

Syracuse University

SURFACE

Syracuse University Honors Program Capstone
Projects

Syracuse University Honors Program Capstone
Projects

Spring 5-1-2014

Investigating the Shelf Life of Benzoyl Peroxide Coated Nozzles for Bone Cement Application

Alysen Leigh Demzik

Follow this and additional works at: https://surface.syr.edu/honors_capstone



Part of the [Biological Engineering Commons](#), and the [Other Biomedical Engineering and Bioengineering Commons](#)

Recommended Citation

Demzik, Alysen Leigh, "Investigating the Shelf Life of Benzoyl Peroxide Coated Nozzles for Bone Cement Application" (2014). *Syracuse University Honors Program Capstone Projects*. 762.

https://surface.syr.edu/honors_capstone/762

This Honors Capstone Project is brought to you for free and open access by the Syracuse University Honors Program Capstone Projects at SURFACE. It has been accepted for inclusion in Syracuse University Honors Program Capstone Projects by an authorized administrator of SURFACE. For more information, please contact surface@syr.edu.

**Investigating the Shelf Life of Benzoyl Peroxide Coated Nozzles
for Bone Cement Application**

A Capstone Project Submitted in Partial Fulfillment of the
Requirements of the Renée Crown University Honors Program at
Syracuse University

Alysen Leigh Demzik
Candidate for B.S. Degree
and Renée Crown University Honors
May 2014

Honors Capstone Project in Bioengineering

Capstone Project Advisor: _____
Dr. Julie M Hasenwinkel

Capstone Project Reader: _____
Dr. Jeremy Gilbert

Honors Director: _____
Stephen Kuusisto, Director

Date: May 6, 2014

Abstract

Two-solution bone cements (TSBC) were developed to address the limitations of current powder-liquid bone cements, however are characterized by a limited shelf life due to spontaneous free radical polymerization. As a solution to pre-polymerization concerns, the initiator benzoyl peroxide (BPO) was removed from the cement solution and incorporated into a thin film coating within the static mixing nozzle, allowing the BPO to integrate with the TSBC as it is mixed for use. Only short-term storage of BPO-coated nozzles and the subsequent effects on bone cement properties is known. The goal of this study was to investigate the effects of time, temperature and light exposure on the thermal characteristics and flexural mechanical properties of BPO-coated nozzle bone cements for a longer period of approximately twelve months. It was hypothesized that with increased time, temperature, and light exposure, the properties of cements made with BPO-coated nozzles would deteriorate. Results revealed a general reduction in thermal and mechanical properties of cements in comparison to standard TSBC and commercial cements. Nozzles stored beyond four months were found to be no longer viable. It was determined that storage time has an effect on cement properties, but effects of storage conditions on cement properties were inconclusive. High variability in test results, most likely due to the inconsistent thin film coating and unpredictable BPO release, indicated that significant improvements must be made to the nozzle coating method. Benzoyl peroxide coated nozzles have the potential to serve as improved alternatives to traditional bone cements, however further investigation into the preparation and shelf life of coated nozzles is required.

Table of Contents

Abstract..... i

Executive Summary..... iii

Acknowledgements..... viii

Introduction..... 1

Materials and Methods..... 6

Results and Discussion.....

Conclusion.....

References.....

Executive Summary

Bone cements are used widely in the fixation of orthopedic implants and in treatment of vertebral compression fractures. The term “cement” implies that the material bonds two things together, where in reality, bone cement should be called “grout”, as it acts as a space-filler that holds the implant against bone. Commercial bone cements are typically made of two-component systems consisting of a liquid and a powder phase that are mixed on site during surgical procedures. In fact, bone cement is the only implant that is manufactured in the operating room. Like all cements, bone cement has an associated setting time, and surgeons must wait until a necessary dough-like viscosity is obtained before it is ready for surgical use.

Reducing both the preparation period within the operating room and the setting time of the bone cement while maintaining the necessary properties is a goal of bone cement research. An alternative two-solution bone cement (TSBC) that reaches this ideal viscosity more quickly following mixing was recently developed, and like commercial bone cements, consists of polymethylmethacrylate (PMMA) dissolved in methyl methacrylate (MMA) monomer. Unlike current bone cements, they incorporate the powder and liquid components together within a two-cartridge system. The initiator of the polymerization reaction, benzoyl peroxide (BPO), is dissolved in the liquid monomer rather than the powder component, so the cement can be pre-made and stored, rather than mixed at the time of use. TSBC is stored in a dual-cartridge mixing system that allows component separation and also shows a reduction in porosity, a characteristic that has been shown to increase cement cracking and fatigue. Concerns

arise with the shelf life and storage of TSBC, as the BPO-containing solution is susceptible to spontaneous free radical polymerization when exposed to heat or light. The decomposition of BPO creates free radicals that can interact with the available MMA molecules. Currently, TSBC requires refrigerated storage.

In attempt to prevent spontaneous polymerization and improve shelf life, investigations into removing the BPO initiator component from the dual-cartridge system, and incorporating it into a film coating within the mixing nozzle that is used for cement delivery have been conducted. This coating allows the BPO to integrate with the TSBC as it is mixed for use, and reduces cement storage to mixing nozzles only. Experiments concerning these BPO-coated nozzles have led to questions regarding the shelf life of the BPO-coated nozzles, specifically.

Only short-term storage of BPO-coated nozzles and the subsequent effects on bone cement properties is known. In previous studies, coated nozzles were tested within one week of preparation, however the storage length of TSBC for commercial use would be for much greater periods. The goal of this study was to investigate the effect of time, temperature and exposure to light on the properties of bone cements for a longer time period of approximately twelve months. Four storage conditions were tested and account for the previously stated effects of light and heat on BPO: covered and uncovered at room temperature, and covered and uncovered at cooled temperatures. Storage periods of twelve, nine, six, four, two and one months were used. Both exotherm and mechanical properties of TSBC produced with BPO-coated nozzles were tested in order to investigate the shelf life and ideal storage conditions of

the nozzles. Exothermic properties include the setting time and maximum temperature reached during curing, while mechanical properties include flexural strength, strain-to-failure, and modulus.

It was hypothesized that with increased storage time, temperature, and light exposure of the coated nozzles, properties of the produced cement would deteriorate. These results were hypothesized due to the expectation that BPO within the coated nozzles may degrade over time. With less BPO available to polymerize, the reaction will slow and be less likely to complete, causing a decrease in maximum temperature and an increase in setting time. With the decrease in polymerization, molecular weight of the cement will decrease and the amount of residual monomer MMA will increase, causing a decline in mechanical properties.

Although results showed high variability amongst condition samples, the study reveals that removing BPO from the bulk of the cement and incorporating it into the mixing nozzle as a thin film resulted in a general reduction in thermal and mechanical properties in comparison to standard TSBC and current commercial cements. Both the reduction in cement properties and high variability in testing results can be attributed to the method of nozzle coating. Coating methods used within this study led to unpredictable BPO release and to inconsistent solution coverage throughout the nozzle, with areas of high solution accumulation with chunks of BPO and areas lacking solution coverage. As bone cement passes through the nozzle, there is significant variation in BPO concentration in the cement that exits. These findings suggest that there are

significant improvements that must be made to the even distribution of the polymer coatings.

Despite variability of cement samples, the majority of mechanical and exotherm testing results reveal that storage time has an effect on cement properties more so than storage conditions. Nozzles stored beyond four months were found to be no longer viable. It can be determined from this study that at some time between four and six months into storage, the BPO within the nozzle thin film coating was degraded to such a degree that a polymerization reaction was not initiated. Future studies would look more closely at the time period between four and six months in order to more precisely determine the point at which coated nozzles have reached the extent of their shelf life. In order to support that an increase in nozzle storage time has a deleterious effect on cement properties, additional data and more repetitious trials would be necessary. A larger number samples would have to be tested in a similar shelf-life study in order to account for the large variability that occurs due to the coated nozzles and the inconsistent BPO release.

Data collected from this long-term study on the storage and shelf life of BPO-coated nozzles provides further insight into the use of two-solution bone cement and its viability as a commercial product. Currently, there exists concerns over the hazards of exposure to fumes during polymerization of commercial powder-liquid cements, and it remains somewhat of a challenge to mix the cement properly while reducing porosity. It is not difficult to see the benefit that could result from a commercially available alternative to existing bone cements that improves upon current preparation and

setting time, and perhaps even the cement's physical and mechanical properties that are so crucial to the longevity of the implant. Benzoyl peroxide coated nozzles have the potential to serve as improved alternatives to traditional bone cements, however further investigation into the preparation and shelf life of coated nozzles is required.

Acknowledgements

Firstly, I would like to thank my advisor, mentor, and Capstone Advisor Dr. Julie Hasenwinkel for allowing me to conduct research in her lab and for offering her guidance and insight throughout both my Capstone Project and my undergraduate career. The knowledge she has imparted and support she has provided has played an enormous role in my experience and achievements at Syracuse University.

I would also like to express my gratitude to the former and current members of the Hasenwinkel laboratory, as the influence each individual has had on my research was invaluable. Specifically, Pushkar Varde, for always being there to help me face a difficult challenge, and to Kayla Huffman, who trained me in many of the required techniques and whose preceding work gave me a road map to my own project.

Thank you to the Renée Crown University Honors Program, for both the enthusiastic encouragement in the endeavor of completing the Capstone Project, and for the Crown Award funding that made my research possible.

Finally, to my family, friends, and especially Garrett Solecki, who motivated, inspired, listened, congratulated and consoled me through the past four years. I would not have accomplished any of this without you.

Introduction

Combined, there are over 1.5 million patients in North America who receive total joint replacements or are treated for vertebral compression fractures each year.^{1,2}

Acrylic poly(methyl methacrylate) (PMMA) bone cements are used widely in implant fixation, whereby bone cement acts as a grout, filling in vacant space around an implant while stabilizing and transferring loads between the implant and the bone. Bone cement also plays a central role in percutaneous kyphoplasty and vertebroplasty surgeries, in which compression fractures of the spine are injected with cement in order to restore vertebrae height and reduce pain.^{3,4}

While acrylic bone cements have been in use for over 60 years and their mechanical and physical properties have been investigated thoroughly throughout literature, there is interest in the development of new cement formulations to improve clinical performance.⁴ Two-solution bone cement (referred to as TSBC) has emerged as an experimental alternative to current commercial powder-liquid cements.

Two-Solution Bone Cement

Two-solution bone cement was developed to address the limitations of commercial cements.⁵ Commercial bone cements are typically made of two-component systems consisting of a liquid and a powder phase that remain isolated until mixed on site in a vacuum-sealed container during surgical procedures. The liquid phase is comprised of the monomer methyl methacrylate (MMA), an activator N, N-dimethyl-p-toluidine (DMPT), and an inhibitor such as hydroquinone. The powder phase consists of a polymer poly(methyl methacrylate) (PMMA), an initiator benzoyl peroxide (BPO), and

a radiopacifier such as ZrO_2 or $BaSO_4$. Powder-liquid cements have an initial phase of very low viscosity, and there is an associated waiting period until the cement reaches an appropriate dough-like viscosity necessary for use. TSBC differs from commercial cements in that it consists of two cartridges with solutions containing equal amounts of MMA and PMMA, but in which BPO and DMPT are in separate cartridges. When mixed together, the two solutions polymerize via a free radical reaction.

Advantages of TSBC

The liquid and powder components are integrated in TSBC, removing the need for vacuum mixing, and creating an initial doughy viscosity that allows for immediate handling. The pre-made solutions of TSBC also increase mechanical performance by eliminating the potential for increased porosity that is associated with the manual mixing of powder-liquid bone cements. Increased porosity is shown to reduce flexural strength and modulus.^{6,7,8} Delivery of TSBC is through a static mixing nozzle that reduces clinical delivery time and allows for simple repeated use of the same cement dose at multiple locations by replacing the delivery nozzle.⁹ Clinical advantages of TSBCs also include increased control of setting characteristics through the optimization of cement initiation chemistry, specifically the concentrations of the initiator and activator^{8,9}, and variable viscosity to meet desired needs by adjusting relative constituent amounts.

Disadvantages of TSBC

Clinically safe and successful powder-liquid bone cements have characteristics such as biocompatibility, maximum curing temperatures below $90^\circ C$, ease of preparation and handling, and setting times that follow the ASTM F451-08 suggestion of

five to fifteen minutes.¹⁰ Setting times of TSBCs are between six to eight minutes with high maximum curing temperatures of approximately 100°C.⁸ The high polymerization temperatures of TSBCs pose an issue, as damage to the surrounding tissue due to thermal necrosis can cause early loosening of an implant as well as protein denaturation.⁷ The higher initial viscosity of the material can also be a major limitation to the use of TSBC in applications that require injection through small cannulas or needles.^{5,8}

Shelf Life and Storage

The BPO-containing solution of TSBC is susceptible to spontaneous polymerization when exposed to heat or light, so storage and shelf life are concerns regarding the alternate two-solution bone cement.¹¹ In attempt to prevent spontaneous polymerization and improve shelf life, investigations into removing the BPO from the dual-cartridge system, and incorporating it into a film coating within the mixing nozzle have been conducted. This would restrict storage to only the mixing nozzle. Experiments concerning these BPO-coated nozzles have led to questions regarding the shelf life of the BPO-coated nozzles, specifically. Investigation into the storage of BPO-coated nozzles may give insight into the viability of commercial usage of two-solution bone cement using a BPO-coated nozzle mixing process.

Study Goals

Only short-term storage of BPO-coated nozzles and the subsequent effects on bone cement properties is known. In previous studies, coated nozzles were tested within one week of preparation⁵, however the storage length of TSBC for commercial

use would be for much greater periods. The goal of this study was to investigate the effect of time, temperature and light on the properties of bone cements for longer time period of approximately twelve months. Over a period of one year, with storage periods of twelve, nine, six, four, two and one months, the exothermic and mechanical properties of TSBC produced with BPO-coated nozzles were tested in order to investigate the shelf life and ideal storage conditions of the nozzles. The setting times, polymerization exotherm during mixing and setting, as well as the flexural properties of the produced cements were recorded.

Four storage conditions were tested for each storage interval to account for effects of light and heat on BPO: covered and uncovered at room temperature, and covered and uncovered at cooled temperatures of approximately 4°C. At each time period, twenty-four nozzles were prepared and stored, with six nozzles for each of the four storage conditions. Control cements containing BPO, used with uncoated nozzles, were prepared and stored for each of the six storage lengths. Coated nozzles covered and stored at a cooled temperature and tested within one week served as an additional control. Cements for coated nozzles were prepared within a week prior to testing.

Hypotheses

Effect of Storage Time on Cement Properties

1. As storage time increases it is hypothesized that the maximum exothermic will decrease and setting time will increase.
2. As storage time increases, it is hypothesized that the flexural strength will decrease, strain-to-failure will increase, and modulus will decrease.

These results were hypothesized due to the expectation that BPO within the coated nozzles may degrade over time. With less BPO available to polymerize, the reaction will slow and be less likely to complete, causing a decrease in maximum temperature and an increase in setting time. With the decrease in polymerization, molecular weight of the cement will decrease and the amount of residual monomer will increase, causing a decline in mechanical properties.

Effect of Storage Conditions on Cement Properties

1. With increased storage temperature it is hypothesized that the effects of increased storage time will be amplified: maximum temperature will decrease, setting time will increase, stress will decrease, strain will increase, and modulus will decrease.
2. With increased exposure to light, it is hypothesized that the effects of increased storage time will be amplified: maximum temperature will decrease, setting time will increase, stress will decrease, strain will increase, and modulus will decrease.

Similar to the effect of increased time on the coated nozzles, an increase in storage temperature and exposure to light will cause the decomposition of BPO. This will likely lead to slower and decreased polymerization of the bone cement causing decreased setting temperatures, longer setting times and decreased mechanical properties.

Materials and Methods

Two-Solution Bone Cement Preparation

All two-solution bone cement used with coated nozzles was prepared using a 0.9:1 polymer to monomer ratio that contained methyl methacrylate (MMA, 99%, Sigma Aldrich), and linear poly(methyl methacrylate) (PMMA, 84,000 g/mol, Monomer-Polymer and Dajac Laboratories). The small end of each cartridge was sealed with a stopper and a solution of 100mL of MMA and 0.7mL of the activator N, N-dimethyl-p-toluidine (DMPT, Sigma Aldrich) that was premixed to ensure full dissolution was distributed equally between both cartridges. Following the solution, 45g of PMMA was added to each cartridge that was then sealed with stoppers and shaken vigorously. A sealed cement cartridge can be found in Figure 1. Cartridges were fixed to a rotating drum for approximately 15 hours, at which point they were removed and stored in a refrigerator at 4°C until testing.



Figure 1: A sealed dual cartridge of bone cement.

Control Cement Preparation

Control cements containing BPO that were injected using an uncoated nozzle were used as a comparison to cements polymerized through coated mixing nozzles. Two control cements were made for each storage period. Control cements were prepared in a dual cartridges at a 1:1 ratio in which each side contains 50mL of MMA and 45g of PMMA, one side contains 1.25g of the initiator BPO, and the other side contains 0.7mL of the activator DMPT. Measured MMA and either BPO or DMPT were premixed in order to allow for complete dissolution. All subsequent preparatory steps followed that of the TSBC method.

Coated Nozzle Solution

The nozzle solution remained consistent for all coated nozzles, with a fixed concentration of 1.25 g BPO per mL MMA. This concentration was determined most effective through previous studies on varied BPO concentration. Measurements for a single nozzle consisted of 5 mL of MMA, 0.25g of PMMA and 0.725 g BPO. Three nozzles were made at one time using a 20mL glass vial. The specified amounts of PMMA and BPO were weighed and remained separate and MMA was measured and pipetted into the vial with a small stir bar. The BPO was added to the monomer and placed on a stir plate until it was completely dissolved, at which point the PMMA was introduced to the solution. The mixture was left stirring on a hot plate at 50°C until complete dissolution was achieved.

Coated Nozzle Preparation

Prepared solutions were incorporated into mixing nozzles to form a thin film coating using a solvent casting method in which the solvent monomer is evaporated throughout the continuous rotation of the nozzle. In order to achieve even solution distribution and evaporation throughout each nozzle, nozzles were fixed horizontally in alternating directions on a rotation drum as seen in Figure 2.



Figure 2: Coated mixing nozzles on the rotating drum during the first 20 hours of the coating process (both ends are sealed with a rubber stopper).

The tip of the mixing nozzle was sealed with a rubber stopper before 5mL of solution was pipetted into the opposite end, which was then also sealed with a rubber stopper. Sealed nozzles were strapped to the drum and left to rotate for approximately 20 hours. The stopper at the tip end of the nozzle was then removed in order to allow for monomer evaporation and the nozzles were left to rotate for another 20 hours at which point the second stopper at the large end of the nozzle was removed. The nozzles

were revolved on the drum for another 20 hours to allow for residual monomer evaporation. After an approximate total of 60 hours on the rotating drum, the nozzles were removed and stored.

Storage Conditions

Four storage conditions were established in order to investigate ideal storage of coated nozzles and account for the effects of light and heat on BPO: covered and uncovered at room temperature, and covered and uncovered at cooled temperatures. Nozzles in covered storage conditions were wrapped in tin foil, as seen below in Figure 3. Nozzles in chilled temperature conditions were stored in the refrigerator at 4°C until testing.



Figure 3: A covered and uncovered coated mixing nozzle before being stored.

Coated nozzles were stored for various time periods before testing to investigate shelf life. Storage intervals of twelve, nine, six, four, two and one months were used. For

each storage interval, twenty-four nozzles were made, with six nozzles for each of four storage conditions. An additional six coated nozzles were made and stored at cooled temperatures for less than seven days and served as an additional control for experimentation.

Experimental Methods

Exothermal Testing

Exothermal testing was performed in order to determine polymerization temperature and setting times of TSBCs. The measurements were taken in accordance with the ASTM F451-08 Standard Specification for Acrylic Bone Cement. Thermal characteristics of cements were investigated using a polyethylene mold consisting of three pieces: (1) a bottom section containing a centered orifice for the insertion of a thermocouple; (2) a central section containing a central circular cavity with a depth of 6mm and a diameter of 60mm and channel for excess drainage; (3) a top section that was secured with six screws after injection of the cement. The mold is shown below in Figure 4.

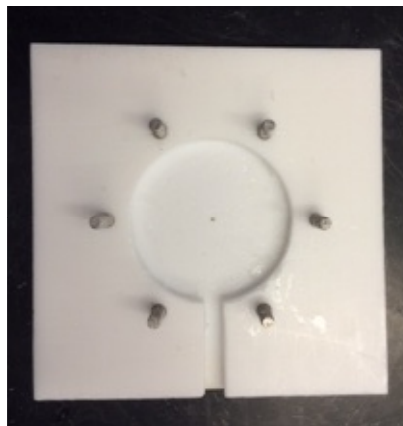


Figure (4): The standard three-part mold used for exothermal testing. The small orifice for the thermocouple can be seen at the center of the bottom mold.

Polymerization temperatures versus time were recorded using a J-type thermocouple (30-gauge). The maximum temperature is defined as the peak of the temperature versus time curing curve, while setting time is defined as the time corresponding to the average temperature between the ambient temperature and maximum temperature. An example of a typical polymerization curve is shown below in Figure 5.

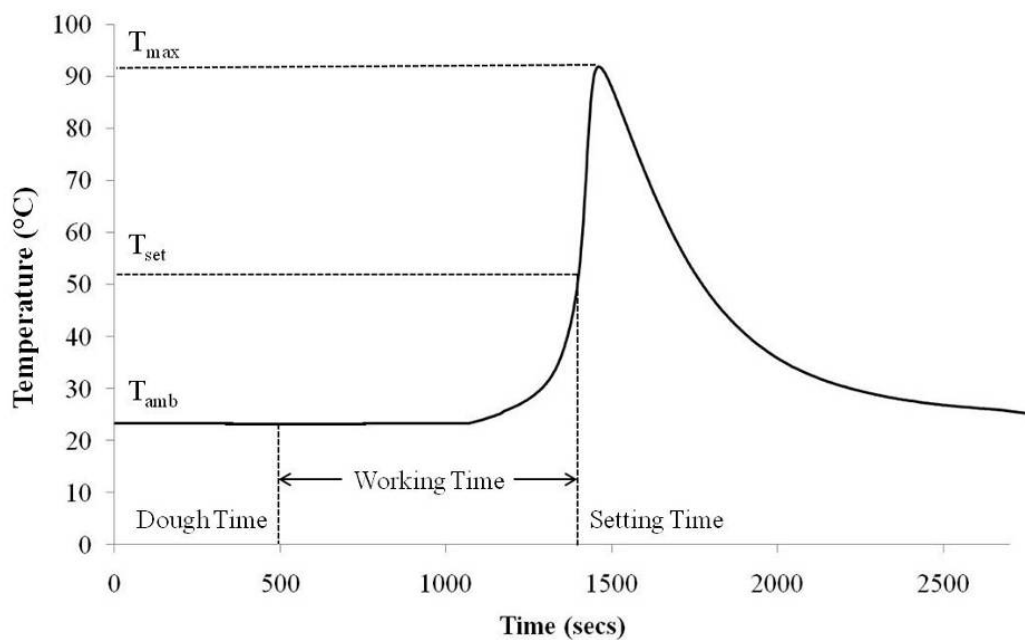


Figure (5): A representative curing curve for acrylic bone cement where T_{max} is the maximum temperature, T_{set} is the setting temperature, and T_{amb} is the ambient temperature. Adapted from (Dunne).

Four trials were conducted for each storage condition within a storage period. Cement cartridges and coated nozzles were removed from storage in the refrigerator prior to testing in order to equilibrate to ambient temperature. Upon reaching room temperature, the cement was injected at the center of the polyethylene mold, which

was then secured and left until the polymerization curve had reached a maximum. Prior experimentation revealed that the initial portion of the cement injected did not completely polymerize, so a small volume of cement was discarded at the beginning of each test before filling the mold.

Three-Point Bend Mechanical Testing

Three-point bend mechanical testing was performed in order to assess the flexural properties of TSBCs. Testing was performed according to ATSM D790-10 Standards using a Sintech MTS System. The three-point bend fixture is shown below in Figure 6. The lowering of the crosshead causes the cement sample to create a load against the central column that is then measured by the connected load cell. Throughout testing, a load-displacement curve is generated. A crosshead speed of 2.54 mm/min was used, with a strain rate of 0.01 mm/mm/min. In order to allow a 10% overhang at each end to prevent sample slippage, the span length was set to 40mm.

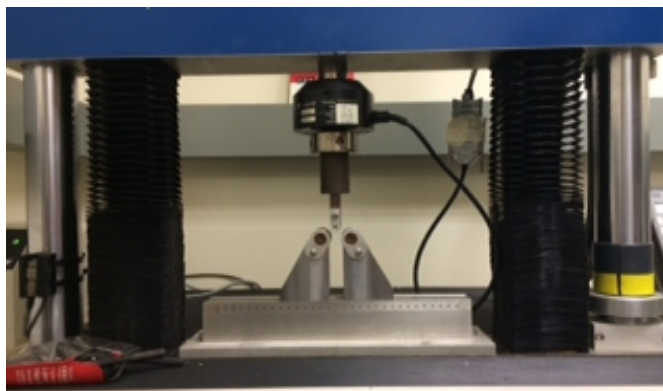


Figure 6: Three-point bend fixture used during flexural testing.

Samples for flexural testing were prepared using a five-part polyethylene mold consisting of six channels that were consistently 75mm in length, 12mm in width, and

4mm in thickness. The flexural sample mold can be seen in Figure 7. Channels were sequentially filled with cement from a single coated nozzle and left to polymerize for an approximate period of two hours. Samples were created from two coated nozzles from each storage period and condition. Inconsistent polymerization led to incomplete samples in several cases. Not all polymerized samples were used for flexural testing due to the presence of cement defects. The samples without major impurities or large surface defects were polished using sand paper of grits 220, 320, 400, and 600. The width and thickness of each polished rectangular sample was recorded before testing, with an average width of 11.5 mm and an average thickness of 3.2 mm.

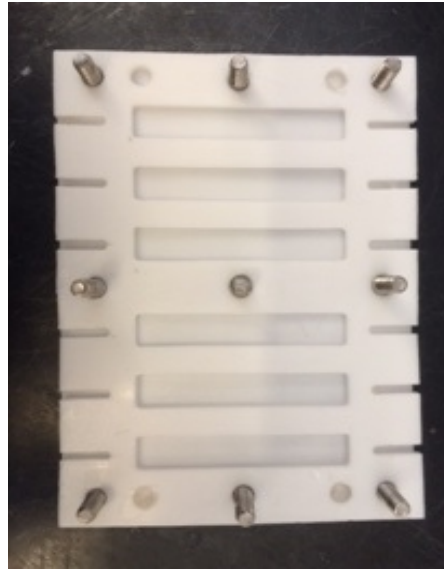


Figure 7: Flexural test sample mold with six channels.

Resulting load-displacement data was used to calculate flexural stress (σ), strain-to-failure (ϵ), and flexural modulus (E) for the cement samples according to equations (1)-(3).

$$\sigma = \frac{3PL}{2bd^2} \quad (\text{Equation 1})$$

$$\varepsilon = \frac{6Dd}{L^2} \quad (\text{Equation 2})$$

$$E = \frac{ML^3}{4bd^3} \quad (\text{Equation 3})$$

Where P was the maximum load, D is the maximum displacement at the center of the sample, L was the span length, and b and d were the sample width and thickness, respectively. M is the slope of the linear region of the load-displacement curve.

Results and Discussion

Bone Cements

Bone cements without BPO were made in order to test the BPO-coated nozzles. Difficulty with the preparation of the bone cements was encountered throughout testing of one and two month storage periods, as well as the one-week control period. It was observed upon storage of the cements and after approximately twenty-four hours in refrigeration after being taken off the rotating drum, that a majority of the cartridges contained cement that appeared to be polymerized near the large rubber stopper. Approximately one fifth to one third of the cement within the cartridge was solidified, and in some cases, a portion of extremely high viscosity cement was observed sitting on top of the solidified cement. A small number of cement cartridges were also discolored. Testing involving several of these cartridges led to decreased or unpolymerized cement samples.

In order to address the cement inconsistencies, new chemicals were ordered and used for bone cement preparation under the premise that existing chemicals,

specifically DMPT, were most likely contaminated. Following chemical replacement, cements were of the desired consistency and facilitated cement polymerization and testing of the four month storage period. While most of the defective cement was recognized upon removal from refrigeration, it is unknown whether cements that may have appeared normal were also affected. Experimental results from exothermal and mechanical testing of one and two months storage conditions as well as the one-week control nozzles could be unrepresentative of the respective conditions due to the described cement defect and could explain inconclusive or unexpected outcomes. The one-week control period should not be considered a point of reference due to the possible skewed results.

Control Cements

Control cements containing BPO were prepared and stored at each pre-determined storage period. Testing of these cements did not occur due to polymerization within the cement cartridges during storage and before testing. Bubbles with approximate diameters between 4mm and 7mm were observed near the large end of the cartridges, indicating that the cement had polymerized. Polymerization of the control cements was unexpected, and it is likely that the same contamination that was proposed to have affected a portion of the bone cements used for nozzle testing also affected the control cements.

Coated Nozzles

It was observed that the coating method of the nozzle led to uneven distribution of the thin film coating. There were regions throughout the nozzle in which solution had

accumulated, and during cement injection, initial cement through the nozzle contained visible BPO debris. Both the exothermal testing procedure and the procedure for creating mechanical samples were modified after this observation by removing a small portion of initial cement before injecting into the respective testing molds.

The preparation of the coated nozzle solutions for all storage periods included the mixing of measured BPO, PMMA and MMA on a 50°C heated stir plate. This method of preparation was in keeping with previous investigations into coated nozzles in order to facilitate dissolution, however heating the solution to such a degree could cause degradation of the BPO prior to storage. It is unknown how the coated nozzle cement samples or test results of the shelf life study were affected by the heating, however the majority of the exothermic and mechanical results seem to be within range of normal values obtained from previous cement studies using coated nozzles.

Coated nozzles prepared and stored for the six month time period were injected, however cements did not polymerize and both exothermal and mechanical could not be completed due to lack of viable samples. In some cases, it was observed that the interior of the nozzles after cement injection were slightly warm to the touch, however not to the degree of temperature increase normally seen during cement curing. It was concluded that the BPO within the nozzle thin film coating had degraded significantly within six months, and consequently, the testing of storage periods at and greater than six months, including nine and twelve months, was determined to be unnecessary.

Exothermal Testing

Maximum Polymerization Temperature

All data collected from exotherm testing of polymerized cement samples was included in calculations, however there were several samples across storage periods that did not polymerize and therefore did not provide exotherm data, remaining high in viscosity and without temperature change throughout the duration of the exotherm test. Table 1 shows the collected data for both maximum temperature and setting time for each storage condition and period, and indicates which samples were not included due to incomplete polymerization. Of the storage periods, samples from the one-month storage period had the most samples that did not polymerize fully during testing, followed by the two-month storage period. This is most likely due to the cement cartridges that were used during one and two month period. Cements made for testing of these periods were polymerizing prior to use, most likely due to contamination of chemicals used.

SAMPLE	Max Temp	Set Time	SAMPLE	Max Temp	Set Time
1CC1			2UR1	66.73	44.86
1CC2	60.94	16.33	2UR2	50.69	31.02
1CC3			2UR3	55.37	17.45
1CC4	56.63	11.36	2UR4	52.83	15.03
1CR1	65.3	8.91			
1CR2	47.92	16.92	4CC1	51.1	11.9
1CR3			4CC2	39.15	16.13
1CR4			4CC3	72.66	5.78
1UC1	63.46	13.13	4CC4	45.08	14.2
1UC2			4CR1	64.84	9.47
1UC3	49.08	23.75	4CR2	53.52	7.22
1UC4	74.82	9.73	4CR3	32.61	18.55
1UR1			4CR4	41.13	17.6
1UR2	70.49	14.27	4UC1	47.46	17.18
1UR3	50.87	37.25	4UC2	75.33	11.95
1UR4	77.04	17.48	4UC3	72.66	5.78
			4UC4	73.8	6.68
2CC1			4UR1	35.06	13.73
2CC2			4UR2	43.16	10.6
2CC3	54.62	19.65	4UR3		
2CC4	82.77	14.7	4UR4	47.92	11.25
2CR1	60.43	8.25			
2CR2	62.15	15.08	C1	43.34	20.67
2CR3	73.97	10.44	C2	39.85	31.82
2CR4			C3	68.1	10.23
2UC1	63.18	18.53	C4	61.52	15.88
2UC2	71.8	17.5			
2UC3	63.29	13.12			
2UC4	68.67	14.97			

Table 1: This table shows exotherm data collected for all samples tested. Data samples are labeled by month (1,2,3,4), condition (Covered cold CC, uncovered cold UC, covered room temperature CR, and uncovered room temperature UR, and sample number (1,2,3,4), respectively. Highlighted rows indicate incompletely polymerized samples. Nine of out of fifty-two samples tested did not completely polymerize.

Average maximum temperatures produced from BPO-coated nozzles at tested storage periods of one week, one month, two months and four months in each of the four storage conditions is shown in Figure 8. Samples made from nozzles stored for six months or greater did not polymerize and are thus not shown in the data. Based on ASTM standards of acrylic bone cements, the maximum acceptable temperature of powder-liquid cements is 90 °C, and previous studies of commercial cements reveal that the average maximum exotherm typically ranges from 71 to 90°C.^{10,12,13} In previous testing of TSBC, maximum temperatures with values of 82 ±16 °C and 77 ±6 °C were found.^{6,14} Results of exotherm testing reveal that maximum temperatures reached by the cement samples polymerized through the coated nozzles, no matter the storage condition, were much lower than those seen in previous exotherm experiments of TSBC, however this is in keeping with the lower temperatures seen in previous testing using BPO-coated nozzles. This result could be due to limitations in the amount of BPO released from the coating as the cement passes through the nozzle during injection. With less BPO available to polymerize, the reaction slows and does not entirely complete, resulting in a lower maximum temperature. Although lower maximum temperatures are desired in bone cements in order to prevent damage to surrounding soft tissues due to thermal necrosis, temperatures that are too low can negatively influence the mechanical properties, the amount of residual monomer present within the cement samples, and prevent the complete polymerization of the samples.

It was hypothesized that the maximum temperature would decrease with storage time and that exposure to light and heat would amplify this effect. Figure 8

illustrates the results of the exotherm tests with regards to maximum temperature in graphical form. Using single-factor analysis of variance (ANOVA) with a p-value less than 0.05 to determine statistical significance, the effects of storage condition by storage period and effects of storage period by storage condition were studied. Analysis revealed that maximum exotherm for four-month storage samples, regardless of condition, was significantly lower than one-month and two-month storage samples. This finding is in support of the hypothesized effects of storage time on exotherm temperatures. Using standard deviation calculations, further analysis following ANOVA results regarding temperatures reached for one, two and four month conditions determined that there was no significant difference in maximum exotherm between one month and two month storage periods. There were no further significant differences found in maximum temperatures across storage times or conditions.

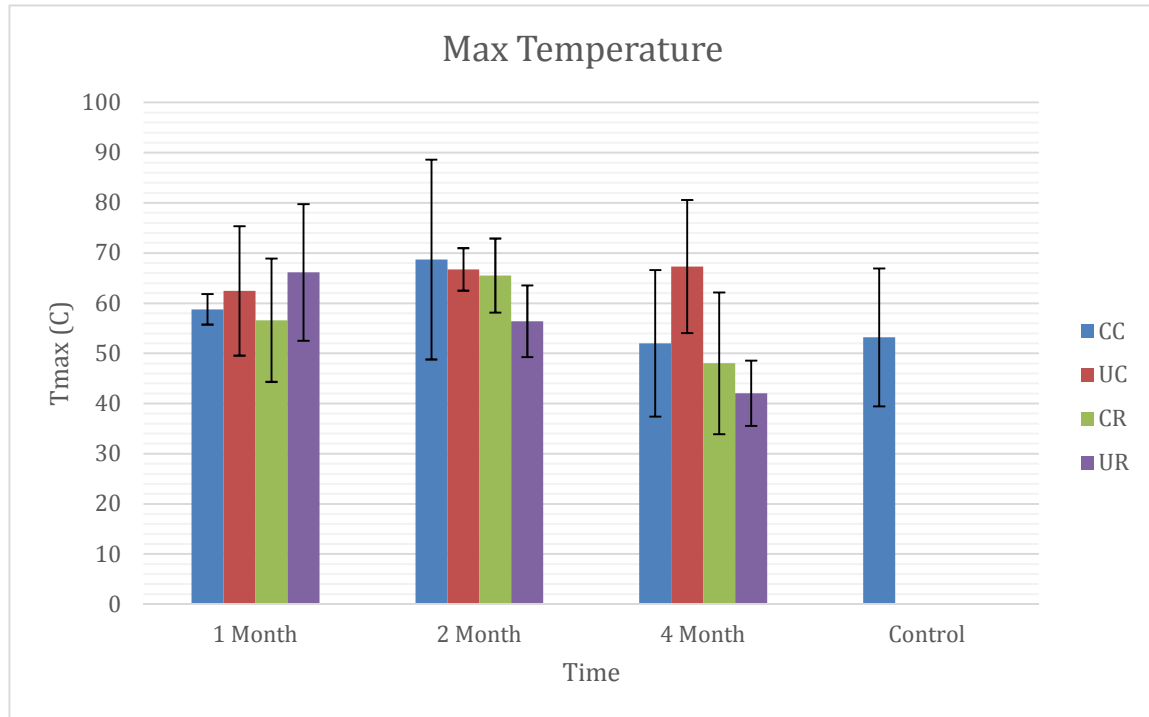


Figure 8: This graph compares the maximum temperatures of cements stored at various storage periods and conditions: covered cold (CC), uncovered cold (UC), covered room temperature (CR) and uncovered room temperature (UR). This graph illustrates that the maximum temperatures at four-month conditions are significantly lower than for one and two month conditions.

The lack of significant differences between maximum temperatures within conditions and storage conditions in turn do not support the hypothesis that storage conditions and times affect maximum exotherm. This can be explained by high variability within samples. It can also be observed in Figure 8 that a majority of the conditions have relatively large standard deviations that overlap with other condition averages, indicating visually that there is not much difference between each storage condition. The high variability is most likely due to the coating methodology of the nozzles. It was observed throughout experimentation that the coating method resulted in an uneven thin-film coating of the nozzles, with regions of high solution accumulation

and regions lacking solution coating. In some cases, this accumulation of solution formed large debris, and during the injection of multiple cements into the exotherm mold, the nozzle had to be trimmed to make a larger opening in order for cement with large debris to continue to flow. This uneven distribution of BPO and large clumps of solution could cause some samples within the same storage conditions to have higher or lower exotherm temperatures. High variability could also be caused in part by the cements used to create samples from coated nozzles, most notably in storage periods of one and two months, as those were the cements of which a number were observed to polymerize prematurely, cause higher numbers of unpolymerized samples, and could have been affected by chemical contamination. The amount of samples that were not included due to incomplete polymerization also led to some storage conditions with sample sizes of less than three, which could explain unexpected temperature averages for certain storage conditions.

Setting Time

According to ASTM standards, setting times between five and fifteen minutes are acceptable.¹⁰ Studies of commercial cements show average setting times of eleven minutes^{8,14} and previous studies for standard TSBC recorded setting times of 6.5 to 8.5 minutes.^{12,13,16} Prior tests on BPO-coated nozzles at similar concentrations show setting times of 8.57 ± 1.70 minutes. The average setting times shown in Figure 9 for BPO coated nozzles at various storage conditions are much longer than that of previously tested TSBC without coated nozzles, which is most likely due to a decrease in available BPO from inconsistent nozzle coatings. If there are limited radicals present to initiate

the polymerizations reaction because BPO remains trapped within film accumulations, then the time it takes for the cement to cure will be extended. While the majority of the setting times are within the ASTM standard accepted range, around twelve to fifteen minutes, there are cements that have setting times that extend upwards of twenty minutes and do not meet the conditions of commercialized cements. An extended cement setting time could allow a surgeon more time for manipulation with a desired viscosity, however TSBC is already characterized by this ideal viscosity upon injection and excess handling time is not as necessary as it is for commercial powder-liquid cements.

Resulting setting times are also greater than that of previously tested BPO coated nozzles, especially in storage periods of one and two months. This result could be explained by the use of cements for these two testing periods that could have been made from contaminated chemicals, causing cement cartridge polymerization prior to testing and possibly unpolymerized or much more slowly-polymerized samples that lead to longer setting times. All successfully polymerized samples made from coated nozzles were included in calculations, while samples that did not completely polymerize and therefore lacked complete data collection were not included. Setting time data for each sample, used to create the graph in Figure 9, as well as an indication of which samples were unpolymerized, can be found in Table 1.

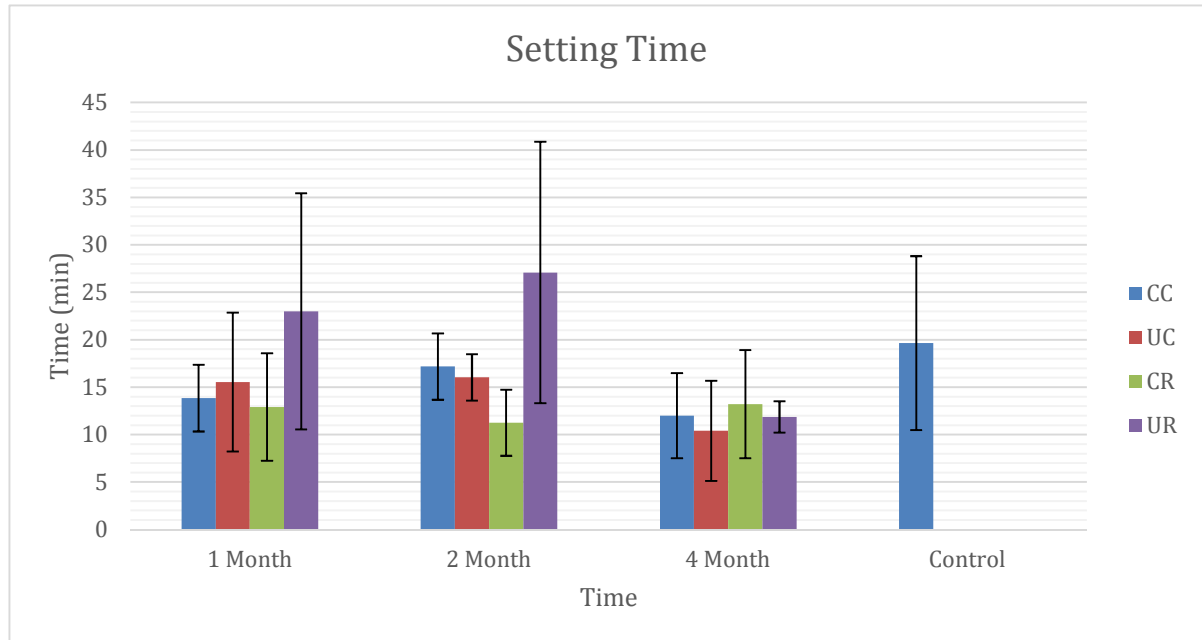


Figure 9: This graph compares the setting times of cements stored at various storage periods and conditions: covered cold (CC), uncovered cold (UC), covered room temperature (CR), and uncovered room temperature (UR). There is no statistically significant difference among groups, most likely due to large standard deviations that can be seen within each condition on the graph.

It was hypothesized that as storage time increased, that setting times would increase, and that exposure to heat or light would increase this effect. The hypothesis is not statistically confirmed through the results, as single factor ANOVA did not indicate any significant differences with a p-value less than 0.05 across storage conditions or storage periods. Although not significantly different, it can be seen across different storage conditions on the graph shown in Figure 9 that samples made from uncovered nozzles stored a room temperature seem to have the greatest setting times, which is in keeping with the proposed hypothesis. Uncovered nozzles at room temperature are exposed to the most light and heat of all storage conditions, allowing for increased degradation of the BPO in the nozzle coating solution that will in turn lengthen the

setting time due to decreased polymerization. Alternatively, and differing from the hypothesis, it seems as though the four-month storage period has an overall shorter setting time, which could be explained by the use of testing cements that were made from new chemicals. No other trends are plainly visible within the graph.

The lack of significant differences between storage periods and conditions is most likely due to the high variability within each data set, which can be observed in the graph shown in Figure 9 in the form of relatively large standard deviations that overlap across the majority of the storage conditions. Variability, similar to that found in the maximum temperature results, was most likely caused by the inconsistent nozzle coating that in turn led to certain samples with greater or lesser amounts of BPO. Polymerization reactions may have occurred more slowly or to a lesser degree due to decreased availability of BPO, increasing the setting times. High variability in setting times could also be caused by varied initial temperatures of the cement mold. When some samples were tested in sequence, not necessarily of the same storage condition, the starting temperature of the mold was slightly higher for subsequent samples, which could affect the average setting times calculated. In addition, the incomplete polymerization of nine total samples, some of which were in the same storage condition and time, created conditions that were characterized by only two samples instead of four, and thus mean setting times, particularly the covered and refrigerated conditions of one month and two month storage periods, may be skewed and misrepresentative of the storage condition as a whole.

Three-Point Bend Mechanical Testing

Mechanical samples for three-point bend testing were determined adequate for testing if they were free of irregularities that could effect flexural performance.

Common imperfections included incomplete polymerization, air bubbles, and BPO clumps. Both the extent and location of the defects were taken into account, as flaws such as air bubbles and BPO clumps within the sample can influence fracture and crack propagation during mechanical testing. Samples with incomplete polymerization often resulted in partial samples that could not be tested, and usually occurred within the first two sample positions in the mold. Samples made from nozzles stored for six months or greater did not polymerize at all, and are thus not shown in the data. Flaws within samples usually occurred within the first three sample positions in the mold, as initial loss of loosely attached BPO clumps cause incomplete polymerization, stickiness, and air bubbles early during injection. Thus, more samples that went through three-point bend testing came from samples injected later into the cement mold, suggesting that there were many cement inconsistencies within nozzle coatings and that the current coating method may need to be improved.

Stress

Recent experiments using TSBC at a ratio of 1.25 g BPO/100 ml MMA with 0.7 ml DMPT/100 ml MMA showed stress values of 80 ± 12 MPa.^{8,15,16} A range of average stresses between 56.6 ± 13.5 MPa to 68.4 ± 12.2 MPa were found in a previous study of BPO coated nozzles.⁵ The stress values found for coated nozzles stored at various periods and conditions, shown in Figure 10, are much lower than that of standard TSBC,

however are in keeping with maximum stresses previously reported for coated nozzles. It should be noted that mechanical stress studies, both of commercial cements and TSBC, have shown high variability among similar cements.¹⁷

It was hypothesized that at increased storage times of coated nozzles as well as at uncovered and room temperature conditions, the resulting stress would decrease. Statistical analysis of the testing data revealed results that both did and did not support the proposed hypotheses. Using single factor ANOVA, it was determined that there is no difference in strength as a function of storage condition within each storage period. The high variability within storage conditions is similar to high variability reported in previous bone cement studies. While traditional bone cement may produce variable samples due to processes such as hand-mixing, inconsistent nozzles coatings leading to incomplete mixing of BPO and other cement components may have similarly resulted in variable samples. In addition, while there are ideally twelve samples for fracture testing, many samples were not included due to cement defects that could affect flexural testing results and smaller sample sizes for certain conditions may not best represent stress values. There could also be samples included in the study that had minute defects that went unnoticed, but may have affected the mechanical properties of the cement sample.

Analysis of effects of storage time by storage condition did reveal that for every storage condition, there is a significant effect of storage time on the strength of the bone cement. This result was determined with p-values of 1.61E-05, 0.00076, 0.001431, and 0.007297 for covered cold, uncovered cold, covered room, and uncovered room

respectively. Calculations showed that samples made from the four-month nozzles have a significantly higher strength than samples made from one and two-month nozzles in the covered cold, uncovered cold, and covered room temperature conditions. This result does not follow the predicted trend of decreasing stress with increasing storage times, which could be attributed to issues, specifically early polymerization within cement cartridges and incomplete sample polymerization, that were seen more often with the testing cements for one and two month periods.

It was determined, however, that there is a significant difference between the one-month and two-month groups for the uncovered room temperature storage condition. It can be seen in the graph of Figure 10 that the maximum stress found in the two-month uncovered room condition is much lower than that of the stress found in the one-month uncovered room condition. This decrease in stress that follows an increase in storage time is in keeping with expected results, and could be due to the fact that uncovered nozzles stored at room temperature is the storage condition with the most exposure to heat and light, and therefore effects of time on cement properties may be more pronounced than in other storage conditions.

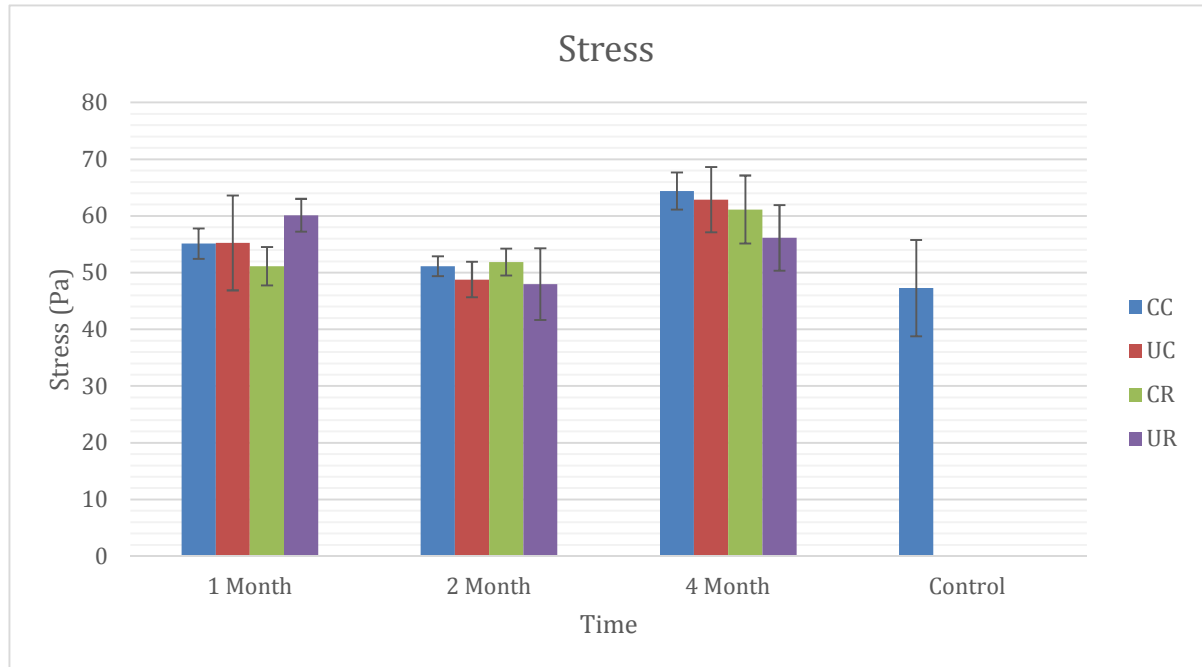


Figure 10: This graph shows the flexural stress values for cements made from coated nozzles stored for various periods at various storage conditions: covered cold (CC), uncovered cold (UC), covered room temperature (CR) and uncovered room temperature (UR). This graph illustrates that stresses are increased for the four-month storage condition.

Strain

Studies on TSBC using 0.7mL of DMPT reported strain-to-failure values of $5 \pm 3\%$.^{8,15,16} The results in Figure 11 show that the majority of the coated nozzle samples tested between one week control period and four months have strain-to-failure values within range of standard TSBC, however the one month period seems to be slightly higher than previously reported cement strain values and two month period seems to be much higher in value. During flexural testing of coated nozzle samples, the majority of samples from the two and one-month storage periods did not break, which could explain the larger reported strain-to-failure values. Samples that did not break during flexural testing in a brittle manner may have an increased amount of residual monomer

present in the cement. It has been shown in literature that an increase in the amount of residual monomer acts as a plasticizer of the polymerized cement matrix.¹⁸ There may be an increase in residual MMA in the cement samples from one and two-month storage conditions because of underactive or contaminated chemicals that may have been present in cements used during those testing periods. Increased residual MMA is not desired in bone cements, as it has the potential to cause chemical necrosis to surrounding tissues.

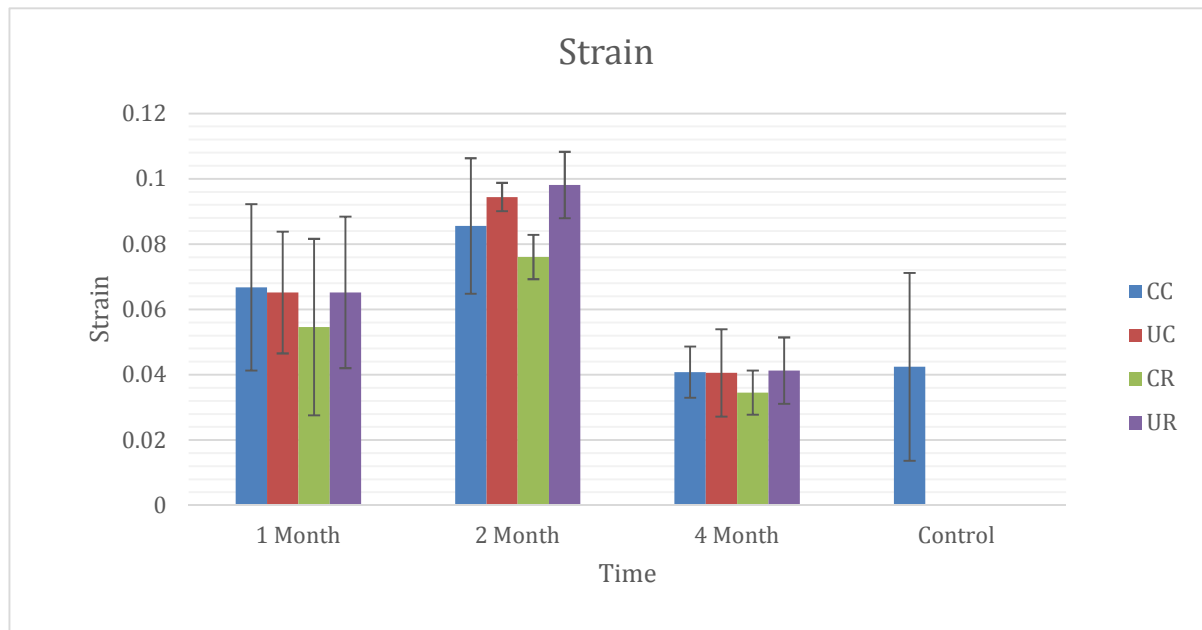


Figure 11: This graph shows flexural strain values for cements made from coated nozzles stored for various periods at various storage conditions: covered cold (CC), uncovered cold (UC), covered room temperature (CR) and uncovered room temperature (UR).

By applying single factor ANOVA to collected strain-to-failure data, it was determined that there is no difference in strain to failure as a function of storage condition within each storage time. This is not in support of the hypothesis that strain values would increase with exposure to heat and light. While not statistically significant,

it can be observed from the graph that strain-to-failure values for samples made from nozzles stored uncovered at room temperature are higher than strain values for cements made from nozzles covered at room temperature across all storage conditions. This observed trend, an increase in strain with an increase in exposure to light, supports the hypothesis that increased light would cause degradation of BPO, in turn causing a decrease in polymerization and an increase in residual monomer that can cause increase in strain. It must be noted though, that the sample sizes are small and the variability is high, so testing with more samples is needed in order to verify this trend with statistically significant results.

Additionally, statistical analysis revealed that for every storage condition, there is a significant, with a p-value less than 0.05, effect of storage time on the strain-to-failure of the bone cement. Specifically, samples made from the four-month nozzles have a significantly lower strain for all storage conditions when compared with two-month and one-month storage conditions. This trend can be seen plainly on the graph shown in Figure 11, with strain values across the four-month period of approximately 4%, which is 2-5% lower than for that of one and two-month periods. This is not in support of the hypothesis that states strain-to-failure values would increase with increased storage conditions. The coating method of the nozzles was consistent across all storage periods, so this outcome may be explained by the difference in cement composition of the four month, as newer chemicals were used.

Modulus

Figure 12 illustrates the results of flexural modulus calculations resulting from the mechanical testing of cement samples made from nozzles stored at increasing time periods stored at each of the four established storage conditions. The flexural modulus found in previous TSBC studies presented values of approximately 2.5 ± 0.25 GPa,^{8,15,16} while a previous study on coated nozzles reported modulus between 1.63 ± 0.421 GPa and 2.22 ± 0.168 GPa.⁵ Modulus values found in this study are less than that of standard TSBC and similar to that of prior tests on coated nozzles. Smaller modulus of bone cement could be beneficial for reducing material stiffness. With a decrease in stiffness, there is improved cement elasticity, allowing for recovery after deformation. Like the increase in strain-to-failure values, the decrease in modulus may be explained by increased residual monomer. In both previous coated nozzles studies and in these results, the coating method of the nozzles may have prevented BPO from mixing and reacting completely with other cement components, decreasing the degree to which the polymerization reaction occurs and causing some of the MMA to be leftover.

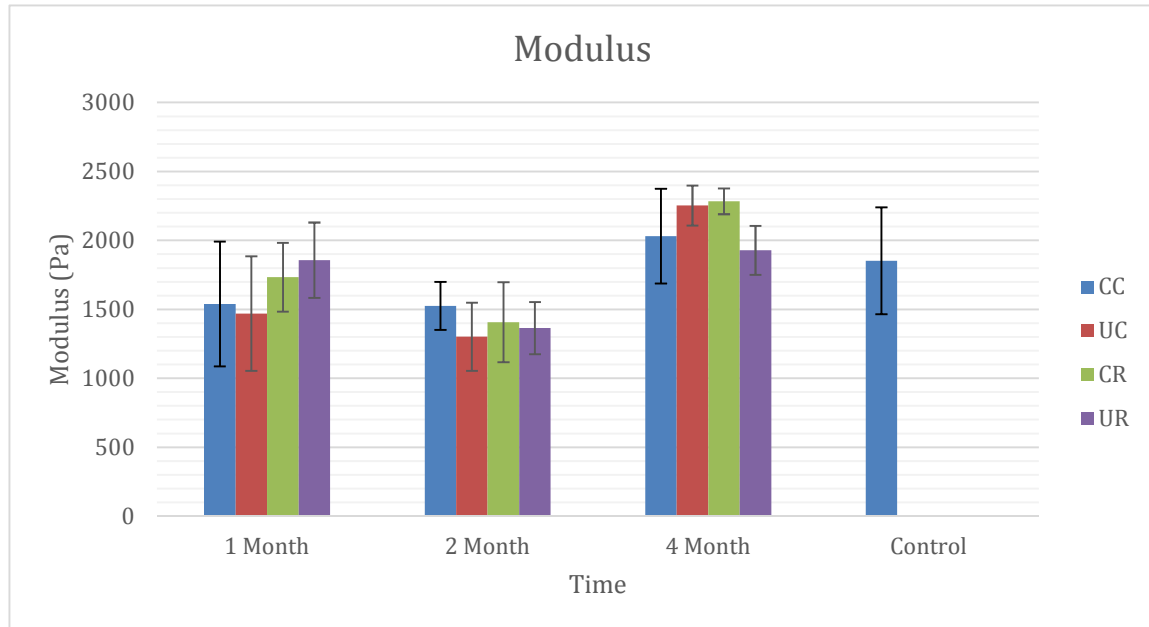


Figure 12: This graph shows the modulus values for cements made from coated nozzles stored for various periods at various storage conditions: covered cold (CC), uncovered cold (UC), covered room temperature (CR) and uncovered room temperature (UR). It can be seen that modulus for the four month period is significantly higher than that of other storage times. In addition, within the four-month period, the uncovered room condition has decreased modulus values when compared with the covered room condition.

It was hypothesized that an increase in temperature and light exposure would decrease modulus, and statistical analysis using single factor ANOVA to determine the effect of storage condition on modulus across a single storage period confirmed this hypothesis for a single storage condition. It was found with a p-value of 0.01, that there is a significant effect of storage condition on the modulus at the four-month storage period. The covered room temperature nozzles produced cements with higher modulus than that of uncovered nozzles stored at room temperature. This is most likely due to the increased exposure of uncovered refrigerated nozzles to light, causing a degradation of BPO that in turn causes decreased cement polymerization, decreased molecular weight, and a decrease in mechanical performance. The remainder of the storage

periods did not show significant difference across storage periods, most likely to do high variability caused by nozzle coatings that do not consistently release BPO into injected cement.

Analysis also revealed that across every storage condition except nozzles that were both covered and refrigerated, there is a significant effect of storage time on the modulus of the bone cement. Samples made from the four-month nozzles have a significantly higher modulus for uncovered room, uncovered refrigerated and covered refrigerated, with reported p-values of less than 0.001. This is not in support of the hypothesis that increased storage times would decrease modulus, and could be explained by the cements used for one and two month periods that may not have allowed full polymerization of cement samples due to contamination of chemical components involved.

Conclusion

Inconsistencies were common in the resulting thermal and mechanical characteristics of cements produced from coated nozzles within each storage condition and storage period. Although results varied amongst condition samples, the study has shown that removing benzoyl peroxide from the bulk of the cement and incorporating it into the mixing nozzle as a thin film resulted in a general reduction in thermal and mechanical properties in comparison to standard TSBC and current commercial cements.

In regards to exothermal testing, the overall results showed lower maximum temperatures and longer setting times than standard TSBC and current commercial cements. Although this could allow for reduction of cellular damage, low temperatures have a large influence on the incomplete polymerization of bone cements and the resulting excess monomer present within the cement that affects mechanical properties and can be toxic to surrounding tissue. Future studies might involve establishing a minimal threshold temperature at which cement properties will deteriorate.

Mechanical testing revealed lower stress, higher strain, and lower modulus for cements made with coated nozzles when compared to standard TSBC and commercial cements. While decreasing the brittle nature of bone cement is desired for some applications, these results were most likely due to an increase in residual monomer that increases plasticity of the cement. Residual monomer is potentially toxic to surrounding tissues in the body, so reducing the amount of unreacted MMA within cements made from coated bone cements, specifically focusing on the consistent release of BPO from

the nozzle, could be the objective of future studies. In addition, mechanical property results of cements made with coated nozzles were most likely impacted by the large amount of mechanical samples that were not suitable for testing due to size and positioning of defects. A majority of samples made with the coated nozzles exhibited air bubbles and BPO clumps. This could be improved with a better nozzle coating technique.

Both the reduction in cement properties and high variability in testing results can be attributed to the method of nozzle coating. Coating methods used within this study led to unpredictable BPO release and to inconsistent solution coverage throughout the nozzle, with areas of high solution accumulation with chunks of BPO and areas lacking solution coverage. As bone cement passes through the nozzle, there is significant variation in BPO concentration in the cement that exits, with loose particles of BPO towards the beginning of the cement stream. This study confirmed previous work with BPO coated nozzles in that the solvent casting technique is a functional method for introducing BPO into bone cement, however has numerous issues such as solution loss, inconsistently coated films, and inconsistent BPO release. These findings suggest that there are significant improvements that must be made to the even distribution of the polymer coatings. Future work may involve investigating alternative coating methods with improved thin films.

Despite variability of cement samples, the majority of mechanical and exotherm testing results reveal that storage time has an effect on cement properties more so than storage conditions. Significant differences between the four-month period and the one

and two-month periods were found in four out of five of the reported cement properties. There was only one case of cement properties varying significantly from one storage condition to another. It was difficult to compare the effects of storage conditions on cement properties due to lack of control cement results and possible contamination of cement chemicals used to make a number of cement samples. In order to support that an increase in nozzle storage time has a deleterious effect on cement properties, additional data and many more repetitious trials would be necessary. A larger number samples would have to be tested in a similar shelf-life study in order to account for the large variability that occurs due to the coated nozzles and the inconsistent BPO release. Due to problems with the cements, further studies would involve repetition of storage conditions and periods using cement that was determined to be viable.

Most notably, it can be concluded from the results that BPO coated nozzles stored beyond four months are no longer viable. Testing of cement samples made with nozzles of the six-month storage period, regardless of storage condition, did not result in any completely polymerized samples, and any change in temperature found in exotherm testing was minute. It can be determined from this study that at some time between four and six months into storage, the BPO within the nozzle thin film coating was degraded to such a degree that a polymerization reaction was not initiated. Future studies would look more closely at the time period between four and six months in order to more precisely determine the point at which coated nozzles have reached the extent of their shelf life. Coated nozzles have the potential to serve as improved

alternatives to traditional bone cements and standard two-solution bone cements, however a more in depth investigation into their shelf life, and more generally, into methods of thin film coating methods, is required.

References

1. Weinstein, J. N. "Balancing Science and Informed Choice in Decisions about Vertebroplasty." *The New England Journal of Medicine* 361 (2009): 619-21.
2. Burton, A. W., L. D. Rhines, and E. Mendel. "Vertebroplasty and Kyphoplasty: A Comprehensive Review." *Neurosurgical FOCUS* 18.3 (2005): 1-9.
3. Lewis, G. "Viscoelastic Properties of Injectable Bone Cements for Orthopedic Applications: State-of-the-art Review." *Journal of Biomedical Materials Research B: Applied Biomaterials* 98B.1 (2011): 171-91.
4. Kuehn, K., W. Ege, and U. Gopp. "Acrylic Bone Cements: Composition and Properties." *Orthopedic Clinics of North America* 36 (2005): 17-28.
5. Huffman, K. R. "A Novel Approach for Incorporating Benzoyl Peroxide in Two Solution Bone Cement." *Masters Thesis, Syracuse University.* (2013)
6. Hasenwinkel, J. M., E. P. Lautenschlager, R. L. Wixson, and J. L. Gilbert. "A Novel High-viscosity, Two-solution Acrylic Cement: Effect of Chemical Composition on Properties." *Journal of Biomedical Materials Research* 47.1 (1999): 36-45.
7. Lewis, G. "Alternative Acrylic Bone Cement Formulations for Cemented Arthroplasties: Present Status, Key Issues, and Future Prospects." *Journal of Biomedical Materials Research B: Applied Biomaterials* 84.2 (2008): 301-19.
8. Rodrigues, D. C., J. L. Gilbert, and J. M. Hasenwinkel. "Two-Solution Bone Cements with Cross-Linked Micro and Nano-Particles for Vertebral Fracture Applications: Effects of Zirconium Dioxide Content on the Material and

- Setting Properties." *Journal of Biomedical Materials Research - Part B Applied Biomaterials* 92.1 (2010): 13-23.
9. Allen, M. J., K. A. Leone, J. M. Hasenwinkel, and J. L. Gilbert. "Tissue Response In Situ Polymerization of a New Two-Solution Bone Cement: Evaluation in a Sheep Model." *Journal of Biomedical Materials Research B: Applied Biomaterials* 79.2 (2006): 441-52.
 10. ASTM F451-08, 2008, Standard Specification for Acrylic Bone Cement. ASTM International, West Conshohocken, PA.
 11. Shim, J. B., S. J. Warner, J. M. Hasenwinkel, and J. L. Gilbert. "Analysis of Shelf Life of a Two-solution Bone Cement." *Biomaterials* 26.19 (2004): 4181-187.
 12. Lewis, G., J. Xu, S. Madigan, and M. R. Towler. "Influence of Two Changes in the Composition of an Acrylic Bone Cement on Its Handling, Thermal, Physical, and Mechanical Properties." *Journal of Materials Science: Materials in Medicine* 18.8 (2007): 1649-658.
 13. Madigan, S., M. R. Towler, and G. Lewis. "Optimisation of the Composition of an Acrylic Bone Cement: Application to Relative Amounts of the Initiator and the Activator/co-initiator in Surgical Simplex®P." *Journal of Materials Science: Materials in Medicine* 17.4 (2006): 307-11.
 14. Hasenwinkel, J. "Optimization of a Novel Two-Solution Poly(methylMethacrylate) Bone Cement: Effect of Composition of Material Properties and Polymerization Kinetics." Diss. Northwestern University, 1999.
 15. Rodrigues, D. C., J. L. Gilbert, and J. M. Hasenwinkel. "Pseudoplasticity and Setting

- Properties of Two-Solution Bone Cement Containing Poly(methyl Methacrylate) Microspheres and Nanospheres for Kyphoplasty and Vertebroplasty." *Journal of Biomedical Materials Research - Part B Applied Biomaterials* 91.1 (2009): 248-56.
16. Rodrigues, D. "Development and Characterization of Multi-Solution Bone Cements Containing Cross-linked PMMA Nanospheres and Nanospherical Polymer Brushes." Diss. Syracuse University, 2010.
17. Bridgens, J., S. Davies, L. Tilley, P. Norman, and I. Stockley. "Orthopedic Bone Cement: DO WE KNOW WHAT WE ARE USING?" *Journal of Bone and Joint Surgery - British Volume* 90-B.5 (2008): 643-47.
18. Vallo, C. I. "Residual Monomer Content in Bone Cements Based on Poly(methyl Methacrylate)." *Polymer International* 49 (2000): 831-38.
19. Dunne N., Ormsby R,W.(2011). MWCNT Used in Orthopaedic Bone Cements, Carbon Nanotubes - Growth and Applications, Dr. Mohammad Naraghi (Ed.)