (23 ± 1 °C) for 24 h and then tested mechanically. The beams were placed on four-point bend fixture with a support span of 20 mm on a mechanical tester (Instron 5967, Norwood, MA). The samples were loaded at a displacement rate of 3.7 mm/min until failure. Flexural strength and flexural modulus of elasticity were calculated using the resulting stress-strain curve.

Degree of Conversion (DC): Fourier Transform Infrared Spectroscopy (FTIR) was used to determine the degree of conversion of LCSM by comparing the change in a stretch associated with silorane ring-opening polymerization (883 cm^{-1} representative of an epoxide ring opening) to standard (one that remained unchanged upon polymerization; the 1257 cm^{-1} of the Si-O bond in CYGEP ring structure). The peak ratios were then calculated. Using lab tape, a delrin washer was affixed to the Attenuated Total Reflectance (ATR) accessory. Approximately 0.1 g of material was placed into the ring that was centered over the ATR crystal. Before curing the resin, one baseline spectrum was collected. The samples were irradiated with a dental lamp for two min from a

distance of 3 mm. Spectra were collected every 30 s, during the two-min light irradiation and continued at this frequency for 10 min post initial light irradiation. Spectra were collected every 3.3 min from 10 – 20 min and every 5 min from 21 – 30 min post initial light irradiation. The resulting degree of conversion curves for the samples yielded the rate of cure and the amount of unpolymerized monomer.

Biocompatibility:

LCSM Discs: Polymer discs were prepared the day of the assay by polymerizing ~80 mg of material in a Delrin® ring mold affixed to a glass slide with lab tape. Discs were light cured using a dental lamp at 40-s increments for a total of 2 min. The polymerized discs were removed from the molds and sterilized using UV light for a total of two h (one h per side) in a laminar hood.

Chemical Cure and P2 Discs: Polymer discs were prepared the day before the assay by polymerizing ~80 mg of material in a Delrin® ring mold affixed to a glass slide with lab tape. The light cured samples were irradiated with a dental lamp at 40-s increments for a total of 2 min. The chemically cured samples were placed in the rings and allowed to polymerize. All samples were allowed to dark cure overnight. On the day of the assay, the polymerized discs were removed from the molds and sterilized using UV light for a total of two h (one h per side) in a laminar hood.

Trypan Blue Assay: The polymerized and sterilized discs were placed into 48-well plate (n=3-4) and pre-washed in growth media for one-h at 35 °C/5% CO_2. The wash media was discarded and replaced with 2 x 10^4 MLO-A5 cells/0.5 mL. After 24 h and 48 h of incubation, cell viability and proliferation were measured using the trypan blue method.[60]

Leachables: Discs were prepared as stated previously and used to test the effect of leachables on MLO-A5 cells. The leachables were extracted from the polymer samples by incubating the discs for 24 h in culture media with serum. After 24 h, the extracts were transferred to a monolayer of MLO-A5 cells (seeded the previous day). The cells were exposed to the extracts for 24 h, after which the cell viability was measured using the methyl thiazolyl tetrazolium (MTT) assay.[60] The cell viability was determined by measuring the optical density of the purple formazan produced by the enzymatic transformation of tetrazolium salt (MTT) by viable cells.

Pull Out Tests:

Ex Vivo: All P2 samples were prepared as described previously. The material was placed in a dental syringe, which was used to deliver the material into an excised rat femur. Then, a titanium rod (22 mm long and 1.5 mm in diameter) was inserted into the femur with the material. The femur was imbedded in the holder with dental cement. The samples were stored in a humidified incubator for 24 h and tested biomechanically.

Commercially available bone cement samples were prepared per the standard instructions and used as a control.

Mimic: P2 samples were prepared as previously stated. The material was placed in dental syringes, which was used to deliver the material into plastic tubing (3 mm diameter), which was pre-scored with holes that had been secured into a cut centrifuge tube with dental cement. Then, a titanium rod (22 mm long and 1.5 mm in diameter) was inserted into the tube with the material. The samples were kept in a humidified incubator for 24 h and tested biomechanically. Commercially available bone cement samples were prepared per the standard instructions and used as a control.

In Vivo Rat Studies:

Sterilization: On the day before surgery, SilMix, filler, and mixing cups were sterilized in a UV cabinet for 4 h. All paper towels, spatulas, and petri dishes were autoclaved in the animal facility prior to the surgery. The balance, speed mixers, mixing baskets, and chemicals (PIH, CPQ, and EDMAB) were transported to the animal facility where they were sterilized by the animal facility staff. All paper towels, spatulas, and petri dishes were autoclaved in the animal facility prior to the surgery.

In Vivo – regular: There previously described P2 sample was placed in a dental syringe. The rats were anesthetized and operated on under aseptic condition. In short, the knees were exposed, and a hole was drilled between the femoral condyles and into intramedullary canal. The bone marrow was disrupted, and the marrow cavity was

irrigated and filled with the biomaterial. A titanium implant (22 mm long and 1.5 mm in diameter) was inserted. The capsule and skin were sutured. The rats were then sacrificed at a specified time point, and the femur with the implant was recovered. The excised femurs were then imbedded in the holder using dental cement and allowed to set for a time between 30 min and 24 h and tested biomechanically. Any control and commercially available bone cement was also tested under the same conditions.

APPENDIX B

CHEMICAL INITIATION TABLE OF ACIDS AND INHIBITORS

Acid wt %	Runs	Gillmore Needle	Time
Hydrochloric Acid			
2.0	2	Failed	8 h
5.0	3	Failed	8 h
10.0	2	Failed	8 h
14.0	1	Failed	8 h
18.0	1	Failed	8 h
Acetic Acid			
5.0	2	Failed	8 h
Phosphoric Acid			
7.8	2	Failed	8 h
Sulfuric Acid			
8.0	2	Failed/Brittle	

Cont.

Acid wt %	Runs	Gillmore Needle	Time
Hydrobromic Acid			
7.5	1	Failed	8 h
Hydroiodic Acid			
6.0	1	Failed	8 h
Trichloroacetic Acid			
9.0	2	Failed	8 h
Trifluoroacetic Acid			
7.0	2	Failed	8 h
p-Toluenesulfonic Acid (*p*TSA)			
11.5	2	Failed	8 h
Aluminum Chloride			
13.0	2	Failed	8 h
Tin (IV) Chloride			
7.4	2	Failed	8 h
Pentafluoropropionic Acid			
4.0	1	Failed	8 h
15.0	1	Failed	8 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
Pentafluoropropionic Acid			
18.0	1	Failed	8 h
Triflic Acid			
2.0	1	Failed/Brittle	
4.0	1	Failed/Brittle	
Hexafluorophosphoric Acid (HFPA)			
2.0	4	Passed	2 min
2.5	5	Passed	1 min
4.0	2	Passed	30 sec
4.6	2	Passed	30 sec
5.0	10	Passed	30 sec
Acetic Acid:HFPA			
2.0 : 4.8	2	Failed	1 h
2.0 : 4.9	1	Failed	1 h
1.2 : 2.8	2	Passed	0.75 h
1.2 : 3.0	2	Failed	1 h
1.0 : 3.2	3	Passed	1 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
Acetic Acid:HFPA			
0.8 : 3.1	1	Passed	0.75 h
1.2 : 5.0	1	Failed/poly in cup	
0.6 : 3.5	2	Failed/poly in cup	
1.2 : 2.7	2	Passed	2 days
1.1 : 4.5	2	Failed/poly in cup	
1.1 : 3.4	2	Passed	2 days
0.8 : 2.7	2	Passed	2 days
2.3 : 2.5	1	Failed	1 h
3.0 : 2.8	2	Failed	1 h
3.1 : 2.1	2	Failed	1 h
1.0 : 3.0	1	Failed	1 h
0.8 : 3.8	1	Passed	1 day
1.3 : 4.8	1	Passed	1 day
1.6 : 3.8	1	Passed	1 day
1.1 : 3.6	1	Passed	1 day
1.1 : 3.5	1	Passed	2 days

Cont.

Acid wt %	Runs	Gillmore Needle	Time
Acetic Acid:HFPA			
1.1 : 5.1	1	Failed/Powder	
1.2 : 4.1	1	Failed/poly in cup	
1.2 : 3.2	1	Passed	2 days
1.3 : 2.6	1	Failed	1 day
PIH:Phosphoric Acid			
3.05:5.37	1	Failed	2 h
1.60:2.24	3	Failed	2 h
3.15:2.21	3	Failed	2 h
3.15:2.21	2	Failed	1 h
0.65:1.63	2	Failed	2 h
0.64:3.21	2	Failed	2 h
0.63:5.03	2	Failed	3 h
0.62:6.21	2	Failed	4 h
3.17:1.59	2	Failed	2 h
3.13:3.13	2	Failed	2 h
3.07:4.90	2	Failed	2 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
PIH:Phosphoric Acid			
3.02:6.29	2	Failed	2 h
4.68:1.56	2	Failed	1 h
4.62:3.08	2	Failed	1 h
4.35:8.70	2	Failed	5 h
8.33:8.33	2	Failed	5 h
2.90:10.14	2	Failed	3 h
4.23:11.27	2	Failed	2 h
4.75:23.95	1	Failed/Powder	
1.60:2.24	2	Failed	3 h
1.97:2.85	2	Failed	3 h
2.23:2.23	2	Failed	6 h
2.98:2.03	2	Failed	6 h
1.60:2.30	4	Failed	5 h
1.60:2.43	4	Failed	22 h
2.97:2.21	4	Failed	5 h
2.93:3.26	4	Failed	4 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
PIH:Phosphoric Acid			
1.60:4.70	4	Failed	4 h
2.98:2.03	4	Passed*	22 h
Phosphoric Acid			
3.23	2	Failed	3 h
6.42	2	Failed	3 h
5.06	2	Failed	3 h
9.25	1	Failed	3 h
1.64	2	Failed	3 h
10.44	2	Failed	3 h
11.76	2	Failed	2 h
25.00	1	Failed/Powder	
PIH:Phosphoric Acid:HFPA			
3.13:2.19:0.63	1	Failed/Powder	
1.60:1.92:0.45	1	Failed/polym in cup	
0.45:1.95:0.13	1	Failed	1 h
0.65:1.97:0.42	1	Failed	6 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
PIH:Phosphoric Acid:HFPA			
0.65:1.94:0.58	1	Failed	6 h
1.19:1.93:0.42	1	Failed	5 h
1.19:1.93:0.61	1	Failed/gelled in cup	
1.51:1.95:0.42	1	Failed	2 h
1.51:1.95:0.45	1	Failed	4 h
1.50:2.05:0.45	1	Failed	1 h
1.19:2.05:0.45	1	Failed	2 h
1.19:1.96:0.45	1	Failed	2 h
Phosphoric Acid:$TiCl_4$			
3.77:2.26	1	Failed	8 h
2.57:2.92	1	Failed	8 h
3.18:3.25	1	Failed	8 h
3.94:2.49	1	Failed	8 h
Water:$TiCl_4$			
0.31:3.54	1	Failed	8 h
0.29:3.03	1	Failed	8 h

Cont.

Acid wt %		Runs	Gillmore Needle	Time
Water:$TiCl_4$				
	1.12:2.95	1	Failed	8 h
	1.14:3.08	1	Failed	8 h
$TiCl_4$				
	6.50	1	Passed, smoking gel	1 h
	2.87	1	Failed	8 h
	0.67	1	Failed	8 h
	0.85	1	Failed	8 h
	2.03	1	Failed	8 h
	2.82	1	Failed	8 h
	3.22	1	Failed	8 h
PIH:$TiCl_4$				
	2.30:0.72	1	Failed/Powder	
	0.34:0.67	1	Failed/Powder	
	0.13:0.68	1	Failed	5 h
	0.16:0.96	1	Failed	5 h
	0.24:1.45	1	Failed	5 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
PIH:$TiCl_4$			
0.23:2.10	1	Failed	5 h
0.26:2.06	1	Failed	5 h
0.67:0.74	1	Failed/Powder	
0.26:3.87	1	Failed/Powder	
Phosphoric Acid:Triflic Acid			
1.92:2.22	1	Failed	8 h
Phosphoric Acid:*p*TSA			
2.29:2.29	1	Failed	8 h
Phosphoric Acid:Trichloroacetic Acid			
2.54:2.25	1	Failed	8 h
Phosphoric Acid:Trifluoroacetic Acid			
2.56:3.59	1	Failed	8 h
2.21:2.87	1	Failed	8 h
PIH:Phosphoric Acid:*p*TSA			
1.56:18.69:1.87	1	Failed	8 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
PIH:Phosphoric Acid:Trichloroacetic Acid			
1.86:2.48:2.59	1	Failed	8 h
PIH:Phosphoric Acid:Trifluoroacetic Acid			
1.85:2.53:3.06	1	Failed	8 h
2.17:2.17:2.57	1	Failed	8 h
2.32:2.32:2.32	1	Failed	8 h
2.16:2.66:2.53	1	Failed	8 h
2.13:3.96:2.56	1	Failed	8 h
2.17:2.48:2.54	1	Failed	8 h
PIH:Trifluoroacetic Acid			
1.62:0.97	1	Failed	8 h
1.92:2.05	1	Failed	8 h
Phosphoric Acid:Ethyl Triflate			
3.46:1.07	1	Failed	5 h
3.35:3.33	1	Failed	5 h
Ethyl Triflate			
0.36	1	Failed	8 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
Ethyl Triflate			
0.71	1	Failed	8 h
6.07	1	Failed	8 h
11.39	1	Failed	8 h
Potassium t-butoxide			
2.98	1	Failed	24 h
**Pyridine:HFPA			
1.2:5.0	1	Failed	0.5 h
1.4:5.5	1	Failed	0.5 h
2.7:6.3	1	Failed	0.5 h
4.1:5.3	1	Failed	0.5 h
2.3:8.3	1	Failed	0.5 h
2.0:8.6	1	Failed	0.5 h
2.1:9.3	1	Failed	0.5 h
4.6:6.9	1	Failed	0.5 h
2.6:9.3	1	Failed	0.5 h
1.9:10.2	1	Failed	0.5 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
**Pyridine:HFPA			
2.0:10.5	1	Failed	0.5 h
2.3:11.1	1	Failed	0.5 h
Pyridine:AA:HFPA			
2.6:1.4:5.9	1	Failed	0.5 h
4.4:2.2:6.6	1	Failed	0.5 h
3.1:2.6:9.4	1	Failed	0.5 h
2.0:4.2:18	1	Failed	0.5 h
BHT:HFPA			
0.9:3.6	1	Failed/polym in cup	
4.6:2.3	1	Failed	0.5 h
5.7:2.8	1	Failed	0.5 h
5.8:4.8	1	Failed/polym in cup	
9.1:3.3	1	Failed	0.5 h
13.0:3.0	1	Failed	0.5 h
16.4:1.8	1	Failed	0.5 h
28.6:2.5	1	Failed	0.5 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
BHT:AA:HFPA			
2.3:2.3:4.0	1	Failed/polym in cup	
3.3:2.7:3.5	1	Failed	0.5 h
12-crown-4:HFPA			
2.6:4.6	1	Failed/polym in cup	
3.2:5.7	1	Failed	0.5 h
8.7:4.4	1	Failed	0.5 h
7.1:6.0	1	Failed	0.5 h
8.6:5.7	1	Failed/polym in cup	
18.3:3.9	1	Failed	0.5 h
12-crown-4:AA:HFPA			
4.4:6.1:8.4	1	Failed	0.5 h
triethyamine:HFPA			
0.4 : 4.1	1	Failed	0.5 h
3.6 : 1.9	1	Failed	0.5 h
1.0 : 5.3	1	Failed	0.5 h
1.4 : 9.4	1	Failed	0.5 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
triethyamine:HFPA			
1.7 : 9.2	1	Failed	0.5 h
3.1 : 12.2	1	Failed	0.5 h
7.8 : 8.6	1	Failed	0.5 h
triethyamine:AA:HFPA			
0.7:2.6:7.3	1	Failed	0.5 h
0.7:3.7:8.5	1	Failed	0.5 h
3.7:5.2:8.2	1	Failed	0.5 h
Acetonitrile:HFPA			
2.5 : 2.3	1	Failed	0.5 h
1.5 : 8.2	1	Failed	0.5 h
3.1 : 19.6	1	Failed	0.5 h
Acetonitrile:AA:HFPA			
1.6:4.3:2.3	1	Failed	0.5 h
1.7:7.7:1.8	1	Failed	0.5 h
1.6:2.3:9.3	1	Failed	0.5 h
1.4:8.9:5.0	1	Failed	0.5 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
Acetonitrile:AA:HFPA			
3.9:5.5:11.2	1	Failed	0.5 h
3.2:15.3:2.2	1	Failed	0.5 h
3.0:15.4:2.2	1	Failed	0.5 h
p-nitroaniline:HFPA			
1.5:6.2	1	Failed/polym in cup	
7.3:3.9	1	Failed	0.5 h
4.1:9.1	1	Failed	0.5 h
3.7:9.0	1	Failed/polym in cup	
5.4:17.8	1	Failed	0.5 h
2,6-dinitroaniline:HFPA			
4.7:2.3	1	Failed/polym in cup	
4.6:2.5	1	Failed/polym in cup	
15.1:3.4	1	Failed/polym in cup	
phenylenediamine:HFPA			
3.6:4.3	1	Failed	0.5 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
2,4-dinitroaniline:HFPA			
3.7:3.2	1	Failed/polym in cup	
Sulfanilamide:HFPA			
4.0:6.2	1	Failed/polym in cup	
2-aminopyridine:HFPA			
1.0:4.0	1	Failed	0.5 h
1.3:4.3	1	Failed	0.5 h
2.2:5.5	1	Failed	0.5 h
4.0:4.5	1	Failed	0.5 h
0.9:8.3	1	Failed	0.5 h
2.0:7.6	1	Failed	0.5 h
2.0:7.7	1	Failed	0.5 h
1.8:7.9	1	Failed	0.5 h
1.8:8.1	1	Failed	0.5 h
1.8:10.1	1	Failed	0.5 h
1.8:10.5	1	Failed	0.5 h
3.5:8.9	1	Failed	0.5 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
2-aminopyridine:HFPA			
1.9:10.8	1	Failed	0.5 h
3.4:10.5	1	Failed	0.5 h
3.4:10.6	1	Failed	0.5 h
1.8:14.8	1	Failed/polym in cup	
1.3:14.1	1	Failed/polym in cup	
2-aminopyridine:AA:HFPA			
4.66:1.37:8.53	1	Failed	4 h
3.97:2.92:5.02	1	Failed	4 h
2.58:1.88:7.39	1	Failed	4 h
3.43:2.17:8.12	1	Failed	4 h
3.89:1.72:8.35	1	Failed	4 h
4.30:1.36:9.16	1	Failed	4 h
4.38:1.57:9.33	1	Failed	4 h
4.04:2.02:9.21	1	Failed	4 h
4.01:1.52:9.26	1	Failed	4 h
3.98:1.55:11.11	1	Failed	4 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
2-aminopyridine:AA:HFPA			
3.46:2.11:9.80	1	Failed	4 h
3.49:2.26:10.20	1	Failed	4 h
3.47:2.09:9.81	1	Failed	4 h
3.46:2.08:9.83	1	Failed	4 h
3.46:2.25:9.84	1	Failed	4 h
3.33:2.12:9.79	1	Failed	4 h
3.44:2.13:10.35	1	Failed	4 h
3.43:2.51:10.39	1	Failed	4 h
3.45:2.10:10.16	1	Failed	4 h
3.43:2.14:10.01	1	Failed	4 h
3.46:2.26:10.36	1	Failed	4 h
3.45:2.27:10.18	1	Failed	4 h
3.67:2.20:10.55	1	Failed	4 h
3.42:2.25:10.71	1	Failed/gelled in cup	
3.46:2.26:10.54	1	Failed	4 h
3.45:2.23:10.69	1	Failed	4 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
2-aminopyridine:AA:HFPA			
3.47:2.29:10.15	1	Failed	4 h
3.47:2.33:10.70	1	Failed	4 h
3.62:2.36:6.36	1	Failed	4 h
3.47:2.07:10.38	1	Failed	4 h
3.41:2.12:10.53	1	Failed/gelled in cup	
3.41:2.18:10.35	1	Failed/gelled in cup	
3.33:2.07:12.72	1	Failed/gelled in cup	
3.28:2.15:13.05	1	Failed/polym in cup	
3.38:2.32:10.36	1	Failed	4 h
3.41:2.32:10.39	1	Failed	4 h
3.41:2.55:10.83	1	Failed	4 h
3.54:2.29:10.40	1	Passed/ gelled in cup	10 min
3.65:2.27:10.98	1	Failed/gelled in cup	
3.72:2.36:10.42	1	Failed/gelled in cup	
3.81:2.44:10.28	1	Failed	4 h
3.83:2.38:9.81	1	Failed	4 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
2-aminopyridine:AA:HFPA			
3.85:2.12:10.06	1	Failed	4 h
3.91:2.33:10.43	1	Failed/smoking gel	
3.97:2.39:10.14	1	Failed/gelled in cup	
4.01:2.37:9.92	1	Failed	4 h
4.36:2.19:10.12	1	Failed	4 h
4.30:2.44:9.86	1	Failed	4 h
4.59:1.95:9.83	1	Failed	4 h
3.40:2.51:11.07	1	Failed/polym in cup	
3.41:2.55:10.83	1	Failed	4 h
3.46:2.60:10.71	1	Failed	4 h
3.53:2.67:10.97	1	Failed	4 h
3.66:2.66:10.37	1	Failed	4 h
3.86:2.15:9.95	1	Failed	4 h
3.78:2.67:9.96	1	Failed	4 h
3.78:2.69:10.13	1	Failed	4 h
3.82:2.20:10.30	1	Failed	4 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
2-aminopyridine:AA:HFPA			
3.32:2.57:10.94	1	Failed	4 h
2.80:2.20:10.11	1	Failed	4 h
2.86:2.13:9.81	1	Failed	4 h
2.84:2.28:10.25	1	Failed	4 h
2.84:2.36:10.19	1	Failed	4 h
2.85:2.29:10.02	1	Failed	4 h
2.86:2.29:9.87	1	Failed	4 h
2.85:2.40:9.83	1	Failed	4 h
2.85:2.54:9.84	1	Failed	4 h
2.69:2.41:9.88	1	Failed	4 h
2.84:2.45:10.08	1	Failed	4 h
2.69:2.29:9.96	1	Failed	4 h
2.96:2.41:9.88	1	Failed	4 h
2.69:2.27:9.84	1	Failed	4 h
2.69:2.43:9.90	1	Failed	4 h
2.58:2.41:9.79	1	Failed	4 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
2-aminopyridine:AA:HFPA			
2.68:2.43:10.03	1	Failed	4 h
2.58:2.41:9.86	1	Failed	4 h
2.55:2.49:9.85	1	Failed	4 h
2.69:2.52:9.84	1	Failed	4 h
2.40:10.16	1	Failed/gelled in cup	
2.58:2.44:9.88	1	Failed	4 h
2.68:2.43:9.95	1	Failed/gelled in cup	
2.71:2.51:9.86	1	Failed/gelled in cup	
2.72:2.44:9.75	1	Failed/gelled in cup	
2.67:2.50:9.61	1	Failed/gelled in cup	
2.68:2.49:9.95	1	Failed/gelled in cup	
2.70:2.50:9.49	1	Failed/gelled in cup	
2.74:2.48:8.99	1	Failed/gelled in cup	
2.88:2.65:8.78	1	Failed/gelled in cup	
2.76:2.47:7.41	1	Failed	4 h
2.77:2.57:8.07	1	Failed	4 h

Cont.

Acid wt %	Runs	Gillmore Needle	Time
2-aminopyridine:AA:HFPA			
2.76:2.56:8.31	1	Failed	4 h
2.74:2.45:8.19	1	Failed	4 h
2.74:2.51:8.13	1	Failed	4 h
2.74:2.52:8.13	1	Failed	4 h

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Publications:

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Kilway, K. V.*, " Development of a novel bone cement" University of Missouri – Kansas City Bone Cement Symposium, Kansas City, MO, October 4, 2014. (My work was included in this presentation.)

Kilway, K. V.*, "Advancements in the development of a novel bone cement" University of Missouri – Kansas City Center of Excellence in Mineralized Tissues Seminar Series, Kansas City, MO, July 16, 2014. (My work was included in this presentation.)

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