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14. ABSTRACT Even though commercial bone cements have not significantly changed in the past 50 years and have been used throughout the world, there are significant drawbacks with the current systems. We have developed a silorane based resin superior to polymethyl methacrylate (PMMA) with many improved properties such as significantly less polymerization stress without an associated reduction in mechanical properties. The specific aims for this project are: Specific Aim 1: Develop a silorane bone cement suitable for in vivo studies and to optimize the formulation of the chemically and mixed cured cement prototypes. Specific Aim 2: Determine the biocompatibility properties and wear debris generation of silorane bone cement prototype. Specific Aim 3: Determine the biological response to silorane bone cement prototype in animal models. By addressing the shortcomings of current PMMA bone cement, the development of the novel silorane bone cement will result in a paradigm shift in orthopedic biomaterials.					
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Yearly Report

Award No.: W81XWH-11-1-0805

Report Date: June 2014

Reporting period: 20-Sept-2011 to 20-May-2014

Principal Investigator: Dr. David Eick (corresponding PI: Dr. Lynda Bonewald)

Award Organization: University of Missouri-Kansas City

Project Title: Bone Repair and Military Readiness

INTRODUCTION:

Even though commercial bone cements have not significantly changed in the past 50 years and have been used throughout the world, there are significant drawbacks with the current systems. These include toxicity, contraction with polymerization, and heat generation. We have developed a silorane based resin superior to polymethyl methacrylate (PMMA) with many improved properties such as significantly less polymerization stress without an associated reduction in mechanical properties. These new resins do not generate cytotoxicity, antigenicity, polymerization stress or significant heat generation. In addition, it appears that this new bone cement is actually supportive of new bone formation. Orthopedic surgeons have had to adapt surgical techniques to account for issues with cementing total joint prostheses and subsequent total joint failures. The cement-bone interface is problematic, as there is no true bonding of cement to bone, only interlay in the trabecular spaces. A cement that can achieve true integration with the bone surface would be advantageous in that it would improve stress transfer to bone and decrease particulate wear. This integration, in turn, could result in improved bone stock if the need for revision arises. Bone infection with prosthetic devices is an increasing major medical problem. As the proposed bone cement prototype polymerizes at a much lower temperature, antibiotics that are sensitive to heat can be added to the cement. Currently, only tobramycin, gentamycin and vancomycin are heat-stable and survive the heat generated by commercially available bone cement during polymerization. Therefore, a wider spectrum of antibiotic availability in bone cement may allow for more appropriate treatment of patients. By addressing the shortcomings of current PMMA bone cement, the development of the novel silorane bone cement will result in a paradigm shift in orthopedic biomaterials.

The specific aims for this project are:

Specific Aim 1: Develop a silorane bone cement suitable for *in vivo* studies and to optimize the formulation of the chemically and mixed cured cement prototypes.

Specific Aim 2: Determine the biocompatibility properties and wear debris generation of silorane bone cement prototype.

Specific Aim 3: Determine the biological response to silorane bone cement prototype in animal models.

Keywords: bone cement, silorane, prosthetic

Original Task Timeline

FY10 Task 1 Develop a silorane bone cement suitable for in vivo studies and to optimize the formulation of the chemically and mixed cured cement prototypes, Subtask 1a. Silanization of filler particles. Months 1-12.

FY10 Task 1: Develop a silorane bone cement suitable for in vivo studies and to optimize the formulation of the chemically and mixed cured cement prototypes, Subtask 1b. Optimize composite formulation with respect to mechanical/handling properties. Months 13-24.

FY10 Task 2: Determine the biocompatibility properties and wear debris generation of silorane bone cement prototype, Subtask 2a. Determine biocompatibility of the optimized chemically initiated silorane bone cement identified in Specific Aim 1 with relevant cell lines (i.e., MLO-A5, MSCs, L929, and HUVEC). Months 1-24.

FY10 Task 3: Determine the biological response to silorane bone cement in animal models, Subtask 3a. Small Animal (Rat) Model. Months 13-18

FY10 Task 3. Determine the biological response to silorane bone cement in animal models, Subtask 3b. Large Animal (Swine) Model. Months 16-24.

Revised Task Timeline

FY10 Task 1 Develop a silorane bone cement suitable for in vivo studies and to optimize the formulation of the chemically and mixed cured cement prototypes, Subtask 1a. Silanization of filler particles. Months 1-12.

FY10 Task 1: Develop a silorane bone cement suitable for in vivo studies and to optimize the formulation of the chemically and mixed cured cement prototypes, Subtask 1b. Optimize composite formulation with respect to mechanical/handling properties. Months 13-24.

FY10 Task 2: Determine the biocompatibility properties and wear debris generation of silorane bone cement prototype, Subtask 2a. Determine biocompatibility of the optimized chemically initiated silorane bone cement identified in Specific Aim 1 with relevant cell lines (i.e., MLO-A5, MSCs, L929, and HUVEC). Months 1-36.

FY10 Task 3: Determine the biological response to silorane bone cement in animal models, Subtask 3a. Small Animal (Rat) Model. Months 13-24

FY10 Task 3. Determine the biological response to silorane bone cement in animal models, Subtask 3b. Large Animal (Swine) Model. Months 24-36.

Revised Grant Chart

Task	Y1Q1	Y1Q2	Y1Q3	Y1Q4	Y2Q1	Y2Q2	Y2Q3	Y2Q4	Y3Q1	Y3Q2	Y3Q3	Y3Q4	Status
Specific Aim 1 a													Completed
b													Completed
Specific Aim 2 a													Extended
Specific Aim 3 a													Completed
b													Extended

Green = Completed

Blue = Extended

OVERALL PROJECT SUMMARY:

Progress for year three from end of year two to date:

FY10 Task 1 Develop a silorane bone cement suitable for in vivo studies and to optimize the formulation of the chemically and mixed cured cement prototypes, Subtask 1a. Silanization of filler particles. Months 1-12. COMPLETED.

Conclusion: The DY5-1TOSU system of glass powder-surface silanation composition appears optimal. The system shows consistently higher strengths and metal-bone adhesion strength upon proper control of the initial formulation moisture content. Silanation with 1TOSU provides dry, organic interface particles that are readily dispersed into Silmix and support high strength, high extent composite cure.

FY10 Task 1: Develop a silorane bone cement suitable for in vivo studies and to optimize the formulation of the chemically and mixed cured cement prototypes, Subtask 1b. Optimize composite formulation with respect to mechanical/handling properties. Months 13-24. COMPLETED

Conclusion: The optimal system is composed of the 65 wt% DY5-1TOSU, 0.40 wt% LMC, and 34.60 wt% LCSM using dry filler and dry comonomers.

FY10 Task 2: Determine the biocompatibility properties and wear debris generation of silorane bone cement prototype, Subtask 2a. Determine biocompatibility of the optimized chemically initiated silorane bone cement identified in Specific Aim 1 with relevant cell lines (i.e., MLO-A5, MSCs, L929, and HUVEC). Months 1-36. COMPLETED EXCEPT FOR WEAR DEBRIS EXPERIMENTS.

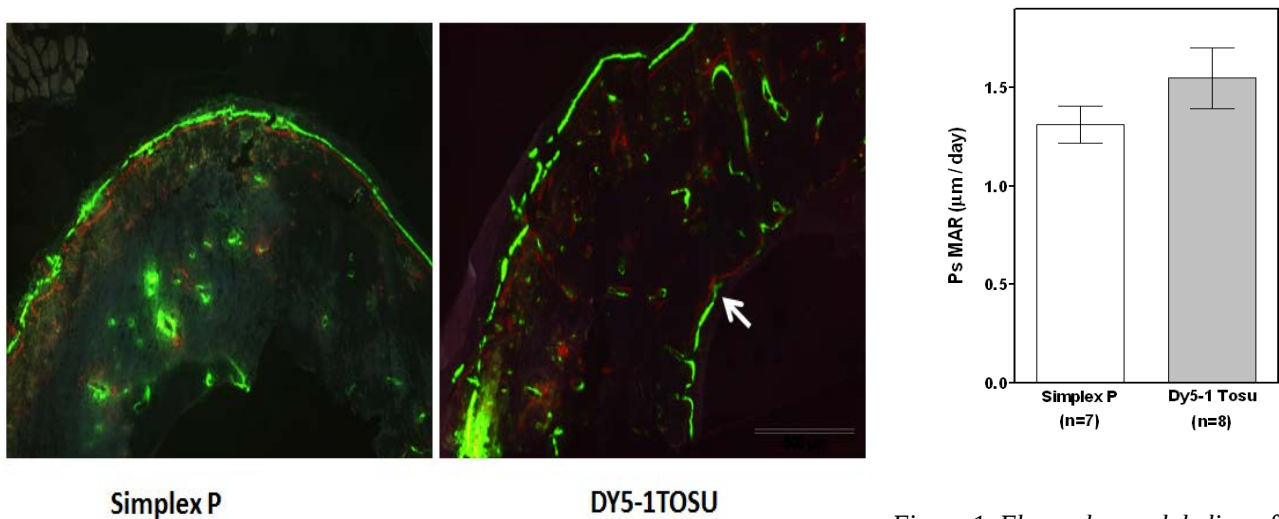
Within the next 3 months wear debris experiments will be performed with the silorane bone cement DY5-1TOSU for comparison to commercially available bone cement. We have now collected the wear debris and have received the cytokine kits (IL-1 β Elisa kit) for testing. Wear debris (5 g of SilMix and 42 g of Simplex P, particle size < 10 μ m) is now available for testing.

FY10 Task 3: Determine the biological response to silorane bone cement in animal models, Subtask 3a. Small Animal (Rat) Model. Months 13-24 COMPLETED EXCEPT FOR HISTOLOGICAL ANALYSES OF IMMUNE REACTION.

Bone formation adjacent to bone cement in vivo.

Experiment 1: Six month old Rat *in vivo* studies for the examination of osseointegration were performed. Simplex P and DY5-1TOSU cements (n= 5-7) were compared. The rats were anesthetized and operated under aseptic conditions. Briefly, the right knee was exposed and a hole was drilled between the femoral condyles and into intramedullary canal. The bone marrow was disrupted. The marrow cavity was irrigated and filled with cement. Then, a titanium rod, 22 mm long and 1.5 mm in diameter, was inserted. The capsule and skin were sutured. We have previously in the last year's report given the results for x-rays showing a periosteal reaction with Simplex P, loss of weight in the first week by rats receiving Simplex P. Injections of fluorochrome intraperitoneally with alizarin red A, and calcein were performed at 2, 4 and 6 weeks PO. The animals were sacrificed at 8 weeks.

Here we show the results of histomorphometry measuring periosteal and endosteal bone formation rates. Endosteal fluorescence double-labeling was observed in DY5-1TOSU group in contrast to no endosteal fluorescent double-labeling in simplex P group (Figure 1). The femurs were longitudinally split and dehydrated in serial ethanol solution. The bone cements in the femur were removed with methyl ethyl ketone. The methyl ethyl ketone was washed off in serial ethanol solution. The samples were dehydrated in serial ethanol solution and infiltrated with acetone and infiltration solution for 5 days placed in embedding solution for polymerization before sectioning.



Simplex P **DY5-1TOSU**

Figure 1. Fluorochrome labeling of cortical bone from 8 mo old rat femur filled with Simplex P or DY5-1TOSU silorane cement. Both periosteal and endosteal double labeling were observed in DY5-1TOSU group. The arrow points to endosteal fluorescence double labeling. The red line: alizarin, the green line: calcein.

Experiment 1: Seventeen 13-month-old rats were used. The right legs were shaved and disinfected with betadine. The skin was incised, and the operation conducted under aseptic conditions (n=9 for Simplex P, n=8 for our bone cement - dry SM Putty DY5-1TOSU 65% filler). The knee joint was exposed, and a 2.2 mm hole was created between the femoral condyles. The bone marrow was reamed and filled with either Simplex P or dry SM Putty DY5-1TOSU 65% filler (SM Putty). The animals were sacrificed after an eight-week period PO. The femurs were harvested and processed for histology. There was no significant difference between the Simplex P and the silorane bone cement (SM Putty) (Figure 2).

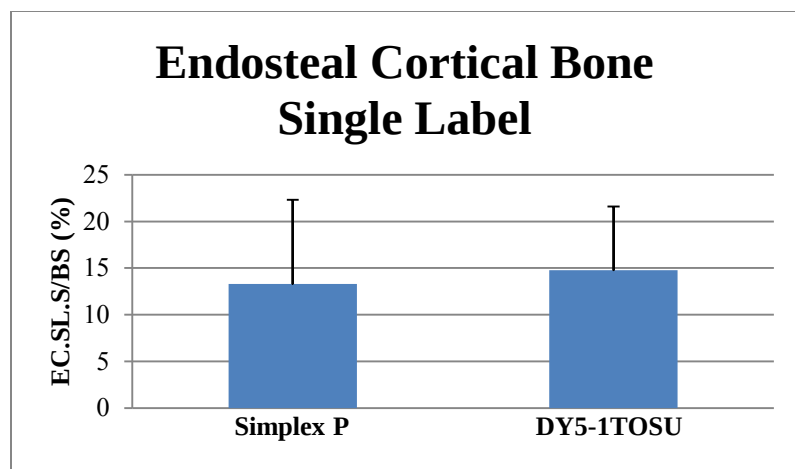


Figure 2. Fluorochrome labeling of cortical bone from 15 mo old rat femur filled with Simplex P or DY5-1TOSU silorane cement. No increase in bone formation was observed on the endosteal surface.

Pull-out strengths of bone cement after eight weeks in vivo:

In this same experiment, the cements were left in the rats for eight weeks to determine effect of time on the pull out strength. Seventeen 13-month-old rats were used. The right legs were shaved and disinfected with betadine. The skin was incised, and the operation conducted under aseptic conditions (n=9 for Simplex P, n=8 for our bone cement - dry SM Putty DY5-1TOSU 65% filler). The knee joint was exposed, and a 2.2 mm hole was created between the femoral condyles. The bone marrow was reamed and filled with either Simplex P or dry SM Putty DY5-1TOSU 65% filler (SM Putty). The animals were sacrificed after an eight-week period PO. The femurs were harvested and immediately tested biomechanically. The pull out strengths for Simplex P (n=7) and our silorane bone cement (n=5) were 6.28 ± 0.44 MPa and 4.94 ± 0.73 MPa, respectively as depicted in the graph below. There was no significant difference between the Simplex P and our silorane bone cement (SM Putty) (Figure 3).

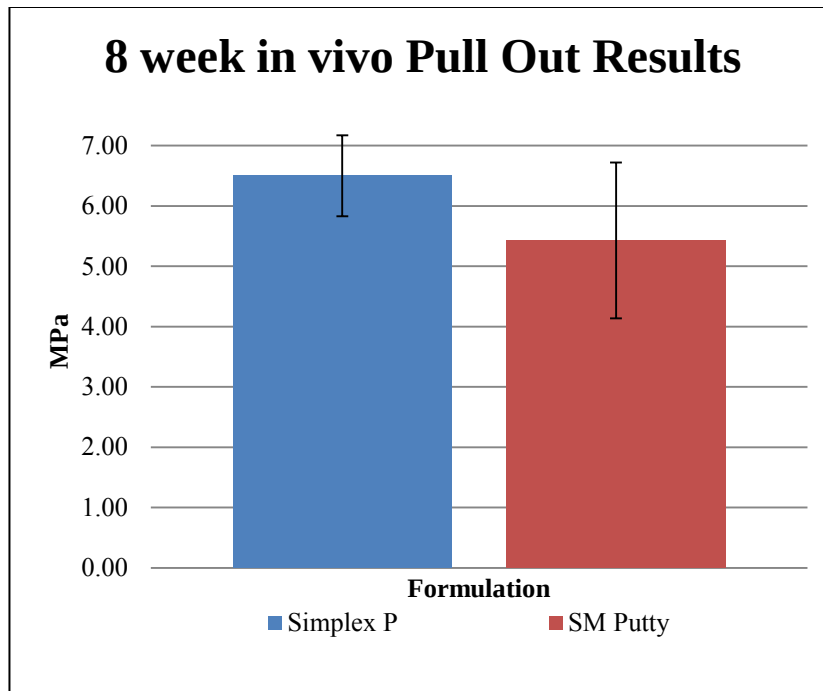


Figure 3. Pull-out strength of different bone cements eight weeks PO in 15 mo old rats.

Conclusion: In summary, *in vivo*, the silorane bone cements are non-toxic, non-inflammatory, and do not inhibit bone formation in contrast to commercially available bone cement which is toxic. However, these silorane bone cements must remain dessicated before use to insure ideal pull out strength. The tissues are now be quantitated for immune reaction.

FY10 Task 3. Determine the biological response to silorane bone cement in animal models, Subtask 3b. Large Animal (Swine) Model. Months 24-36.

Amount of reagents prepared for Swine experiments: Approximately 40 g of cement is required per swine femur and the cement is 35 wt% SilMix, then 15 g of SilMix is required per femur. If 6 swine legs are used, then we will need a total of 90 g of SilMix. There will also be 3 legs for practice (2 *ex vivo* and 1 with a dead pig) for an additional 45 g. There is currently 200 g of dry SM available, which is enough for the swine studies (135 g needed). At this time, there should be a minimum need for dry SM and addition rat studies.

Development and approval of the swine animal protocol. Drs. Bonewald and Kilway traveled to Columbia, Mo Oct. 28, 2013 to meet with Dr. Tim Sidranski and tour the facilities. A plan was made regarding the swine studies. Dr. Eric Walters was put in charge of assisting us with these studies. In January of 2014, an animal protocol was submitted to the Columbia, Mo IACUC based on Oct-Nov discussions. In March, the IACUC wanted to change 1) our pigs to minipigs or 2-3 month old Landrace pigs and 2) facilities. We responded with 1) minipigs are very expensive and our budget would not cover and 2-3 month old pigs are growing and the titanium rod would be overgrown making pull-out studies difficult and 2) that we would need to visit the new facility. A skype meeting with Columbia's ACUC and veterinarians was organized on July 17, 2014 to discuss their concerns regarding the pig protocol. After 8/14/14 meeting with Eric Walters, final revisions/additions were made and resubmitted on 8/21/14. It was approved

9/10/14 and approved by DOD ACURO on 9/19/14.

Development of Pull-Out Apparatus for Pig Femur: Due to the larger femur size of the swine, it was not feasible to modify the pull-out setup used for the rats. A completely new apparatus for the swine pull-out test was developed with Bret Lesan in the engineering department. The prototype should be done and ready for investigational testing by the middle of October.

Obtaining equipment for the swine surgery. We have the following available: surgical guides/reamers, Weitlander retractors, x-ray stand (UM Columbia), manual aspirator system, titanium rods (unetched/will be acid etched) and IV stand. We are still working on purchasing sufficient Simplex P, delivery systems for cements, access to anesthetics/analgesics/antibiotics, and peripheral vascular cuff. We have enlisted the services of Charles E. Wiedmeyer DVM, PhD, DACVP, Associate Professor - Veterinary Clinical Pathology, University of Missouri, Columbia to perform the blood chemistry and cbc.

Consultations with Orthopaedic Surgeons on consistency and handling properties of the silorane cement. We met with three orthopaedic surgeons: Dr. Suhel Kotwal – Orthopaedics, Truman Medical Center, Kansas City MO; Dr. Jonathan Dubin – Orthopaedics, Truman Medical Center, Kansas City MO; and Dr. Donna Pacicca – Orthopaedics, Children’s Mercy Hospital, Kansas City MO. Their observations and comments on the silorane cement are found below.

Dr. Suhel Kotwal: The handling time was fine and had two phases, similar to commercial cement. The silorane was grainy compared to commercial cement, which is due to the higher filler content in the silorane. He liked the low curing temperature. He said he would be able to add a variety of antibiotics to the cement when implanting joints in high-risk patients or revisions.

Dr. Jonathan Dubin: He thought the handling time might be too slow for certain applications but could also potentially cut down surgical times by five minutes or so. We explained that the handling time could be adjusted with the amount of catalyst used. He was very interested in the low curing temperature of the cement. He said it would allow for the incorporation of more kinds of antibiotics and even antifungals. The antibiotics could be used with implantation of joint replacement, but he was more interested in the silorane cement with antibiotics as a space or beads for localized treatments of infections. He was also interested in its use to help stabilize/strengthen osteoporotic bone for screw augmentation.

Dr. Donna Pacicca: The handling time was OK, similar to commercial cement. The silorane was grainy compared to commercial cement. She said surgeons are taught “grainy” cement is bad because it creates voids, so we would need to explain to them “grainy” here is just the higher filler content. She liked the darker color of the silorane cement, as it would make it easy to distinguish bone from cement. The low toxicity and the ability to use a wide variety of antibiotics was appealing, especially in pediatrics. When asked if she would use the silorane cement, she said “if it was proven safe and effective then definitely yes”.

Comments on administrative and logistical matters.

Problems with getting IACUC approval as described above.

KEY RESEARCH ACCOMPLISHMENTS:

- Performed pull-out tests on aged rats of 13-15 months. No significant differences were observed between Simplex P and DY5-1TOSU.

- Performed bone formation histology on 6-8 month and 13-15 month old rats. Bone formation may be occurring in the younger rats, but no significant differences were observed in the older rats.
- Have generated the Simplex P and DY5-1TOSU wear debris for testing.
- Developed a silorane bone cement that has equivalent pull-out strength to commercially available bone cement, but is non-toxic, non-inflammatory, non-exothermic, has low shrinkage, and is potentially osteogenic.
- Received both IACUC and ACURO approval for performing the swine experiments.
- Received a Fastrack Award to fund an FDA consultant for the pig studies and to begin the antibiotic experiments (see below).
- Have planned and executed our Bone Cement Symposium for October 4, 2014. The symposium was quite a success and will be reported at the next quarterly report.

CONCLUSIONS:

We have developed a novel silorane bone cement with excellent properties that is ready for in vivo large animal testing. While conducting the swine studies it will be determined if wear debris from this cement will have any inflammatory or osteoclast activation/resorption properties. We are hopeful that this technology will soon be licensed. We also plan a resubmission of the SBIR for the commercialization of the silorane bone cement. In contrast to commercially available bone cement which is toxic, the silorane bone cement does not cause any weight loss, bone loss, or inflammation in vivo. With the improved biocompatibility, reduced exothermicity, good handling properties, incorporation of antibiotics/growth factors, and potential for osseointegration/osseointegration, this material has potential to be used for screw augmentation, total hip/knee joint replacement, and other orthopedic and dental applications. The reduced curing temperature of approximately 26° C of the dual initiated silorane composite makes it possible to carry and deliver a wide range of antibiotics and potentially growth factors, which previously could not be used in PMMA bone cements. We have overcome our previous issues with strength to produce a material that is on par with commercial bone cement. The development of the silorane bone cement is very promising for application for human use.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

INVESTIONS, PATENTS AND LICENSES: A patent cooperative treaty (PCT) has been published for the innovative chemical initiator systems by UMKC and Nanova will have an exclusive license (still under negotiation).

REPORTABLE OUTCOMES: We have developed a silorane bone cement that has equivalent pull-out strength to commercially available bone cement, but is non-toxic, non-inflammatory, non-exothermic, low shrinkage, and potentially osteogenic that will hopefully be commercialized.

OTHER ACHIEVEMENTS:

Fastrack Award received.

This award for \$50,000 was received from the University of Missouri System. There are two main goals: 1) Determine the effects of adding low and high dose antibiotic on the properties of

the silorane cement and determine the stability of heat labile antibiotics in silorane cements. Cephazolin will be tested because it is the antibiotic of choice by orthopaedic surgeons and cannot be added to commercially available bone cement, and tetracycline, another very heat sensitive antibiotic. The effects of the antibiotic on the strength and mechanical properties of the bone cement will be determined. Conversely, the effects of the cement on antibiotic activity and elution will be determined. 2) Hire an FDA consultant to advise on toxicology studies for the swine experiments in order to file an investigational device exemption (IDE) to secure FDA approval for clinical trials. We will develop a plan with an FDA consultant, which will include a comprehensive program that includes the series of timelines, critical milestones and cost parameters. It will allow us to efficiently and effectively move from the IDE, to the collection of clinical performance data to 510(k) approval and commercialization and eventually licensing.

We have started the antibiotic elution studies with Vancomycin. Vancomycin was tested in the silorane cement with different amounts of antibiotic (up to 20 wt%) and LMC (up to 0.91 wt%). See Tables 1 and 2. The samples with the samples with the highest Vancomycin (~20 wt%) were very dry and crumbled. The sample with the regular amount of LMC failed completely. Sample with very high LMC (over 2X the normal amount) passed the 1 lb GNT at 45 min. Both of the 15 wt% Vancomycin samples passed, the high LMC at 45 min and the regular between 45-60 min. Samples with literature amounts of Vancomycin (consistent with 1 g or 5 g to 40 g of BC) with regular LMC amounts were tested. Both of these samples passed the 1 lb GNT between 45-60 min. This material is now being tested for vancomycin elution by HPLC-Mass Spectrometry.

Table 1: Vancomycin Sample Weights.

Sample	Vancomycin (g)	LMC (g)	1TOSU-DY5 (g)	SM (g)	LIS (g)	Total (g)
Regular	0.00000	0.01079	1.75384	0.89484	0.03874	2.69821
20%-A	0.55222	0.00933	1.43572	0.73256	0.03172	2.76155
20%-B	0.53871	0.02450	1.39784	0.71323	0.03088	2.70516
15%-A	0.39868	0.00904	1.46838	0.74923	0.03244	2.65777
15%-B	0.40026	0.01809	1.46838	0.74923	0.03244	2.66840
2.4%-A	0.05555	0.00933	1.46834	0.74920	0.03244	2.31486
11.11%-A	0.28250	0.01019	1.46838	0.74922	0.03244	2.54273

Table 2: Vancomycin Sample wt%'s and Polymerization Results.

Sample	% Vancomycin	% LMC	% 1TOSU-DY5	% SM	% LIS	Results
Regular	0.00	0.40	65.00	33.16	1.44	Pass 45-60min
20%-A	20.00	0.34	51.99	26.53	1.15	FAIL
20%-B	19.91	0.91	51.67	26.37	1.14	Pass 45-60min
15%-A	15.00	0.34	55.25	28.19	1.22	Pass 60min
15%-B	15.00	0.68	55.03	28.08	1.22	Pass 45min
2.4%-A	2.40	0.40	63.43	32.36	1.40	Pass 45-60min
11.11%-	11.11	0.40	57.75	29.47	1.28	Pass 45-

A						60min
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Degrees obtained that are supported by this award:

Bradley David Miller defended and published his dissertation entitled, "Synthesis and Analysis of Siloranes for use as a Biomaterial and Extended Twisted Molecular Ribbons" in 2013. He has been working as a visiting assistant professor at William Jewell College in Liberty, MO.

Employment received based on experience/training supported by this award.

REFERENCES: none

APPENDICES: none