Microencapsulation of Octylcyanoacrylate for Applications as a Healing Agent in a Self-healing Bone Cement

by

Alice Bradbury Welsh Brochu

Department of Biomedical Engineering

Duke University

Date: ______________________

Approved:

___________________________

William Reichert, Supervisor

___________________________

Gabriel Lopez

___________________________

Stephen Craig

Thesis submitted in partial fulfillment of
the requirements for the degree of Master of Science in the Department of
Biomedical Engineering in the Graduate School
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ABSTRACT

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Abstract

Total joint replacement (TJR) surgeries are performed on thousands of patients every year, yet these implants are subject to failure following prolonged exposure to the harsh environment of the body as well as the complex loading patterns seen in biological joints. The generation of wear debris from both the articulating surfaces and the poly(methyl methacrylate) (PMMA) bone cement used to anchor the replacements in place serves to accelerate wear and subsequent failure of the device. Self-healing approaches that employ an encapsulated healing agent embedded in a catalyst-containing matrix have been developed to restore mechanical function to materials that undergo crack damage; following capsule rupture, and healing agent release and polymerization serves to halt microcrack propagation. However, existing encapsulated systems do not adhere to biomaterials constraints. In this work, interfacial polymerization of polyurethane (PUR) in an oil-in-water emulsion was used to achieve encapsulation of octylcyanoacrylate (OCA), a medical grade adhesive used in sutureless surgeries. The optimized encapsulation procedure was determined by studying the effects of solvent, surfactant, and temperature on the final product. The average size and size distribution, capsule shell thickness, percent fill and reactivity of encapsulated agent, and shelf life of these capsules were studied and are now suitable for incorporation into PMMA and assessment as potential healing agent systems.
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1. Specific Aims

In order to introduce the concept of self-healing materials to the biomaterials field, several aims must be met.

1. Examine the current approaches of material self-healing and determine which method could be applied to an existing implant to design the first self-healing biomaterial. While numerous approaches to self-healing materials have been suggested, not all of these techniques are appropriate for biomaterials or employ materials that comply with biomaterials constraints. Our first aim will be to select the most feasible method for the initial attempts at engineering a self-healing biomaterial.

2. Assess the feasibility of our proposed material design and application. Once a suitable method of and application for self-healing in biomaterials has been identified, the feasibility of the method must be assessed. In this project specifically, the development of a reproducible and effective method of encapsulating OCA within PUR will be vital in meeting this aim. Various characterizations of the resulting capsules (diameter, shell thickness, content, content reactivity, process survivability, etc) will also be required to determine the feasibility of our concept.
2. Introduction

Biological materials such as bone, skin, and muscle, when healthy, undergo *in situ* self-healing through a cycle of consumption and regeneration that prevents the accumulation of defects due to tissue aging and fatigue. Healing and biomaterials are most commonly linked through the tissue response to the presence of an implant [1-3]. Ratner has coined the term “biomaterials that heal” to describe biomaterials that actively promote wound healing [4] as opposed to those aimed at passivity or inertness. While the biology and chemistry of healing have significant impacts on biomaterial performance, biological healing does not address the physical repair of biomaterials that experience mechanical and chemical breakdown as they are subjected to loading and degradation effects *in vivo*. Developing synthetic biomaterials with the intrinsic ability to autonomously repair mechanical and chemical damage would be particularly important for implants that replace tissues that are also capable of self-repair.

The *in situ* repair of synthetic materials for engineering applications first requires the ability to detect the damage — however, *in situ* detection and repair of biomaterials is particularly difficult due to the lack of adequate *in vivo* imaging techniques to detect failures and suitable minimally invasive methods to repair the damage. Overall, only substantially damaged or compromised biomaterials can be detected *in situ*, and if detected are generally retrieved and replaced [5-11].
The development of synthetic materials that autonomously repair in situ on the microscopic level before suffering macroscopic failures would significantly extend the lifetime of a given structure or device. This is precisely the motivation behind the newly emerging class of “self-healing materials” that are endowed with the intrinsic ability of self-repair in response to damage arising from physical and chemical stresses within its use environment [12-17]. Generally speaking, self-healing materials are designed to sense, halt, and even reverse damage, ideally without requiring the application of external physical or chemical stimuli. These materials hold the potential for significantly extending material lifetimes by avoiding failures initiated by accumulated microcracks. To date, the majority of the research conducted on self-healing bulk materials has employed composites, adhesives, and cements intended for traditional engineering applications [12-18].

Orthopedic, dental, and cardiovascular implants that undergo cyclic loading patterns are the primary candidates for improvement via self-healing techniques. Due to its long history of usage, extensive characterization, lack of post-polymerization machining and modifications, and the need for improvement [8, 11, 19, 20], PMMA bone cement was selected as the initial application of self-healing to biomaterials. Due to their reactivity with water and anions present in the body, medical grade cyanoacrylates were selected as a potential biocompatible healing agent. These materials currently have
applications in sutureless surgeries [21] but could also be applied to self-healing materials.

Here we describe the initial research performed to encapsulate OCA within a PUR shell for use in a matrix of PMMA bone cement. We begin with descriptions of the various failure modes of commercially-available bone cement followed by a summary of the self-healing methods that have been developed and the successes seen with such methods. The evolution of the encapsulation procedure as well as the influence of agitation rate, surfactant, and temperature are investigated and discussed, the initial reactivity and percent fill of the capsules are assessed, and future directions presented.
3. Background

3.1 Bone cement is commonly used to stabilize total joint replacements

3.1.1 Total joint replacements are a common implant procedure

While these implants are well-designed there are still a variety of failure modes that jeopardize the overall success of the replacements. In 2006 alone in the United States over 750,000 THR and TKR procedures were performed [22]. Based on the aging population and overall average increase in weight of the general public, future demand for primary hip replacement procedures is expected to increase by 171% by 2030 [22, 23], representing a significant number of potential beneficiaries of any steps taken to improve current joint replacements.

PMMA bone cement is a space-filling matrix that forms mechanical interlocks between the stem of the implant and the surrounding boney tissue [24, 25]. Bone cement consists of two components: low molecular weight PMMA powder plus an initiator (e.g. benzoyl peroxide), and liquid MMA monomer. Mixing the two components forms a slurry that initiates polymerization yielding a workable dough that is applied to the implant, which hardens into a solid mass after the implant is inserted into the boney tissue [24].
Table 1: Commercial bone cement composition

<table>
<thead>
<tr>
<th>Powder component</th>
<th>Liquid component</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMMA (67%)</td>
<td>MMA (98%)</td>
</tr>
<tr>
<td>PMMA/PS copolymer (21%)</td>
<td>N,N-dimethyl-p-toluidine (≤2%)</td>
</tr>
<tr>
<td>Benzoyl peroxide (2%)</td>
<td>Hydroquinone (75 ppm)</td>
</tr>
<tr>
<td>Barium sulfate (10%)</td>
<td></td>
</tr>
</tbody>
</table>

Cemented total joint replacements are commonly used to provide superior long-term survival of the implant through bone integration and by minimizing stress shielding but lose effectiveness following wear and microcracking [6, 7]. Minimizing the generation of wear particles associated with cemented joint replacements through development of a first generation self-healing bone cement would contribute significantly to the extension of the implant lifetime and improve patient quality of life.

3.1.2 Joint replacements fail following accumulation of microdamage sustained throughout years of cyclic usage

3.1.2.1 Crazing and crack formation

Microscopic examination of cyclically loaded polymeric implants often reveals the presence of crazed microcracks characterized by a network of fibrils that span the crack edges [26]. Crazes and microcracks form to relieve internal or external stresses in areas of high stress concentration, such as along a pre-existing crack, at the surface of the polymer, or at a void within the polymer. A crack will propagate if the stress on the system is reduced by its growth. As propagation occurs, the fibrils joining the bulk surfaces of the polymer can either behave in a ductile manner by recruiting fresh
material from the edge of the bulk surface to maintain the fibril connections, or the fibrils can behave in a brittle manner and proceed to fracture.

3.1.2.2 Wear and wear particle formation

Wear is the loss of bulk material through adhesion, abrasion, erosion, fretting, and/or fatigue [27, 28]. Wear damage in biomaterials is common to the articulating surfaces in TJRs and is regarded as the primary failure mode that influences the long-term performance of such implants [8, 19, 20, 26-29]. Particles that break off from the bulk material during wear comprise both mass loss from the original implant as well as debris that serves as added abrasion. Attempts by the immune system to consume and remove these wear particles often leads to a state of a chronic inflammation. It has also been observed that wear debris cleared from the joint space by lymph or blood flow collects in the lymph nodes and other organs of the immune system [28, 30, 31].

The current gold standard for articulating surface applications is UHMWPE [19]. With a molecular weight between 2 and 6 million, UHMWPE imparts toughness through crystallinity between 39 and 75% and the extensive intertwining of the extremely long molecular chains that interlock the crystalline domains, restricting motion to the amorphous polymer regions [11]. The capacity of UHMWPE to resist cyclic fatigue can be augmented by direct compression molding and hot isostatic pressing to give better fatigue resistance and smooth surface finish than that afforded by extrusion techniques [5, 11, 32, 33]. However, it is currently estimated that for each day
of patient activity approximately 100 million microscopic UHMWPE wear particles are released into the joint space [22], representing significant mass loss and sources of abrasion and chronic inflammation.

The currently available alternatives to UHMWPE in TJRs are the limited use of alumina and zirconium as the articulating surfaces [34]. While these ceramics demonstrate excellent strength, stiffness, and lubricity, they have poor toughness and resistance to crack propagation. Attempts have been made to improve the surface properties of metals through the application of diamond-like, titanium nitride, and chromium nitride coatings, but poor adhesion of these coatings to the metal substrate also results in debris that compromises the implant [31].

3.2 Several approaches to self-healing have been proposed and investigated

If traditional composite materials that merely retard failure may be considered zeroth generation self-healing materials, then taxonomically-speaking, first generation self-healing materials may be used to describe approaches that “halt” and “fill” damage, whereas second generation self-healing materials describe those that strive to “fully restore” the pre-failed material structure.

3.2.1 Zeroth generation self-healing materials: composites that retard but do not repair mechanical damage

Composite materials are attractive because they can be designed to improve material stiffness and strength by dispersing stiffer or stronger particulates or fibers into
the softer polymer matrix [26, 33]. By employing materials with different mechanical properties for the matrix and additive phases, the overall properties of the composite will reflect the most desirable traits of each material. For example, glass fibers added to an epoxy matrix forms an intermediately stiff and strong, but more compliant, fiber-glass. Dispersed particulates and fibers of composite structures can also increase the toughness, impact strength, and wear resistance of the base matrix by absorbing a greater fraction of the load, by inhibiting pathways for crack propagation, and resisting void formation. Properly designed composite materials thus have the capacity to retard mechanical failure but do not have the ability to repair damage. As such, traditional composites behave like a “stuck zipper” that is hard to un-zip, but also one that cannot be re-zipped. This capacity gives traditional composite materials a “zeroth-order” self-healing status.

Huang and Ramakrishna provide an excellent and comprehensive review of biomedical composite materials [35]. In spite of a long history of development in load-bearing applications, only a few of these composite devices have progressed to widespread clinical use [35]. The lack of success with composite biomaterials may be attributed to the deleterious effects of placing the material in an environment that accelerates water absorption and compromises adhesion between the dispersed and matrix phases; i.e. a 37\(^\circ\)C, cyclically loaded, aqueous, high-salt polyelectrolytic environment.
Selecting a chemically inert nonpolar matrix polymer has the advantage of resisting water absorption, but it also increases the difficulty of finding a dispersed phase that bonds well with the matrix material. Strong interface bonding is necessary for efficient transferring of loads between the two phases and the prevention of voids or crack formation at the interfaces, which can serve as stress concentrators that initiate crack propagation [26]. Finding suitable materials to disperse in nonpolar and chemically inert UHMWPE thus has proven to be problematic. For example, carbon fiber-reinforced UHMWPE has shown poor results clinically because the carbon fibers did not bond well with the UHMWPE matrix, serving as stress concentrators and sources of crazing [33]. Consequently, it has been reported that fatigue cracks propagate up to eight times faster in carbon-reinforced UHMWPE than in pure UHMWPE [33].

Self-reinforcing materials are a special class of composites consisting of a matrix and reinforcing additive made from the same material [36]. Ideally, the compatibility between dispersed and matrix phases made of the same material will allow for composite strengthening through improved interface bonding. This approach is also attractive because cytotoxic adhesion promoters such as silanes are not required [29]. It may also be more cost-effective and practical because only the properties of a single material must be considered. Self-reinforced rods, plates, pins, screws, and tacks have all been produced from self-reinforced PLA, PGA, and their copolymers and are most commonly used as osteofixation devices in craniomaxillofacial implants [29, 35]. These
self-reinforcing screws have successfully been used for fixation of mandibular fractures [36]. Perhaps the commercially available bone cements could also be considered self-reinforced materials because PMMA powder is used to reinforce the newly polymerized MMA monomer.

### 3.2.2 First generation self-healing materials: composites that irreversibly repair but do not restore damaged matrix

An emerging area of composite research is the development of dispersed components that respond to the presence of damage by releasing a healing agent. This “first generation” of self-healing materials employs the process of matrix repolymerization to replace the damaged original material with a cured replacement. Here, matrix repolymerization is analogous to gluing together the two sides of an open zipper, which will close the zipper but is irreversible and does not restore its original zipped structure.

Kessler and Murphy both provide excellent reviews and in-depth discussions of matrix repolymerization self-healing systems that have been investigated in recent years [12, 37]. Two common matrix repolymerization systems employed to date rely on a ruthenium-based Grubbs’ catalyst to induce ROMP of a DCPD monomer [12-14] or various reactive epoxy resins that induce curing of a bisphenol-A epoxy matrix [15]. Microencapsulated PDMS self-healing systems have also been investigated [38, 39]. While many groups have successfully utilized these systems, the following is meant to discuss illustrative examples of the feasibility of the approach.
Figure 1 illustrates the matrix repolymerization method utilizing microencapsulated healing agent dispersed in a catalyst-embedded polymer matrix. When a propagating crack encounters a microcapsule, it causes the capsule shell to rupture, releasing the healing agent into the crack plane via capillary action or crack closure following unloading. Crack formation also exposes embedded catalyst that initiates curing of the healing agent released from the capsules into the crack area. The reaction binds the surfaces of the crack together, halting its progression through the material [12-14, 38, 40-42]. The reverse case of microencapsulated catalyst and phase-separated healing agent has also been explored [38, 39].

Figure 1: Encapsulation of a reactive healing agent can be used to achieve self-healing in materials ([43], adapted from [14])
Healing agent-filled hollow cylinders and branched network structures also have been employed to increase the efficiency of healing agent delivery to the defect area. Bleay et al. investigated a variety of small diameter hollow glass fibers that were capable of simultaneously storing healing agent and providing structural reinforcement to an epoxy matrix. However, this group encountered problems with release of healing agent from smaller diameter fibers [12]. Toohey et al. and Lee et al. have proposed three-dimensional networks capable of autonomously healing following repeated damage [16, 17] but in both approaches the vascular network must be replenished with monomer healing agent between each damage event. Regardless of whether one employs microcapsules, hollow fibers, or network structures, the encapsulating vessel must be large enough to release a sufficient amount of healing agent to repair the defect but small enough to not negatively impact matrix material mechanical properties.

White et al. were able to successfully encapsulate DCPD within a polymer matrix containing Grubbs’ catalyst. Following the formation of microcracks and subsequent ROMP reaction, this group demonstrated up to 75% recovery of the virgin fracture toughness following 48 hours of healing time [14]. Healing efficiency was assessed by comparing the critical loads at fracture of healed and virgin specimens following TDCB testing. A more detailed description of TDCB testing can be found in various references, including [13, 37, 44]. This group also found that the average critical load for virgin self-healing matrices containing DCPD and Grubbs’ catalyst was 20% higher than the control
group containing no microspheres or embedded catalyst, indicating improved matrix
toughness through the inhibition of craze formation and subsequent crack propagation.

Andersson et al. described the self-healing capabilities of PDMS with UF
microcapsules following tear testing [38]. These investigators showed that the samples
were able to consistently recover more than 70% of the original tear strength of the
polymer. In research conducted by Blaiszik et al., UF microcapsules containing epoxy
resins (Epon 828 and 862) and solvents for use in self-healing applications were
successfully engineered [15]. These microcapsules were shown to satisfy several
desirable requirements for self-healing materials, including processing survivability,
thermal stability, and efficient in situ rupture for the delivery of the encapsulated
healing agent. Table 2 summarizes the catalyst and matrix materials previously used
with repolymerization self-healing techniques.
<table>
<thead>
<tr>
<th>Matrix Material</th>
<th>Healing Agent</th>
<th>Catalyst</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vinyl ester</td>
<td>HOPDMS and PDES (phase separated in matrix)</td>
<td>DBTL in PUR microcapsules</td>
<td>[38, 39]</td>
</tr>
<tr>
<td>Epon 828 cured with DETA</td>
<td>DCPD in UF capsules</td>
<td>Grubbs’ catalyst in paraffin wax microspheres</td>
<td>[45, 46]</td>
</tr>
<tr>
<td>PMMA</td>
<td>DCPD in UF capsules</td>
<td>Grubbs’ catalyst</td>
<td>[47]</td>
</tr>
<tr>
<td>Epon 828 cured with DETA</td>
<td>Epon 862 or Epon 828 diluted with cholorobenzene, phenylacetate, ethyl phenylacetate in UF capsules</td>
<td>None</td>
<td>[15]</td>
</tr>
<tr>
<td>Epon 828 cured with DETA</td>
<td>DCPD in UF capsules</td>
<td>Grubbs’ catalyst</td>
<td>[12-14, 48, 49]</td>
</tr>
<tr>
<td>YD-115 cured with KH-816</td>
<td>DCPD in UF capsules</td>
<td>Grubbs’ catalyst</td>
<td>[48, 49]</td>
</tr>
<tr>
<td>YD-115 cured with KH-816</td>
<td>ENB in UF capsules</td>
<td>Grubbs’ catalyst</td>
<td>[48, 49]</td>
</tr>
</tbody>
</table>

With any self-healing system, the healing process must occur on the same time scale as the event that initiates and propagates the damage. Unfortunately, the mechanics of healing agent release into the crack region and the kinetics of healing agent curing occurs more slowly than the cyclic loading frequencies that are experienced in many industrial and biomedical settings. With current systems the incorporation of mechanically stable curing periods is essential to achieve adequate healing [38]. Clearly, incorporating curing periods is not feasible with many biomedical applications of constant or semi-constant cyclic loading, such as in orthopedic, dental, and
cardiovascular applications proposed as candidates for self-healing applications. As a result, the competition between crack propagation and self-repair represents a recurring and significant challenge for extension into biomaterials.

There are also a number of widely recognized, general technical challenges associated with implementing first generation self-healing materials in engineering applications. These include identifying catalyst and resin systems that maintain chemical reactivity while encapsulated, can be deployed in response to the presence of microcracks, will infuse adequately into these cracks upon release, will progress to a fully cured product, and will form stable chemical bonds within the matrix material. While promising results have been demonstrated, effective self-healing materials require careful optimization of the physio-chemical characteristics of the catalyst, the healing agent, the encapsulation/release vehicle materials, and the matrix. Clearly, full optimization is application-specific; however, this field is still very new and the published work to date has primarily focused on characterizing self-healing model systems rather than designing materials for specific applications.

The only self-healing material of this design nearing commercial application is not polymer-based, but a self-healing concrete being developed at the University of Michigan [18, 50]. This self-healing concrete is designed to bend under tensile strain via the formation of tiny microcracks while, alternatively, existing concrete forms large cracks under the same forces. The mixture contains dispersed depots of dry cement...
within the concrete matrix that are exposed to water and carbon dioxide as cracks form. The reaction of these components forms a calcium carbonate “scar” that fills the defect and halts crack propagation. It was demonstrated that self-healing concrete is capable of withstanding strains up to 5% (compared to 0.01% tensile strain that causes failure of standard concrete) and will recover most, if not all, of its mechanical strength after deformation [18].

While first generation systems are conceptually straightforward and have received the greatest attention for engineering applications to date, there still remain a number of concerns that have limited their more widespread usage (Table 3). Clever solutions for many of these challenges have been demonstrated for non-biological, ex vivo materials, but the constraints are much more severe for in vivo applications.

Table 3: Technical concerns associated with first self-healing generation approaches

<table>
<thead>
<tr>
<th>Technical concern</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healing agent/catalyst consumption</td>
<td>[12, 17]</td>
</tr>
<tr>
<td>Healing agent stability in microcapsules</td>
<td>[13-15]</td>
</tr>
<tr>
<td>Microcapsule shell process survivability</td>
<td>[14, 15]</td>
</tr>
<tr>
<td>Release of healing agent from capsule</td>
<td>[14, 40]</td>
</tr>
<tr>
<td>Capsule/matrix interface bonding</td>
<td>[14, 15, 40]</td>
</tr>
<tr>
<td>Healing agent viscosity and volatility</td>
<td>[15, 48, 49]</td>
</tr>
<tr>
<td>Healing agent must infiltrate crack plane quickly but not diffuse away from area too quickly</td>
<td>[48]</td>
</tr>
<tr>
<td>Uneven healing agent/catalyst distribution or ratios in matrix</td>
<td>[13, 17, 45, 51, 52]</td>
</tr>
<tr>
<td>May require excessive healing times*</td>
<td>[13, 38, 43]</td>
</tr>
<tr>
<td>Complex loading patterns limit healing opportunities*</td>
<td>[43]</td>
</tr>
<tr>
<td>Functional lifetime of encapsulated agent*</td>
<td>[43]</td>
</tr>
<tr>
<td>Toxicity of healing agent/catalyst system*</td>
<td>[43]</td>
</tr>
</tbody>
</table>

* indicates concerns specific to biomaterials
3.2.3 Second generation self-healing materials: materials that reversibly restore damaged matrix

A “second generation” self-healing material, as defined here, reversibly restores a damaged material to its original, undamaged state. This is analogous to the act of opening (introducing damage) and then closing (initiating repair) a zipper. Except in the melt, reversible repair of most polymers is kinetically inaccessible because the energy barrier to molecular rearrangement is high and the molecular dynamics are slow. Consequently, all second generation self-healing polymer systems currently under investigation concern either the chemistry of weak bonds that are reversible at low temperature or techniques that input the energy necessary for molecular rearrangement. Regardless of temperature and absolute time scale, however, the two approaches are united in that local reversibility (i.e. bond reforming reactions) must be significantly faster than global processes (e.g. polymer flow and macroscopic deformation).

3.2.3.1 Second generation self-healing materials based on shape restoration

Second generation strategies for self-healing materials might involve hybrid architectures that possess both reversible and irreversible interactions. Upon application of a force, the ensemble of weak reversible interactions preferentially yields (providing deformability), while the stronger irreversible interactions remain intact (providing scaffolding). Ideally, when the force is removed, the scaffold of irreversible interactions guides the reassembly of the reversible interactions, restoring the material to its original undeformed state (Figure 2). Shape restoration materials therefore differ from “shape
memory materials” because they are driven by end group associations rather than by an energy-consuming transition between two states.

The molecular basis for this strategy finds its roots in dynamic covalent chemistry, which comprises the reversible making and breaking of covalent bonds, and supramolecular chemistry, which imparts the capability of self-assembly or self-organization using highly directional and reversible non-covalent interactions that dictate the overall mechanical properties of a material [53]. The dynamic dissociation and reassociation of stress-bearing bonds allow for rapid conformational changes that affect the properties of shape restoration materials [54, 55]. These properties depend on
the characteristics of the association, the overall flexibility of the molecule, and the environment of the system [55]. Current dynamic covalent methods comprise a wide range of well-known reaction types [56], and retro Diels-Alder reactions [41], disulfide exchange reactions [57, 58], and hydrazone linkages [59] are all potentially compatible with biological environments. Reversible supramolecular interactions in polymers are generally achieved through numerous mechanisms [54, 60, 61], including: hydrogen bonding, exemplified by the ureidopyrimidinone unit of Sijbesma and Meijer [60]; metal coordination chemistry, for example silver complexation by tridentate heterocyclic peptides [62] or calcium-bridged carboxylates [63]; or π-stacking, such as that seen with triphenylene motifs [64]. Other synthetic supramolecular motifs are known to function in biological environments, but an even greater repository of useful structures is likely found in biology itself; for example, peptide-peptide, ligand-receptor, and nucleic acid-based interactions [65-70].

3.2.3.2 Second generation self-healing materials based on application of heat or light

A somewhat exotic but nonetheless interesting example of heat-induced defect repair is polymer flow caused by projectile puncture. Research conducted at the NASA Langley Research Center described self-healing characteristics of EMAA, Affinity EG 8200, and PB-g-PMA-co-PAN that utilize the heat produced by a penetrating projectile (e.g. a bullet) to induce localized melting and rapid reassociation of polymer chains, inducing repair [12, 71, 72]. While such materials may have military and space
exploration applications, this approach does not seem well-suited for biomaterials applications [73]. Interested readers are encouraged to view a short NASA Real World Mathematics eClip, accessed online on May 19, 2010, at:


In contrast to repair by localized polymer flow are self-healing systems that repair defects by reforming bonds upon the application of heat, radiation, or electric fields. As with the shape restoration strategies in the preceding section, a primary challenge is to identify chemistries in which localized bond forming takes place on time scales that are much shorter than both material flow/deformation and crack propagation. One demonstrated example of such chemistry is the Diels-Alder reaction, in which a diene and a dienophile undergo cycloaddition to form the Diels-Alder adduct that can dissociate at high temperatures via the retro-Diels-Alder reaction [74]. As such, polymers containing Diels-Alder chemistries have reversible covalent bonds that can be formed and broken by thermal cycling. These polymers are particularly useful because they minimize free radical formation that could lead to undesirable chain reactions resulting in improper structural recovery [75]. Chen et al. were able to design a matrix able to repeatedly heal under mild conditions (i.e. 75°C for 3 hours) without requiring a catalyst, additional monomer, or special surface treatments of the fracture plane [76, 77]. This group found that the energy needed to break the Diels-Alder adducts was much lower than the energy required to break other covalent bonds, demonstrating the
preferential failure of these interactions. Recovery of approximately 57% of the original fracture load was reported. The group proposed using this material for electronic packaging applications where cracking occurs due to differences in the thermal index of expansion [76].

Repair by the application of light is also of considerable interest. The Urban research group has developed a self-healing material consisting of oxetane-substituted chitosan precursors incorporated into polyurethane networks [78]. These systems use ultraviolet light to recombine free radicals to form crosslinks and have been found to repair surface scratch damage in less than one hour. These materials have been proposed for use in automotive coatings that are both damaged and exposed to ultraviolet light during common usage. This system also may be employed regardless of ambient temperature or humidity, further enabling its widespread use. Unfortunately, development of self-healing biomaterials based on energy-dependent mechanisms will be limited to surface-accessible environments such as cutaneous repair unless it can be tied to a deeply-penetrating heat or radiation source.

In comparison to first generation self-healing materials, the second generation approach is more compatible with “soft”, low-modulus material applications. In addition, there exists the possibility of pseudo-infinite cycling and potential advantages that come with a single-component (as opposed to composite) system. Nevertheless, the
utility of second generation materials currently lags that of first generation materials, and a number of significant challenges exist (Table 4).

### Table 4: Technical concerns associated with second generation approaches

<table>
<thead>
<tr>
<th>Technical concern</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity of materials*</td>
<td>[43]</td>
</tr>
<tr>
<td>Diffusion of reversible crosslink materials out of scaffold*</td>
<td>[43]</td>
</tr>
<tr>
<td>Lack structural properties to sustain significant loads</td>
<td>[75, 79]</td>
</tr>
<tr>
<td>Inadvertent triggering of reversibility mechanism</td>
<td>[43]</td>
</tr>
<tr>
<td>Formation of free radicals</td>
<td>[76, 77]</td>
</tr>
<tr>
<td>Potential for improper structural recovery</td>
<td>[43]</td>
</tr>
<tr>
<td>Design complexity</td>
<td>[43]</td>
</tr>
<tr>
<td>Requires external application of forces</td>
<td>[12]</td>
</tr>
<tr>
<td>Polymer remains in failed state indefinitely</td>
<td>[43]</td>
</tr>
<tr>
<td>No autonomous method to recognize damage</td>
<td>[12]</td>
</tr>
<tr>
<td>Slow time scale of healing</td>
<td>[76]</td>
</tr>
<tr>
<td>Accessibility of implant to employ techniques that require energy input*</td>
<td>[43]</td>
</tr>
</tbody>
</table>

* indicates concerns specific to biomaterials

### 3.3 Introduction of self-healing approaches to biomaterials

While second generation fully-reversible approaches may be more elegant and ultimately yield better long term-solutions, the first generation approach is most feasible for immediate extension into biomaterials due to the improved mechanical properties of such materials. Arguably, self-healing bone cement represents the simplest and most
straightforward mechanically loaded first generation self-healing biomaterial to design and test.

Following the matrix repolymerization paradigm outlined previously (Figure 1), self-healing bone cement would consist of the PMMA matrix plus an embedded catalyst and a dispersed microencapsulated healing agent. Figure 3 illustrates how one might incorporate a self-healing capability into the two-component bone cement; e.g., incorporating encapsulated healing and its catalyst into the powder component. Mixing the two components polymerizes the PMMA and distributes the encapsulated healing agent and healing agent catalyst throughout the resulting PMMA matrix. Because the cement is mixed in the operating room and applied directly to the implant there are also no post-polymerization manufacturing or machining processes that must be followed to produce implants of specific sizes and geometries, further simplifying material design.
Apply to implant

Figure 3: Potential method of incorporation of capsules into PMMA bone cement [43]

The only report to date of developing a self-healing biomaterial of any sort came from Biggs et al. [47] who incorporated the matrix repolymerization formulation of White et al. [14] directly into a commercial two-component PMMA bone cement. The Biggs study consisted of encapsulating DCPD monomer in UF microcapsules [80], and blending the microcapsules and organo-metallic Grubbs’ catalyst with the PMMA powder component. Mechanical testing of the cured cement was used to demonstrate that the self-healing material formulation increased fracture crack resistance by a substantial 4-8 fold compared to the unmodified formulation. Clearly, Grubbs’ catalyst was efficient in catalyzing ROMP of DCPD, and thus effective in sealing microcracks in PMMA matrix; however, DCPD and Grubbs’ catalyst are mildly and acutely toxic,
respectively [81, 82]. UF may also be a problem due to the potential for formaldehyde leaching. These toxicity considerations would render the formulation of White et al. undesirable for biomaterials applications. That said, the prospect of self-healing bone cements remains a straightforward and attractive biomaterial prospect, but only if an appropriately non-toxic formulation can be identified.

3.4 Candidate materials

For the purposes of this research there is no need to formulate a new bone cement matrix material and the currently used PMMA material will make an excellent first candidate matrix material for self-healing in biomaterials.

By selecting OCA as the encapsulated healing agent, the overall matrix design outlined in Figure 1 is now simplified to a matrix containing solely encapsulated healing agent. OCA, a medical-grade cyanoacrylate used in as a tissue adhesive in sutureless surgeries, has never been used as a healing agent in self-healing materials but possesses many of the attributes favored for biocompatible healing agents (including low cytotoxicity and desirable mechanical properties) [21]. An embedded catalyst for the healing agent is no longer required because polymerization of the released OCA will be triggered by the presence of bodily fluids that will bathe the bone cement.

3.5 Emulsions

Previous papers by self-healing materials groups utilize various types of emulsions to produce microcapsules although other methods of encapsulation, such as
air suspension, multi-orifice centrifugation pan coating, electrostatic atomization, spray
drying and congealing, and solvent evaporation, also exist [83, 84]. Here we describe
emulsions in further detail to provide an introduction to the methods employed in this
work.

3.5.1 Types of emulsions

Emulsions are heterogeneous systems containing at least one immiscible liquid
phase dispersed within another; the dispersed phase takes the form of droplets with
diameters as small as hundreds of nanometers [85]. In these emulsions, one phase, the
discontinuous phase, is referred to as the disperse phase and the bulk phase in which
the droplets are dispersed is called the continuous phase. In general, the components of
the emulsions are an oily or organic phase and an aqueous phase; depending on which
phase is the disperse phase in these systems they are referred to as oil-in-water and
water-in-oil emulsions. Various microemulsions (oil-in-water-in-oil, water-in-oil-in-
water, etc) have also been reported and have gained importance in fields such as
controlled release and drug delivery [86-90] (Figure 4).
Figure 4: Numerous types of core-shell structures exist and are utilized for varying applications, including controlled release and drug delivery.

The term “interfacial polymerization” is used to describe the formation of the capsule shell at the oil and water interface. As the immiscible phases are agitated, a chain extender is added to the solution and accumulates at the interface between the oil and water phases where it is available to trigger polymerization of the shell material dissolved within the organic phase [83, 91]. The formation of a shell gives the permanent sequestering of one phase from the other, resulting in a population of capsules within the continuous phase.

Many factors contribute to the formation of a stable emulsion including the ratio of the volumes of the immiscible phases, the temperature, pH, mode of addition of one phase to the other, and perhaps most importantly, the type and amount of surfactant selected [92, 93]. These variables can be classified into three categories: (1) formulation variables that are associated with the equilibrium state of the system and include the temperature and pressure of the system, (2) composition variables that deal with the
quantities, proportions, and ratios of the various emulsion components, and (3) emulsification protocol characteristics such as the way the emulsion is made and modified or how the formulation and composition variables are changed as a function of time or space [94].

3.5.2 Role of surfactants

Surfactants, or emulsifiers, are amphiphilic (containing both water- and oil-loving regions) molecules that generally fit into one of several categories. An emulsifier promotes the formation of an emulsion, makes the emulsion easier to prepare, controls the resulting particle size, stabilizes the emulsion, and controls the type of emulsion that is formed [92, 94]. The main four types of surfactants and emulsifiers are (1) anionic, (2) cationic, (3) non-ionic, and (4) amphoteric. Between the two immiscible phases of an emulsion there exists a large interface that surrounds the dispersed phase. Emulsifiers adsorb at this interface, reducing the interfacial tension and promoting stability. As the amount of surfactant is increased, the total amount of surface area of the interface between the oil and water phases can also be increased because there is enough surfactant available to stabilize the increased interface area. This increase will result in the formation of smaller particles within the emulsion (Figure 5). It has been suggested that various stabilizers lead to preferential curvature of the interphase between the two immiscible phases and would also determine the emulsion type formed [95].
The properties of the surfactant, namely its hydrophile-lipophile balance (HLB), are crucial in determining the properties of the resulting emulsion. The HLB is a measure of the hydrophilicity of an emulsifier and can be used to predict its behavior in an emulsion. Generally speaking, the HLB is the balance of the size and strength of the opposing hydrophilic and lipophilic groups within the surfactant molecules [92, 93, 96]. Because every oil phase emulsifies optimally at a specific HLB, knowing this property allows one to considerably narrow down the field of available surfactants to select one ideal for the desired application. Depending on the type of application, the HLB number of the surfactant is an excellent characteristic to use to select the appropriate surfactant (Table 5, reproduced from [92, 95]). However, these values were determined for non-ionic surfactants and ranges may vary throughout the different classes of surfactants (anionic, nonionic, amphoteric, etc); many new artificial surfactants with unique behaviors have been introduced since this time and may not strictly adhere to
these guidelines. Table 6 includes the HLB for common surfactants, including those studied throughout the course of this research.

### Table 5: HLB Ranges and Applications

<table>
<thead>
<tr>
<th>Range of HLB Values</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-6</td>
<td>W/O emulsifier</td>
</tr>
<tr>
<td>7-9</td>
<td>Wetting agents</td>
</tr>
<tr>
<td>8-18</td>
<td>O/W emulsifier</td>
</tr>
<tr>
<td>13-15</td>
<td>Detergent</td>
</tr>
<tr>
<td>15-18</td>
<td>Solubilizer</td>
</tr>
</tbody>
</table>

### Table 6: Common Surfactants and Their HLB Values

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Surfactant Type</th>
<th>HLB Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tween 65</td>
<td>Non-ionic</td>
<td>10.5</td>
<td>[85, 93]</td>
</tr>
<tr>
<td>Gum arabic</td>
<td>Non-ionic</td>
<td>10-12</td>
<td>[96, 97]</td>
</tr>
<tr>
<td>Brij 52</td>
<td>Non-ionic</td>
<td>12.9</td>
<td>[85, 93]</td>
</tr>
<tr>
<td>Tween 20</td>
<td>Non-ionic</td>
<td>16.7</td>
<td>[85, 93]</td>
</tr>
<tr>
<td>Polyoxyethylene 40 stearate (Myrj 52)</td>
<td>Non-ionic</td>
<td>16.9</td>
<td>[85, 93]</td>
</tr>
<tr>
<td>Pluronic F-68</td>
<td>Non-ionic</td>
<td>29</td>
<td>[85, 93]</td>
</tr>
</tbody>
</table>

The free energy of an emulsion can be reduced by shrinking the surface area between the two phases; this is achieved through the coalescence of the drops [94]. The prevention of this coalescence is paramount to the successful formation of a stable interface at which interfacial polymerization of the capsule material can occur. Surfactant selection greatly influences the amount of free energy of the system, controlling the continued separation of the two phases, enabling encapsulation reactions to occur.
4. Methods

4.1 Preparation of polyurethane prepolymer

PUR was selected as a potential capsule material for this application due to its long history of use in biomaterials as well as its successful application for the encapsulation of water-reactive isophorone diisocyanate (IPDI) by Yang et al. for potential self-healing applications [98-100]. Briefly, a toluene diisocyanate (TDI) prepolymer was prepared for use in the microencapsulation procedure. Toluene 2,4-diisocyanate (109.25 g) was dissolved in cyclohexanone (750 mL) in a 3-necked round bottom flask. The mixture was suspended in an oil bath at 80 °C and agitated with a magnetic stirrer. 1,4-butanediol (BD, 20.315 mL) was slowly added to the stirring TDI/cyclohexanone solution at a rate of 2mL/min and then the flask was purged with N₂ and allowed to react for 24 h. Following this reaction time, the mixture was distilled at 100 °C under vacuum for 4-5 h to remove the excess cyclohexanone, water, and TDI from the system. A viscous yellow polyurethane prepolymer remained in the flask following the completion of this process. Gas permeation chromatography (GPC) was used to determine the number- and weight-average molecular weights (Mₙ and Mₙ) respectively, and therefore the polydispersity index (PDI) of the prepolymer. Mₙ was found to be 2489 and Mₙ was 3639 giving a PDI of 1.462. Although these molecular
weights are higher than those reported by Yang et al., our PDI value was comparable to their reported value of 1.33 [98].

4.2 Encapsulation of OCA

4.2.1 Acetone as the dispersed phase

At room temperature, gum arabic surfactant (11.25 g) was dissolved in deionized water (75 mL) in a 250 mL beaker. The solution was agitated for 1 h with a digital mixer (VWR PowerMax Elite Dual-Speed Mixer) before beginning the encapsulation procedure. The aqueous phase was suspended in a hot water bath at 50 °C just before the organic phase was added to begin the encapsulation process.

To prepare the organic solution, the prepolymer (3 g) was dissolved in acetone (5 mL) at room temperature. OCA (1 mL) was dissolved in a volume of acetone (10 mL) separate from the PUR solution. Just before addition to the aqueous phase, the OCA and PUR solutions were mixed slowly. The mixture was gradually added to the stirring aqueous phase 1mL at a time using a pipette. After all of the organic phase was added to the aqueous phase, 1,4-BD (3 mL) was added to the stirring mixture dropwise via a syringe at a rate of 1mL/min to act as a chain extender to polymerize the PUR shell material. The reaction progressed for 1h at which time the agitator was switched off, the suspension of microcapsules rinsed with deionized water, and vacuum filtered.
Capsules made with this protocol were agitated at 500, 700, 900, and 1100 rpm during the 1 h polymerization step to study the effects of agitation rate on the properties of the resulting capsules. A Denton Desk IV vacuum sputter coater was used to deposit a 10 nm layer of gold onto the samples of each type for SEM imaging. Capsules were also crushed between two glass slides to assess the reactivity of the encapsulated agent.

**4.2.2 Methyl ethyl ketone as the dispersed phase**

The preparation of the surfactant solution remained constant from the procedure using acetone to the procedure using methyl ethyl ketone (MEK). However, slight variations were made to study the effect of the amount of surfactant on the resulting microspheres. Solutions were prepared with 0.5, 1, 3, 5, 7, 10, 12, and 15% gum arabic in the aqueous phase.

To prepare the organic solution, the prepolymer (3 g) was dissolved in MEK (10 mL) at room temperature. OCA (1 mL) was dissolved in a volume of MEK (2 mL) separate from the PUR solution. Just before addition to the aqueous phase, the OCA and PUR solutions were mixed slowly. The mixture was gradually added to the stirring aqueous phase 1mL at a time using a pipette. After all of the organic phase was added to the aqueous phase, 1,4-BD (3 mL) was added to the stirring mixture dropwise via a syringe at a rate of 1mL/min to act as a chain extender to polymerize the PUR shell material. The reaction progressed for 1h at which time the agitator was switched off,
suspension of microcapsules rinsed with deionized water, and vacuum filtered. A Denton Desk IV vacuum sputter coater was used to deposit a 10 nm layer of gold onto the samples of each type for SEM imaging. Capsules were also crushed between two glass slides to assess the reactivity of the encapsulated agent.

4.2.3 Pluronic F-68 as surfactant with MEK as dispersed phase

At room temperature, Pluronic F-68 surfactant (1.84 g) was dissolved in deionized water (90 mL) in a 250 mL beaker. The solution was agitated for 1 h with a digital mixer (VWR PowerMax Elite Dual-Speed Mixer) before beginning the encapsulation procedure. The aqueous phase was suspended in a hot water bath at 40 °C just before the organic phase was added to begin the encapsulation process.

To prepare the organic solution, the prepolymer (3 g) was dissolved in MEK (10 mL) at room temperature. OCA (4 mL) was dissolved in a volume of MEK (8 mL) separate from the PUR solution. Just before addition to the aqueous phase, the OCA and PUR solutions were mixed; the organic solution was continuously added to the stirring aqueous phase using a 25 mL pipette. After all of the organic phase was added to the aqueous phase, 1,4-BD (3 mL) was added to the stirring mixture dropwise via a syringe at a rate of 1 mL/min to act as a chain extender to polymerize the PUR shell material. The reaction progressed for 1 h at which time the agitator was switched off, the suspension of microcapsules rinsed with deionized water, and vacuum filtered. A
Denton Desk IV vacuum sputter coater was used to deposit a 10 nm layer of gold onto the samples of each type for SEM imaging. Capsules were also crushed between two glass slides to assess the reactivity of the encapsulated agent.

4.3 Incorporation of capsules into a PMMA matrix

A commercial bone cement (components summarized in Table 1) was used to assess the process survivability of the capsules. The powder component of the bone cement (10 g) was mixed with the liquid monomer (4.7 g) according to the manufacturer’s instructions. PUR capsules (1 g) were added to the PMMA dough and vigorously stirred to disperse the capsules within the matrix material. The cement dough was molded into small disks and the curing process completed. Small samples of the resulting composites were analyzed using SEM to visualize the capsules within the matrix.
5. Results

Many factors within the encapsulation procedure influence the resulting
emulsion and subsequent capsule formation. Figure 6 summarizes the factors that affect
the emulsion stability that were altered in this research and the numerous characteristics
used to assess the resulting capsules.

![Diagram of variables and characteristics](image)

Figure 6: Variables considered and characteristics used to assess the capsules resulting
from various protocol modifications
5.1 Capsules made with acetone do not possess many of the desired characteristics for ideal capsules

5.1.1 Capsule size, morphology, and population uniformity

To determine the effect of agitation rate on the resulting microcapsules, polymerization of the PUR was performed at 500, 700, 900, and 1100 rpm. It is well-documented that increasing agitation rate will result in the formation of smaller capsules [98, 101]; as the discontinuous phase is dispersed at a faster agitation rate, the droplets to break into increasingly smaller particles before the interfacial polymerization of PUR takes place. The average capsule diameter is plotted against the agitation rate used to make those capsules in Figure 7. It should also be noted that the size distribution of the capsules decreases at increasing agitation rates while all other factors are held constant. The numerical values from these graphs are also summarized in Table 7.
Figure 7: Increasing agitation rate results in decreasing average capsule diameter of PUR capsules made with acetone solvent and 13 wt% gum arabic surfactant.

Table 7: Average capsule diameter and standard deviation at various agitation rates

<table>
<thead>
<tr>
<th>Agitation Rate (rpm)</th>
<th>Average Capsule Diameter (µm)</th>
<th>Standard Deviation (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>455</td>
<td>206</td>
</tr>
<tr>
<td>700</td>
<td>302</td>
<td>109</td>
</tr>
<tr>
<td>900</td>
<td>152</td>
<td>72</td>
</tr>
<tr>
<td>1100</td>
<td>119</td>
<td>73</td>
</tr>
</tbody>
</table>

While the capsules appeared to have normal, spherical morphologies when viewed through a light microscope (Figure 8A) SEM images showed somewhat fragile capsules that did not appear fully-formed (Figure 8B). Interestingly, this encapsulation procedure resulted in the formation of two distinct densities of spheres – a layer that
floated to the surface of the reaction mixture following the cessation of stirring, and another layer that settled on the bottom of the reaction beaker.
Figure 8: (A) Light microscope and (B) SEM image of PUR capsules made with acetone solvent and 13 wt% gum arabic surfactant at 700 rpm
5.1.2 Capsule shell thickness

SEM images were also used to approximate the average shell thickness of these capsules (Figure 9, capsules made at 500 rpm following acetone encapsulation protocol). Although SEM does not always allow for perfect cross-sections of the capsules to be photographed, the thicknesses calculated here tally well with those reported with other self-healing encapsulated systems [12, 98]. For capsules made with acetone, average capsule thickness ranged from $9 \pm 4 \ \mu m$ to $17 \pm 4 \ \mu m$ depending on the agitation rate with slower rates resulting in thicker capsules.

![Figure 9: Shell thickness can be clearly seen in fractured PUR capsules made with acetone solvent and 13 wt% gum arabic surfactant at 500 rpm](image-url)
5.1.3 Content reactivity

It was also found that although the fluid released from the capsules made at 500 rpm with acetone as the organic solvent was initially able to bond two glass microscope slides together (day 0), this capability was lost over time (day 7) (Figure 10).

![Day 1 and Day 7 images](image.png)

Figure 10: Reactivity of encapsulated healing agent is lost over time in PUR capsules made with acetone solvent and 13 wt% gum arabic surfactant at 500 rpm

5.1.4 Capsule content

While samples of each capsule type were crushed between slides to verify the release of active healing agent, a method was required that would quantify the amount of healing agent encapsulated when compared with the amount of solvent and shell material present in the final capsules. Various groups within the self-healing field have
described the use of thermogravimetric analysis (TGA) to study the thermal properties of their capsules [98]. Known weights of each sample were heated at a controlled rate until all of the material had been vaporized. The decomposition of the material was recorded and from this information, the rate of mass loss was also determined.

Comparisons between the decomposition rates of samples of the pure solvent, pure healing agent, pure shell material, and capsules made under various conditions were made to approximate the percent fill of the capsules.

Capsules made following the acetone protocol at 500 and 900 rpm were analyzed using TGA. Pure samples of OCA and PUR were also analyzed and used for comparisons with the capsules (Figure 11). Samples were heated at a rate of 10 °C/min under N₂. By analyzing the rate of weight loss it was determined that the capsules were approximately 17% OCA and 78% PUR regardless of the agitation rate used. The weight that was unaccounted for was attributed to residual solvent content, voids within the capsules, and slight human error throughout the calculations.
Figure 11: TGA was used to determine the content of the PUR capsules made with acetone solvent and 13 wt % gum arabic surfactant by studying (A) percent weight remaining and (B) rate of weight loss.
5.2 Use of methyl ethyl ketone as a solvent yields improved capsule morphology but not uniformity

Following solubility studies with a variety of organic solvents (Table 8), MEK was selected for the encapsulation procedure due to its immiscibility with water and its ability to dissolve both PUR and OCA. It was also hypothesized that an improved solvent would encourage the production of capsules with uniform density. Figure 12 is an SEM image of capsules made following same protocol as that in Figure 8B using MEK as the solvent instead of acetone.

Table 8: Solvents tested for use with modified encapsulation procedure

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility in Water</th>
<th>Dissolves PUR?</th>
<th>Dissolves OCA?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>Miscible</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Octanol</td>
<td>Immiscible</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>Immiscible</td>
<td>Poorly</td>
<td>Yes, but lowers reactivity</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>Miscible</td>
<td>Yes</td>
<td>Yes, but lowers reactivity</td>
</tr>
<tr>
<td>Hexane</td>
<td>Immiscible</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>Immiscible</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Butanol</td>
<td>Immiscible</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Toluene</td>
<td>Immiscible</td>
<td>Poorly</td>
<td>Yes</td>
</tr>
<tr>
<td>THF</td>
<td>Miscible</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>DCM</td>
<td>Immiscible</td>
<td>Poorly</td>
<td>Yes</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Immiscible</td>
<td>Poorly</td>
<td>Yes</td>
</tr>
<tr>
<td>Xylene</td>
<td>Immiscible</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Ethyl ether</td>
<td>Immiscible</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>MEK</td>
<td>Immiscible</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
While the overall morphology of the capsules was greatly improved (as evidenced by the fully-formed, more consistent resulting spheres), two distinct and separate densities of capsules were still produced through the encapsulation procedure. As the surfactant selection is extremely important to the overall characteristics of an emulsion, and because the solvent and healing agent had both been modified from the Yang protocol [98], further investigations into the influence of gum arabic on the emulsions were conducted. From this point onward all further optimizations were performed using MEK as the solvent.

Figure 12: PUR capsules made with MEK solvent and 15 wt% gum arabic surfactant at 500 rpm have improved morphology and are fully-formed
5.3 Increasing surfactant concentration decreases average capsule diameter and size distribution

The amount of gum arabic was varied to examine the effects of weight percent of surfactant on the morphology and uniformity of the resulting capsules made with MEK solvent. As shown in Figure 13, lower weight percentages of gum arabic (0.5 and 1 weight percent) yielded large capsules, nice spherical morphologies, and little polymeric debris. A few of these capsules had an interesting cratered appearance that may be attributed to lack of surfactant in some regions. At progressively higher concentrations of gum arabic (7, 10, 15, and 15 weight percent), smaller, round capsules are still formed but the amount of polymer debris remaining in the final capsule product and fragility of the existing capsules were concerning.
Figure 13: Varying surfactant amount changes size and morphology of the resulting PUR capsules with MEK solvent and (A) 0.5, (B) 1, (C) 3, (D) 5, (E) 7, (F) 10, (G) 12, and (H) 15 wt% gum arabic at 500 rpm
As expected, the increase in amount of surfactant decreased both the average capsule diameter as well as the size distribution of the resulting capsule population (Table 9 and Figure 14). All of these experiments were performed at the same agitation rate (500 rpm) during the interfacial polymerization step.

Table 9: Increasing surfactant decreases capsule size and distribution when all other factors remain constant

<table>
<thead>
<tr>
<th>Weight Percent of Surfactant</th>
<th>Average Capsule Diameter (µm)</th>
<th>Standard Deviation (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>962</td>
<td>337</td>
</tr>
<tr>
<td>1</td>
<td>665</td>
<td>265</td>
</tr>
<tr>
<td>3</td>
<td>531</td>
<td>202</td>
</tr>
<tr>
<td>5</td>
<td>367</td>
<td>124</td>
</tr>
<tr>
<td>7</td>
<td>391</td>
<td>127</td>
</tr>
<tr>
<td>10</td>
<td>358</td>
<td>93</td>
</tr>
<tr>
<td>12</td>
<td>308</td>
<td>105</td>
</tr>
<tr>
<td>15</td>
<td>113</td>
<td>57</td>
</tr>
</tbody>
</table>
However, all surfactant concentrations still resulted in the formation of two distinct capsule population densities. These results encouraged further investigations into the type of surfactant used, not merely the amount of surfactant used.

**5.4 Capsules made with MEK solvent and Pluronic F-68 as the surfactant meet the desired capsule requirements**

The procedure described using MEK as the dispersed phase was modified to use 1 weight percent of the following surfactants: Tween 20, Tween 65, Brij 52, Pluronic F-68, and Myrj 52. These preliminary experiments resulted in the selection of Pluronic F-68 as the most viable surfactant for our application.
5.4.1 Capsule size

Following this surfactant selection, the effects of the amount of surfactant (Table 10 and Figures 15 and 16), reaction temperature (Figures 16 and 17), and agitation rate (Table 11 and Figures 17 and 18) were studied and the results summarized in the following figures and tables.

Table 10: Effect of weight percent of Pluronic F-68 surfactant on resulting size of PUR capsules made with MEK solvent at 500 rpm

<table>
<thead>
<tr>
<th>Weight Percent of Surfactant</th>
<th>Average Capsule Diameter (µm)</th>
<th>Standard Deviation (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>349</td>
<td>106</td>
</tr>
<tr>
<td>2</td>
<td>342</td>
<td>99</td>
</tr>
<tr>
<td>3.5</td>
<td>174</td>
<td>77</td>
</tr>
<tr>
<td>5</td>
<td>164</td>
<td>55</td>
</tr>
</tbody>
</table>
SEM analysis of the various surfactant weight percentages was performed and based on the excellent morphology of capsules fabricated with lower weight percent Pluronic F-68 (Figure 16), 2 weight percent was selected as the optimal concentration of surfactant to be used for the encapsulation of OCA in PUR using MEK as the solvent.
Figure 16: Higher weight percentages of Pluronic F-68 give increasingly poor morphologies as seen in (A) 1, (B) 2, (C) 3.5, and (D) 5 weight percent; PUR capsules made with MEK solvent at 500 rpm at room temperature

The same procedure was performed at room temperature, in a water bath at 40 °C, and in a water bath at 70 °C with 2% Pluronic F-68 by weight used as the surfactant in the aqueous phase. Capsules formed at room temperature were slightly misshapen, with elongated morphologies and greater size distribution (Figure 16B), while capsules
made at 40 °C have an extremely narrow distribution and regular spherical morphology (Figure 17A). When the protocol was performed at 70 °C, no capsules were formed.

Figure 17: Increasing agitation rate decreases PUR capsule size in capsules made with MEK solvent and 2 wt% Pluronic F-68 surfactant at 40 °C and (A) 500, (B) 700, (C) 900, and (D) 1100 rpm
The encapsulation procedure with 2 weight percent Pluronic F-68 at 40 °C was repeated at agitation rates of 250, 500, 700, 900, and 1100 rpm to determine the effects of agitation rate on capsule characteristics (Table 11 and Figures 17 and 18).

Table 11: Effects of agitation rate on resulting PUR capsule size with MEK solvent and 2 wt% Pluronic F-68 at 40 °C

<table>
<thead>
<tr>
<th>Agitation Rate (rpm)</th>
<th>Average Capsule Diameter (µm)</th>
<th>Standard Deviation (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>513</td>
<td>290</td>
</tr>
<tr>
<td>500</td>
<td>220</td>
<td>74</td>
</tr>
<tr>
<td>700</td>
<td>166</td>
<td>47</td>
</tr>
<tr>
<td>900</td>
<td>111</td>
<td>29</td>
</tr>
<tr>
<td>1100</td>
<td>89</td>
<td>21</td>
</tr>
</tbody>
</table>

Figure 18: Increasing agitation rate results in decreasing capsule diameter of PUR capsules made with MEK solvent and 2 wt% Pluronic F-68 at 40 °C
It is also interesting to note that while the standard deviation decreases here as agitation rate increases, the standard deviation is a much smaller proportion of the average capsule diameter than those presented using the acetone as the dispersed phase using gum arabic as the surfactant. As seen in Table 7, the standard deviation of capsules made using the gum arabic ranged from about 36 to 60% of the average capsule diameter whereas the standard deviation of those capsules made with Pluronic F-68 surfactant ranged from 23 to 34% of the average capsule diameter. This further supports the assertion that Pluronic F-68 is better suited to form stable emulsions for the purpose of encapsulating OCA within a PUR shell.

5.4.2 Capsule thickness

SEM was again used to analyze the thickness of the capsules made using Pluronic F-68 as the surfactant. The average shell thicknesses were found to range from $10 \pm 5 \, \mu m$ to $3 \pm 1 \, \mu m$ depending on the agitation rate, with slower agitation rates (500 rpm) producing the thickest shells.

5.4.3 Reactivity of the encapsulated agent

The contents released from capsules made at 500 and 700 rpm were able to bond two glass microscope slides together at day 0 (Figure 19) as well as day 7, but the capsules made at faster agitation rates were unable to do this. A stereoscope (Bausch & Lomb) was used to view the capsules made at all agitation rates as they were crushed.
and/or sliced open with a scalpel blade. Fluid release was still visualized at 2 weeks post-fabrication in capsules made at all agitation rates even though the capsules made at higher agitation rates were not capable of bonding two glass microscope slides together.

![Microscope slides glued together](image)

**Figure 19:** Microscope slides are glued together by the contents of crushed PUR capsules made with MEK solvent and 2 wt% Pluronic F-68 at 500rpm and 40 °C

### 5.4.4 Capsule content

TGA was again used to determine the percent fill of the capsules. Known weights of each sample were heated at a controlled rate until all of the material had been vaporized. The decomposition of the material was recorded and from this the rate of mass loss was also determined. Comparisons between the decomposition rates of samples of the pure solvent, healing agent, and shell material and capsules made under various conditions were made to approximate the percent fill of the capsules.
Capsules made following the MEK protocol using Pluronic F-68 surfactant at 500, 700, 900, and 1100 rpm were analyzed using TGA. Pure samples of OCA and shell material were also analyzed and used for comparisons with the capsules (Figure 20). Samples were heated at a rate of 10 °C/min under N₂. The composition of the capsules made at the different agitation rates were determined by analyzing the rate of weight loss (Table 12). Residual solvent (up to 7%) was also found in some of the capsules and may account for some of the missing weight. This could also be attributed to volume of capsules occupied by air as well as slight human errors in the calculations.

**Table 12: Percent fill of PUR capsules made with MEK solvent and 2 wt% Pluronic F-68 at 40 °C, day 1 post-fabrication**

<table>
<thead>
<tr>
<th>Agitation Rate (rpm)</th>
<th>Percent OCA</th>
<th>Percent Shell</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>51</td>
<td>39</td>
</tr>
<tr>
<td>700</td>
<td>58</td>
<td>34</td>
</tr>
<tr>
<td>900</td>
<td>54</td>
<td>40</td>
</tr>
<tr>
<td>1100</td>
<td>52</td>
<td>38</td>
</tr>
</tbody>
</table>
Figure 20: TGA was used to determine the content of the PUR capsules made with MEK solvent and 2 wt % Pluronic F-68 surfactant at 40 °C, day 1 post-fabrication by studying (A) percent weight remaining and (B) rate of weight loss.
Capsules were also tested at one month post-fabrication as well to study their shelf life. As indicated in Table 13 and Figure 21, capsules tested at one day and one month post-fabrication have slightly different compositions but these decreases in core content tally well with the reported literature of other types of capsules used in self-healing matrices [98]. This decrease in core content corresponds with a slight increase in shell wall material.

Table 13: Effects of one month dry storage time on PUR capsules made with MEK solvent and 2 wt % Pluronic F-68 surfactant at 40 °C

<table>
<thead>
<tr>
<th>Agitation Rate (rpm)</th>
<th>Percent OCA, One Day</th>
<th>Percent OCA, One Month</th>
<th>Percent Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>51</td>
<td>46</td>
<td>10</td>
</tr>
<tr>
<td>700</td>
<td>58</td>
<td>56</td>
<td>4</td>
</tr>
</tbody>
</table>
Figure 21: Composition of capsules changes slightly following one month of storage. TGA was used to determine the content of the PUR capsules made with MEK solvent and 2 wt % Pluronic F-68 surfactant at 500 and 700 rpm and 40 °C, 1 month post-fabrication by studying (A) percent weight remaining and (B) rate of weight loss.
5.4.5 Residual fluid from encapsulation procedure

TGA was also used to analyze the fluid remaining following the encapsulation procedure performed at 500 rpm. The weight loss rates, as shown in Figure 22, indicate that the residual fluid is almost entirely deionized water with small traces of MEK still present, as indicated by the small peak and dip around 75 °C.

Figure 22: TGA of fluid remaining following completion of the procedure producing PUR capsules using MEK solvent, 2 wt% Pluronic F-68 surfactant at 500 rpm and 40 °C
5.5 *Capsules survive incorporation into a PMMA matrix and could therefore be considered for this application*

Following characterization of the capsules, preliminary experiments were performed to determine if the capsules could survive incorporation into a PMMA bone cement matrix. The mixing of the components, exposure to liquid MMA, and elevated temperatures associated with the curing of the matrix are of particular concern to maintaining the integrity of the capsule shell and contents.

Capsules were observed in the matrix via stereoscope and SEM (Figure 23), verifying they possess the properties necessary for being used in this application. Capsules were visible within the bulk matrix (Figure 23A) as well as in the crack planes (Figure 23B). Capsules in these images were made at 500 rpm.
Figure 23: PUR capsules made with MEK solvent and 2 wt% Pluronic F-68 at 500 rpm and 40 °C were observed to survive incorporation into PMMA matrix and are visible at (A) the surface of the bulk matrix and (B) in a crack plane within the matrix.
6. Discussion

6.1 A water-immiscible solvent is required to give desired capsule morphologies

The formation of a stable emulsion depends greatly on the immiscibility of the two phases of interest. As was the case in the first protocol used, two phases with moderate or high miscibility cannot be used to fabricate capsules with reliable morphologies. While acetone is an excellent solvent for both PUR and OCA, it is miscible with water and therefore will not produce a stable emulsion with defined interfaces at which interfacial polymerization of PUR can occur to yield shell wall formation. This would account for the appearance of the capsules that are not yet fully-formed – the acetone/water interface is so fleeting that complete polymerization does not have time to occur. Although the reactivity of the encapsulated healing agent was assessed by crushing the capsules between two glass coverslips and through TGA studies, the use of a solvent immiscible with water (MEK) ultimately yielded improved capsules. The variability of the results following single input variable adjustments serves as a reminder of the delicate balance found in most emulsions; even the slightest modifications can drastically change the resulting system and the functionality of the final product.

Due to the compromised integrity of the shell wall of capsules made with acetone, as the interior of the capsules were exposed to air, any OCA in solution
remaining in the capsules that were not fully formed was able to evaporate, slowly reducing the healing capacity of the capsule population in general. This would account for the gradual loss of healing capacity between day 0 and day 7 post-fabrication. Capsules made with MEK retain their reactivity at day 7 and TGA results indicated a large percentage of the OCA is still present following 1 month of dry storage.

It was initially hypothesized that the presence of the holes in some of the capsules created with the acetone solvent was also causing the production of what appeared to be two distinct capsule population densities; the hollow, incomplete capsules were floating to the top while the denser filled capsules were sinking to the bottom of the reaction vessel. Unfortunately, selection of a new solvent did not result in the formation of a uniform population of microcapsules as initially believed. Ultimately, the formation of a complex microemulsion was hypothesized to be the cause of these separate capsule populations and was supported via SEM (Figure 24). This internal structure also greatly resembles that seen in Figure 4.
Figure 24: Inner morphology of PUR capsule made with MEK solvent and 2 wt% gum arabic at 500 rpm

It has been reported that when the amphiphilic surfactant equally “likes” the organic and aqueous phases, two-phase splitting can occur and result in the formation of various microemulsions with strangely organized mediums of percolated micelles or bicontinuous and sponge-like structures [94]. Generally the microemulsion contains most of the surfactant of the system and similar amounts of the organic and aqueous phases; these sponge-like structures would account for the heavier and denser particles seen sinking within the reaction beaker following the interfacial polymerization of the PUR shells. Therefore, the MEK encapsulation protocol was modified to use a new surfactant, Pluronic F-68, with the increased hydrophilicity known to promote the formation of oil-in-water emulsions.
6.2 More hydrophilic surfactants yield improved OCA encapsulation

Although gum arabic was successfully employed to encapsulate water-reactive IPDI within PUR [98], changing the reaction temperature, solvent, and healing agent necessitated a change in the surfactant choice as well.

As with any emulsion, too much surfactant will essentially make two immiscible liquids miscible in each other, blurring the phase boundaries and hindering proper capsule formation, while too little surfactant will fail to stabilize the emulsion. Incremental increases in the weight percent of surfactant revealed interesting changes in the shell walls of the capsules formed.

Utilizing the HLB scale system and the requirements outlined in Table 5, Pluronic F-68 was selected as the replacement surfactant in the system. With an HLB of 29, Pluronic F-68 is very hydrophilic and will therefore be more likely to form an oil-in-water emulsion [85]. Preliminary experiments resulted in the formation of a single population density of capsules although their morphologies were irregular (Figure 16). Although this HLB does not fit into the 10-18 range described by Griffin and Davies [92, 93, 95], when surfactants within this range (Brij 52, Myrj 52, Tween 65, and Tween 20) were employed instead of gum arabic, no capsule formation was seen. Instead, a large chunk of polymer formed in the reaction beaker following the addition of BD. Increasing the amount of surfactant did not help to disperse the organic phase to
prevent solid polymer formation nor did varying the temperature of the reaction. All of these surfactants are more lipophilic and even though they have been reported to form oil-in-water emulsions, for our applications they did not promote such an emulsion. However, this research is not describing the process of creating a MEK-in-water emulsion but rather a PUR/OCA/MEK-in-water emulsion and the complex nature of the organic phase is clearly affecting the properties of the surfactant.

Pluronic F-68 is a block copolymer of polyoxyethylene and polyoxypropylene and its performance as an emulsifier is strongly tied to the temperature at which it is being employed [85, 94]. Furthermore, non-ionic surfactants containing propylene oxide, butylene oxide, nitrogen, or sulfur exhibit behavior that has not yet been related to composition [93]. Experiments performed at increased temperature, 70°C, resulted in the formation of no capsules, merely a mass of polymerized PUR, indicating the temperature selected was above the functional range of Pluronic F-68 for our application. At 40°C however, uniform capsules of a single population were created; this temperature was therefore determined to be the optimum temperature for our application. This increased reaction temperature could also increase the polymerization rate between the BD and the PUR, further improving the microcapsule morphologies.
6.3 Importance of emulsion composition

The introduction of the organic phase into the aqueous phase also plays an important role in the way the emulsion develops. In initial experiments that resulted in the formation of two distinct capsule population densities, the organic phase was added to the aqueous phase very slowly, 1mL at a time using a micropipette. It is possible that in the time required for all of the organic phase to be added, the emulsion characteristics changed; the ratio of organic phase to aqueous phase varied from 1:75 for the first milliliter added to 12:75 for the last milliliter added. Because the composition and emulsification protocol variables of emulsions are identified as two of the 3 major categories of emulsion variables [94], it is possible that this gradual shift in proportion of organic to aqueous phase resulted in various microemulsions and local instabilities within the reaction vessel.

Following the selection of Pluronic F-68 as the new surfactant, the mode of addition was modified so that the organic phase was added to the aqueous phase in a continuous stream using a large 25 mL pipette. The residual polymer left floating on the surface of the emulsion liquids was eliminated and the only products were capsules of one population, bolstering this hypothesis.
6.4 TGA analysis

While the initial TGA experiments performed here suggested that the capsules made using the acetone protocol did not contain much OCA, this result is not unexpected or discouraging given the somewhat poor morphology of the capsules and considering the capsules were analyzed 6 weeks post-fabrication. The percent fills reported are low compared to other studies [98], it is unsurprising given that the capsules weren’t fully formed (Figure 8B) and were tested almost 6 week post-fabrication.

As indicated in Table 12 and Figure 20, the percent fill of the capsules made using MEK as the organic phase and Pluronic F-68 as the surfactant is greatly improved. Also, analysis of a sample of the residual encapsulation fluid (Figure 22) indicates that the OCA used in the procedure is no longer present at the end of the encapsulation process, further supporting the assertion that OCA is successfully encapsulated following this method. It is also important to note here that polymerized shell material from crushed microcapsules was tested as the pure capsule sample here; the degradation rates were found to be slightly different for polymerized shell material and PUR prepolymer. An encapsulation protocol containing no OCA was used to fabricate empty PUR capsules that were then crushed, rinse, and dried thoroughly before TGA testing.
6.5 Incorporation into a bone cement matrix

The successful incorporation of these OCA-containing PUR capsules is encouraging and supports the feasibility of our material design. Damage to microcapsules was observed following prolonged contact with liquid MMA, but by changing the mixing order so that the capsules were added to the PMMA dough after the polymerization of the liquid MMA by the benzoyl peroxide had already begun, the capsules survived the curing of the matrix. The curing temperature of PMMA has been reported as anywhere from 40-110 °C with an average of 67.5 °C [102] and the TGA data indicated that roughly 95% of the shell material was still present at 200 °C; therefore, it was not believed that the temperature of curing should compromise the capsule shell, and our results support this hypothesis. While the healing capabilities of this matrix have yet to be assessed, further optimizations can now be pursued with the hopes that an even better formulation can be developed and successfully employed for our desired purpose.
7. Future Directions

7.1 Further optimizations to the encapsulation protocol

Though the capsules currently being tested have promising properties and meet the requirements for the ideal capsule, there are other avenues to investigate that could lead to an even more ideal final product. Further experiments are planned to see if the temperature could be increased above 40°C and still yield capsules with desirable characteristics. It may also be possible to increase the reaction time of the BD and PUR to compensate for using a lower reaction temperature to increase the durability and strength of the capsule shells. The pH of the emulsion has also been considered as an important factor in the emulsion stability and may also be investigated for our applications [94]. Furthermore, a mid-range hydrophilic surfactant, such as PVA, is being studied as a potential surfactant.

Based on the residual fluid analysis results, the amount of OCA used in the procedure is going to be increased to find the maximum amount that may be encapsulated before trace amounts of OCA is present in the residual encapsulation fluid. It is hypothesized that increasing the amount of OCA used in the protocol will further increase the percent fill of the capsules because more OCA will be available for encapsulation. Several air bubbles were observed within the capsules under the stereoscope immediately following rinsing and filtering and may account for some of
the missing weight percentages calculated. As Figure 22 indicated, the residual fluid remaining following the successful completion of the encapsulation procedure does not contain OCA; therefore, it may be possible to significantly increase the amount of OCA used in the organic phase without resulting in presence of residual OCA following the encapsulation reaction. Future encapsulation procedures will increase the amount of OCA while decreasing the volume of MEK it is dissolved in to keep the overall volume of organic materials constant.

Dye encapsulation studies will also be performed to help with the visualization of the release of the capsule contents. This will be especially helpful during the mechanical testing of the bulk matrix so that capsule failure will be readily obvious.

7.2 Further analysis of the shelf life of capsules

PMMA bone cement is designed to be used off-the-shelf and although commercially available brands do have expiration dates it is important that the capsules produced here have a shelf life that is equal to or exceeds the shelf life of the PMMA. Also, because joint replacements are often used more than 10 years, stable encapsulated agent should also be present in the implant throughout its usage lifetime.

TGA can be utilized to examine the shelf life of the capsules over several months following a variety of storage conditions [98]. Retesting of the samples will be performed at time points of 3 and 6 months post-fabrication to examine how the weight
percentages of the various capsule components (i.e. shell wall, solvent, and encapsulated agent) change over time. However, it is also anticipated that capsule lifetime will be increased following incorporation into a PMMA matrix because any trace evaporation through the capsule wall will be decreased or halted altogether.

7.3 Bond strength analysis

The strength of the bond formed by polymerization of the released capsule contents should also be quantified. In their intended application, these capsules will not be required to bond together large surfaces, like the glass microscope slides used in our preliminary studies, but rather small microcracks propagating through a matrix. However, calculating the strength of the bond will be useful in further characterizing the microcapsules. Such studies could be performed by gluing slides together with a known weight of capsules (and therefore known amount of OCA) and studying what shear force is required to break the bond between the slides using microstrain analysis (MSA). These results could be useful in determining the ability of the encapsulated glue to hold a microcrack together within a PMMA matrix under physiological loading conditions.

7.4 Single capsule compression studies

The mechanical properties of an individual capsule shell wall will also be determined using single capsule compression studies [103, 104]. These tests will be used
to look at the effect of microcapsule diameter, shell thickness, and percent fill of the final properties of the capsules; the results may also be used to model whether or not a propagating crack in a PMMA matrix will rupture the capsules. Keller and Sottos used a stepper actuator to apply a constant displacement to the surface of a microcapsule via a compression punch [103] and measured the increase in load as the capsule was compressed. The shell wall was considered ruptured at the displacement at which the load began to decrease. From these measurements, their UF capsules were determined to have an elastic modulus of 3.7 GPa.

White et al. have also reported that the stiffness of the capsule with relation to the surround matrix material is also extremely important and affects the stress state around the propagating crack and the sphere itself. When the spherical inclusion, the microcapsule, is more compliant than the surrounding matrix, the crack propagation is attracted towards the capsule [14]. This attraction to and subsequent rupture of the capsule is the basis on which this system was developed and is therefore crucial in the success of the project.

MSA will be used to study the individual capsule properties to determine which agitation rate and resulting shell thickness provides the best mechanical properties for this application. The MSA also has an environmental chamber that could be used to
study the properties of the capsules at elevated (i.e. body) temperature to give a better understanding of the effects exposure to the usage environment on the capsule.

7.5 Healing efficiency via TDCB and hip-like simulator studies

Following extensive characterization of the capsules, their properties within a PMMA matrix must be examined. Tapered double cantilever beam (TDCB) studies have been used previously to assess healing efficiency of developed encapsulated healing agent systems [13, 14, 37, 44]. While this type of test is valuable to assess the functionality of the capsules once they are embedded into a matrix, the loading and healing conditions (i.e. single damage event healed over 24-48 hours) are not applicable to biomaterials.

The characterization protocols for acrylic bone cement are well established and outlined in ISO 5833, DIN ISO 527-1, ASTM D5045, and ASTM F 2118-01a [105, 106]. ISO 5833 recommends a constant strain rate compression test for the determination of the compressive strength and compressive modulus, and a four-point bending test for determining the bending strength and the bending modulus [107]. In the compression tests, cylindrical specimens are cast in a 6 mm diameter mold and cut to 12 mm lengths. Specimens are loaded between platens with a constant crosshead speed of 20 mm/min. In the four point bending tests, specimens are cast in a 3.3 mm x 75 mm x 10 mm rectangular mold; specimens are loaded across a standard four-point test rig (60 mm
outer loading point separation, 20 mm inner loading point separation) and subjected to a constant crosshead speed of 5 mm/min. It is anticipated that in both cases, tests will be run until sample failure on a Tinius Olsen tensile tester in compression mode at 23°C using dry specimens prepared 24 hours before testing.

However, given the design of the self-healing system (utilizing the reaction of OCA with anions in the body), the effects of moisture and elevated temperature must also be investigated because these factors will contribute significantly to the behavior of PMMA and it is necessary to study the effects of the encapsulated healing agent on the final formulation. Giddings et al. reported that at body temperature, PMMA samples exhibited ductile crack initiation, suggesting that the in vivo mechanism of cement fracture is different from the room temperature in vitro model of PMMA crack initiation. Furthermore, the group reported a decrease of 20-22% in the elastic modulus in samples tested at body temperature as opposed to room temperature [25]. Each of these findings would have bearing on our results as well.

7.6 Cell culture studies

Following the successful incorporation of the capsules into PMMA, the cytotoxicity of the material must be compared to that of commercially-available bone cement. Given that all the materials used in the self-healing formulation have
widespread usage in biomaterials it is hypothesized that the self-healing bone cement will not provide any increase in cytotoxicity.

Recommended standards for biocompatibility testing of bone cement are less specific than are recommendations for mechanical testing, mostly referring to the ISO-10993 Guidelines for Biological Evaluation of Medical Devices Part-1; however, first level biological testing of biomaterials generally includes elusion and cell growth cytotoxicity testing. In this study we will follow the protocols of Alt et al. who evaluated the cytotoxicity of antibacterial acrylic bone cement using elution and cell live-dead testing [108].

7.7 BCA as a potential capsule material

Although the results using PUR as the capsule material for this self-healing system are promising, it may be more advantageous to employ an in situ polymerization technique to form the capsule wall. Butylcyanoacrylate (BCA) is another type of medical grade cyanoacrylate employed during sutureless surgeries. Because of its longer hydrocarbon tail, BCA reacts more quickly than OCA and the resulting polymer film is stronger, but more brittle, than the polymerized films formed by OCA.

BCA has been employed in the drug delivery field as a capsule material for various water-soluble drugs [109, 110]. In an oil-in-water emulsion, when the organic phase containing the dissolved BCA comes in contact with the surfactant-containing
aqueous phase, the BCA will polymerize at the solvent-water interface, resulting in capsule formation. Elevated temperatures and a chain extender are not required so the process is simplified somewhat; these characteristics make BCA an attractive capsule material for the encapsulation of OCA.

Preliminary encapsulation procedures have been performed using a protocol modified from Wohlgemuth et al. (Figure 25) [109]. While capsules are formed during the emulsification process, they have not yet been successfully filtered without rupturing; these results are promising and indicate that perhaps the reaction time or amount of BCA used should be increased to yield more robust capsules. For these experiments, the OCA was stained with an oil-soluble dye so that it would be distinguishable from the BCA capsules. Upon filtering, the capsules, which were initially white, ruptured and the release of purple contents was observed, suggesting that OCA was successfully encapsulated within BCA shells.
Figure 25: BCA capsules represent an excellent potential capsule material if their material strength can be improved
8. Conclusion

A number of first generation and shape restoration techniques exist that are feasible, realistic, and reasonably effective but have not been extended to biomaterials, a field that would greatly benefit from the development of such materials. Interfacial polymerization of PUR was successfully carried out to encapsulation OCA. The resulting capsules were assessed for their usefulness as healing agent reservoirs for a self-healing PMMA bone cement based on capsule morphology and uniformity, diameter, shell thickness, content reactivity, percent fill, and process survivability. While the preliminary results reported here are promising, demonstrating feasibility of the material design, future work will focus on the mechanical properties of a PMMA/PUR capsule composite bone cement. Similar to the self-healing materials field in general, the first self-healing, load-bearing biomaterials to arise will likely employ an irreversible healing agent system rather than a more complex reversible system.
<table>
<thead>
<tr>
<th>Terms and Abbreviations</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Affinity EG 8200</td>
<td>Ethylene octene</td>
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<tr>
<td>BD</td>
<td>Butanediol</td>
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<tr>
<td>Brij 52</td>
<td>Polyoxyethylene (2) cetyl ether</td>
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<tr>
<td>DBTL</td>
<td>Di-n-butylin dilaurate</td>
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<tr>
<td>DCPD</td>
<td>Dicyclopentadiene</td>
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<td>DETA</td>
<td>Diethylenetriamine</td>
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<td>EMAA</td>
<td>Surlyn</td>
</tr>
<tr>
<td>ENB</td>
<td>5-Ethylidene-2-norbornene</td>
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<td>Epon 828</td>
<td>Diglycidyl ether of bisphenol-A</td>
</tr>
<tr>
<td>Epon 862</td>
<td>Diglycidyl ether of bisphenol-F</td>
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<td>GPC</td>
<td>Gas permeation chromatography</td>
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<tr>
<td>HLB</td>
<td>Hydrophilie-lipophile balance</td>
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<td>HOPDMS</td>
<td>Hydroxyl end-functionalized polydimethylsiloxane</td>
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<td>IPDI</td>
<td>Isophorone diisocyanate</td>
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<td>KH-816</td>
<td>Cycloalipathic amine</td>
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<td>MEK</td>
<td>Methyl ethyl ketone</td>
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<td>MSA</td>
<td>Microstrain analysis</td>
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<td>Myrij 52</td>
<td>Polyoxyethylene (40) stearate</td>
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<tr>
<td>OCA</td>
<td>Octylcyanoacrylate</td>
</tr>
<tr>
<td>PB-g-PMA-co-PAN</td>
<td>Poly(butadiene)-graft-poly(methyl acrylate-co-acrylonitrile)</td>
</tr>
<tr>
<td>PDES</td>
<td>Polydithiioxysiloxane</td>
</tr>
<tr>
<td>PDI</td>
<td>Polydispersity index</td>
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<td>PDMS</td>
<td>Polydimethylsiloxane</td>
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<td>Polyglycolide</td>
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<td>Polylactic acid</td>
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<td>Pluronic F-68</td>
<td>Polyoxyethylene-polyoxypropylene block copolymer</td>
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<tr>
<td>PMMA</td>
<td>Poly(methyl methacrylate)</td>
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<tr>
<td>PUR</td>
<td>Polyurethane</td>
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<tr>
<td>PVA</td>
<td>Poly(vinyl alcohol)</td>
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<tr>
<td>ROMP</td>
<td>Ring-opening metathesis polymerization</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
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<tr>
<td>TDCB</td>
<td>Tapered double cantilever beam</td>
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<tr>
<td>TDI</td>
<td>Toluene diisocyanate</td>
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<tr>
<td>TGA</td>
<td>Thermogravimetric analysis</td>
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<tr>
<td>THA</td>
<td>Total hip arthroplasty</td>
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<tr>
<td>TJR</td>
<td>Total joint replacement</td>
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<tr>
<td>TKA</td>
<td>Total knee arthroplasty</td>
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<td>Tween 20</td>
<td>Polyethylene glycol sorbitan monolaurate</td>
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<td>Tween 65</td>
<td>Polyoxyethylenesorbital Tristearate</td>
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<tr>
<td>UF</td>
<td>Urea formaldehyde</td>
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<tr>
<td>UHMWPE</td>
<td>Ultra high molecular weight polyethylene</td>
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<td>YD-115</td>
<td>Butyl diglycidyl ether of bisphenol-A</td>
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References


