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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.

15. SUBJECT TERMS

Bone cement, silorane, prosthetic

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Final Report

Award No.: W81XWH-11-1-0805

Report Date: October 18, 2015

Principal Investigator: Dr. J David Eick (corresponding PI: Dr. Lynda F 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. 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 were:
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, toxicity, exothermicity, osteogenesis, prosthetics

OVERALL PROJECT SUMMARY:

- **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. Date completed: Sept. 20, 2012. Subtask 1b. Optimize composite formulation with respect to mechanical/handling properties. Date completed Sept. 2013.
- **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. Date completed: Sept. 20, 2012. Subtask 2b. Determine biocompatibility of wear debris. Date completed Dec. 19, 2014- Feb. 2015.
- **Task 3:** Determine the biological response to silorane bone cement in animal models, Subtask 3a. Small Animal (Rat) Model. Date completed Dec. 19, 2014. Subtask 3b. Large Animal (Swine) Model. Date completed June 20, 2015.

There were four major delays in the progress of the work. The first occurred when we could not get the silorane cement to efficiently polymerize unless moisture was reduced in the starting monomer. The second was when we did not realize that moisture would prevent the 60% filled silorane bone cement to sufficiently polymerize in vivo. This occurred with the first pull-out experiment. This was corrected by increasing the filler to 65% along with better drying of the monomer prior to cement formulation. The third major delay came with setting up the swine experiments when changes had to be made on location. A fourth delay occurred for the unexpected long time needed to get animal handling protocols approved.

KEY RESEARCH ACCOMPLISHMENTS:

All goals as listed above were met:

- 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/silorane and support high strength, high extent composite cure.
- 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 co-monomers.
- *In vitro,* the silorane bone cements are non-toxic and non-inflammatory, as well as stimulates osteogenesis.
- *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 desiccated before use to insure ideal pull out strength.
- A Bone Cement symposium was held October 4, 2014 to discuss and disseminate information on the silorane bone cement. Dr. Tim Topoleski, University of Maryland, was the guest speaker. Thirty-four scientists and orthopaedic surgeons from around the area shared their knowledge, research and experience during the Bone Cement Symposium. Considerable enthusiasm was generated for use of the silorane bone cement, mainly based on its low exotherm and lack of toxicity. Consensus was achieved on the use of heat sensitive antibiotics and potentially for antifungals for bone infections potentially through antibiotic containing pre-

molded stabilizer, cement beads, and antibiotic containing nails. (The symposium program and summary of round table discussion is attached.)

- Wear debris was prepared from commercially available bone cement and DY5-1TOSU samples (5 g of SilMix and 42 g of Simplex P, particle size < 10μm) and effects on macrophage-like RAW 264.7 cells evaluated using cytokine kits (IL-1β Elisa kit). A modest effect of Simplex P was observed, but none with the silorane cement.

Abbreviations: Lamoreaux catalyst (LMC); light-cured SilMix (LCSM); yttrium aluminosilicate glass (DY5); barium boroaluminosilicate glass (M12); [(9,9-diethyl-1,5,7,11-tetraoxaspiro[5.5]undec-3-yl)methyl]trimethoxysilane (1TOSU); [3-(9,9-diethyl-1,5,7,11-tetraoxaspiro[5.5]undec-3-yl)propyl]trimethoxysilane (3TOSU); and 2-(3,4-epoxycyclohexyl)-ethyltrimethoxysilane (ECHE). SilMix [1:1 combination of bis[2-(3{7-oxabicyclo[4.1.0]heptyl}ethyl]methylphenyl silane (PHEPSI) and 2,4,6,8-tetrakis (2-(7-oxabicyclo[4.1.0] heptan-3-yl)ethyl)-2,4,6,8-tetramethyl-1,3,5,7,2,4,6,8-tetra-oxatetrasilocane (CYGEP)].

CONCLUSION:

We have developed a novel silorane bone cement with excellent properties. 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/osseoinduction, 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, antifungals, and potentially growth factors, which previously could not be used in PMMA bone cements. The development of the silorane bone cement is very promising for application for human use.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

Peer-Reviewed Scientific Journals:

Effect of Moisture on Cationic Polymerization of Silicone Epoxy Monomers R. A. A. Upul Ranaweera, Thomas P. Schuman, Rongpeng, Wang, Bradley D. Miller, and Kathleen V. Kilway, Journal of Applied Polymer Science 2015 (04/2015), 132(15), DOI: 10.1002/app.41831 10pgs. (Includes acknowledgement of Federal Support).

Silorane resin supports proliferation, differentiation, and mineralization of MLO-A5 bone cells in vitro and bone formation in vivo. J. David Eick, Cielo Barragan-Adjemian, Jennifer Rosser, Jennifer R Melander, Vladimir Dusevich, Rachel A. Weiler, Bradley D. Miller, Kathleen V. Kilway, Mark R Dallas, Lianxiang Bi, Elisabet L Nalvarte, Lynda F. Bonewald Journal of Biomedical Materials Research. Part B, Applied biomaterials. 04/2012; 100(3):850-61. (Includes Acknowledgement of Federal Support) PMID: 22278990.

Estimation of properties of a photoinitiated silorane-based composite with potential for orthopaedic applications. Jennifer R. Melander, Rachel A. Weiler, Bradley D. Miller, Thomas P. Schuman, Kathleen V. Kilway, Delbert E. Day, Mariano Velez, J. David Eick Journal of Biomedical Materials Research. Part B, Applied biomaterials. 11/2011; 100(1):163-9 (Includes Acknowledgement of Federal Support) PMID: 22102398.

Development of a Novel, Non-Toxic, Non-Exothermic, Osteogenic Bone Cement Lianxiang Bi, Kathleen V. Kilway, Jennifer R. Melander, Elizabeth M. Menuey, Rachel A. Weiler, Jennifer L. Rosser, Anita Xie, Yukiko Kitase, Thomas P. Schuman, J. David Eick, and Lynda F. Bonewald Submitted to Nature Materials (Includes Acknowledgement of Federal Support).

Comparison of silorane bone cement with commercial cement in a swine femoral implant model Kathleen V Kilway, Donna Pacicca, Rachel A Weiler, Elizabeth M. Menuey, Jennifer L Rosser, Anita Xie, Terrance McIff, Damon Mar, J David Eick, Thomas P Schuman, Eric Walters, Michael Fink, LF Bonewald, In preparation. (Includes Acknowledgement of Federal Support).

Abstracts:

Novel Silorane Bone Cements Exhibit Similar Mechanical Properties but None of the in vivo Inflammatory Effects of Commercial Bone Cement. Bi, Lianxiang; Eick, J. David; Kilway, Kathleen V.; Weiler, Rachel A.; Miller, Bradley D.; Schuman, Thomas P.; Bonewald Lynda F. SU0065 ASBMR, Baltimore, MD, October 4-7, 2013. (Includes Acknowledgement of Federal Support)

The Optimization and Effect of a Platinum Catalyst on the Mechanical and Handling Properties of Novel Silorane Bone Cements while maintaining Osteogenic Capacity. Kilway, Kathleen V.; Weiler, Rachel A.; Bi, Lianxiang; Eick, J. David;, Miller, Bradley D.; Schuman, Thomas P.; Bonewald Lynda F. MO0054, ASBMR, Baltimore, MD, October 4-7, 2013. (Includes Acknowledgement of Federal Support)

Evaluation of Different Silorane-Based New one Cements, Bunnell, T.J.; Bi ,L. ; Bonewald, L., Abstract #846, International Association for Dental Research/American Association for Dental Research, 91st General Session, Seattle, WA, March 20-23, 2013. (Includes Acknowledgement of Federal Support)

Dual-initiated Silorane Formulations for Use as a Bone Cement Alternative Kilway, K. V.; Eick, J. D.; Bi, L.; Weiler, R. A.; Miller, B. D.; Bunnell, T. J.; Melander J. R.; Schuman, T. P.; Bonewald, L. F., Poster # 1232, Orthopaedic Research Society 2013 Annual Meeting, San Antonio, TX, January 26-29, 2013. (Includes Acknowledgement of Federal Support)

Measuring Strain in Bone Cement with Carbon Nanotubes Melander J. R.*; Holmes, R. R.; Yao, X.; Weiler, R. A.; Eick, J. D abstract # SBC2012-80620, American Society of Mechanical Engineers 2012 Summer Bioengineering Conference, Fajardo, Puerto Rico, June 20-23, 2012.

Polymerization Stress and the Influence of TOSU Addends on Methacrylate Composites Holmes, R. R.*; Melander J. R.; Weiler, R. A.; Schuman, T. P.; Kilway, K. V.; Eick, J. D abstract # SBC2012-80627, American Society of Mechanical Engineers 2012 Summer Bioengineering Conference, Fajardo, Puerto Rico, June 20-23, 2012.

TOSU Addends Maintain Mechanical Properties while Decreasing Polymerization Stress Melander J. R.*; Holmes, R. R.; Weiler, R. A.; Miller, B. D.; Kilway, K. V.; Schuman, T. P.; Eick, J. D., "Abstract # 973, American Association for Dental Research 41st General Session, Tampa, FL, March 21-24, 2012.

Biocompatibility of a Chemically Initiated Silorane Resin Miller BD, Weiler RA, Melander JR, Nalvarte EL, Kilway KV, Bonewald LF, and Eick JD. Poster Presentation, 89th Annual Meeting & Exhibition of the International Association for Dental Research, San Diego, CA, March 2011.

Physical Properties of Filled Chemically Initiated Silorane Biomaterials Weiler RA, Melander JR, Miller BD, Kilway KV, Bonewald LF, and Eick JD. Poster Presentation, 89th Annual Meeting & Exhibition of the International Association for Dental Research, San Diego, CA, March 2011.

Handling Properties and Exothermicity of Chemically Initiated Silorane Biomaterial Melander JR, Weiler RA, Miller BD, Kilway KV, and Eick JD. Poster Presentation, 89th Annual Meeting & Exhibition of the International Association for Dental Research, San Diego, CA, March 2011.

Flexural Properties of Silorane Bone Cement Melander JR, Weiler RA, Miller BD, Kilway KV, and Eick JD. Poster Presentation, ASME 2011 Summer Bioengineering Conference, Farmington, PA, June 2011.

Improving the Strength of a Silorane Bone Cement Melander JR, Weiler RA, Miller BD, Kilway KV, and Eick JD. Poster Presentation, Missouri Musculoskeletal Conference, July 2011.

Oral Presentations:

Generation of a Novel Bone Cement to Fight Bone Infection, L. F. Bonewald "Center for Biomedical Science and Engineering Distinguished Campus Seminar, Missouri Science and Technology, Rolla, MO, May 7, 2015. (Includes Acknowledgement of Federal Support)

Biological Effects of Silorane Bone Cement, L. F. Bonewald, Speaker, Bone Cement Symposium, Kansas City, MO., Oct. 4, 2014. (Includes Acknowledgement of Federal Support)

Development of a novel bone cement, K. V Kilway, University of Missouri – Kansas City Bone Cement Symposium, Kansas City, MO, October 4, 2014Kilway,

Advancements in the development of a novel bone cement, K. V Kilway, University of Missouri – Kansas City Center of Excellence in Mineralized Tissues Seminar Series, Kansas City, MO, July 16, 2014.

Designing Materials Interfaces, T. P. Schuman, University of Cincinnati, OH, November 1, 2013.

Toward Biocompatible Bone Cements, T. P. Schuman, University of Southern Mississippi, MS, October 2013.

Silorane Composites for Orthopaedic Applications, Part III, K. V Kilway, University of Missouri – Kansas City Center of Excellence in Mineralized Tissues Seminar Series, Kansas City, MO, June 20, 2012.

Influence of the Composite Filler-to-Matrix Interface on Bulk Properties, Thomas P. Schuman, Invited speaker, Missouri State University, Springfield, MO, 1 February 2012.

INVENTIONS, PATENTS AND LICENSES:

Kilway, K. V.; Bonewald, L. F.; Schuman, T. P. Curators of the University of Missouri, USA) **Biomaterial Compositions,** U.S. Patent US20130210953 A1, 2013. (Includes Acknowledgement of Federal Support)

Thomas P. Schuman, "**EPOXY PHOSPHONATE ESTER AS A COUPLING AGENT FOR TRANSITION METAL AND METAL OXIDE SURFACES**", patent application in process (at Missouri S&T, at similar stage as our cement patent application). (Includes Acknowledgement of Federal Support).

REPORTABLE OUTCOMES:

The optimal silorane bone cement prototype was found to be comprised of the 65 wt% DY5-1TOSU, 0.40 wt% LMC, and 34.60 wt% LCSM using dry filler and dry co-monomers.

The silorane bone cement prototype is non-toxic, non-inflammatory, and stimulates osteogenesis *in vitro*. The pull out strength of the silorane bone cement in the rat model was found to be comparable to commercially available bone cement. The prototype also has a low curing temperature (26 °C) allowing for a wider variety of antibiotic and antifungal incorporation than current bone cements.

The silorane prototype has potential use as a bone cement, spacer, and antibiotic/antifungal beads.

OTHER ACHIEVEMENTS:

Daniel Rodman – undergraduate researcher, BA in Chemistry, graduated July 2012, (still worked for us through October 2012) now employed by SpecChem.

Caitlyn Reger - undergraduate researcher, BA in Chemistry, graduated December 2013 (still worked for us through June 2014) employed by Vince and Associates Clinical Research.

James Cash - undergraduate researcher, BA in Chemistry, graduated July 2012 (still worked for us through December 2012) now attending chiropractic school at Logan College of Chiropractic University Programs in St. Louis, MO.

Andrew Kraft - undergraduate researcher, BA in Chemistry, graduated May 2013 (cum laude), currently pursuing an MD at a Caribbean Medical School.

Leila Suleiman - undergraduate researcher, BS in Chemistry, graduated December 2013, employed by Water One.

Katelyn Kephart - undergraduate researcher, BA in Chemistry, graduated May 2013, employed by Cerner.

Khristle Tolbert - undergraduate researcher, BS in Chemistry graduated May 2013, lab intern at Integrity Home Care.

Jamandeep Kaur - undergraduate researcher, pursuing a BA in Chemistry/Biology.

Ashley Harkleroad - undergraduate researcher, BS in Chemistry graduated May 2015, Laboratory tech for CFS West Holdings Inc., Overland Park, KS.

James Reed III - undergraduate researcher, BA in Chemistry, graduated August 2014, currently pursuing BS in Physics.

Danielle Waggerman - undergraduate researcher, BA in Chemistry, graduated August 2014.

Afsaneh Zare Mohazab - undergraduate researcher, pursuing a BA in Chemistry.

Amanda Derewenko - undergraduate researcher, BS in Chemistry, graduated May 2015, currently employed as a Senior Administrative Assistant and Lab Tech at Stowers Institute for Medical Research

Jennifer R. Melander - PhD in Oral Biology and postdoctorate at UMKC, is currently employed as an Assistant Professor in the Department of Biological Systems Engineering at the University of Nebraska – Lincoln.

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 December 2013. He was employed as a visiting assistant professor at William Jewell College in Liberty, MO from August 1, 2013 – July 30, 2014 and is working as a high school science teacher in the Greater Kansas City area.

Rachel Ann Weiler - defended her dissertation entitled, “The Study of Initiation Systems and Formulations for the Development of a Novel Silorane Biomaterial” and will be graduating in December 2015.

James Bryan **–** synthesized monomers and will be graduating with a MS in Chemistry in December 2015.

Ranaweera Upul Ranaweera - was a postdoctoral fellow during the project for one year. He has obtained one publication thus far and has two more manuscripts, one of which is currently held awaiting patenting of a surface modification technique. Ranaweera was instrumental in demonstrating the moisture effect on monomer polymerization rate and effective polymerization methodology (published manuscript). He joined a group at University of Cincinnati as a postdoctoral fellow.

Nicholas Jentsch - was an undergraduate student taking research hours. Nick assisted with TOSU molecule syntheses and spectroscopic characterizations. He is a graduate student at the University of Southern Mississippi working on his doctoral degree funded by a competitively awarded NSF fellowship.

REFERENCES: None

APPENDICES:

1) Manuscript: Silorane resin supports proliferation, differentiation, and mineralization of MLO-A5 bone cells in vitro and bone formation in vivo. J David Eick, Cielo Barragan-Adjemian, Jennifer Rosser, Jennifer R Melander, Vladimir Dusevich, Rachel A Weiler, Bradley D Miller, Kathleen V Kilway, Mark R Dallas, Lianxiang Bi, Elisabet L Nalvarte, Lynda F Bonewald Journal of Biomedical Materials Research. Part B, Applied biomaterials. 04/2012; 100(3):850-61. (Includes Acknowledgement of Federal Support).

2) Manuscript: Estimation of properties of a photoinitiated silorane-based composite with potential for orthopaedic applications. Jennifer R Melander, Rachel A Weiler, Bradley D Miller, Thomas P Schuman, Kathleen V Kilway, Delbert E Day, Mariano Velez, J David Eick Journal of Biomedical Materials Research. Part B, Applied biomaterials. 11/2011; 100(1):163-9 (Includes Acknowledgement of Federal Support).

3) Manuscript: Development of a Novel, Non-Toxic, Non-Exothermic, Osteogenic Bone Cement Lianxiang Bi, Kathleen V. Kilway, Jennifer R. Melander, Rachel A. Weiler, Jennifer Rosser, Anita Xie, Yukiko Kitase, Elizabeth Menuey, Thomas P. Schuman, J. David Eick, and Lynda F. Bonewald, To be submitted to Nature Materials. (Includes Acknowledgement of Federal Support and see attachment).

4) Manuscript: Comparison of silorane bone cement with commercial cement in a swine femoral implant model. Donna Pacicca,* Kathleen V. Kilway*, Rachel A. Weiler, Jennifer L. Rosser, Anita Xie, Elisabeth Menuey, Terrance McIff, Damon Mar, J. David Eick, Thomas P. Schuman[5], Eric Walters, Michael Fink, and Lynda F. Bonewald, In preparation. (Includes Acknowledgement of Federal Support and see attachment).

5) Program for Bone Cement Meeting
See attachments entitled Bone Cement Symposium Program Book and Bone Cement Symposium Announcement.

6) Minutes from Bone Cement Meeting
See attachment entitled Bone Cement Symposium Round Table Notes.

7) Fast Track Award received and completed. This award for $50,000 was received from the University of Missouri System. In summary, silorane bone composites are non-toxic and have better biocompatibility than commercially available BisGMA-TEGDMA and PMMA bone cements, silorane bone composites support bone cell differentiation, silorane composites have acceptable handling properties and equivalent *in vivo* pull-out strength as commercial PMMA bone cement and the antibiotic, vancomycin, has excellent elution profiles compared to methacrylate bone cements.

Appendices

Manuscript

Silorane resin supports proliferation, differentiation,

and mineralization of MLO-A5 bone cells in vitro and bone formation in vivo

Society For Biomaterials

Silorane resin supports proliferation, differentiation, and mineralization of MLO-A5 bone cells *in vitro* and bone formation *in vivo*

J. David Eick,[1] Cielo Barragan-Adjemian,[1] Jennifer Rosser,[1] Jennifer R. Melander,[1] Vladimir Dusevich,[1] Rachel A. Weiler,[2] Bradley D. Miller,[2] Kathleen V. Kilway,[2] Mark R. Dallas,[1] Lianxing Bi,[1] Elisabet L. Nalvarte,[1] Lynda F. Bonewald[1]

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Abstract: Methyl methacrylate used in bone cements has drawbacks of toxicity, high exotherm, and considerable shrinkage. A new resin, based on silorane/oxirane chemistry, has been shown to have little toxicity, low exotherm, and low shrinkage. We hypothesized that silorane-based resins may also be useful as components of bone cements as well as other bone applications and began testing on bone cell function *in vitro* and *in vivo*. MLO-A5, late osteoblast cells, were exposed to polymerized silorane (SilMix) resin (and a standard polymerized bisGMA/TEGDMA methacrylate (BT) resin and compared to culture wells without resins as control. A significant cytotoxic effect was observed with the BT resin resulting in no cell growth, whereas in contrast, SilMix resin had no toxic effects on MLO-A5 cell proliferation, differentiation, nor mineralization. The cells cultured with SilMix produced increasing amounts of alkaline phosphatase (1.8-fold) compared to control cultures. Compared to control cultures, an actual enhancement of mineralization was observed in the silorane resin-containing cultures at days 10 and 11 as determined by von Kossa (1.8–2.0 fold increase) and Alizarin red staining (1.8-fold increase). A normal bone calcium/phosphate atomic ratio was observed by elemental analysis along with normal collagen formation. When used *in vivo* to stabilize osteotomies, no inflammatory response was observed, and the bone continued to heal. In conclusion, the silorane resin, SilMix, was shown to not only be non cytototoxic, but actually supported bone cell function. Therefore, this resin has significant potential for the development of a nontoxic bone cement or bone stabilizer. © 2012 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 100B: 850–861, 2012.

Key Words: siloranes, bone stabilization, mineralization, MLO-A5 cell line, osteotomy

How to cite this article: Eick J. David, Barragan-Adjemian C, Rosser J, Melander JR, Dusevich V, Weiler RA, Miller BD, Kilway KV, Dallas MR, Bi L, Nalvarte EL, Bonewald LF. 2012. Silorane resin supports proliferation, differentiation, and mineralization of MLO-A5 bone cells *in vitro* and bone formation *in vivo*. J Biomed Mater Res Part B 2012:100B:850–861.

INTRODUCTION

Bone cements have been used for decades in the fixation of prosthetic devices. Polymethyl methacrylate (PMMA)-based cement is a well-recognized conventional bone cement that provides reasonably good clinical results; however, severe problems are still associated with its use, such as, cytotoxicity, thermal injury, respiratory and cardiovascular complications in addition to polymerization shrinkage, which can affect the stability of the implant.[1–5] The interaction between resin and bone causes internal stress that can lead to gap formation between the PMMA and the bone.[6]

Silorane-based resins have been developed by 3M-ESPE[7] for the production of dental composite materials. These resins have proved to have superior characteristics to bisGMA/TEGDMA (bisphenol A glycidyl methacrylate and triethylene glycol dimethacrylate), the two usual monomer components of dental composites. The term "silorane" was introduced to represent hybrid monomer systems that contain both siloxane and oxirane structural moieties. Siloranes contain a cyclosiloxane backbone, which imparts hydrophobicity[8]; in addition, they contain cycloaliphatic oxirane sites that have high reactivity and present less shrinkage during polymerization than methacrylates.[9,10] Some cyclosiloxanes have been reported to undergo cationic ring-opening polymerization with volume expansion.[11] These resins exhibit excellent biocompatibility. Cytotoxicity ratings are as good as or better than those for typical methacrylate dental monomers, such as bisGMA-based polymer. They also are nonmutagenic.[12–14] Marginal integrity and microleakage of silorane-based restorative systems are reported to be superior to methacrylate-based systems.[15] Shear bond strength and other mechanical properties have also been studied and

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found to be better than the methacrylate resins.[16–20] It was also shown that a silorane-based dental composite can effectively bond to bone.[21]

In order to begin to develop better bone cements, we analyzed the effect of silorane-based resins on bone cell function *in vitro* and *in vivo*. One aim of the present study was to analyze the effect of silorane-based resin on bone cell proliferation, differentiation, and mineralization. MLO-A5 cells were used as an *in-vitro* model for bone formation. MLO-A5 cells are a post-osteoblast/pre-osteocyte-like cell line established from the long bones of 14-day-old mice expressing the large T-antigen driven by the osteocalcin promoter.[22] These cells express extremely high levels of alkaline phosphatase and osteocalcin, as well as, osteopontin, periostin, bone sialoprotein, and PTH type 1 receptor compared to primary osteoblasts and osteoblast cell lines.[22] Previously, we had shown that the MLO-A5 cells mineralize in culture, forming sheets not nodules, and that this mineralized matrix contains a ratio of calcium to phosphorus similar to bone.[23] These cells will mineralize in the absence of beta glycerolphosphate (βGP) in 6–7 days, but this process is accelerated by the addition of an external source of phosphate. Spectra obtained by Fourier transform infrared spectroscopy, FTIR, of these cultures were shown to be very similar to normal bone.[22,23] The MLO-A5 cells appear to be a good model for *in vitro* lamellar bone formation. These cells were used for the present study in order to obtain insight into the potential mechanisms by which bone would form in the presence of silorane-based resins in comparison to the effect of a methacrylate composite, bisGMA/TEGDMA (BT). A second aim of the study was to examine the effects of silorane resin on bone cell function *in vivo* and to determine if the resin elicited an inflammatory response. We chose to use the standard femoral osteotomy approach in mice. The silorane resin was used to stabilize the osteotomy was up to one month for radiographic and histological analysis. Overall, silorane resin had little or no negative effects on bone cell function.

MATERIALS AND METHODS

Preparation of resins

The resins were prepared as described previously.[17,24,25] Briefly, the silorane-based resin SilMix, is a 1/1 wt/wt mixture of two silicon-containing oxiranes, bis[2-(3{7oxabicyclo[4.1.0]heptyl})-ethyl]methylphenyl silane (PHEPSI)[26] and 2,4,6,8-tetrakis(2-(7-oxabicyclo[4.1.0]heptan-3-yl)ethyl)-2,4,6,8-tetramethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane (CYGEP)[27] (see Figure 1). A conventional methacrylate-based matrix resin bisGMA (BisGMA/TEDGMA 50/50) used in dental composites was used as a control. For the drop method, the silorane (SilMix) and methacrylate (Z250: bisGMA/ TEGDMA) resins were obtained from 3M-ESPE (St. Paul, MN, and Seefeld, Germany). For the rest of the samples, the methacrylate monomer system (BT) was a 1:1 mixture by weight of two methacrylates, bisGMA (purity: 93%, Esstech, Inc.) and TEGDMA (purity: 97%, Sartomer). With the exception of the resin drop samples, the silorane monomers (SilMix) were synthesized using an adapted procedure for PHEPSI[26] and CYGEP[27] resulting in a >95.8% purity as determined by ^{1}H NMR spectroscopy. All resin samples were prepared at room temperature (~ 20°C) and under yellow light in order to prevent premature polymerization. The photoinitiator system (see Figure 1) used for all the resins consisted of phenyl[*p*-2-hydroxytetradecyloxyphenyl]iodonium hexafluoroantimonate (PI, Gelest, Inc., Tullytown, PA); camphorquinone (CQ, Aldrich, Milwaukee, WI), and ethyl-4-dimethylaminobenzoate (EDMAB, Fisher Scientific / ACROS, Pittsburgh, PA). The photoinitiator and monomer systems were combined using a speed mixer and mixed for periods of 5–15 minutes depending on the amount of material. The final mass composition was 0.15% EDMAB, 1.0% CQ, 3.0% PI, and 95.85% SilMix and the BT composition was 0.15% EDMAB, 1.0% CQ, 3.0% PI, and 95.85% BisGMA/TEGDMA. Resins were prepared the same day and used within a 2-h period after preparation.

Resin polymer characterization

To ensure that resin polymerization was complete, the degree of conversion (DC) of the SilMix and BT resins was analyzed using FTIR spectroscopy (Perkin–Elmer Spectrum One, ATR sampling mode) analysis. A Delrin mold was fixed on the FTIR instrument; the resin (80 mg) was added to the mold, and the resin polymerized via light cure with a 3M curing lamp, (3M XL3000, St. Paul, MN, 450 mW/cm^2 light intensity) for three 40-sec intervals. The solid discs ($n = 6$) were detached from the mold, and half were subjected to 2 h sterilization below laminar hood UV light (1 h per side). The other half were allowed to dark cure. FTIR spectra were collected from the unpolymerized resin at 2 min after light cure and at 4 h after light cure (polymer with dark cure) ($n = 3$). The FTIR spectra were baseline corrected, and the DC was calculated for each polymer sample using a polymerization dependent peak [BT: 1638 cm^{-1} (C=C), and SilMix: 883 cm^{-1} (oxirane ring opening)], which was compared to an internal standard [BT-1608 cm^{-1}(phenyl), and SilMix 1258 cm^{-1}(C—O in ring)]. The DC was calculated as the difference in the peak ratios from the unpolymerized resin assuming that the unpolymerized resin spectra represented no (0%) polymerization.

Culture of MLO-A5 cells with resin

Approximately 50 μL of the resin was dropped into the center of a NUNC brand Thermanox coverslip (Electron Microscopy Science Hatfield, PA) and polymerized with a 3M curing lamp (450 mW/cm^2 light intensity) for three 40-sec intervals. Thermanox coverslips with polymerized resin drops of 5-mm diameter or without resin for control were used in triplicate. The coverslips were placed in 24-well plates, and MLO-A5 cells were plated at a density of 3.5×10^4 cells/cm^2 in α-MEM containing 5% fetal bovine serum (FBS) and 5% calf serum (CS). Cells were cultured for 24, 48, and 72 h, then washed with PBS, and harvested with trypsin-EDTA. The cell number was measured using a Coulter Counter (Z1 Coulter particle counter, Beckman Coulter Fullerton, CA). In these experiments, it was observed that cells did not attach well to the resin drop

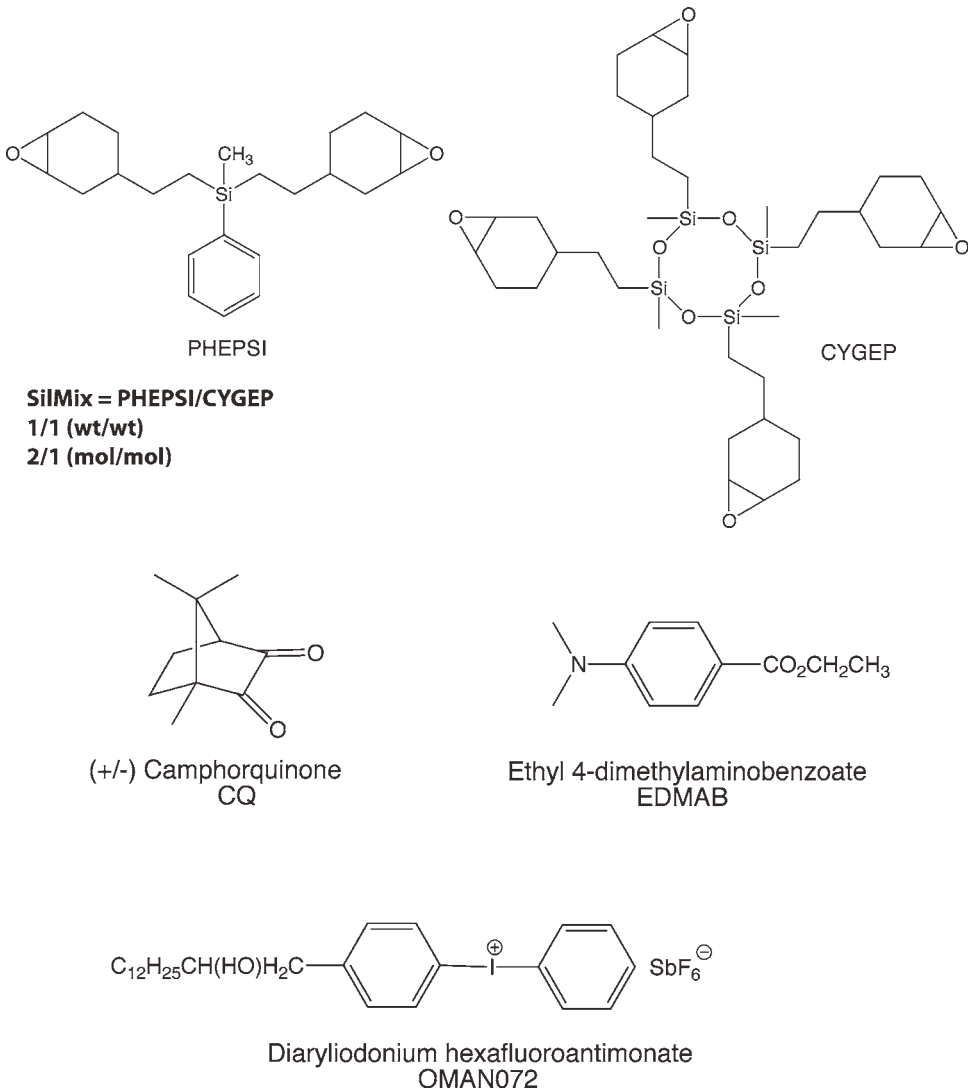

FIGURE 1. Chemical structure of siloranes and photoinitiator system used for the resins. The silorane resin used for the *in vitro* bone cell assays and *in vivo* is composed of SilMix a 1/1 wt/wt ofPHEPSI/CYGEP.

surfaces; therefore a second experimental design generated discs of SilMix and BT polymer of 9 mm diameter by 0.7 mm thickness. Discs were prepared by placing 80 mg of the freshly mixed resin into Delrin ring molds (McMaster-Carr, Elmhurst, IL), which were fixed on glass slides. The resin was light cured (3M curing lamp, 450 mW/cm^2 light intensity) for three 40-sec intervals at a distance of 1 mm from the top of the sample. Solid polymer discs were detached from the molds and sterilized under laminar hood UV light for 1 h on each side. The discs, which covered the entire bottom surface of 48-well culture plates, were placed in the wells prior to addition of MLO-A5 cells at a density of 3.5×10^4 cells/cm^2. After 24 and 48 h of incubation, cell attachment and proliferation were assessed by measuring the cell number using the Coulter counter assay and the Trypan blue dye exclusion (TBE) assay.

The cell monolayer was washed with 0.5 mL of PBS, and then pooled with the respective supernatants. Trypsin/EDTA (0.2 mL) was added to the cell layer and incubated for 2–3 min at 37°C/5% CO_2. Meanwhile, the cells in the supernatants were pelleted and treated with 0.05 mL Trypsin/EDTA at 37°C/5% CO_2. Trypsinized cell suspensions were pooled (1.25 mL), then centrifuged for 2 min (5000 rpm). The obtained cell pellet was re-suspended in 100 μL of PBS. In each microcentrifuge tube, 20 μL of 0.4% trypan blue dye was added, mixed thoroughly, and allowed to stand for 3–5 min at room temperature. A hemacytometer was loaded with 10 μL cell suspension, and cells were counted under a microscope. Similar procedures were performed with cells grown on the polystyrene control wells.

Cell viability in response to polymer extracts

To study the effect of leachables on cell viability, sterilized discs were inserted into 48-well plates and washed with 0.5 mL of culture media for 1 h at 37°C/5% CO_2. The used media was discarded, and fresh media (0.5 mL) was added to the polymer discs as well as the control wells ($n = 4$) and incubated for 48 h at 37°C/5% CO_2. In parallel and in

the same plate, the 1 h pre-incubated wells were seeded with 0.5 mL of 3.5×10^4 cells/mL and grown for 48 h. Then, the media in these wells containing cells were removed and replaced with 0.5 mL of conditioned media exposed to the discs (assumed to contain leachables from the polymer discs). After incubation for 24 h at 37°C/5% CO_2, cell viability was measured using the MTT assay. For the MTT assay, 50 μL of 5 mg/mL of MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide, Sigma M5655] in phosphate buffered saline (PBS) were added to the culture plates and returned to the incubator. After 4 h of incubation time, the supernatants with unreacted MTT were discarded, and the purple formazan crystals in the cells were dissolved by adding dimethyl sulfoxide (DMSO, 0.5 mL). Formazan/DMSO aliquots were read at 550 nm in a 96-well plate reader (Molecular Devices Corp., Menlo Park, CA).

Culture of cells for mineralization. Due to low attachment of cells on resin surfaces, mineralizaton analysis was carried out using resin drops. Approximately the resin (50 μL) was dropped into the center of a NUNC brand Thermanox coverslip (Electron Microscopy Science Hatfield, PA) and polymerized with a 3M curing lamp, (450 mW/cm^2 light intensity) for three 40-sec intervals. To rule out any effects of cells potentially settling in the middle of the well and being displaced by the resin for successive experiments, resin (20–30 μL) was placed either in the center of the coverslip or toward the side, but not touching the edge of the coverslip. After polymerization, the sample and Thermanox discs were washed with PBS and placed in 24-well plates for sterilization under laminar hood ultraviolet light for ~ 1–2 h, before use.

Thermanox coverslips with polymerized resin drops or without resin for control were used in triplicate. The coverslips were placed in 24-well plates, and MLO-A5 cells were cultured as described previously.[22] MLO-A5 cells were plated at a density of 3.5×10^4 cells/cm^2 in α-MEM containing 5% fetal bovine serum (FBS) and 5% calf serum (CS). Upon confluency, designated day 0, media was removed, and the cells were incubated in mineralizing media, α-MEM with 10% FBS, 4 m*M* of β-glycerolphosphate, βGP, and 100 μg/mL of ascorbic acid. Media were changed every two days through 11 days.[23]

Alkaline phosphatase assay

MLO-A5 cells were cultured on cover slips for 6 days under mineralization conditions as described above and analyzed for alkaline phosphatase enzyme activity. Briefly, cells were fixed with 10% buffered formalin for 10 min and washed with PBS two times. Fresh solution containing 0.033% NBT (nitro blue tetrazolium) and 0.017% BCIP (5-bromo-4-chloro-3-indolyl phosphate) in ALP buffer (100 m*M* sodium chloride, 5 m*M* magnesium chloride, 100 m*M* Tris-HCl, pH 9.5) was added to the cultures and incubated at 37°C for 20 min. The purple stained area was measured using a semiautomated imaging system as described previously.[22,23]

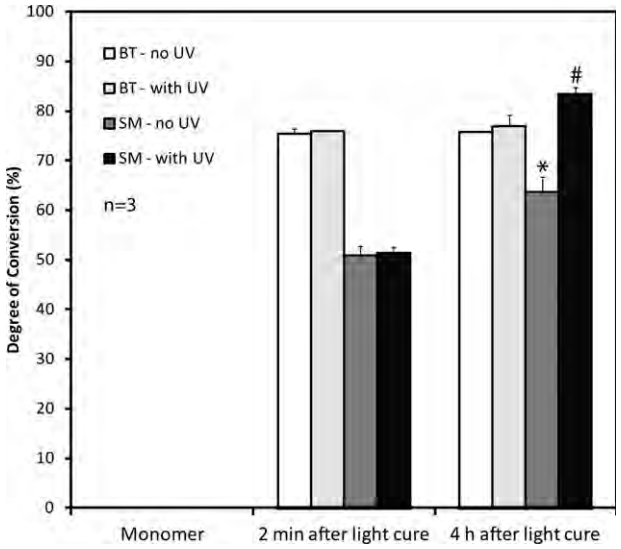

FIGURE 2. Overall degree of conversion for bisGMA/TEGDMA (BT) without ultraviolet light, UV (open bars) or with UV sterilization treatment (dotted bars) and SilMix (SM) polymers without UV (hatched bars) and with UV (gray bars). The DC of 4 h after light cure and UV sterilized polymers are representative of the DC of discs used for the cell proliferation tests ($n = 3$). *Significant change ($p < 0.05$) in the DC of the SM without and with UV light treatment at 4 h, as well as the DC of the SM from 2 min to 4 h. #Significant increase ($p < 0.05$) in the DC of SM-with UV relative to BT-with UV using three-way ANOVA.

Immunohistochemical staining for collagen type 1

MLO-A5 cells were plated on coverslips and cultured as described above. After 6 days in culture, the cells were washed with PBS (two times), then fixed with 95% ethanol for 5 min and washed with PBS (three times). The cultures were then incubated with blocking solution, (PBS + 1% horse serum + 0.05% NaN_3) for 2 h at room temperature, followed by incubation with polyclonal antibody to type I collagen, LF-67, that recognizes the C-telopeptide of collagen type 1 (the antibody was kindly provided by Dr. Larry W. Fisher, National Institutes of Health, Bethesda, MD, USA). A 1:400 dilution in PBS + horse serum was added for 1 h at room temperature, followed by incubation with Cy-3 conjugated donkey anti-rabbit IgG in blocking solution, 1:250 for 1 h and followed by washing with PBS (six times). The cells were then examined, and photos were taken using fluorescence microscopy (Nikon eclipse E800 microscope).[23]

Scanning electron microscopy (SEM)

MLO-A5 cells were cultured on coverslips for 24 and 48 h as well as 6 and 10 days. At the end of the culture, the cells were gently washed with PBS, and fixed with 10% formalin for 20 min, washed again with PBS and dehydrated in a graded series of ethanol, and dried using hexamethyl disilazone (HMDS) for 5 min. After dehydration, the coverslips were attached to SEM stubs and sputter-coated with gold-palladium. The gold-palladium-coated cultures were examined using a FEI/Philips XL30 field emission environmental scanning electron microscope. An accelerated voltage in a range of 15 to 25 KeV was used for the secondary and

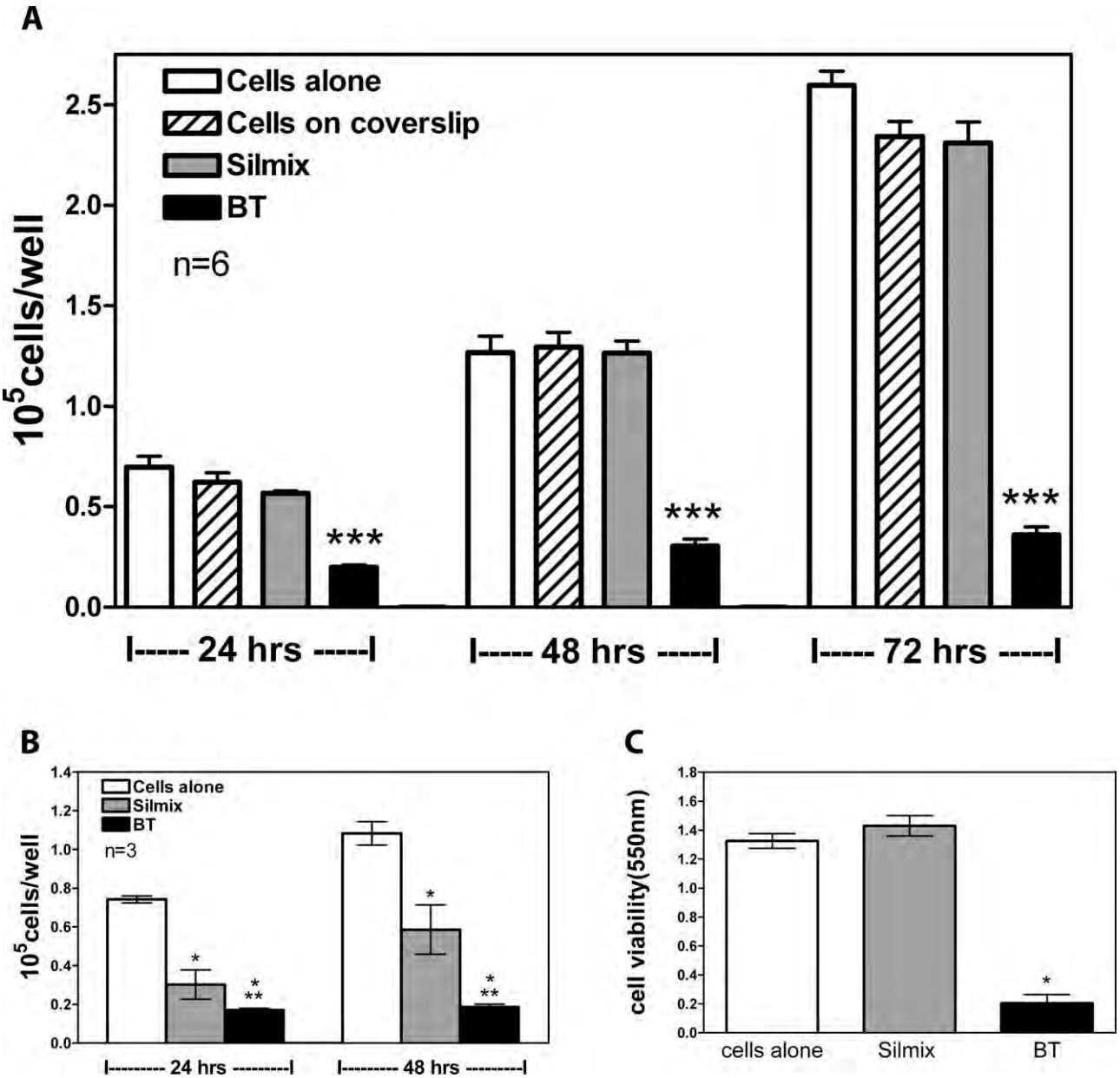

FIGURE 3. Effects of silorane and BT resins and resin leachables on MLO-A5 cell proliferation. Effects on cell number using resin drops (A) and resin discs (B). Silorane and BT disc media conditioned for 48 h added to MLO-A5 cells (C). ***Significantly different from SilMix ($p < 0.001$); *significantly different from cells alone ($p < 0.05$; $n = 3$); **significantly different from SilMix ($p < 0.05$) using one-way ANOVA and Tukey post test ($n = 3$).

backscatter electron imaging. For X-ray microanalysis EDS, the cultures were carbon-coated and examined with 15 KeV accelerating voltage. X-ray spectra and maps for calcium and phosphorus distribution were acquired.[23]

Von Kossa staining for phosphate quantification

The MLO-A5 cultured cells were washed with PBS and fixed with 10% buffered formalin for 10 min The samples were washed with water several times before a 2% silver nitrate solution was added and the plates exposed to UV light for 20 min and followed by rinsing with water. Five percent sodium thiosulfate was added for 3 min before rinsing. A modified van Gieson stain was then used as a counterstain following the von Kossa stain. This stain consisted of five parts 1% acid fuchsin and 95 parts picric acid, which was added for 5 min followed by washing with 95% ETOH (two times), 100% ETOH (two times), and then air drying before analysis. The mineralized area and total area were measured using a semiautomated imaging system as described previously.[22,23] Briefly, the area of von Kossa-stained matrix was quantified by automated image analysis using a video analysis program (Jandel Scientific, San Rafael, CA) linked to a video screen camera (CCD/RGB; Sony Corp., Park Ridge, NJ) and microscope (model BH2; Olympus Corp., Precision Instruments Division, Lake Success, NY) equipped with metallurgical lenses.

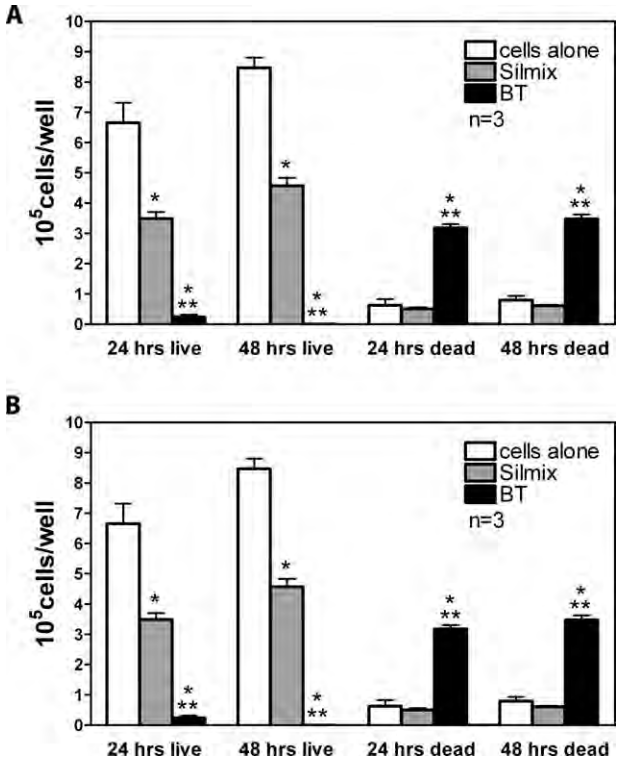

FIGURE 4. Effects of silorane and bisGMA/TEGDMA (BT) resins on MLO-A5 cell proliferation and attachment to polymer disc surfaces. (A) Effects of SilMix and BT on live and dead cell number. (B) In comparison to BT, most of the cells in wells with SilMix were viable with a percentage of live cells greater than 87% and similar to the controls. Compared to the respective time, *significantly different from cells alone ($p < 0.05$); **significantly different from SilMix ($p < 0.05$) using one-way ANOVA and Tukey post test.

Alizarin red staining for calcium quantification

The MLO-A5 cells were cultured for mineralization and fixed in formalin as mentioned previously above. Fixed cultures were washed three times with TBS (Tris-buffered saline) and then stained with 4 n*M* alizarin red S dye (AR-S) for 5 min. Cultures were then rinsed with water followed by a 15-min wash with TBS to reduce nonspecific AR-S stain. The mineralized areas were measured using a semiautomated imaging system as described previously.[23]

Stabilization of osteotomized murine femuri with SilMix

Surgical procedures. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of authors' institution. Eight 12-week old C57black6 mice were housed in the animal care facility under a 12-h/12-h light/dark cycle. The mice were anesthetized with 3.5% isoflurane and maintained with ketamine/dexdormitor (75/0.25 mg/kg body weight, intraperitoneally). The skin over the right hind limb was shaved, swabbed with betadine, and draped under aseptic conditions. Using the sterile instruments, a 1.5-cm skin incision was made on lateral aspect of thigh extending from the vastus lateralis muscle to the patellar ligament insertion, preserving the patellar ligament. The patella was retracted medially with the knee extended. The knee was slowly flexed to expose the intercondylar notch. The intramuscular septum between the vastus lateralis and hamstring muscles was separated using blunt dissection, and the periosteum was incised to expose the femur. A transverse fracture of the femur was created using an electrical round saw. A 0.7-mm K-wire was gently inserted into the intercondylar entry point, through the fractured femur, to the appropriate depth (approximately the level of lesser trochanter), which served as intramedullary fixation for the fracture to prevent angulations or displacement. SilMix resin (50–70 μl) was applied around the fracture site and cured using a dental curing lamp for 20 sec (three times. After polymerization of SilMix resin, the stability of the fixed femur was evaluated, then the K-wire was removed. The capsule and skin were sutured with 4–0 nylon. The animal was allowed to fully recover in a separate cage on a warming pad and was allowed activity *ad libitum*. An analgesic (buprenorphine hydrochloride, 0.05

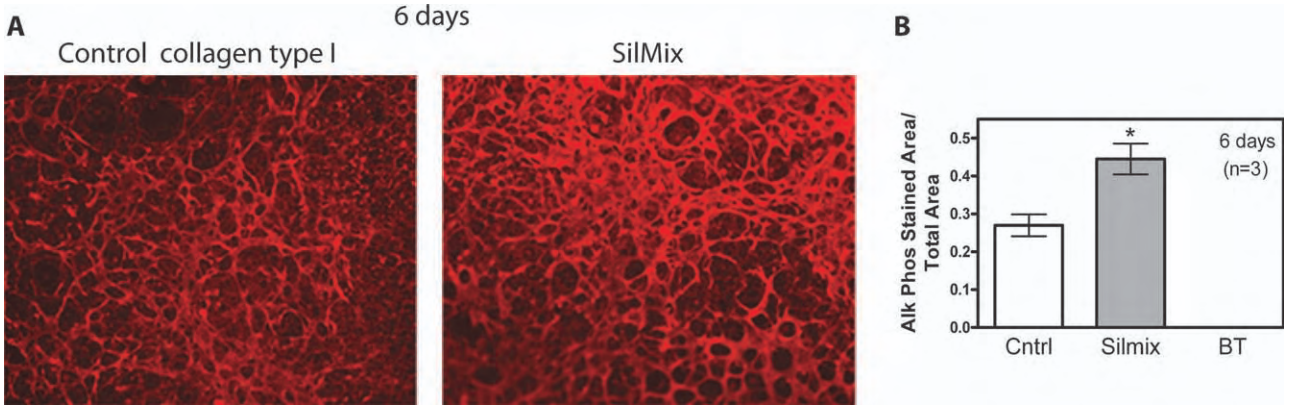

FIGURE 5. Effects of silorane on collagen matrix formation and alkaline phosphatase. Immunohistochemical staining of collagen type 1 fibers in the control (left) and SilMix (right) revealed an intact collagen network that appeared thicker in the SilMix resin drop cultures (A). (×10 magnification). No negative effects were observed on collagen matrix formation at 6 days of culture. At 6 days, significantly elevated alkaline phosphatase was observed in the SilMix containing cultures compared to the control. No alkaline phosphatase was detected in the BT cultures at 6 days (B). *Significantly different from cells alone ($p < 0.05$) and from BT ($p < 0.001$) using one-way ANOVA and Tukey post test. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

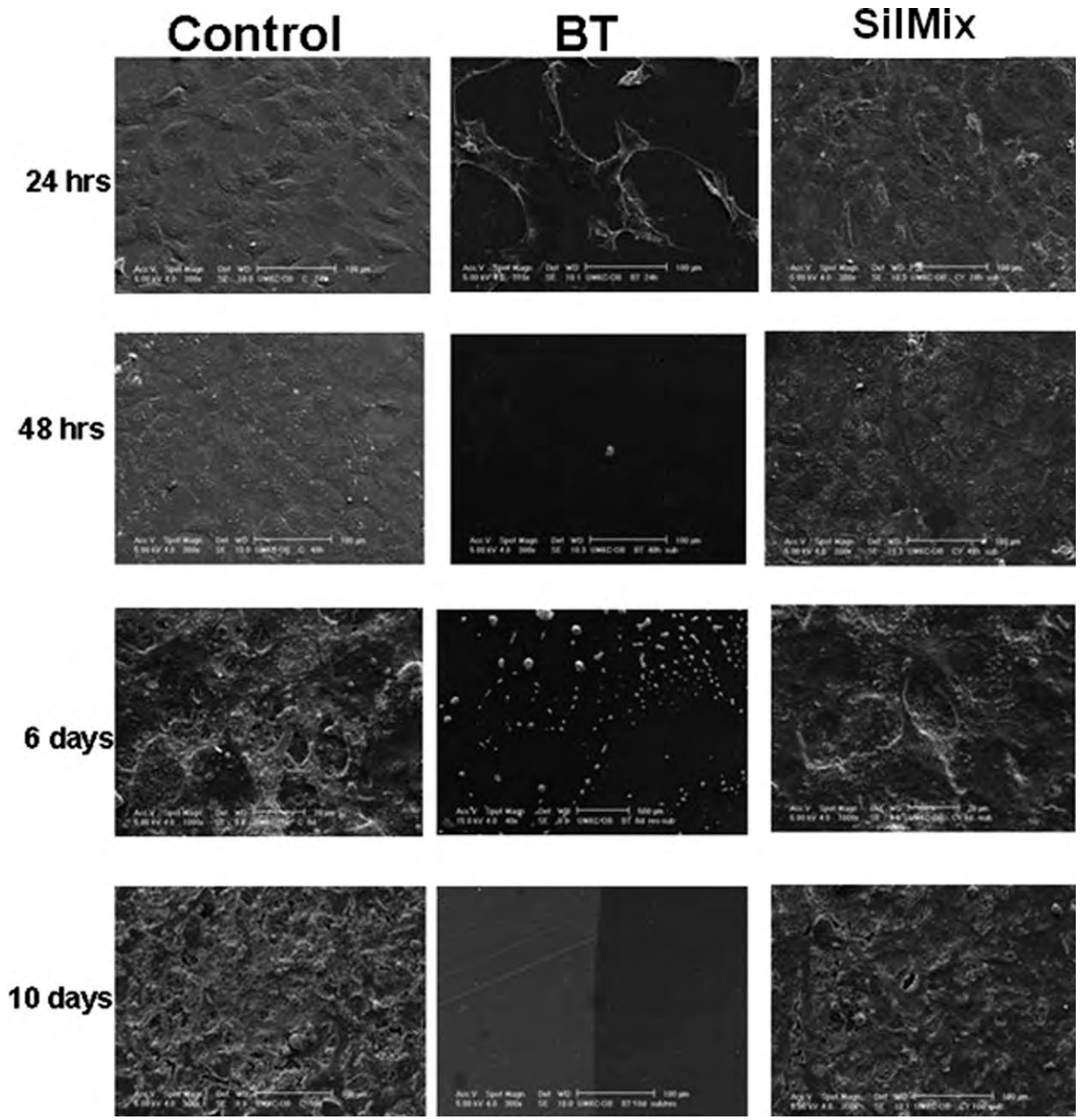

FIGURE 6. Secondary electron micrographs of MLO-A5 cells cultured for 24 and 48 h, and 6 and 10 days on cover slips with resin drops. At 24 h, the morphology of cells exposed to the SilMix appears normal, but with some membrane ruffling. The BT exposed cells appear necrotic. By 48 h, no cells are visible in the BT cultures. In contrast, the SilMix cultures show normal cell growth and matrix formation and appear healthy at 10 days of culture (scale bars = 100 μm except for 6 days at 20 μm).

mg/kg) was administered subcutaneously twice per day during three postoperative days.

Radiographic evaluation. At days 0, 7, 14, 21, and 28 postsurgery, the animals were anesthetized with ketamine/dexdormitor (75/0.25 mg/kg body weight, intraperitoneally). Radiographs of the femora were obtained using a Faxitron MX-20 (Faxitron X-Ray LLC, Lincolnshire, IL) at 26 KV and 10 sec. The fracture healing, angulation, or displacement of SilMixresin stabilized osteotomized femora was evaluated.

Histological assessment. The animals were sacrificed at days 7 ($n = 4$) or 28 ($n = 4$) postsurgery. The SilMix resin stabilized femora with surrounding tissues were harvested, fixed in 10% buffered formaldehyde for 2 days, decalcified in 14% EDTA for 3 weeks, and then incubated with 15% and 30% sucrose, serially, for 2 days. The samples were embedded for frozen sections allowing retention of both bone and resin, and the sections were cut longitudinally at 12 μm. The serial sections were stained with hematoxylin and eosin. The newly formed bone at the fracture site was evaluated.

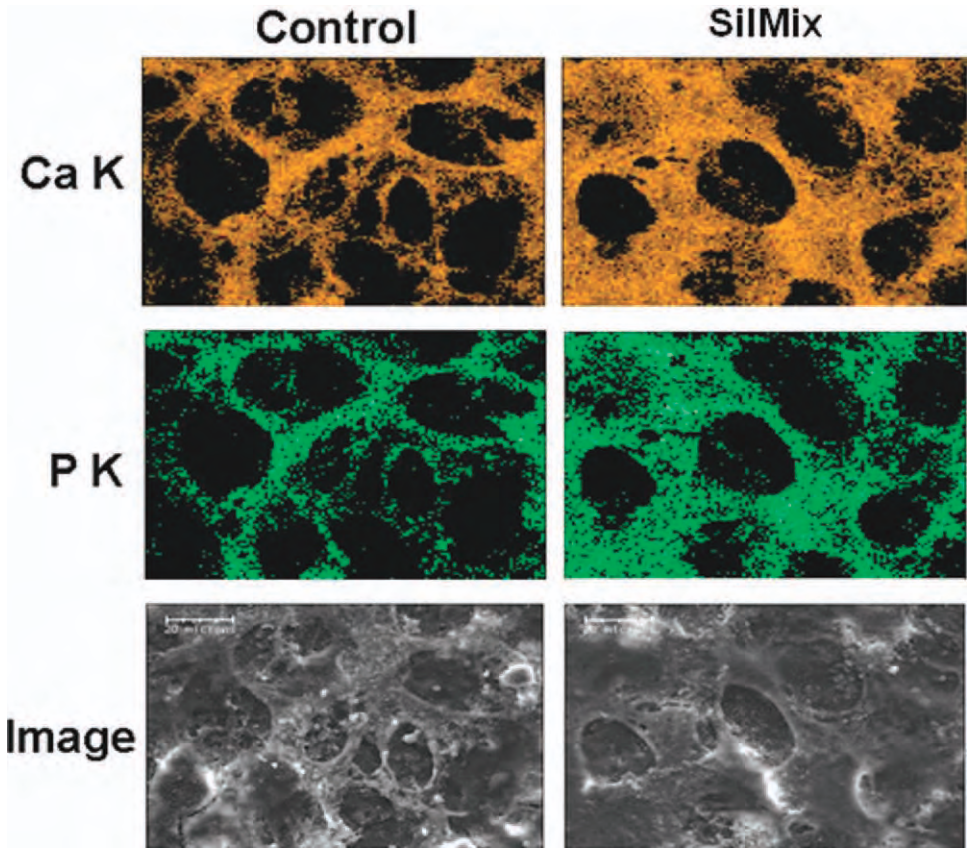

FIGURE 7. Elemental analysis (EDS) of the mineralized honeycomb-like matrix formed by the MLO-A5 cells at 11 days of culture on cover slips with resin drops. The calcium component pattern colorized as orange (Ca K) completely matches or overlays with the phosphate component pattern colorized as green (P K) and is consistent with the micrograph image (Image). Again, by this approach the cell matrix formed in the SilMix containing wells appears more mineralized (scale bars = 20 μm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Statistical Analysis

Statistical significance was determined either using the one-way ANOVA and Tukey post test or in some cases the three-way ANOVA for significance at the $p < 0.05$ level. Experiments were repeated a minimum of two times with similar results.

RESULTS

The chemical structure of the silorane that was used in this study is shown in Figure 1. The degree of polymerization of the SilMix and the BT resins can be found in Figure 2. Peak ratios of a spectral peak associated with polymerization (883 cm^{-1} representing ring-opening in siloranes) with an unchanging peak (1257 cm^{-1} in curing siloranes) were calculated for each material. For the BT specimens, degree of polymerization was calculated, based on the 1637 cm^{-1} (C=C) associated with polymerization with respect to 1714 cm^{-1} (C=O). For the first set of *in vitro* experiments, resin drops were placed either in the center or off-center in culture wells. No significant differences were observed depending on drop placement. The cell number was higher in the wells containing silorane resin drops at 24, 48, and 72 h as compared to a greater than 50% reduction in methacrylate BT containing wells [Figure 3(A)]. In contrast to the resin drop cultures, there were pronounced decreases in cell numbers for cells grown on the polymer disc surfaces [Figure 3(B)]. In order to test if this effect was due to toxic leachables, extraction of the disc resins was performed. The amount of formazan produced by cells in the presence of SilMix disc extracts was similar to levels of formazan produced by control cells (tissue culture grade polystyrene conditioned media); however, BT resin extracts generated considerable toxicity [Figure 3(C)]. This shows that no toxic component was released by the SilMix resin in contrast to the BT resin.

Using the trypan blue dye exclusion method, the number of live cells on SilMix surfaces were significantly lower ($p < 0.05$) than the controls [Figure 4(A)]. The number of dead cells were also lower but not significantly different from the number of dead cells in the controls. Upon calculation of the percent of live and dead cells, the percentage of live cells obtained with SilMix was similar to the controls [Figure 4(B)]. However, the number and percent of live cells for BT was very low at 24 h with most cells dead at 48 h.

Because the cells did not adhere well to the resin discs, the resin drop approach was used to examine osteoblast differentiation and function. Collagen type 1 is essential for

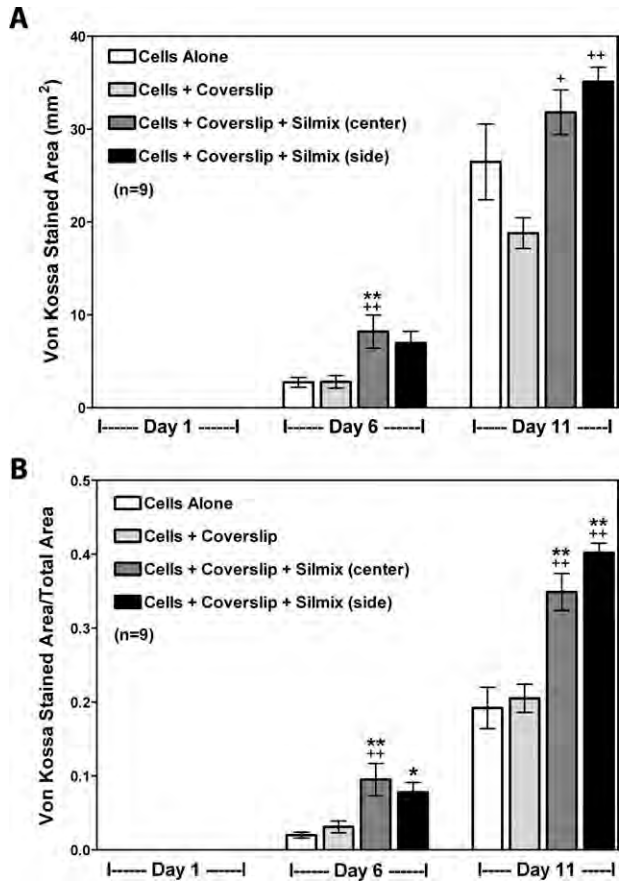

FIGURE 8. Quantitation of mineral formed in cultures on cover slips with resin drops using von Kossa staining. A good correlation is observed between each stain for phosphate in (A) and per total area measured in (B). $^{+}$Significantly different from cells + coverslip ($p < 0.05$); *significantly different from cells alone ($p < 0.05$); $^{++}$significantly different from cells + coverslip ($p < 0.01$); **significantly different from cells alone ($p < 0.01$) using one-way ANOVA and Tukey post test.

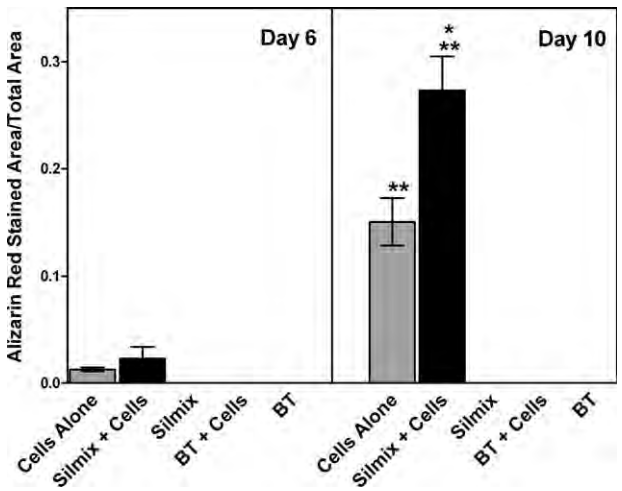

FIGURE 9. Quantitation of mineral formed in cultures using Alizarin red staining for calcium at 6 and 10 days on cover slips with resin drops. Whereas the BT treated cultures had no detectable mineral, surprisingly, the SilMix cultures had greater staining for mineral than control cultures. Neither of the cover slips with just the resins, exhibited any background staining. *Significantly different from cells alone ($p < 0.01$); **Significantly different from BT + cells ($p < 0.001$) using one-way ANOVA and Tukey post test.

the normal mineralization of bone.[28] Immunofluorescent staining for type 1 collagen was performed on MLO-A5 cells at day 6 of culture (Figure 5). The pattern is clearly fibrillar at 6 days and of greater intensity in the silorane containing culture (right panel) compared to control (left panel) [Figure 5(A)]. Alkaline phosphatase activity at 6 days was significantly higher in culture wells with silorane than in the control [Figure 5(B)].

Analysis of the ultrastructure of the cultures using SEM was also performed [Figure (6)]. The top row shows the cells after 24 h in culture. In contrast to the cells cultured

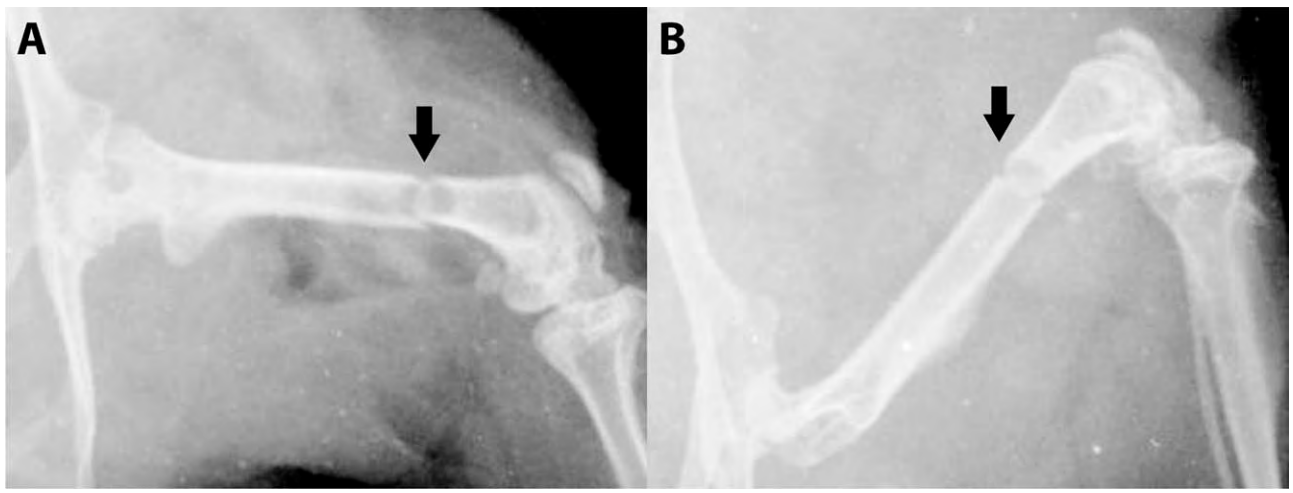

FIGURE 10. Radiographs of the SilMix resin stabilized murine femori at day 0 (A) and 28 (B) postsurgery. There was no sign of displacement of the femoral fracture (arrow). The fracture gap was coalescing at 28 days (magnification = ×2).

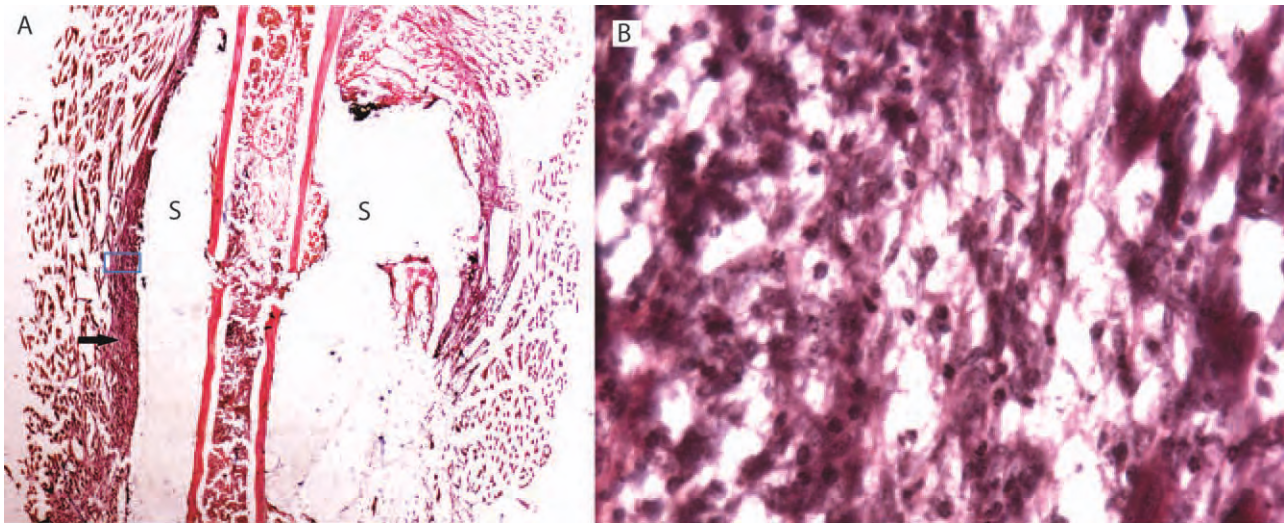

FIGURE 11. Histological section showing SilMix resin (S) encasing the murine osteotomized femur. The arrow shows granulation tissue between muscle tissue and biomaterial and filling the fracture gap (A). No inflammatory response was observed at 7 days postsurgery. The blue box is the area of magnification as reflected in B (original magnification: A, ×1; B, ×40).

in the BT-containing wells, which were dying at 24 h and absent by 48 h, the control cultures (left panel), as well as silorane containing cultures(right panel), showed high cell confluency. After 6 days under mineralization conditions, the cells in the silorane containing cultures appeared similar to controls, with a well-formed honeycomb-like matrix. These cells cultured up to 10 days showed the formation of a mineralized matrix covering the entire well. The mineralized honeycomb-like matrix formed by the MLO-A5 cells in the presence of silorane was analyzed for mineral content using energy dispersive spectroscopy (EDS) to obtain calcium and phosphorus distribution maps (Figure 7). Calcium (A) and phosphorus (B) were co-localized within the mineralized structures of the matrix (C-SEM). Quantification of mineralization was performed using both von Kossa (Figure 8) and Alizarin Red staining (Figure 9). Von Kossa detects phosphate, whereas alizarin red detects calcium.[29] As can be seen in Figure 8, von Kossa staining increased with extended time in culture in the control wells and the wells containing silorane. There was a complete absence of staining in the wells containing BT.

In these cultures in which the resin was centered in the well, the mineralized matrix appeared to build up around the silorane resin drop. Next, mineralization assays were performed on cells grown on Thermanox discs with the silorane drop placed in the center as compared to the sides of the disc. No significant effects were observed on mineralization whether quantified using the total stained area or the total stained area divided by the total area in the well which included the resin drop (Figures 8 and 9). No significant

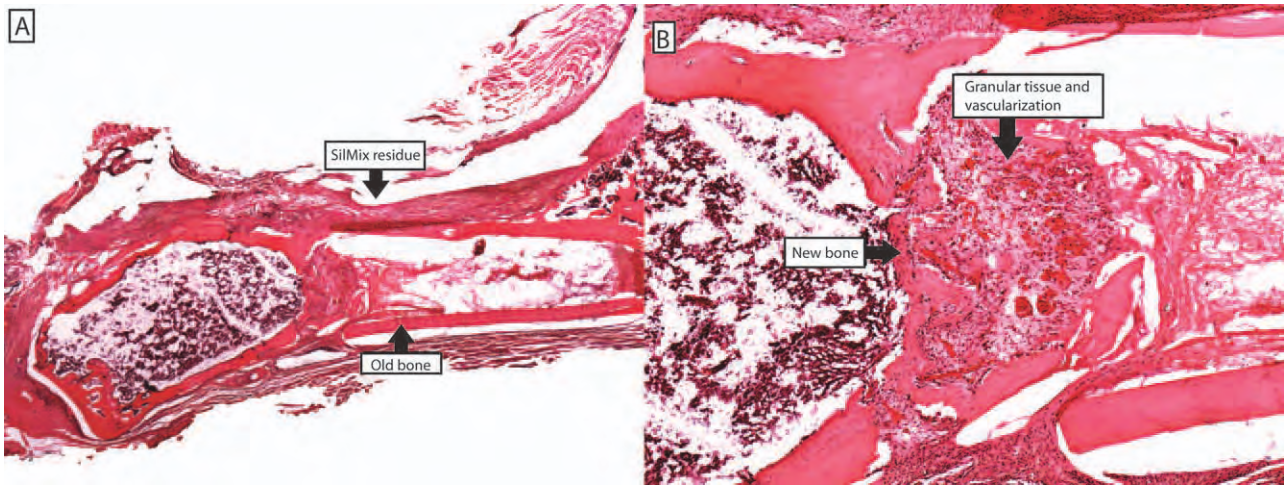

FIGURE 12. Histological section showing SilMix resin residue around the fracture site, newly formed woven bone filling the fracture gap, many blood vessels present in the newly formed woven bone area at 28 days. No inflammatory reaction was observed (original magnification: A, ×1; B, ×4).

difference was found whether the drop was placed in the center or at the side of the coverslip. Therefore, the position of the resin had no effect on cellular differentiation and mineralization.

Radiographic assessment of osteotomies of femora (transverse straight-line fracture) was performed by X-ray at days 0, 7, 14, 21, and 28 post-operation. Radiographs postsurgery showed that there was no sign of displacement of the femoral fracture at 28 days (Figure 10). The fracture gap became opaque, and no external callus was observed at 28 days postsurgery (other time points are not shown). The osteotomized femurs were encased by SilMix resin, and there was no displacement of the stabilized fractured bones. Furthermore, no inflammatory response was observed around the fracture sites (Figure 11). One to two layers of cells were observed between the bone and material at day 7 postsurgery (Figure 11). The granulation tissue containing fibroblasts and interspersed small blood vessels were seen between the muscle tissue and biomaterial. Histological evaluation showed that there was no sign of displacement of the femoral fracture at 28 days postsurgery (Figure 12). SilMix resin residue was observed around the fracture site. The fracture gap was filled by newly formed woven bone. Blood vessels were present in the newly formed woven bone area. No inflammatory reaction or external callus was observed at the fracture site.

DISCUSSION

We have previously developed silorane-based resins superior to methacrylate-based resins based on enhanced biocompatibility and significantly less polymerization stress without an associated proportional reduction in mechanical properties.[7,8] Siloranes are now being used as matrix resins to produce low stress/shrinkage dental composites with reduced cytotoxicity and genotoxicity.[12] Clearly, the siloranes are superior with respect to a lower exothermic temperature, less shrinkage, and less toxicity, compared to methacrylates representing a major step forward for use beyond dental composites. Therefore, we hypothesized that siloranes may be an improvement and serve as replacements of methacrylates used in orthopedic applications, such as bone cements, and performed *in vitro* and *in vivo* experiments to begin to test this hypothesis.

In this study, we demonstrated that silorane-based resins are nontoxic to bone cells and support parameters of bone formation both *in vitro* and *in vivo*. The mineralized, formed matrix was composed of collagen type I in a honeycomb-shaped structure, and the mineral component contained calcium and phosphate in a normal ratio compared to controls and normal bone. Surprisingly, our experiments showed that regardless of placement of silorane in culture wells the bone cells mineralized similarly, if not to a greater extent than control wells. This result may be due to the fact that the resin drop itself displaced surface area and increased cell number per area. Whereas this could explain the increase in mineralized matrix, it could not explain the increase in alkaline phosphatase. Further experiments are required to validate this observation.

Surfaces on prosthetic devices and bone cements can have either beneficial or detrimental effects on bone cells.[30] Many materials have ideal structural properties to function as implants, cements, or scaffolds but do not have the necessary biocompatibility or bioactivity. Conversely, many materials have neutral or enhancing biological properties but lack the necessary mechanical properties. Materials can be toxic, neutral, or can even support bone growth, especially with the inclusion of growth factors.[31] In this study, low bone cell attachment to silorane surfaces was observed. We have shown that surfaces can modulate the differentiation of osteoblasts,[32] and Boyan and coworkers have shown that surface roughness and microtopography plays a role in bone cell function and mineralization.[33,34] Therefore, biocompatibility and induction of bone growth become important issues. Even though low bone cell attachment was observed, this property could be improved using approaches such as surface etching.

Also, in this study, we demonstrated that when the silorane resin was placed around a bone defect, a femoral osteotomy, no inflammation was observed. Surprisingly, at 28 days, the bone began to heal in the absence of callus. This result raises the question as to whether this approach could be used in patients to stabilize bone. Pediatric orthopedic surgeons are often faced with fracture situations where the bone is either too small to support plate stabilization, or too close to the physis such that fracture stability cannot be achieved without jeopardizing the integrity of the physis. An inert stabilizer that is not toxic to physeal cartilage could be ideal in this setting. Other fracture applications might include patients with severely osteoporotic bone, where screws may not achieve good integration, or in patients with significant contractures (as in cerebral palsy or stroke) that do not allow for standard nail or plate insertion without creating further injury. Other uses include battlefield situations or natural disasters, where fractures could be stabilized before transport for more permanent treatment. This material would be easy to remove from the fracture site for definitive treatment. Therefore, in addition to being a substitute for methyl methacrylate in bone cement, silorane resin could function as a bone stabilizer. These concepts are undergoing further testing in our laboratory.

CONCLUSION

In conclusion, bisGMA/TEGDMA was toxic and totally inhibited bone cell growth while the low toxicity of silorane resin supported bone cell differentiation, matrix formation, and mineralization. In addition, all of the experimental methods, such as von Kossa and Alizarin red staining, and the SEM and EDS analyses, were in agreement and complementary with regard to quantitation and mineral composition. These studies clearly show that the silorane is superior to the bisGMA/TEGDMA with regard to support of bone cell function and has the potential to be used as a component of bone cement or as a bone-stabilizing material.

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Manuscript

Estimation of properties of a photoinitiated silorane-based composite with potential for orthopaedic applications

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Estimation of properties of a photoinitiated silorane-based composite with potential for orthopaedic applications

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Abstract: We have synthesized a filler-reinforced silorane composite that has potential applications in orthopaedic surgery, such as for a bone stabilizer. The purpose of the present work was to develop a method for estimating four properties of this material; namely, maximum exotherm temperature, flexural strength, flexural modulus, and fracture toughness. The method involved the use of mixture design-of-experiments and regression analysis of results obtained using 23 formulations of the composite. We validated the estimation method by showing that, for each of four composite formulations that were not included in the method development, the value of each of the aforementioned properties was not significantly different from that obtained experimentally. Our estimation method has the potential for use in the development of a wide range of orthopaedic materials. © 2011 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 100B: 163–169, 2012.

Key Words: orthopedic biomaterials, design-of-experiments, mechanical properties, handling properties

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INTRODUCTION

There is a wide range of methods used to stabilize fractured bones, including plaster casts, splints, external fixators, and intramedullary pinning.[1] These methods are adequate for the majority of fractures, but, in some cases, such as comminuted fractures of small bones, additional methods are needed. Successful bone fracture outcomes depend on adequate stabilization during the healing process.[2–4] One of those additional methods involves the use of a bone stabilizer.[5] It has been suggested that composites, based on a silorane resin as the matrix, are potential candidates for polymeric bone stabilizers. With this type of stabilizer, the polymer is applied directly to the bone and polymerizes directly on it, thereby obviating the need for ample and healthy bone for placement of pins and/or rods, and allowing the stabilization of the fracture without joint immobilization.[6,7] There are limited data on the properties of these silorane resin-reinforced composites[8–15] due to the recent development of siloranes for dental applications.[9,16] Furthermore, novel silanized filler particles have not been explored to achieve a solid resin/filler particle interface and subsequent improved mechanical properties.[17–19] This situation may be rectified by developing validated methods for estimating their properties. In the present contribution, we give details of such a method, with reference to four properties of particular importance to materials to be used as bone stabilizers, namely, maximum exotherm temperature (T_{max}), flexural strength (σ_B), flexural modulus (E_B), and fracture toughness (K_{IC}).

MATERIALS AND METHODS

Preparation of composites

The matrix for these composites was prepared in house using chemicals obtained from Cambridge Isotopes, Gelest, Aldrich, and Alfa Aesar. The matrix was a silorane resin (SilMix, SM) that comprised 50 Wt/Wt % of (bis[2-(3{7-oxabicyclo[4.1.0]heptyl})-ethyl]methylphenyl silane) (PHEPSI) and 50 Wt/Wt % 2,4,6,8-tetrakis(2-(7-oxabicyclo[4.1.0]heptan-3-yl)ethyl)-2,4,6, 8-tetramethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane (CYGEP; Figure 1). To formulate the composites, three different fillers were added to the matrix, with the choice of filler being based on four criteria, namely, interfacial compatibility with the matrix, low cytotoxicity, a refractive index close to that of the matrix, and no inhibition of polymerization. The fillers used were a glass, yttria alumino-silicate; 15.0 Wt % Y_2O_3, 5.0 Wt % Al_2O_3, and 80 Wt

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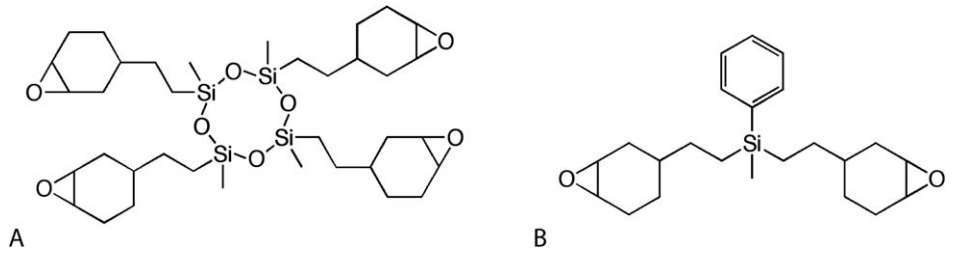

FIGURE 1. Components of silorane resin (SilMix, SM). (A) CYGEP and (B) PHEPSI. Note the epoxide groups (C–O–C) at the extremities of the structures.

% SiO_2 (DY5), another glass, barium boroaluminosilicate; 54.5 Wt % SiO_2, 5.9 Wt % Al_2O_3, 10.5 Wt % B_2O_3, and 29.1 Wt % BaO (M12), and alumina nanorods ($\sim$ 30 nm width $\times$ 450 nm length) prepared from boehmite nanorods. The surface of each of the fillers was modified with 2-(3,4-epoxycyclohexyl) ethyl trimethoxysilane (ECHE-TMS) by refluxing with 1 vol/vol % ECHE-TMS dissolved in methylisobutylketone. The composites were photoinitiated using an initiator composed of 0.15 Wt % ethyl *p*-dimethylaminobenzoate (Acros, Pittsburgh, PA), 1.0 Wt % camphorquinone (Sigma Aldrich, St. Louis, MO), and 3.0 Wt % *p*-(octyloxyphenyl)-phenyliodonium hexafluoroantimonate (Gelest, Morrisville, PA). In preliminary studies, we found that photoinitiated silorane polymerization was not inhibited when filled with up to 50 Wt % of the ECHE-TMS surface modified glasses or up to 5 Wt % of the ECHE-TMS surface modified alumina nanorods.[20]

We used a commercially available mixture design-of-experiments software (Design Expert 7; Stat-Ease, Minneapolis, MN) to analyze the characteristics of the composite formulations tested (Table I). Replicates were added to the tested formulations to lower and balance the leverages of each data point. Each of these formulations was prepared by mixing the filler and the resin using a high-speed mixer (FlackTek, Landrum, SC) until visual inspection every 5 min confirmed complete mixing. The composite was allowed to rest 10–15 min after mixing, until visible air bubbles were removed and then used immediately to prevent premature polymerization. The properties of composite formulations 4 and 6 were not determined because they failed to polymerize.

Maximum exotherm temperature

Exotherm temperature was measured using a K-type thermocouple (Omega, Stamford, CT) affixed to a glass slide and slightly bent so that the tip of the thermocouple was positioned in the center of an acetal resin (Delrin®) washer (McMaster-Carr, Aurora, OH), which was also affixed to the glass slide with lab tape (Figure 2). Each composite formulation (0.6 g) was mounded to completely cover the tip of the thermocouple. The sample was then irradiated [12 mm diameter tip, 450 mW/cm^2 (Cure Rite, Dentsply Caulk, Milford, DE) at a distance of 3 mm] using a dental curing lamp (3M XL3000, St. Paul, MN) for 2 min. Specimens were inspected after testing, and results were excluded from further study if the tip contacted the glass slide or was not entirely covered with the composite. Temperature data were recorded using a data logger (OM-PLTC, Stamford, CT) at 1 Hz for 30 min postirradiation.

Flexural strength and modulus

Flexural specimens (25 mm $\times$ 2 mm $\times$ 2 mm) were formed in borosilicate glass tubes (VitroCom, Mountain Lakes, NJ) coated with silicone spray mold release (Mark V Laboratory, East Granby, CT) as per ISO specification 4049.[21] A pipette

TABLE I. Compositions of the Composites Formulated in Development of Material Property Estimation Method

Formulation number	Volume fraction			
	SM	DY5	M12	Nanorod
1	0.7312	0.0000	0.2688	0.0000
2	0.7754	0.1709	0.0390	0.0147
3	0.9848	0.0000	0.0000	0.0152
4	0.7108	0.2684	0.0000	0.0208
5	0.8733	0.1180	0.0000	0.0087
6	0.7108	0.2684	0.0000	0.0208
7	0.8863	0.0000	0.1049	0.0088
8	0.7201	0.1435	0.1258	0.0106
9	0.7352	0.0000	0.2433	0.0216
10	1.0000	0.0000	0.0000	0.0000
11	0.8355	0.0877	0.0768	0.0000
12	0.8355	0.0877	0.0768	0.0000
13	0.9164	0.0379	0.0332	0.0125
14	1.0000	0.0000	0.0000	0.0000
15	0.7815	0.1085	0.0951	0.0148
16	0.7877	0.0452	0.1521	0.0150
17	0.7044	0.2956	0.0000	0.0000
18	0.8863	0.0000	0.1049	0.0088
19	0.7044	0.2956	0.0000	0.0000
20	0.8578	0.0412	0.0874	0.0136
21	0.7312	0.0000	0.2688	0.0000
22	0.9848	0.0000	0.0000	0.0152
23	0.7201	0.1435	0.1258	0.0106
24	0.8733	0.1180	0.0000	0.0087
25	0.7352	0.0000	0.2433	0.0216
Additional composite formulation 1	0.7352	0.0000	0.2433	0.0216
Additional composite formulation 2	0.7886	0.0462	0.1485	0.0167
Additional composite formulation 3	0.7247	0.0839	0.1855	0.0059
Additional composite formulation 4	0.9154	0.0813	0.0000	0.0033

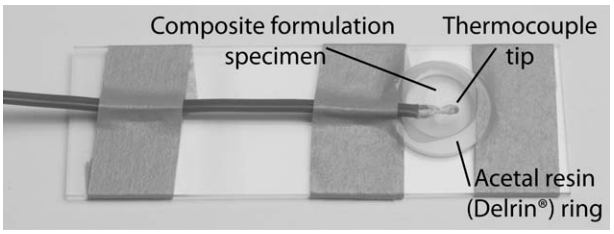

FIGURE 2. A photograph of the experimental setup for determining the maximum exotherm temperature.

was used to fill the molds with resin. The specimen was irradiated [12 mm diameter tip, 450 mW/cm^2 (Cure Rite, Dentsply Caulk, Milford, DE) at a distance of 3 mm] using a dental curing lamp (XL3000; 3M, St. Paul, MN) for 2 min along the top surface at three consecutive regions for 40 s each, 40 s in a scanning motion along the bottom of the glass mold, and then the specimen was removed from the glass. The method of photoinitiating specimens and induction of any overlapping regions have been shown to not have an effect on flexural properties.[22] The specimens were stored in phosphate-buffered saline (PBS), at 23 ± 1°C, for 24 h, after which, the specimen was loaded, until fracture, at a displacement rate of 3.7 mm/min in a four-point bend fixture with a support span of 20 mm on a BOSE mechanical tester (EnduraTEC ELF 3300, Eden Prairie, MN). Specimens with visible surface flaws, bubbles, or undistributed filler particles were excluded from the study. The resulting stress–strain curve was used to determine flexural strength (σ_B) and flexural modulus of elasticity (E_B).

Fracture toughness

The configuration of the specimens used in the fracture toughness tests was the same as that used in the flexural strength/modulus tests. Using steps detailed in ASTM D 1708-06a,[23] a 0.15-mm slotting cutter (Malco, Cranston, RI) was used to create a 0.6 mm deep notch on one face of the test specimen. The specimen was stored in PBS, at 23 ± 1°C, for 24 h after which it was loaded, on a materials testing machine (ELF 3300), at a displacement rate of 1.0 mm/min, until fracture. Specimens with visible surface flaws, bubbles, or undistributed filler particles were excluded from the study. The fracture toughness (K_{IC}) of the composite was determined as a function of the maximum load incurred before failure according to the standard.

Property estimation method

There were two steps in the method. In the first, for a given material property, the equation given below was fitted to the body of experimental results obtained from the 23 tested composite formulations. This equation relates the material property to the volume fractions of the fillers. In other words, the equation is of the form:

$$\begin{aligned} Y = a + b[\mathrm{SM}] + c[\mathrm{DY5}] + d[\mathrm{M12}] + e[\mathrm{NR}] + f[\mathrm{SM}][\mathrm{DY5}] \\ + g[\mathrm{SM}][\mathrm{M12}] + h[\mathrm{SM}][\mathrm{NR}] + i[\mathrm{DY5}][\mathrm{M12}] + j[\mathrm{DY5}][\mathrm{NR}] \\ + k[\mathrm{M12}][\mathrm{NR}] + l[\mathrm{SM}][\mathrm{DY5}][\mathrm{M12}] + m[\mathrm{SM}][\mathrm{DY5}][\mathrm{NR}] \\ + n[\mathrm{SM}][\mathrm{M12}][\mathrm{NR}] + o[\mathrm{DY5}][\mathrm{M12}][\mathrm{NR}] \end{aligned} \quad (1)$$

where [ ] denotes volume fraction of the filler or resin, and *a*–*o* are the corresponding coefficient estimates (Table II). The values of these coefficients, the standard error associated with each coefficient, and the coefficient of multiple determination for the equation were determined using a commercially available regression analysis software (Design-Expert 7.1.6, Stat-Ease, Minneapolis, MN).

In the second part, we synthesized four additional composite formulations whose compositional details were within the range of those used in the development of the estimation method (additional composite formulations 1–4 in Table I). For each of these additional formulations, T_{max}, σ_B, E_B, and K_{IC} were determined using the methods detailed above. In addition, for each of these additional formulations,

TABLE II. Equation Coefficients and Standard Errors for Model Output

	Maximum exotherm temperature		Flexural strength		Flexural modulus		Fracture toughness	
Component	Coefficient estimate	Standard error	Coefficient estimate	Standard error	Coefficient estimate	Standard error	Coefficient estimate	Standard error
A-SilMix	115.16	3.92	73.33	3.42	2.29	0.07	0.46	0.04
B-DY5	67.56	4.10	80.79	5.65	5.10	0.12	0.95	0.04
C-M12	92.87	5.56	104.86	26.11	3.92	0.54	0.74	0.06
D-Nanorods	6610.09	4630.80	−20672.71	70507.21	1324.03	1465.76	119.27	50.67
AB	27.60	21.91	−106.13	296.65	−8.01	6.17	−0.11	0.24
AC	37.91	26.29	−262.66	248.78	11.72	5.17	0.61	0.29
AD	−6703.78	4864.27	21175.63	74266.22	−1390.08	1543.91	−122.86	53.22
BC	49.72	29.79	103.19	414.16	13.96	8.61	1.06	0.33
BD	−7579.50	4783.65	21090.45	75838.89	−1549.26	1576.60	−127.25	52.34
CD	−7453.12	5005.54	21755.33	76913.36	−1391.98	1598.94	−127.10	54.77
ABC			814.65	2385.21	−36.34	49.59		
ABD			4231.67	6154.37	629.28	127.94		
ACD			6254.09	7321.42	−209.52	152.20		
BCD			−7323.69	8737.34	111.92	181.64		
R^2	0.9090		0.8111		0.9902		0.8474	

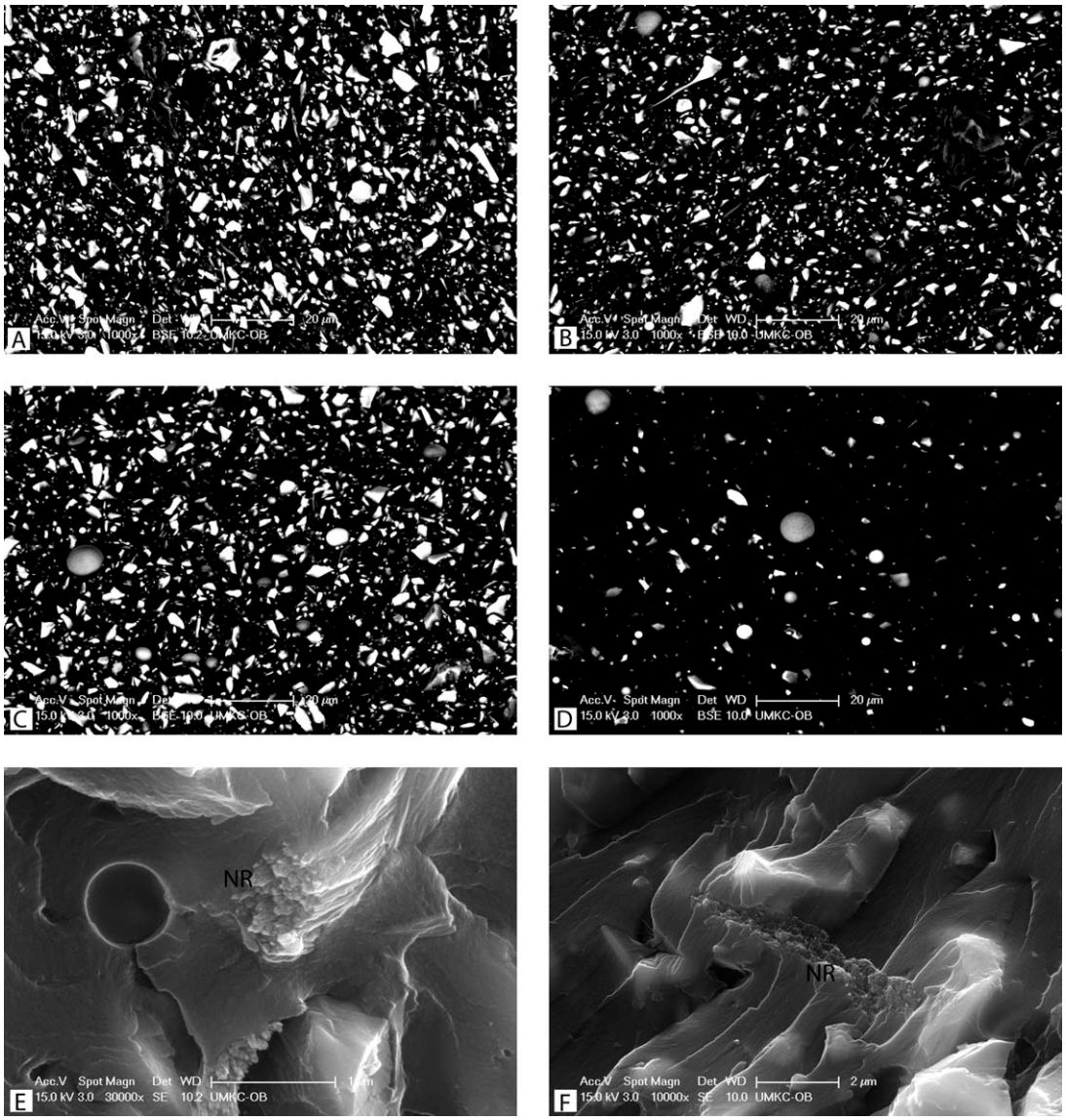

FIGURE 3. Morphologies of the fracture surface of flexural test specimens of additional composite formulations 1–4. Images (1000×) of additional composite formulations 1–4 (panels A–D) show uniform distribution of the glass filler particles (DY5 and M12). Higher magnifications of composite 1 (panel E, 30,000×) and composite 2 (panel F, 10,000×) reveal agglomerations of nanorod filler particles.

morphological details, in particular, the distribution of the filler particles within the matrix, were obtained using a scanning electron microscope (SEM; Field-Emission Environmental Scanning Electron Microscope FEI/Phillips XL30 ESEM-FEG, Phillips Electron Optics, FEI Company, Hillsboro, OR). SEM analysis of the fracture surfaces of flexural test specimens was used to verify if the filler particles were adequately distributed throughout the composite.

Statistical analysis

For each of the four additional composite formulations, a predicted material property obtained using Eq. (1) was compared with that obtained experimentally (T_{max}, $n = 3$; σ_B, $n = 8$; E_B, $n = 8$; and K_{IC}, $n = 8$) using a one-sample *t*-test (PASW Statistics 18, IBM Corporation, Somers, NY).

RESULTS

For each of the material properties determined, the fit of Eq. (1) to the experimentally obtained values for the 23 formulations was (1) good for formulations in which the filler was either of the two glass particles (Table II), which is attributed to good dispersal of these materials in the matrix (Figure 3); (2) poor for formulations in which the filler was alumina nanorods (Table II), which is attributed to agglomeration of these materials in the matrix (Figure 3); and (3) poor when combinations of the volume fractions of the fillers in the composites were considered (Table II). The property estimation method was considered validated (Table III).

DISCUSSION

We have identified a biocompatible silorane resin with potential applications in many health fields, such as

TABLE III. Predicted and Observed Results of Additional Composite Formulations

	Maximum exotherm temperature (°C)		Flexural strength (MPa)		Flexural modulus (GPa)		Fracture toughness (MPa $m^{1/2}$)	
	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted
Composite 1	74.0 (4.0)[a]	76.6 (68.2–85.1)	59.9 (4.6)	72.0 (64.3–79.7)	4.96 (0.09)	5.45 (5.29–5.61)	0.86 (0.07)[a]	0.84 (0.75–0.93)
Composite 2	83.7 (1.5)	88.4 (83.1–93.6)	66.5 (5.6)	77.0 (27.4–126.6)	4.26 (0.07)	4.58 (3.55–5.61)	0.83 (0.06)[a]	0.84 (0.78–0.90)
Composite 3	77.3 (2.1)	83.7 (73.1–94.3)	61.5 (2.5)	98.5 (41.0–155.9)	4.66 (0.08)	5.13 (3.94–6.33)	0.86 (0.03)[a]	0.87 (0.76–0.99)
Composite 4	110.7 (10.1)[a]	103.5 (94.9–112.2)	60.0 (3.9)	65.6 (15.7–115.6)	2.73 (0.04)	1.78 (0.74–2.82)	0.59 (0.05)	0.53 (0.44–0.63)

For the observed data, numbers in parentheses are standard deviation.
For the predicted values, numbers in parentheses are 95% confidence intervals.
[a] $p > 0.05$ between predicted and observed measurements.

orthopaedics. For instance, the silorane could provide an alternative fracture stabilization technique for comminuted fractures. Previous studies have shown the silorane is biocompatible and has good mechanical properties. However, the effect of various fillers on the maximum exotherm temperature, flexural strength, flexural modulus, and fracture toughness of silorane composites has not been determined. The goal of this article was to develop a method for estimating these properties in the silorane composite.

Although their functions are different, the desirable properties of the silorane composite have been adapted from values given in acrylic bone cement standards. The ISO standard for acrylic bone cement[24] requires a maximum exotherm of less than 90°C, a flexural strength greater than 50 MPa, and a flexural modulus greater than 1.8 GPa. Fracture toughness is also important to predict increased fracture resistance. Fracture toughness ranges depending on test method from 1.0–2.3 MPa $m^{1/2}$ for commercially available acrylic bone cements.[25–27] However, all of these measurements are greatly dependent on test method. The results of this study indicate that the silorane composites containing a combination of fillers (DY5, M12, and/or nanorods) met the suggested bone stabilizer requirements with exotherms as low as 65°C, flexural strength up to 63.8 MPa, and flexural moduli up to 5.16 GPa. However, the fracture toughness of photoinitiated silorane composites (0.42–0.96 MPa $m^{1/2}$) was lower than the range for commercially available acrylic bone cements. One solution identified by the developed method that meets the ISO 5833[24] criteria contains 85.8% silorane, 13.1% DY5, 0.0% M12, and 1.1% nanorods. The predicted properties of this formulation are an exotherm of 88.1°C, flexural strength of 74.0 MPa, and a flexural modulus of 3.7 GPa. However, it must be noted that the properties calculated in this study were conducted on smaller specimens to accommodate available material size. To fully understand the ability of this novel material to meet bone cement standards, tests must be conducted on the optimized material using standard methods.

The flexural properties, in particular flexural strength, were the least accurately predicted from the model. Although a similar trend was seen in the predicted versus observed modulus values, the range of observed strengths for the four additional composites was small (59–66 MPa), and the values were lower than the predicted values (72–99 MPa). This suggests premature failure of the test specimens, although specimens with visible flaws (voids, filler agglomerations, etc.) were excluded from the study. Further analysis, such as high-magnification observation of fracture surfaces, was not utilized to identify flawed specimens, but may have excluded additional samples from the study, resulting in greater mechanical property values. The flexural strength of the additional composite that did not contain nanorods (composite 4) was the closest to fitting its predicted value. Furthermore, the standard error of many of the composites containing nanorods (denoted by D in Table II) was greater than the estimated coefficient. These results may be due to incomplete mixing of the nanorods in the composite, which showed some agglomeration in SEM analysis (Figure 3).

Inhomogeneity of filler distribution may lead to stress concentrations. These stress concentrations would not greatly affect the global properties, such as modulus or exotherm. However, they could cause premature failure, thus lowering the fracture strength of the resulting composite.

It is known that mixture design-of-experiments method suffers from combinatorial explosion when dealing with multi-component mixtures.[28,29] Combinatorial explosion describes the inability to compute the outcome due to the intractability of the number of inputs; in other words, too many inputs (components) gives far too many outcomes (possible behaviors) to compute. Solvason et al.[29] further described how the visualization of multiple components in the design space is problematic. Given a response change over the design space, it is difficult to determine which one component (or combination) causes that response, or how much each component affects the overall response. Internal points within the design space cannot be predicted using the response coefficients if we cannot discern the sizes of the coefficients accurately. Check points, such as those used in this study, help to discern the accuracy of the model for prediction. More points in general also assist and can be chosen to be more orthogonal with respect to the other components. To develop the predictive equations in this study, we used material property values obtained from 23 composite formulations. The number of formulations was determined through analysis of the leverage of each formulation on the resulting model. Overall, leverages were decreased by adding replicates to below 0.5, with a homogenous range from 0.22 to 0.48. Due to combinatorial explosion, however, we cannot assess all behaviors using the model. Therefore, the solution given in this study is simply a predictive tool.

Further limitations of regression analysis include the fact that the solution may not fit the data and/or the detail of information between model points may not be fitted well (if there is a strong nonlinear response component). In this study, however, we do not expect a strong, nonlinear response. Also, the fitting was assessed using the additional composite formulations to determine fitting error (as opposed to experimental errors which are assessed by repeated testing at constant conditions). A way to further test the fitting, though, would be to transform the data using a suitable function to 'flatten' the response, fit to a model, and then transform the solution for comparison. If different functions are used, however, it can be difficult to compare them mathematically; thus, this process may be more of an academic exercise than being useful for validating a particular model.

Overall, based on the fact that the majority of standard errors for the coefficient estimates were reasonably low, and the observed properties for the additional composites were within the predicted ranges, the method of prediction developed in this study appears to be valid for determining maximum exotherm temperature, flexural strength, and flexural modulus based on the additional composites. In contrast, the standard errors of the estimates of the coefficients for properties of composites that contained nanorods (D, AD, BD, etc.) were quite large; thus, caution should be taken when predicting properties of nanorod-containing composites.

As with any study, there are limitations. As mentioned previously, the flexural strength results may indicate incomplete filler dispersion. Future studies will incorporate advanced mixing techniques, such as ultrasound, and verify fracture site filler particle homogeneity with SEM analysis to improve future models. Furthermore, a more accurate model of the physiological environment, such as placing specimens in contact with bone, could be used to provide more meaningful exotherm measurements. This study was conducted on photoinitiated materials because this is the method currently used in the commercial silorane product, and we wanted to limit the number of variables. Although this study focused solely on photoinitiated materials, the ideal silorane composite for orthopedic use will be chemically initiated. Photoinitiation requires an external light source which is not ideal for the majority of orthopedic applications. The chemically initiated silorane will likely have a slower polymerization speed, so it is likely that as the silorane composite formulation is optimized the peak exotherm will also be decreased.

CONCLUSIONS

The objective of this study was met through the development of a method of predicting the mechanical and maximum exotherm temperature properties of the photoinitiated silorane composite. The silorane composite investigated in this study met the suggested material properties (exotherm, flexural strength, flexural modulus, and fracture toughness) for a bone stabilizer. For some of the composite formulations that contain alumina nanorods, the predicted values are up to 60% greater than observed flexural strength values, and flexural modulus predicted values are 10% greater than those observed. It is suggested that this is due to poor dispersal of the nanorods in the matrix. Improved filler distribution may be able to improve model accuracy.

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Manuscript

Development of a Novel, Non-Toxic, Non-Exothermic, Osteogenic Bone Cement

Title: Development of a Novel, Non-Toxic, Non-Exothermic, Osteogenic Bone Cement

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Abstract

A novel bone cement has been generated replacing poly(methyl methacrylate) with a silorane resin. A dual cure polymerization system was developed using a light initiation system combined with Lamoreaux's catalyst. The silorane bone cement has similar mechanical properties to commercially available bone cement but has a low exotherm of 26 °C, is non-toxic to bone cells and supports bone cell differentiation and mineralization *in vitro*. *In vivo*, the silorane bone cement does not cause any weight loss, bone loss, or inflammation in 9 or 12 month old rats. Comparable femoral pull-out strength to commercial bone cement was obtained *ex vivo* and *in vivo*. The reduced curing temperature makes it possible for inclusion of antibiotics, antifungals and growth factors that are heat or chemically destroyed by PMMA polymerization. This material has potential to be used for orthopedic and dental applications based on its comparable strength, improved biocompatibility, reduced exothermicity, and potential to support osteogenesis.

Introduction

Poly(methyl methacrylate), PMMA, is a biologically compatible acrylic glass also known as 'Plexiglass'. PMMA has been used as the major component of bone cements to anchor artificial joints since it was first introduced in orthopaedic surgery by Sir John Charnley in 1970 (6). Although PMMA is non-toxic, methyl methacrylate, the constituent monomer of PMMA, is an irritant and potential carcinogen (11). The leached monomer causes toxicity, as well as blood pressure lability, hypoxia, and mental confusion (13-15) and increased systemic levels of *gamma*-glutamyltranspeptidase that can lead to anorexia, nausea/vomiting, and/or occasional temperature spikes (12). In addition bone cement has high exothermic properties (5, 9) and i*n vitro* tests have shown that the curing temperature can reach over 100 °C due to heat release from MMA monomer polymerization (5). Coupled with poor osteointegration (2, 10), many efforts have been made to develop an alternative to PMMA bone cement with reduced exothermicity and improved biocompatibility while maintaining the handling, and mechanical properties of bone cements (2, 5, 16, 17).

Antibiotics are being incorporated into bone cements with greater frequency. There are approximately 200,000 hip implant cases and 300,000 knee implant cases each year in the United States (2, 3). Over the period of 2002 to 2006, about 85% of primary total knee joint replacements and more than 70% of total hip joint replacements were cemented (4, 5). With this increased number of implants and replacements, bone infection with prosthetic devices is an increasing major medical problem. To date mainly tobramycin, gentamycin and vancomycin are sufficiently heat-stable to survive the heat generated by commercially available bone cement during polymerization. Therefore, a wider spectrum of antibiotics that can remain stable after high heat is needed to combat newer and more resistant strains of bacteria.

The cement-bone interface is problematic as there is no true bonding of cement to bone, only interlay in the trabecular spaces. Orthopedic surgeons have had to adapt surgical techniques to account for issues with cementing total joint prostheses and subsequent total joint failures. 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.

Silorane resin is used currently used in commercially available dental composites (Filtek Silorane Low Shrink) (). 3M ESPE US. Filtek™ Silorane Low Shrink Posterior Restorative. *http://solutions.3m.com/wps/portal/3M/en_LK/3M-ESPE-APAC/dental-professionals/products/category/composites/filtek-ls/(accessed May 5, 2015).* It is non-toxic, non-mutagenic (4-6), does not shrink and is non-exothermic (). Silorane is a hybrid monomer system that contains both siloxane and oxirane structural components, a one to one mixture of CYGEP and PHEPSI (), both of which are non- toxic compounds. Siloranes contain a cyclosiloxane backbone which imparts hydrophobicity [7] and cycloaliphatic oxirane sites with high cationically catalyzed reactivity [8,9]. Silorane based dental restorative systems shrink less than methacrylates during polymerization, have less microleakage(11) and have better shear bond strength and other mechanical properties (12-17).

Previously we had shown that light polymerized silorane was non toxic to bone cells, supported markers of bone cell differentiation and osteogenesis *in vitro* and *in vivo* supported bone healing with no toxic effects (Eick et al, 2012). To date, silorane has not been used in bone cements because it can only be light, not chemically polymerized. For orthopaedic applications, this non-toxic resin requires chemical polymerization. Here we describe a chemical cure system for polymerization of the silorane.

In addition, here we also describe the generation of a new bone cement with a unique chemistry and composition, whereby methyl methacrylates (PMMA or BisGMA) are replaced by the silorane base resin. Due to the different chemistry, glass fillers replace hydroxyapatite fillers as used with PMMA cements. The silorane formulation has similar mechanical properties to commercial bone cements while remaining

non-toxic *in vitro* and *in vivo*. Silorane wear debris does not generate a cytokine response *in vitro*. As the silorane formulation has a low exotherm allowing incorporation of a broader spectrum of antibiotics, it will expand the use of bone cements for many orthopedic applications.

Results:

Development of chemically polymerized, non-cytotoxic silorane bone cement.

Resin. The base resin consists of a mixture of two siloranes, which is a generic term for compounds that contain Si-C and Si-O bonds (1). Our silorane resin is a 1:1 combination of bis[2-(3{7-oxabicyclo[4.1.0]heptyl}ethyl]methylphenyl silane (PHEPSI)(33) and 2,4,6,8-tetrakis(2-(7-oxabicyclo[4.1.0]heptan-3-yl)ethyl)-2,4,6,8-tetramethyl-1,3,5,7,2,4,6,8-tetra-oxatetrasilocane (CYGEP) (34) and hereafter referred to as SilMix (Figure 1). Both of the components contain aliphatic epoxide groups, which polymerize through a cationic pathway. We have found that there is less polymerization stress and shrinkage than that of free-radical polymerizing methacrylate containing compounds [42-43] (Figure 1). While SilMix has been prepared previously, we have been able to modify accepted procedures in order to produce bulk quantities of the material at purities greater than 95.8% as determined by ^{1}H NMR spectroscopy.

Initiation system:
Previously, only light initiation systems [36-37, 44-45] have been tested, which are not ideal for orthopedic applications. An alternative initiation system, which would allow for optimization and improvement of all aspects of the polymerization process needed to be developed. Several special considerations, such as cytotoxicity and exothermicity were taken into account when developing the chemical initiation system. A dual cured polymerization system was identified (and patented) using a light initiation system (1.19 wt% *p*-(octyloxyphenyl)phenyliodonium hexafluoroantimonate), 0.40 wt% camphorquinone, and 0.06 wt% ethyl *p*-dimethylaminobenzoate) and Lamoreaux's catalyst, which consists of a platinum-chloride complex with ether and aldehyde linkages derived from octanol.

Fillers:
A variety of fillers have been developed and used in order to improve the strength and stiffness of the silorane composites. In collaboration with MO-SCI Corporation (Rolla, MO), several types of ceramic fillers, including alumina nanorods, barium boroaluminosilicate glass (M12), and yttria aluminosilicate glass (DY5) have been selected, modified and incorporated into the silorane resin with the hope to effectively provide radiopacity, tailor handling properties, lower curing temperature, and improved mechanical strength and modulus[47]. According to the preliminary data on mechanical properties and exothermicity, DY5 was selected as the filler for optimizing the silorane bone cement.

Surface modified Fillers:
The surface modification of fillers is a critical step in improving the mechanical properties of the resulted composite. These modifications were made to the glass fillers to provide for dispersion wetting, interfacial particle-to-matrix adhesion, and reduced polymerization stress. Silanes can react with absorbed moisture or –OH groups on the filler surface and thus form chemical bonding through sequential hydrolysis and adsorption-condensation reactions. ECHE-TMS (2-(3,4-epoxycyclohexyl) ethyl trimethoxysilane) has been extensively used for silorane composites, because the epoxy groups of the ECHE-TMS molecules can join the ring-opening polymerization of the silorane matrix and thus form covalent bonding between the filler to the polymer matrix. High polymerization shrinkage and stress is a major deficiency of current PMMA bone cement and it was found in our previous work that the addition of TOSUs into silorane resin systems could effectively reduce the internal stress during polymerization [48]. The organic moiety provides wetting and interfacial strength properties while adding the potential to reduce internal stress in the composite. Since group length has a direct effect on interfacial bond and thus composite strength, the tether

length between the reactive group and the surface active ligand is one design aspect that was considered. The preliminary mechanical testing data indicated that compared to the silorane composites containing non-modified fillers, both the TOSUs modified fillers and ECHE-TMS modified fillers reinforced silorane composites had significantly improved mechanical properties and the TOSUs modification achieved better reinforcing effect than ECHE-TMS modification.

Mechanical properties, handling properties and exothermicity
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 (61.6 ± 3.7 °C). (See Table 4).

Karl Fisher test for effects of moisture
A study to investigate moisture effects and metal implant surface modification effects were done (Figure 2). Moisture content in the initial formulation was measured and varied through saturation with water and by drying to low moisture concentrations to observe the effect on moisture [JAPS reference]. As synthesized and utilized Silmix in all trials to date had moisture contents near saturation, ~1.8%, whereas a saturated moisture concentration was ~1.9%. Azeotropic drying reduced initial moisture content to 1.0 (dried) and 0.3% (ultra-dried), respectively.

Biological effects, in vitro and in vivo, of the silorane bone cements

In vitro effects of silorane bone cement on bone cell viability and function:
The silorane-based cements were compared to light cured silorane and to three commercially available bone cements for bone cell number (Fig. 3A) and for their osteoblast toxicity (3B). The percent dead cells in Osteobond bone cement group were 28.6±4.6; 28.3±2.6 in Palacos; 14.6±1.9 in Simplex P; 4.2±0.4 in LC; 5.5±0.6 in M12-U; 5.7±0.8 in M12-1; 6.4±0.7 in M12-3; 3.7±0.4 in M12-E; 4.7±0.5 in DY5-U; 4.4±0.2 in DY5-1 and 3.8±0.8 in DY5-E. There were statistically significant differences between silorane-based bone cements group and commercial bone cements group. The cell number was less in the silorane samples but little toxicity indicating less cell proliferation. This was most likely due to the poor attachment of bone cells to the surfaces of the disc compared to culture dish plastic. While toxicity was observed with the commercially available bone cements, no toxicity was observed with the silorane formulations compared to control group. We also tested the effects of the cements on osteoblast function, such as generation of alkaline phosphatase (Fig. 3C) and mineralization (Fig. 3D). The levels of alkaline phosphatase and mineralization in silorane-based bone cements groups were significantly higher than that in Simplex P group ($p<0.05$). The silorane bone cements actually promoted osteoblast differentiation and function suggesting osteogenic activity in vitro.

Effects of Simplex P and Silorane DY5-1 wear debris on Interleukin 1-beta, IL-1β, production.
To determine if the wear debris from the silorane cement would illicit an inflammatory reaction, the production of IL-1β by RAW 264.7 macrophage cells was performed (Fig. 3E). LPS at concentration of 10ng/ml significantly induced IL-1 β (128 pg/ml) which is 19-fold higher than that of control (media alone). Both cements at $5x10^5$ particles induced IL-1 β, but Simplex P showed significantly higher potency with a 5 fold increase (32 pg/ml) while a non-significant 1.5 fold increase for Silorane DY5-1 (11 pg/ml) was observed.

In vivo effects of silorane bone cement in a rat model.
Sixty six 6-month-old rats were used, twenty-two for Simplex P, fourteen for M12-ECHE, fifteen for DY5-ECHE, and fifteen for DY5-1TOSU cement. The rats were anesthetized and operated on under aseptic conditions. For the short term study to examine the inflammatory response histologically, the rats were sacrificed at week one post operation (PO). For long term study to determine the osseointegration, the rats

were sacrificed at week eight PO. Prior to sacrifice, the body weight of rats was taken and the rats weighed at week 1, 2, 4, 6, and 8 PO and compared to that before surgery. The X-rays of femora of rats were taken at week 1, 4, and 8 PO. Injections of fluorochrome intraperitoneally with alizarin red A, and calcein were performed at 4 and 6 weeks PO. The rats injected with commercial bone cement showed significant weight loss at one week post-surgery (Fig. 4A, $P<0.001$). No significant reduction in weight was observed in rats carrying the silorane bone cements. Radiographs of femora were taken at week 1, 4, and 8 PO. The radiographs of rat femora filled with commercial bone cement Simplex P showed periosteal reaction and bone loss at week 4 and 8 PO in contrast to silorane-based M12-ECHE, DY5-ECHE and DY5-1TOSU bone cements which showed no periosteal reaction (Figure 4B). DY5-1TOSU is shown in Fig. 4B, and the same lack of reaction was observed in M12-ECHE and DY5-ECHE (data not shown). The femora of rats filled with Simplex P or DY5-1TOSU cement and sacrificed at week one PO were processed, sectioned and stained with H&E. The histological examination shows that there are many inflammatory cells next to commercial bone cement Simplex P in contrast to DY5-1TOSU cement where few inflammatory cells could be observed. Endosteal fluoresence double-labeling was observed in DY5-1TOSU group in contrast to no endosteal fluorescent double-labeling in the Simplex P group (Figure 5).

Pull out tests to determine strength of silorane bone cement compared to commercially available bone cement.

First, an in vitro mimic pull out test was devised before moving into the animal models. No significant differences were observed (Fig. 6A). Before using live animals, pull out tests were conducted on excised rat femora. The silorane-based bone cements M12-ECHE, M12-1TOSU, M12-3TOSU, DY5-ECHE, DY5-1TOSU were investigated. The pull-out strength for Simplex P was 5.4±0.2 MPa, 5.4±0.4 MPa for M12-ECHE, 5.3±0.8 MPa for M12-1TOSU, 6.4±0.6 MPa for M12-3TOSU, 5.8±0.5 MPa for DY5-ECHE and 5.4±0.3 MPa for DY5-1TOSU. The values of pull-out strength of silorane-based cements were similar to that of Simplex P or even higher under these conditions. There were no statistically significant differences among Simplex P, M12-ECHE, M12-1TOSU, DY5-ECHE and DY5-1TOSU.

Next mechanical testing using the rat femora ex vivo was performed. The ex vivo pull-out test using femora of 6-months-old rats was used to determine the pull-out strength of different silorane based bone cements compared to Simplex P (Fig. 6B). Statistically significant differences were found between the Simplex P and the Silorane bone cements with ECHE surface-treated M12 and the 3TOSU surface-treated M12 modified glass fillers with the silorane cements having lower pull-out strength ($p < 0.001$). No statistical difference was found between the Simplex P and the M12-1TOSU and DY5-ECHE surface-treated cements (data not shown). These results suggest that the silorane bone cements are affected by the wetting conditions of ex vivo rat femora to a greater extent than the Simplex P cement. When these cements were tested in vivo, the silorane cement became slippery due to the blood from the medullary wound. Therefore, the effect of moisture (i.e., degree of dryness) was investigated (see Figure 2) along with the increase of filler for improved strength.

In vivo Pull-out strengths of bone cements.

Fifteen 13-month-old rats were used to examine integration of DY5-1TOSU cement with different treatments/modifications which included non-dried 60% filler, dried 60% filler, dry 65% filler, and dry 65% filler with the titanium rod pre-dipped. The right leg was operated on under aseptic condition and a dried titanium rod was inserted into all groups except for the dry DY5-1TOSU 65% filler (n=3/formulation). The animals were sacrificed at one week PO. The femurs were harvested and immediately tested biomechanically. The pull-out strength for Simplex P was 4.1± 1.3 MPa, 1.7 ± 0.7 MPa for original DY5-1TOSU 60% filler, 2.6 ± 0.6 MPa for dry original DY5-1TOSU 60% filler, 4.4 ± 0.9 MPa for dry Putty DY5-1TOSU 65% filler, and 4.8± 0.9 MPa for dry DY5-1TOSU 65% filler with pre-dipped rod. (Fig. 6C). Therefore the pull-out strength of dry DY5-1TOSU 65% filler groups was similar to the Simplex P. No

differences were observed between pre-coating and uncoated titanium rods. For the next set of experiments the dry DYt-1TOSU was used.

Five 13-month-old rats were used to test Simplex P, dry DY5-1TOSU 65% filler, and dry DY5-1TOSU 65% filler with the titanium predipped in the cement. This additional step was included again to determine if pre-dipping the titanium rod improved pull-out strength. Both legs were operated on under aseptic conditions (n=3/formulation). The femur was filled with cements and the animals were sacrificed 24 hrs. PO. The pull-out strength for Simplex P was 6.1 MPa, 4.7 MPa for dry DY5-1TOSU 65% filler, and 4.0 MPa for dry dipped DY5-1TOSU 65% filler (Figure 6D). There were no statistically significant differences among Simplex P and silorane cements. Based on these results, the Simplex P and dry DY5-1TOSU 65% filler cements were left in the rats for eight weeks to determine effect of time in vivo on the pull out strength. Twelve 11-month-old rats were operated on under aseptic conditions (n=7 for Simplex P, n=5 for our bone cement - dry SM DY5-1TOSU 65% filler). The animals were sacrificed at 13 months after an eight-week period PO. The femurs were harvested and immediately tested biomechanically. The pull out strengths for Simplex P and the silorane bone cement were 6.3 ± 0.4 MPa and 4.9 ± 0.7 MPa, respectively. There was no significant difference between the Simplex P and our silorane bone cement (Fig. 6E).

Discussion

A novel bone cement has been generated by utilizing the components of dental composites, known for their non-toxic, non-exothermic properties with low shrinkage and high strength. This is been accomplished through a unique dual cure approach. The chemistry used to generate this novel silorane cement is distinct from that used for traditional bone cements. PMMA bone cements typically consist of two parts, namely powder and liquid. The powder portion contains prepolymerized PMMA beads, methacrylate-styrene-copolymer, benzoyl peroxide as initiator and barium sulfate or zirconium oxide as radiopacifier (7, 8). The liquid portion is usually composed of methyl methacrylate (MMA), N-dimethyl-p-toluidine, which is an activator to accelerate the polymerization, and hydroquinone, which is an inhibitor to avoid self-polymerization of the monomer before use. Since its debut, the composition of PMMA has not changed substantially. The fillers used for commercial bone cement include acrylate based plastics and opacifiers. In contrast, the silorane cement contains glass as a filler.

Other types of bone cements have been created to address the shortcomings of commercial cement. Calcium phosphate bone cements are able to degrade in the human body and possess very good biocompatibility and handling properties, however they can only be used for non-load bearing locations (18, 19). A BisGMA-TEGDMA based bone cement (Cortoss™) has been cleared by the FDA for vertebral augmentation with the goal to replace current products. The Cortoss™ bone cement exhibits less exothermic reaction, reduced shrinkage, and comparable mechanical properties to other PMMA products (20-25) and appears superior to PMMA bone cement (26, 27). However, there are still concerns regarding leachable monomers and the biocompatibility of BisGMA composites (28-30). Their commercial uses have been significantly impeded after two independent studies, showed vertebroplasty did not provide any benefit over the control group (without treatment) (31, 32).

Curing of the silorane monomers showed variation of cure rate, final cure extent, and strength, which were optimized in rate, extent, and strength by a moderate moisture content (JAPS reference). The as-synthesized monomers possessed moisture contents of near-saturation (~0.17 wt.-%), which led to slow polymerization rates due to chain transfer, low polymerization extent of reaction. The highest water concentrations also reduced final polymer mechanical strengths.

Cationic initiation of epoxy polymerization occurs with participation of moisture. Excess moisture causes chain transfer of active polymerization and results in decreasing polymerization efficiency and these effects are observed more or less as a function of monomer chemical structure. The moisture effect on polymerization is, however, a key ingredient in cationically catalyzed polymerizations where the proper amount of water allows fastest, effective monomer conversion. The key to consistency in resulting polymerization rate and polymer properties was therefore established by control of the initial moisture concentration. Moisture, if minimized in the initial formulation, was ideal for the cement since as moisture diffuses into the hydrophobic silorane slowly and in low concentrations, the silorane polymerization and strength were enhanced. These results have been borne out with in vivo rat bone pull out strengths, which show consistent, high strength.

As a first step before performing expensive in vivo animal experiments, the bone cements were tested *in vitro* on bone cell viability, proliferation, alkaline phosphatase expression and mineralization. While the commercial bone cements had negative effects on each of these parameters, none of the silorane bone cements had any negative or toxic effects on bone cells. In fact, the silorane cements actually stimulated alkaline phosphatase activity and mineralization in culture. This was a surprise as normally this effect is only observed with growth factors and not biomaterial surfaces. This results support the prediction that the silorane cements may actually stimulate or support bone formation. This was the case for the nine month old, but not the fifteen month old rats as shown by fluorochrome labeling. Another concern of biomaterials is the generation of wear debris which can generate an inflammatory reaction. Whereas the wear debris

from both commercial bone cement and silorane cement did stimulate some reaction, the IL-1b levels were modest and not significant. This result suggests that neither commercial bone cement nor silorane cement will generate a significant inflammatory response *in vivo*.

For the *in vivo* studies employing the rat femoral implant model, x-rays were taken at weeks 1, 4 and 8. A periosteal reaction was observed in the animals receiving the commercial bone cement, but none was observed with the three silorane cements. Also, a significant decrease in body weight was observed in the animals receiving the commercial bone cement but not the silorane cements at week 1. These data show that the effects of the silorane cements are better tolerated by the animals compared to commercial cement. Histologically no significant differences were observed after eight weeks *in vivo* suggesting that any negative effects by the commercial cement were resolved. A modest increase in endosteal bone formation was observed with the silorane cements compared to the commercial cement in nine month old animals but not in fifteen month old animals. This may reflect the differences between a growing and an aging skeleton.

The ultimate test for bone cement functionality is pull-out strength. Again, preliminary testing was performed first in a mimic system followed by ex vivo femori before implanting in animals. No differences were ever observed between the commercial cements and the silorane cements in the mimic system. The bone cements were also tested in excised rat femora, and although the silorane cements were less than commercial cement, no significant differences were observed (data not shown). Significant differences were observed between the commercial cement and two of four silorane cements after one week in vivo. Additional in vivo results suggested that the silorane cements were sensitive to the effects of moisture. Next experiments were performed using dried silorane and increasing the filler from 60 to 65%. These changes returned the pull-out strength of the silorane cement to that of commercial cement in vivo. No significant differences were observed between the dried silorane cement with or without pre-dipping the titanium rod in cement. After eight weeks in vivo, no significant differences were observed between the commercial cement and the optimally formulated silorane cement.

In conclusion, we have developed a novel silorane bone cement with excellent properties. The silorane bone cement does not cause any weight loss, bone loss, or inflammation *in vivo* while providing equivalent bone pull out strength. With the improved biocompatibility, reduced exothermicity, good handling properties, and potential for osseointegration/osseoinduction, 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 antifungals and growth factors, which previously cannot be used in PMMA bone cements.

Materials and Methods

General procedures. The composites of the silorane bone cement are listed in Figure 1 and the silorane compositions in Table 1. Proton NMR spectra were recorded on a Varian AC 400 MHz spectrometer. Carbon NMR spectra were recorded on a Varian AC 400 spectrometer operating at 100 MHz. All commercial chemicals and solvents were used as supplied unless otherwise stated. Anhydrous toluene (>99.8%), 4-vinylcyclohexene-1,2-epoxide (98%), and octanol (>99%) were purchased from Aldrich. 2,4,6,8-Tetramethylcyclotetrasiloxane (99%) was purchased from Alfa Aesar. Methylphenylsilane (>95%) and platinum octanal/octanol complex (2.0-2.5% Pt in octanol) were purchased from Gelest. The chloroplatinic acid hexahydrate (99.9% Pt) and Wilkinson's catalyst (>99%) were purchased from Strem Chemicals. Bis[2-(3{7-oxabicyclo[4.1.0]heptyl})ethyl]methylphenyl silane (PHEPSI) was synthesized from an adapted procedure by Crivello.[41] **(**Crivello, J.V.; Bi, D. *J Polym. Sci. Part A: Polym Chem.* 1994, *32*, 683-697.) 2,4,6,8-Tetrakis(2-(7-oxabicyclo[4.1.0]heptan-3-yl)ethyl)-2,4,6,8-tetramethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane (CYGEP) was prepared from an adapted procedure by Aoki.[82] (Aoki, S. *U.S. Patent 6,255,428* 2001.) Lamoreaux's catalyst was synthesized from an adapted procedure by Lamoreaux.[74] (Lamoreaux, H. F. *U.S. Patent 3,220,972* 1965.) The silorane is comprised of the CYGEP and PHEPSI monomers combined at a 50:50 weight ratio. Once the two monomers are combined, SilMix is stable at 6 °C for for 2 yr. The LMC is prepared, tested, distributed into 0.5 mL vials, placed under argon, and stored in the freezer at -15 °C. Glass fillers (listed in Table 2.) were provided by MoSci Corporation (Rolla, MO). Three types of filler modifications were tested (Table 3) and the amount of surface modification was calculated based on specific surface area per mass of glass powder, an estimated surface area per silane of 40Å^2, and the molecular mass of the silane. The calculated amount of silane was added to glass powder dispersed in toluene and refluxed for 5 hr. Isolation of powder and drying at 125ºC for 10hr afforded dry glass powder. Surface modification was quantified by infrared spectroscopy, thermogravimetric analysis, and X-ray photoelectron spectroscopy.

Quality testing of co-monomers:
Each monomer undergoes quality testing with a corresponding control, which had passed previously, before combined as SilMix. The SilMix containing the newly synthesized monomer was combined with the *p*-(octyloxyphenyl)phenyliodonium hexafluoroantimonate) (PIH), camphorquinone (CPQ), and ethyl *p*-dimethylaminobenzoate (EDMAB) in a high-speed mixer until no particles are seen (approximately 15 min). Next ECHE modified M12 glass filler is added and mixed in the high-speed mixer for 20 min. The material is allowed to cool to room temperature, and then the Lamoreaux Catalyst (LMC) is added using a needle and syringe (by weight on a balance) and mixed by hand for approximately 30 sec. The material (~0.1 g) is then placed in a Delrin ring that was affixed to a glass slide with lab tape. The Gillmore Needle test (GNT) was performed at 15 min increments for 1 hr or until the sample passed (pass = no marks or indentations from 1 lb GNT). If a sample did not pass within the 1 hr, then the sample failed the quality control test. The final composition tested contained 38.03 wt% SilMix, 1.19 wt% PIH, 0.40 wt% CPQ, 0.06 wt% EDMAB, 60.00 wt% filler, and 0.32 wt% LMC.

Resin preparation:
Silmix is a comonomer system consisting of PHEPSI and CYGEP in a 1:1 by wt ratio. The monomers were combined in a high-speed mixer for 15 min. For neat light-cured SilMix (LCSM): SilMix (SM) was blended with the *p*-(octyloxyphenyl)phenyliodonium hexafluoroantimonate) (PIH), camphorquinone (CPQ), and ethyl *p*-dimethylaminobenzoate (EDMAB) in a high-speed mixer until no particles were visible (between 30 min to an hour depending on the amount of material). The final composition of the LCSM was 95.85 % SM, 3.0 % PIH, 1.0 %, CPQ, and 0.15 % EDMAB (by total weight of sample). For filled Light Cured samples: LCSM was combined with the filler at the wanted amount and mixed in a high-speed mixer between 15 and 30 minutes depending on the amount of material. For Prototype 2 samples (silorane bone cement): Light-cured SilMix (LCSM) was prepared as stated previously. A portion of the LCSM

was combined with the glass filler and mixed in the high-speed mixer (approximately 25 min). The material was allowed to cool to room temperature, and then the Lamoreaux Catalyst (LMC) was added using a needle and syringe (by weight on a balance) and mixed by hand for approximately 30 sec.

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

*Generation of wear debris***:**
Wear debris was prepared from commercially available bone cement (SimplexP) and DY5-1TOSU material. Each cement was polymerized, milled, and screen classified to produce particle size distributions < 10 μm diameter. SEM was used to characterize the samples.

Evaluation of the biocompatibility of the different silorane-based new bone cements:
The discs of light cured neat silorane (LC), silorane-based bone cement with M12 unmodified (M12-U) or ECHE surface-treated M12 (M12-E) or 1TOSU surface-treated M12 (M12-1) or 3TOSU surface-treated M12 (M12-3) or ECHE surface-treated DY5 (DY5-E) or 1TOSU surface-treated DY5 (DY5-1) glass fillers and commercial bone cements discs: Simplex P (Stryker Corp.), Palacos and Osteobond (Zimmer Inc.) of 9 mm diameter by 0.7 mm thickness were prepared. The discs were sterilized under UV light for 1 h on each side. The MLO-A5 cells were seeded on the discs in 48-well plate at a density of $3.5x10^4$ cells/cm^2 in a-MEM containing 5% fetal bovine serum (FBS) and 5% calf serum (CS) at 37°C/5% CO_2, and cultured for 24 hrs (4 samples/group) and examined using the Trypan blue dye exclusion (TBE) assay. The control wells without disc were served as positive control. After 24 h of incubation, the cells were harvested and counted using the Trypan blue dye exclusion (TBE) assay.

Alkaline phosphatase:
The silorane-based bone cement with DY5 unmodified (DY5-U) or DY5-ECHE (DY5-E) glass fillers and commercial bone cement discs: Simplex P (Stryker Corp.) of 9 mm diameter by 0.7 mm thickness were prepared. The sterilized discs were inserted into 48-well plate. The MLO-A5 cells were seeded on the discs in 48-well plate at a density of $3.5x10^4$ cells/cm^2 in a-MEM containing 5% fetal bovine serum (FBS) and 5% calf serum (CS) at 37°C/5% CO_2 and cultured until confluence. At confluence after plating (day 0), the culturing medium was replaced with mineralization medium, α-MEM with 10% FBS + 4 mM β-Glycerophosphate + 50 μg/mL ascorbic acid. The mineralization medium was replaced every 2 days. After 6 days in mineralization medium, the levels of alkaline phosphatase were examined. Cells were lysed with 0.05% Triton-X by two freeze-thaw cycles. ALP activity was assayed with 1.5 M 2-amino-2-methyl-1-propanol (AMP) buffer (pH 10) and quantified against p-nitrophenol phosphate standard curve. Absorbance at 405 nm was recorded in triplicate and normalized by protein concentrations (ALP (nM)/protein (ug)/Min).

Mineralization:
The silorane cements discs: DY5-U, DY5-ECHE and Simplex P discs were sterilized. The MLO-A5 cells were seeded on the discs in 48-well plate at a density of $3.5x10^4$ cells/cm^2 in a-MEM containing 5% fetal bovine serum (FBS) and 5% calf serum (CS) at 37°C/5% CO_2 and cultured until confluence. The control wells without disc were served as positive control. At confluence after plating (day 0), the culturing medium was replaced with mineralization medium, α-MEM with 10% FBS + 4 mM β-Glycerophosphate + 50 μg/mL ascorbic acid. The mineralization medium was replaced every 2 days. The mineralization

medium was replaced every 2 days. After 12 days in mineralization medium, the cells were fixed in 10% buffered formalin and stained with 2% Alizarin-S stain and then destained with 10% cetylpyridinium chloride. Absorbance at 570nm was recorded in triplicate.

The effect of wear debris on the production of IL-1β in RAW264.7 cells:
Raw264.7 cells were seeded on a 96-well plate at a density of 1x105 cells. After 24 hours, the cells were treated with 100 uL of two different cement debris solutions, Silorane DY5-1 and Simplex P with indicated particle numbers per well ($1x10^5$~$5x10^6$ particles/well). Lipopolysaccharide (LPS, Sigma-Aldrich) was used as a positive control (0~10ng/ml). Cultures were incubated at 37°C for 48 hours in 5% (v/v) CO_2 in air. Supernatants from two wells were pooled (n=3 each), and the concentrations of one of cytokines, IL-1β were assayed using a commercially available kit (Mouse IL-1β ELISA Kit, Ray Biotech, Inc.), according to manufacturer's protocols. The results were expressed as pg/ml at OD 450 nm. The experiment was repeated twice.

Animals and surgical procedure:
All animal surgical procedures were performed at the University of Missouri-Kansas City in compliance with the NIH Guide for Care and Use of Laboratory Animals (1985). The ninty-eight Sprague Dawley rats were maintained in an animal care facility for 10 days to acclimate to diet, water, and housing under a 12 h/12 h light/dark cycle. In general, the rats were anesthetized by intraperitoneal injection with 3.5% isoflurane/ketamine/ dexdormitor (75/0.25 mg kg^{-1} body weight). The surgical area of rat's knee was disinfected with betadine. With a sterile instrument, a 1.5 cm medial parapatellar incision was made to expose the knee joint. The patella was retracted laterally with the knee extended. A 2.2 mm hole was drilled into the intercondylar notch with a drillbit, to penetrate the subchondral cortical bone and gain access to the femoral intramedullary canal. The marrow cavity was disrupted by inserting a threaded hand drill proximally through the entire length of the diaphysis to approximately the level of the lesser trochanter. A guide implant was placed into the ablated cavity to ensure that the canal was an appropriate size to accommodate the definitive implant. The cavity was flushed with 10 ml of sterile saline for removal of loose marrow contents. Following irrigation, commercial bone cement Simplex® P, or different silorane-based cements was introduced into the intramedullary canal, and then a titanium implant, 22 mm long and 1.5 mm diameter was implanted in a retrograde manner, respectively. The capsule and skin was sutured with 4-0 nylon suture. Reversal of anesthesia was achieved with atipamezole at a dose of 0.5 mg kg^{-1}. Subcutaneous injections of the analgesic buprenorphrine at 0.05 mg kg^{-1} were given daily for 3 post-operative days. The condition of the surgical wound, food intake, activity and clinical signs of infection and/or neurological compromise were monitored daily. The body weight of rats was weighed, radiographies were performed and injections of fluorochrome intraperitoneally with alizarin red A, and calcein were performed at different time points PO.

X-rays:
The rats were anesthetized by intraperitoneal injection with 3.5% isoflurane/ketamine/ dexdormitor (75/0.25 mg kg^{-1} body weight). Radiographs from lateral view of femur including knee and hip joints were taken at 1, 4, and 8 weeks post-surgery (Faxitron X-ray; Faxitron Bioptics, LLC, Tucson, AZ). The images were taken to assess the position of the titanium implants.

Histology:
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, and placed in methyl methacrylate solution for polymerization before sectioning. The sections were cut at 10 um.

Mimic pull out system:

A mimic pull out system was designed to test pull out strength before proceeding to the use of rat femora. In order to mimic rat femur, plastic tubing of 3 mm diameter with holes was used. The silorane-based bone cement with M12-ECHE, (M12-E), M12-1TOSU (M12-1), M12-3TOSU (M12-3), DY5-ECHE (DY5-E), or DY5-1TOSU (DY5-1) glass fillers were investigated. The plastic tubes were filled with different bone cements and a titanium rod of 22 mm long and 1.5 mm in diameter inserted. The pullout test was conducted at a displacement rate of 0.25 mm/min to failure with the force in Newtons. The values were calculated by dividing the force at the point of failure by the surface area of the implant in the plastic tubing.

Mechanical Testing using the rat femora Ex Vivo Pull-out test:
The rat femoral ex vivo test was performed using femora excised from stored, frozen male Sprague–Dawley rats (approximately 6 months old) (n=10 rats, 20 femora). A hole was drilled into the intercondylar notch with a Dremel drill bit to penetrate the subchondral cortical bone and gain access to the femoral intramedullary canal. The marrow cavity was disrupted by inserting a threaded hand drill proximally through the entire length of the diaphysis to approximately the level of the lesser trochanter. A guide implant was placed into the ablated cavity to ensure that the canal was an appropriate size to accommodate the definitive implant. Four silorane bone cement formulations, containing either M12-ECHE, M12-1TOSU, M12-3TOSU or DY5-ECHE, were compared with commercial bone cement Simplex P (served as positive control). The cements were introduced into the intramedullary canal and then a titanium implant, 22 mm long and 1.5 mm diameter, was implanted in a retrograde manner. The femora implanted with titanium rods fixed with bone cements were kept in an incubator at 37 °C for 24 hrs. The pull-out strength was calculated by dividing the force at the point of failure by the surface area of the implant in the femur.

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Author roles:

LB, performed the in vitro and in vivo experiments, KK, JM, RW, EM, and JR for assistance with animal surgery, AX for histological analysis, YK for wear debris experiments, TS, DE, LFB conceived of concept, oversaw the in vitro and ex vivo experiments, and wrote the manuscript.

References: ***to be included last.***

Tables

Table 1. Silorane Composition

Sample ID	%SM	%PIH	%CPQ	%EDMAB	%DY5	%LMC
Regular (60% filled)	38.03	1.19	0.40	0.06	60.00	0.32
Putty (65% filled)	33.16	1.04	0.35	0.05	65.00	0.40

Table 2: Glass Fillers from MoSci Corporation.

a)

Batch Formula Composition Filler	Y_2O_3	Al_2O_3	SiO_2	Na_2O	total
DY5	15	5	80	0	100

b)

Batch Formula Composition Filler	Na_2O	K_2O	MgO	CaO	BaO	TiO_2	ZrO_2	ZnO	B_2O_3	Al_2O_3	SiO_2	SO_3	Fe_2O_3	Sb_2O_5	total
M-12	0	0	0	0	29.1	0	0	0	10.5	5.9	54.5	0	0	0	100

Table 3. Filler modifications

Modification	Abbreviation
2-(3,4-epoxycyclohexyl)ethyltrimethoxysilane	ECHE
[(9,9-diethyl-1,5,7,11-tetraoxaspiro[5.5]undec-3-yl)methyl]trimethoxysilane	1TOSU
[3-(9,9-diethyl-1,5,7,11-tetraoxaspiro[5.5]undec-3-yl)propyl]trimethoxysilane	3TOSU

Table 4. Summary of material properties of silorane cements with different glasses compared to ISO 5833 Standard.

	M12-3TOSU	M12-ECHE	DY5-3TOSU	DY5-ECHE	Simplex P	ISO 5833 Standard
Exothermicity (°C)	26 ± 0.5	26 ± 0.5	26 ± 0.5	26 ± 0.5	61.6 ± 3.7	≤90
Handling time (mins)	8-10	8-10	8-10	8-10	6-10	3-15
Flexural modulus (GPa)	4.3	3.8	3	2.6	2.2	≥1.8
Flexural strength (MPa)	46	40.5	38	35.5	71.5(61.2)	≥50
Compressive strength (MPa)	n/t	83	77	91	78	≥70
Cytotoxicity (% cell death)	<5%	<5%	<5%	<5%	15%	n/a

n/t = not tested

Figures:

Figure 1: **Chemical components of the silorane cement**. A). Structures of co-monomers Cygep and Phepsi. B). Structures of light initiated system, PIH, EDMAB, CPQ. C). Structure of surface filler modifiers ECHE, 1TOXU, 3TOXU for the barium boroaluminosilicate glass (M12) and yttria aluminosilicate glass (DY5) glass fillers.

Figure 2. **Karl Fischer titration results (top) and cure extent (bottom) for ultra-dried Silmix (SM), dried SM, as received SM (shipped), and saturated SM resin formulations.** The cure extent was measured as relative FTIR absorption of oxirane to ether band conversion as a function of time of the Silmix polymerization as a function of water concentration in the initial formulation.

Figure 3. **In vitro effects of cements on viability and differentiation of bone cells, MLO-A5.** Three commercially available bone cements were tested: Osteobond, Palacos, and Simplex P. LC = light cured, M12-U=M12 unmodified glass, M12-1 = M12-1TOSU, M12-3 = M12-3TOSU, M12-E = M12-ECHE, DY5-U = unmodified glass, DY5-1 = DY5-1TOSU, DY5-EC= DY5-ECHE. A). Effect of cements on cell number. There were significantly fewer cells in the wells containing the cements compared to controls and there were greater numbers of dead cells in the wells with the commercial bone cements. B). Effects of cements on percent live/dead cells. There was a significant decrease in percent live cells and significant increase in percent dead cells in the wells containing the commercial cements. There were no significant differences between control and the silorane cements. C.) Effects of cements on alkaline phosphatase, a marker of bone cell differentiation. There was a significant increase in alkaline phosphatase activity in the wells containing the silorane cements. D.) Effect of cements on mineralization. Similar to the findings with alkaline phosphatase activity, there was also an increase in mineralization in the wells containing the silorane cements. E) Effect of bone cement wear debris on cytokine, IL-1b production. The particles at 5 x 10^6 had a modest effect on LPS production. * $p< 0.05$.

Figure 4. **In vivo effects of cements on rat weight and skeleton.** A). The changes of body weight of rats with commercial bone cement Simplex P, and silorane-based M12-ECHE, DY5-ECHE, DY5-1TOSU bone cement at different time points PO. *$P<0.001$. B). Radiographs of rat femora filled with Simplex P taken at 1 (SP-1w), 4 (SP-4w) and 8 (SP-8w) weeks PO. The images SIL-1w, SIL-4w, and SIL-8w represent the rat femur filled with DY5-1TOSU. M12-ECHE and DY5-ECHE not shown. A periosteal reaction (arrows) of rat femur filled with commercial bone cement Simplex P was observed at week 4 (SP-4w) and 8 (SP-8w) PO. There was no periosteal reaction in any of the silorane-based cement groups.

Figure 5: **In vivo effects of cements on bone formation and bone marrow.** A-D). H&E staining of cortical bone, trabecular bone, and marrow from the surgically operated femur and the non-operated femur from animals receiving either commercial bone cement or silorane bone cement DY5 dried, 65% filler, Simplex P or DY5-1TOSU. A periosteal reaction was observed in cortical bone from both commercial and silorane bone cement (A). No periosteal reactions were observed on the periosteum of the non-operated contralateral control (B). Marrow was present surrounding trabecular bone in the silorane containing femur but less marrow and more empty spaces were observed in the femurs receiving commercial cement (C). In the contralateral controls a consistent great marrow cellularity was observed with the commercial cement compared to the silorane cememt (D). E-F). Fluorochrome labeling of cortical bone of rat femur filled with Simplex P or DY5-1TOSU silorane cement. Both periosteal and endosteal double labeling were observed in DY5-1TOSU group (E2) compared to the commercial bone cement (E1) in the nine month old rats. The arrow points to endosteal fluorescence double labeling. The red line: alizarin, the green line: calcein. No effects were observed in the periosteal mineral apposition rate in nin month old rats (E3). In 15 month old rats, no significant differences were observed in endosteal single label surface over total surface (F1) and a slight decrease was observed with the silorane cement compared to the commercial cement for the periosteal mineral apposition rate (F2).

Figure 6: **Pull out strengths of bone cements in an in vitro mimic system and in vivo.** A). In vitro pull-out strength (MPa) of different bone cements using a mimic pull-out system. There were no statistically significant differences between Simplex P and silorane-based bone cements. B). Ex vivo pull out strengths using excised rat femurs after 1 week in vivo. The M12-ECHE and M12-3TOSU were significantly less than Simplex P but there were no significant differences between M12-1TOSU and DY5-ECHE. C). Pull-out strength of different bone cements comparing drying and increasing amount of filler. The DY5 containing 65% filler was equivalent to Simplex P. Predipping the titanium rod in cement did not have a significant effect. D). Pull-out strength of DY% 65% filler dried with and without predipping the titanium rod in cement After 24hr PO, though less, no significant differences were observed between the Simplex P and DY5-1TOSU cements. E). Pull-out strength of Simplex P and DY5-1TOSU with 65% glass filler eight weeks PO. No significant differences were observed in 13 month old rats. *$P<0.05$

Figure 1.A: Structures of co-monomers

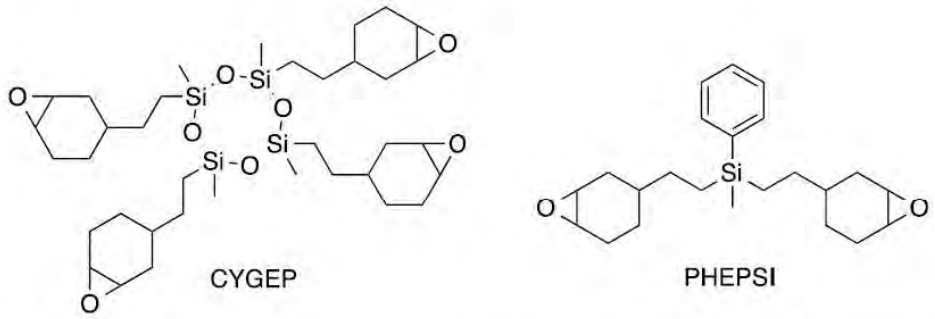

Figure 1.B: Structures of light initiated system (3 wt% PIH, 1 wt% CPQ, and 0.15 wt% EDMAB)

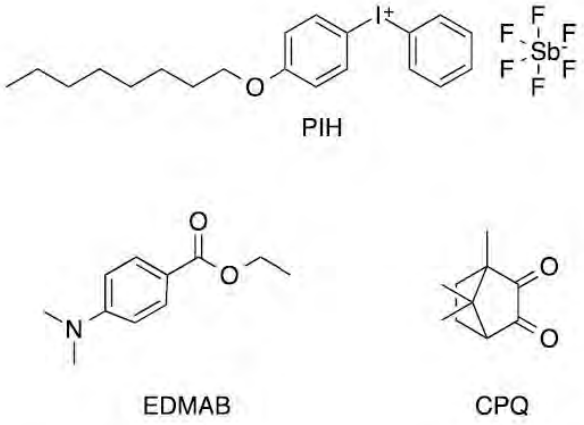

Figure 1.C: Structures of filler modifiers

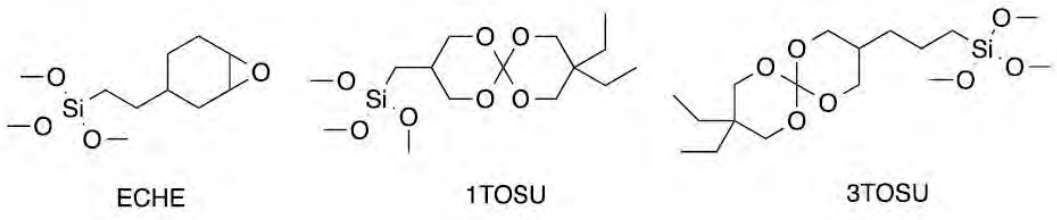

Figure 1

Entry	Formulation Description	Average water, weight% (mg/g) ± Standard Deviation
1	Ultra-Dried SM	**0.0291 ± 0.0057**
2	Dried SM	**0.1029 ± 0.0082**
3	As-Received SM	**0.1781 ± 0.0165**
4	Saturated SM	**0.1932 ± 0.0133**

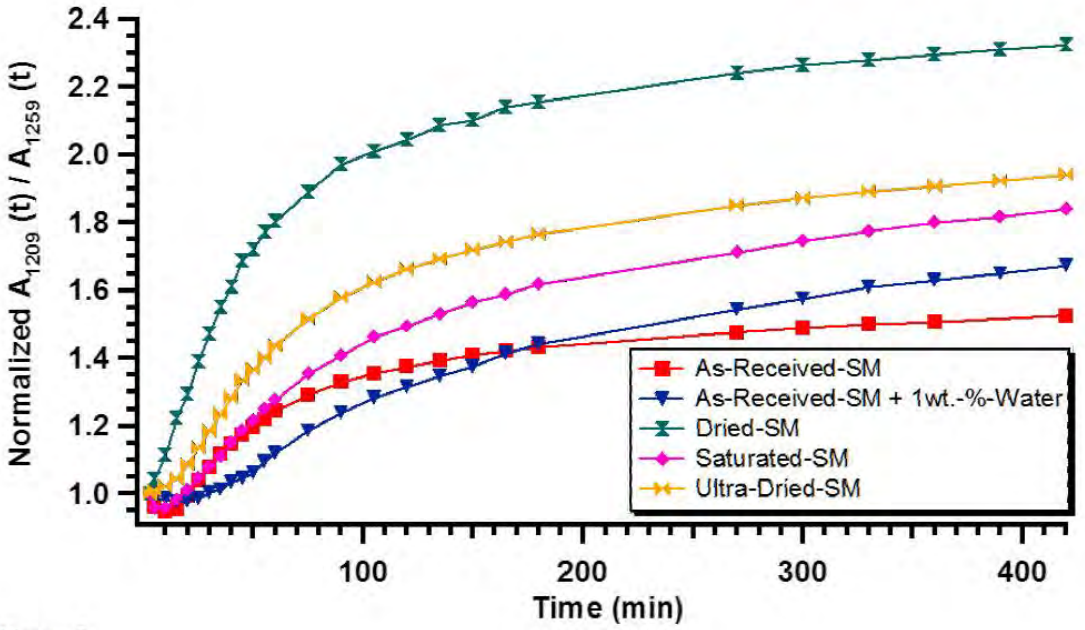

Figure 2.

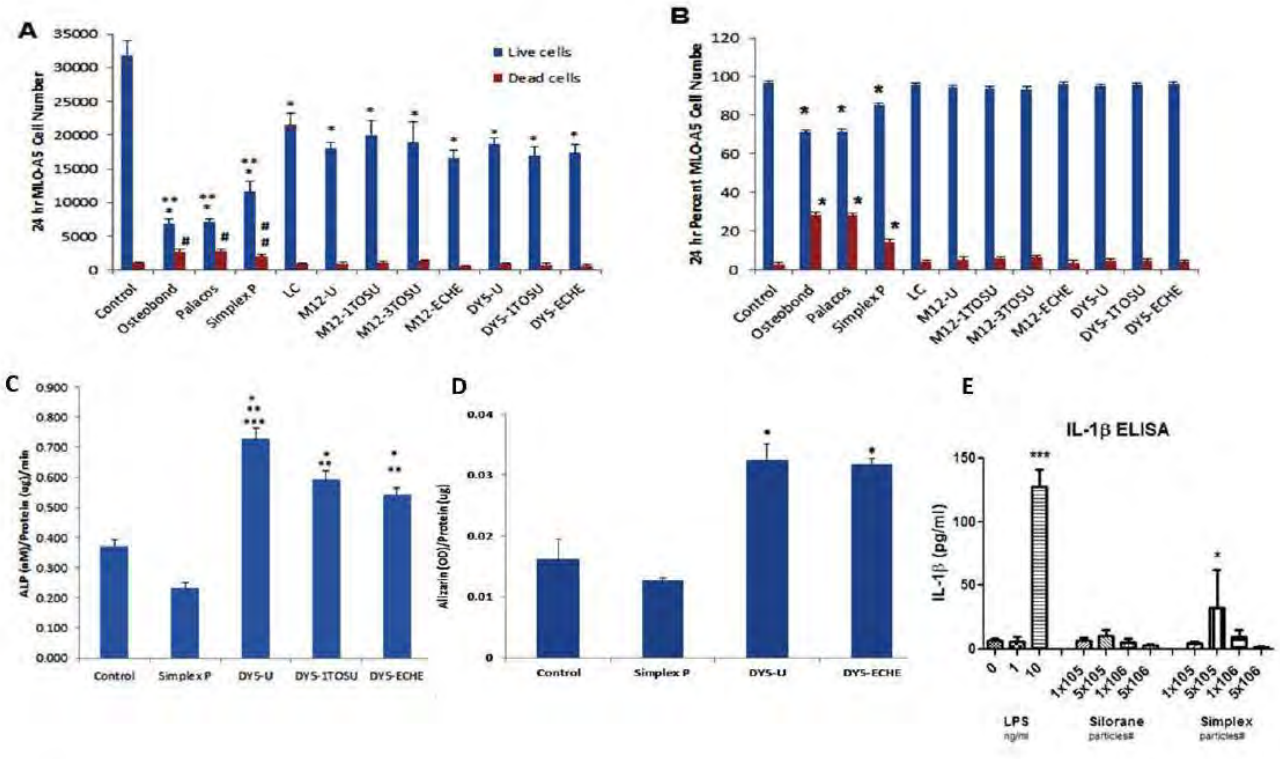
A
24 hr MLO-A5 Cell Number
Live cells
Dead cells
Control
Osteobond
Palacos
Simplex P
LC
M12-U
M12-1TOSU
M12-3TOSU
M12-ECHE
DYS-U
DYS-1TOSU
DYS-ECHE
B
24 hr Percent MLO-A5 Cell Numbe
C
ALP (nM)/Protein (ug)/min
D
Alizarin (OD)/Protein (ug)
E
IL-1β ELISA
IL-1β (pg/ml)
LPS
ng/ml
Silorane
particles#
Simplex
particles#

Figure 3

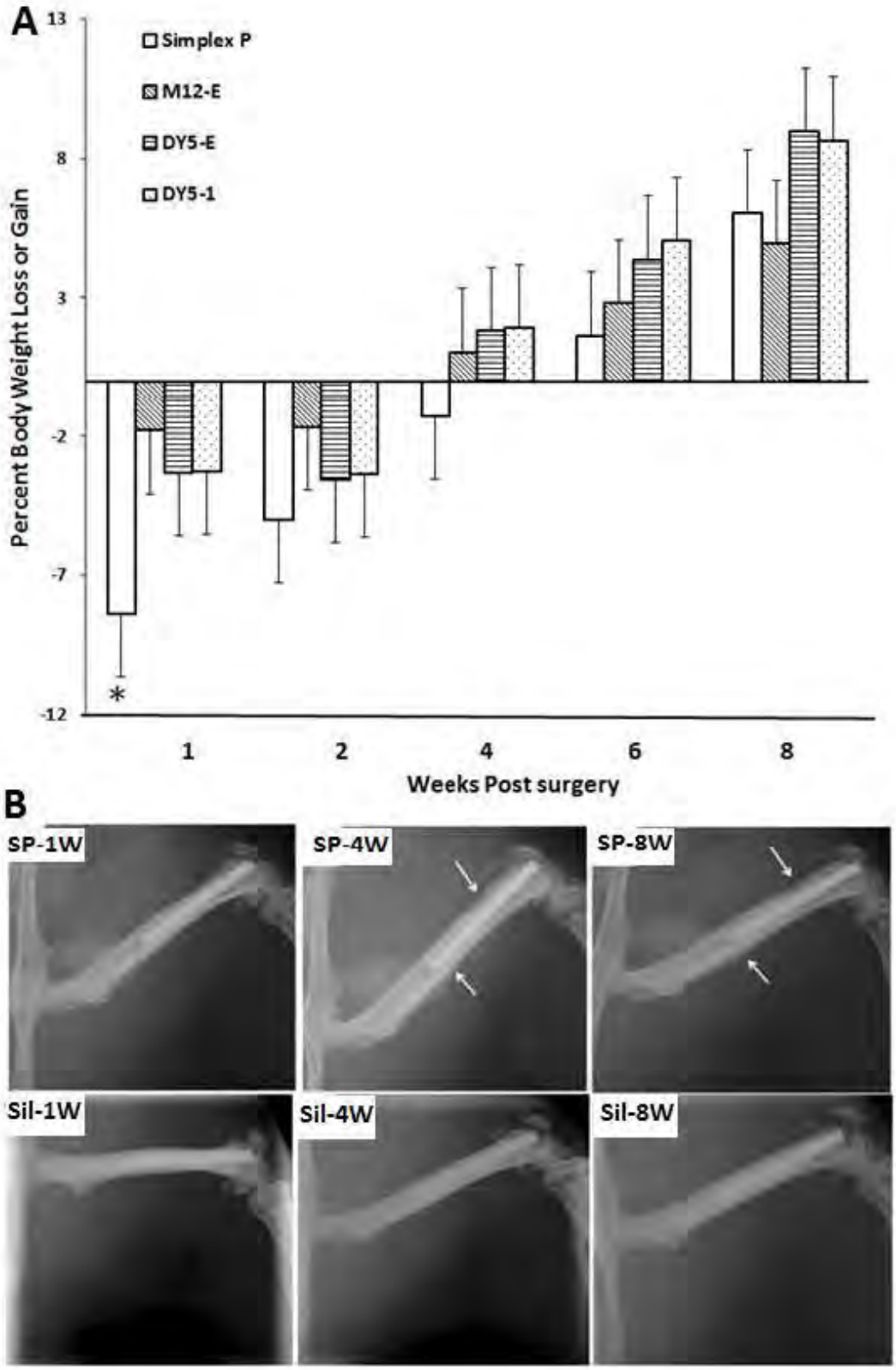
A
Simplex P
M12-E
DY5-E
DY5-1
Percent Body Weight Loss or Gain
13
8
3
-2
-7
-12
*
1
2
4
6
8
Weeks Post surgery
B
SP-1W
SP-4W
SP-8W
Sil-1W
Sil-4W
Sil-8W

Figure 4

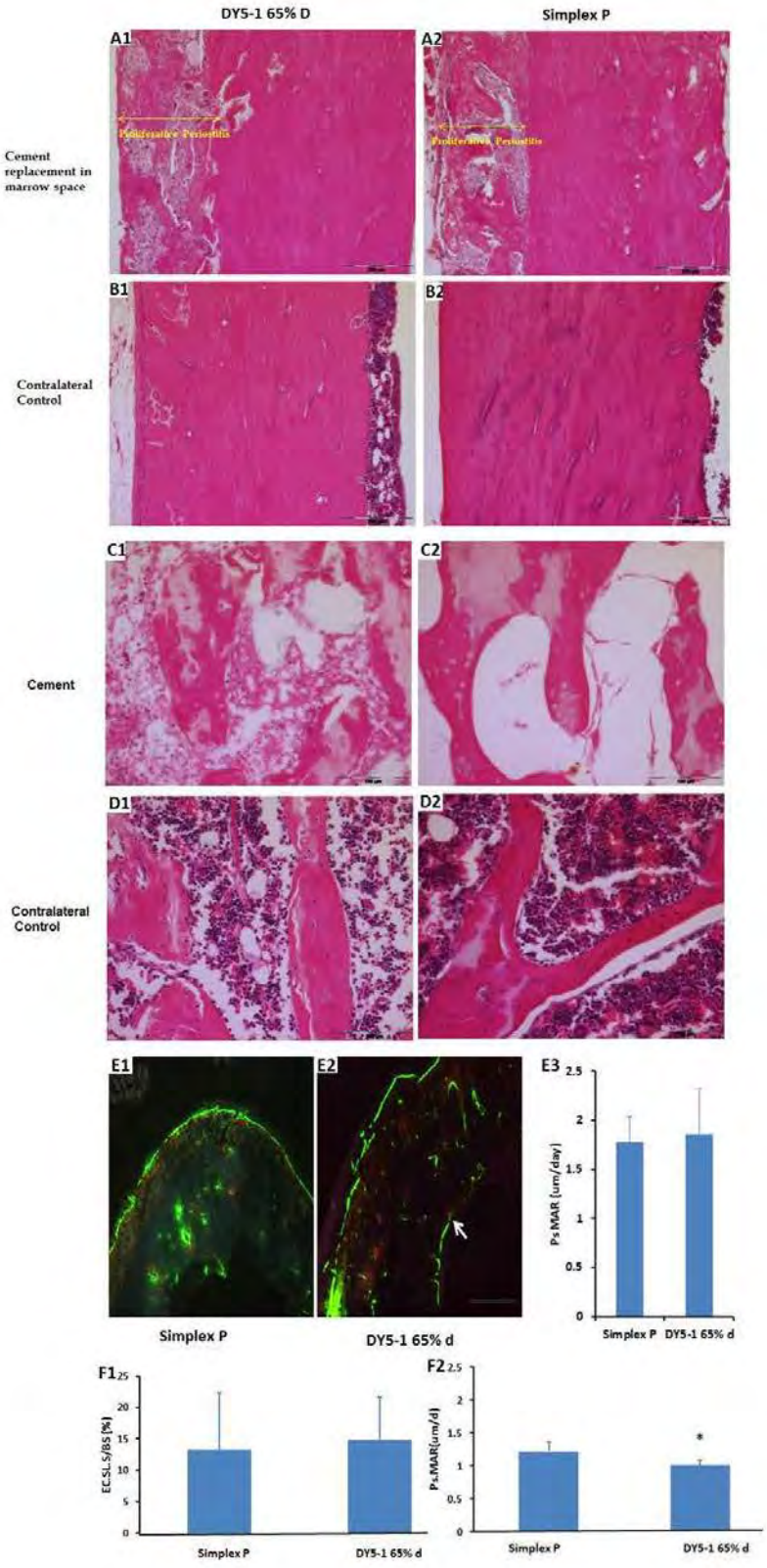
DY5-1 65% D
Simplex P
A1
A2
Cement replacement in marrow space
Contralateral Control
B1
B2
Cement
C1
C2
Contralateral Control
D1
D2
E1
E2
E3
Ps.MAR (um/day)
Simplex P
DY5-1 65% d
F1
EC.Sl.S/BS (%)
F2
Ps.MAR(um/d)
*

Figure 5

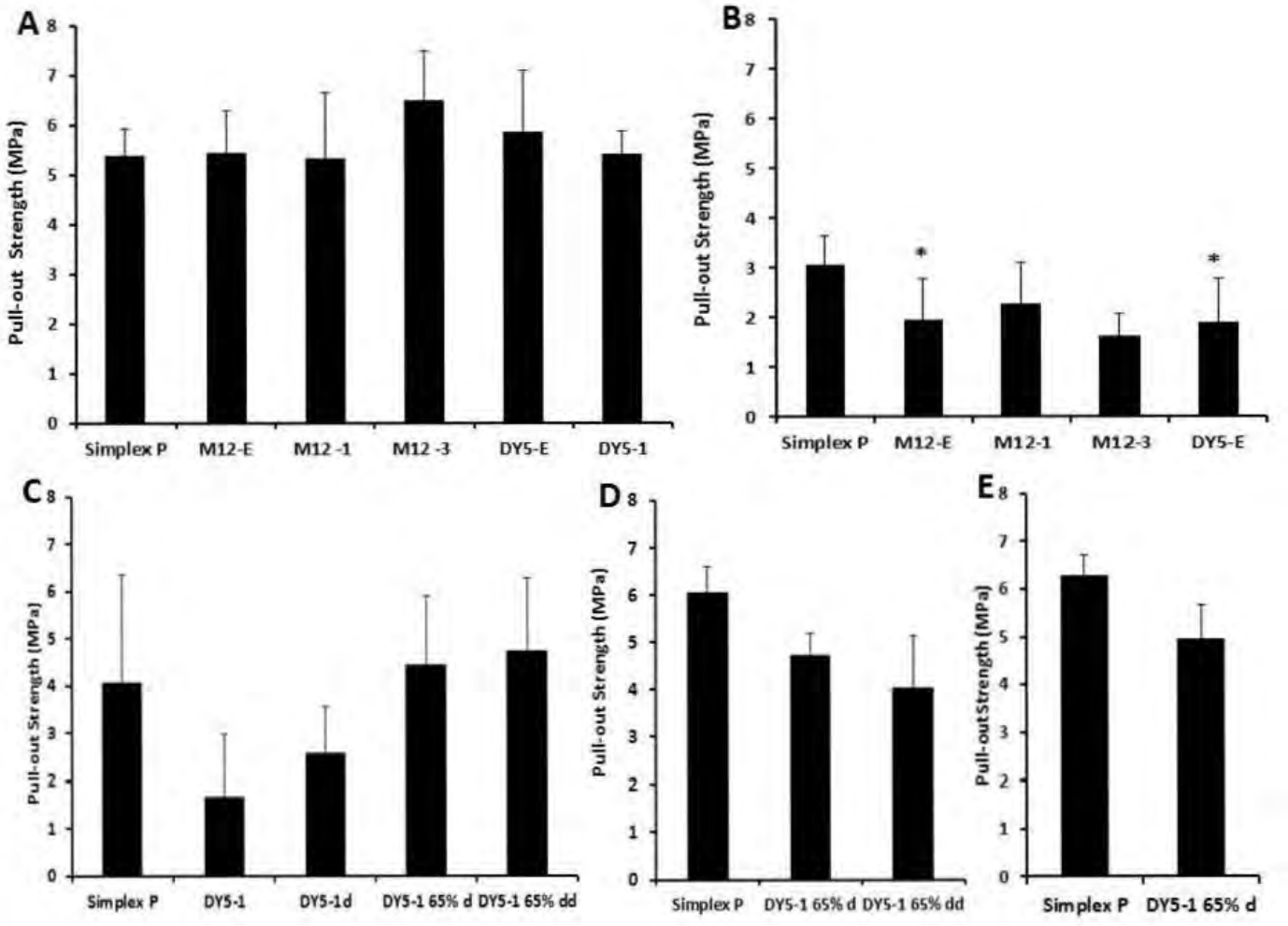
A
Pull-out Strength (MPa)
Simplex P
M12-E
M12 -1
M12 -3
DY5-E
DY5-1
B
Pull-out Strength (MPa)
Simplex P
M12-E
M12-1
M12-3
DY5-E
C
Pull-out Strength (MPa)
Simplex P
DY5-1
DY5-1d
DY5-1 65% d
DY5-1 65% dd
D
Pull-out Strength (MPa)
Simplex P
DY5-1 65% d
DY5-1 65% dd
E
Pull-outStrength (MPa)
Simplex P
DY5-1 65% d

Figure 6

Manuscript

Comparison of silorane bone cement

with commercial cement in a swine femoral implant mode

In preparation:

Comparison of silorane bone cement with commercial cement in a swine femoral implant model.

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Abstract

A novel bone cement, where toxic methylmethacrylate is replaced with non-toxic silorane resin, was tested in a large animal model, 9-11 month old Landrace female swine. Previous studies showed that the silorane cement had equivalent pull-out strength in the rat femoral titanium rod implant model with no toxicity, yet evidence of greater bone formation when compared to commercial cement. In this study, we show equivalent pull-out strength between commercial cement and the silorane cement in a porcine femoral titanium rod implant model. After 8 weeks in vivo, the pull out force of the titanium rods ranged from 405 to 770 Newtons. The separation occurred between the titanium rod and the cement for the commercial sample, but with the silorane cement, two out of three of the separations occurred between the bone and cement. Histological analysis showed plexiform bone formation in these animals, representative of a growing skeleton. No significant differences were observed in periosteal bone formation nor in cortical width, however, a significant increase in bone formation rate and mineral apposition rate was observed in the silorane cement samples as compared to the commercial bone cement. No inflammation or indication of toxicity was observed in the marrow or bone surfaces in either the commercial or silorane bone cement. No visible differences were observed in spleen, heart, lung, pancreas, or kidney. These studies show that the silorane bone cement has equivalent pull-out strengths to commercial bone cement, but actually promotes new bone formation.

Introduction

Though commercially available bone cement has excellent biomechanical and handling properties, it is toxic to cells and tissues. We proposed that it may be feasible to replace the toxic methylmethacrylate in commercial bone cements with the non-toxic resin, silorane, used in dental composites (Melander 2011; Eick et al, 2012). First we tested the hypothesis that silorane resin would have no detrimental effects on bone, using bone cell lines in vitro and osteotomy stabilization in vivo (Eick et al, 2012). No toxic effects were observed and surprisingly, the silorane resin supported parameters of bone formation as it increased alkaline phosphatase and mineralization in vitro. As dental silorane composites are light-cured, it was necessary to determine a method for chemical polymerization. This was accomplished by using a Lamoureux's catalyst (Kilway et al, 2013). The chemically polymerized silorane cement was tested in vivo in rats and found to be non-toxic, positive on bone formation, and had similar pull-out strength as compared to commercially available bone cement (Bi et al, submitted). Here we show the results of testing the silorane bone cements in a large animal model, the swine femoral titanium rod implant model.

Materials and Methods:

Bone Cements:

The DY5-1TOSU system of glass powder-surface silanation composition appears optimal as determined by our previous studies using the rat femoral implant model (Bi et al, submitted). 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 co-monomers. 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. As a control, Simplex P commercially available bone cement was used.

Surgical Procedure for Pig Femoral Implant

Fourteen female Landrace/Large White (Choice Genetics) pigs (approximately 9 month-old) were anesthetized with a mixture of 200 mg of ketamine, 1 ml of 1% atropine, and 10 ml of diazepam, intramuscularly. The pigs were endotracheally intubated and maintained on a closed circuit anesthesia unit with a 5% isoflurane oxygen mixture, and continuously received intravenous Ringer's solution for volume substitution, as well as the antibiotic Cefazolin. The pigs were placed in a left lateral decubitus position and the area of the right knee joint shaved and prepped with alternating povidone/iodine solution and alcohol, three times. All procedures were performed under aseptic conditions. A 5-cm medial parapatellar incision was made to expose the knee joint. The patella was retracted laterally with the knee extended. The knee was slowly flexed to expose the intercondylar notch. A 10-mm hole was made in the intercondylar notch using a stainless steel guide and cannulated reamer system (Arthrex low profile femoral reamer), to penetrate the subchondral cortical bone and gain access to the femoral intramedullary canal. The marrow cavity was reamed through the entire length, 120-130 mm, of the diaphysis to approximately the level of the lesser trochanter. A guide implant was placed into the ablated cavity to ensure that the canal was of an appropriate size to accommodate the

definitive implant. The cavity was flushed with 100 ml of sterile saline for removal of loose marrow contents. Following irrigation, the canal was dried with a lap sponge in order to maintain hemostasis. Silorane bone cement or commercial bone cement was slowly introduced into the intramedullary canal, via a large syringe which was slowly backed out of the canal. Next, a titanium rod measuring 100 mm in length and 6.5 mm in diameter was inserted into the cement-filled femur. Once the cement had hardened, the capsule was repaired with 2-0 Vicryl interrupted figure of eight sutures and subcutaneous tissue approximated with 2-0 Vicryl interrupted buried suture. Skin was sutured with buried 3-0 Monocryl subcuticular suture. For pain management, Buprenex, 0.03 mg/kg, was administered intramuscularly to the pig, and 2 fentanyl patches (150ug/hr) were placed on the pig's back, immediately postoperatively. The pigs were orally given Cephalexin (22mg/kg) for 7 days post-operatively. Animals were allowed activity *ad libitum,* and were weighed weekly to monitor weight loss. The incision was monitored for any inflammatory response and movement of operated limb was monitored every day. X-ray was taken immediately post operation and at post-operative week 4. Blood samples (50mls) were obtained at week 1 and week two. Injections of fluorochromes were performed at 4 weeks (calcein at 15mg/kg) and 7 weeks (alizarin complexone dehydrate at 15mg/kg) postoperative to label active bone surfaces. Animals were euthanized by intravenous injection of Euthasol 8 weeks post surgery. Samples of soft tissue such as heart, liver, lung, spleen, kidney and pancreas were photographed and collected for histological examination. The femurs, both operated and contralateral control, were harvested, denuded of soft tissue, and cut at the diaphyseal shaft approximately 1-2 cm proximal to the end of the titanium rod. A 1 cm cross section was collected and placed in 10% neutral buffered formalin for histology. A similar bone section was taken from the non-operated, non-treated control femur. The remainder was frozen at -20^0C for biomechanical testing.

Specimen Preparation for Pull-Out Experiments.

Originally we had planned to expose approximately 13-14mm at the distal end of the rod for attachment for pull out studies. However, as several of the rods were more deeply embedded, more than 13-14 mm had to be removed and this resulted in a significant

loosening of some of the rods prior to testing. Thus it was decided to expose the rod 13-14 mm at the midshaft. The condyles were trimmed and excess soft tissue was removed prior to “potting” the bone in a metal cylindrical fixture surrounded by Bondo (3M 265, 140 grams grey/4 grams red), to secure it for pull out. (See Figure 1, left side).

To consistently thaw the frozen bones, they were placed at room temperature for 5hrs, then placed in a 4°C refrigerator overnight (~10hrs). The bones were then brought to room temperature 1-2hrs prior to potting. The bone shaft was marked at ~5.5cm proximal to surgical entry to indicate potting height. Additionally, the top of the bone was wrapped with paper towel to prevent Bondo attaching to the bone surface, which allowed for easier harvesting of bone samples for further histological analysis.

The inside of the metal fixture was coated with Vaseline for easy removal post-pull out. To ensure proper alignment of the rod when potting the bone, the rod/bone was suspended by clamping the rod into the materials testing system (MTS), such that the rod would remain perpendicular during the potting process. The bone was then lowered outside the fixture to determine the bottom of the fixture and the instrument zeroed. Once zeroed, the bone was lowered into the fixture and the stage was adjusted to ensure the specimen was centered or reasonable close. The stage was also free to move during the pull out test which should further help achieve alignment. With the bone and rod secured in the clamp, hanging above the fixture, 150 gm of Bondo was poured into the fixture and the bone lowered into the Bondo. Additional Bondo was then added until the 5.5cm line was reached, with the bulk of the Bondo at or below this point. The surface was then smoothed and the screws located in the sides of the fixture were tightened by hand. Flat screws were used to secure the Bondo and bone, instead of pointed screws, so as to minimize any additional load on the bone. Approximately 300 grams of Bondo was used per sample, prepared in two 150 gram batches, and it was allowed to polymerize for 40 minutes prior to pull out testing. (See Figure 1, right side).

Determination of heat generated by potting of specimen in Bondo, a heat generating material.

Using a LaserGrip 630, a non-contact infrared thermometer, the curing temperature of the Bondo surface was measured, as well as any heat transferred to the metal potting fixture, or to the external bone surface ~2mm above the Bondo surface.

Mechanical testing:

The samples were run on a MTS 858 Mini Bionix II with a 14 kN load cell. The failure detector, sampling frequency, load rate, and displacement limit were set as the values specified below. The initial load was measured for five seconds prior to starting the test and then averaged together to obtain a baseline. Failure detector: 25% F_{max}; Sampling frequency: 100 Hz; Load rate: 0.25mm/sec; Total displacement: 5mm.

Histological Analyses:

Freshly dissected femurs were trimmed of most of muscles, and approximately 1cm cross sections of bone were manually cut with a hack saw, 1-2 centimeters proximal to the end of the Ti rod, and were immediately fixed into 100-200ml 10% buffered Formalin (fisher, cat.23-245-685) at 4°C for 48-60hrs, then changed to 70% EtOH for an additional 24h at 4°C. Using a dental hand drill with diamond disk attachments, an approximately 1/3 piece of each cross section was transversely cut, so that the piece contained representative areas in which the cement was both attached, and not attached to the endosteal bone surface. The contralateral controls were taken from the same region. Samples were dehydrated in graded EtOH from 70%, 80%, 95% (2 changes) to 100%(3 changes) using an automated tissue processor by 5-7h each step. Following dehydration, infiltrate samples in a 20ml glass vial filled with 15ml reagent and change daily with acetone, acetone mixed with different ratio methyl methacrylate (MMA, sigma, M55909), and MMA for 5days total. Samples were then embedded in a 20ml glass vial, containing a 5ml pre-polymerized base, by adding 14ml freshly made MMA. Polymerize at -20C for 3-5 days. When fully polymerized, vials are removed from the freezer, broken and rinsed with water to remove any glass shards. The plastic block is trimmed with a Buehler ISOMET 1000 precision saw and the trimmed blocks mounted on a Thermo Scientific Microm HM355S microtome for cutting into 5um thick sections using a Dorn &Hart microedge Tungsten carbide D profile knife.

H&E stain: The plastic was removed from sections using ethylene glycol monobutyl ether acetate (Fisher Scientific, E181-4) with three changes of 20min each, followed by 10% EDTA for two minutes to remove mineral. The standard H&E staining was performed.

Measurement of cortical bone thickness: Three non-adjacent sections per animal were used for quantitation. Multiple x1 images of non-stained plastic sections were taken using the Meta Morph montage function on a Nikon E800 microscope, followed by 'stitching' of the images. The stitched images were entered into the Analysis Software to measure the cortical bone thickness (See Figure 6).

Imaging of fluorochrome label and performance of dynamic histomorphometry.
Bone formation rates and mineral apposition rates were performed as described previously (Bi et al, submitted). A time period of three weeks representing between calcein injection and alizarin injection was used for the calculations. Three non-adjacent slides were used per animal to analyze trace labels using the Osteomeasure system and software. A Nikon E800 fluorescent microscope was used with a DAPI-FITC-TRITC triple band filter. Using the x4 lens, 4 fields were quantitated along the endosteal surface and moving towards periosteal surface. The starting point was one field from the border of the bone adjacent to cement and not adjacent to cement (See Figures 7, 8 and 9). For the control leg, the starting point is one field from the middle of the cortex.

Results:

Surgical outcomes: Out of 14 surgeries performed, two were euthanized due to surgical error, one was euthanized due to an accident in which the non-operated leg fractured, and one died coming out of sedation. Therefore, there was a loss of four animals. For the remaining animals, sacrifice was performed at 8 weeks PO. A visual inspection at the time of sacrifice by the veterinarian did not reveal any obvious pathology. Images of each organ were also taken, but none showed any pathology (data not shown). Results of blood chemistry profiles showed no abnormal readings (data not shown).

Results of pull out tests: Originally we had planned to expose the distal end of the rod for attachment for pull out studies, however, this resulted in a significant loosening of some of the rods prior to testing. This reduced the sample size to n=3 for the silorane cement and n=4 for the commercial cement. Thus it was decided to expose the rod at the midshaft (Figure 1). Also, to insure there was no significant effect of the exothermic rise in temperature of the Bondo on the bone, surface temperatures were recorded (Table 1). The pull-out tests revealed no significant differences between the silorane cement and the commercial cement (Figure 2 and Table 2). For the commercial cement, the displacement first occurred between the cement and the titanium rod (n=4), however for the silorane cement, displacement occurred between the cement and bone in two of the samples and between cement and the rod in one of the samples (Table 2).

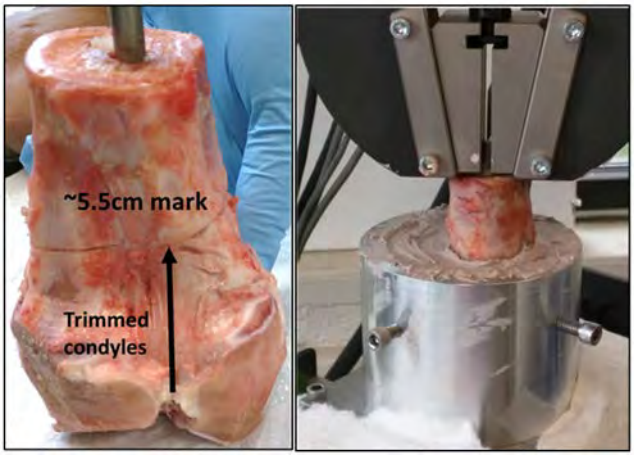
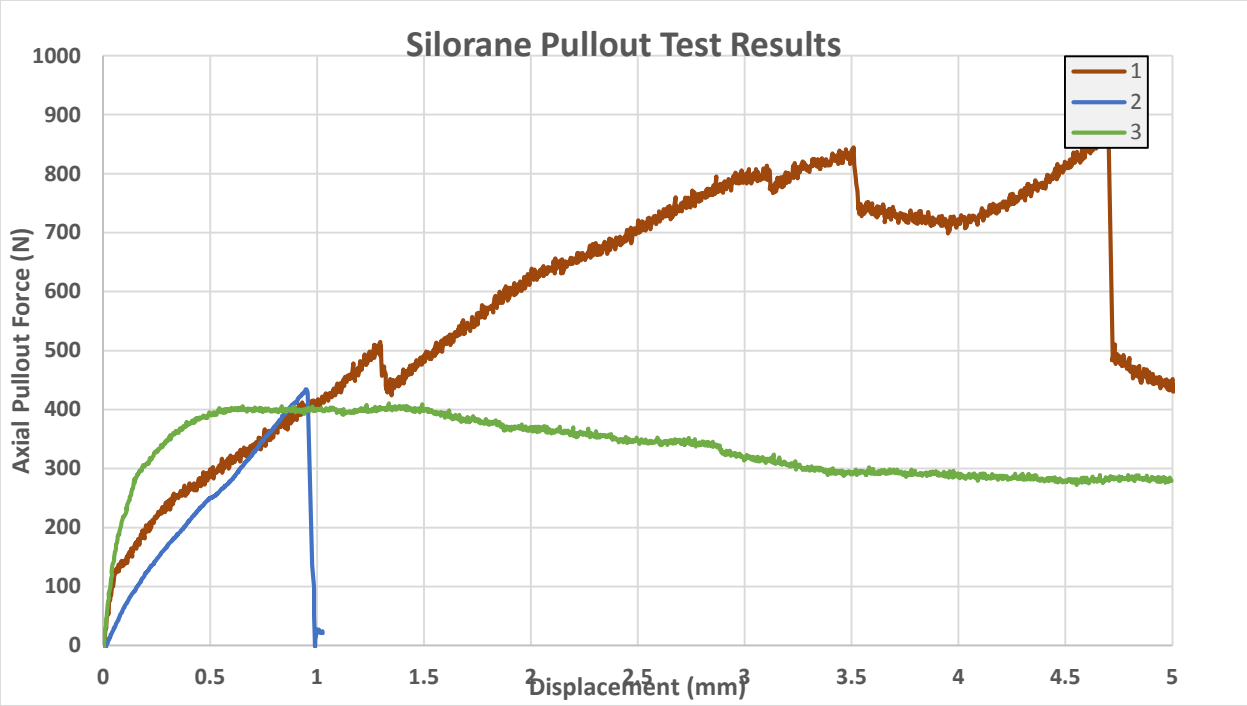


Figure 1: Image showing the location of the titanium rod surrounded by cement, trimmed condyles, and level to where Bondo was applied (5.5 cm from femoral condyle (left). Also shown is the potted sample attached to the MTS 858 Mini Bionix II before pull out (right).

Table 1. This shows the temperature of the Bondo as compared to the temperature of the bone.

	Temp (°C)		
Time	Bondo	Bone	Pot
2:30	40.1, 35.3	25.6, 29.2*	26.2
2:37	52.2, 45.5	32.7	26.8
2:41	41.9, 38.6	32.4	27.6
2:56	35.1, 33.7	31.2	26.8

*After Bondo was removed from the bone



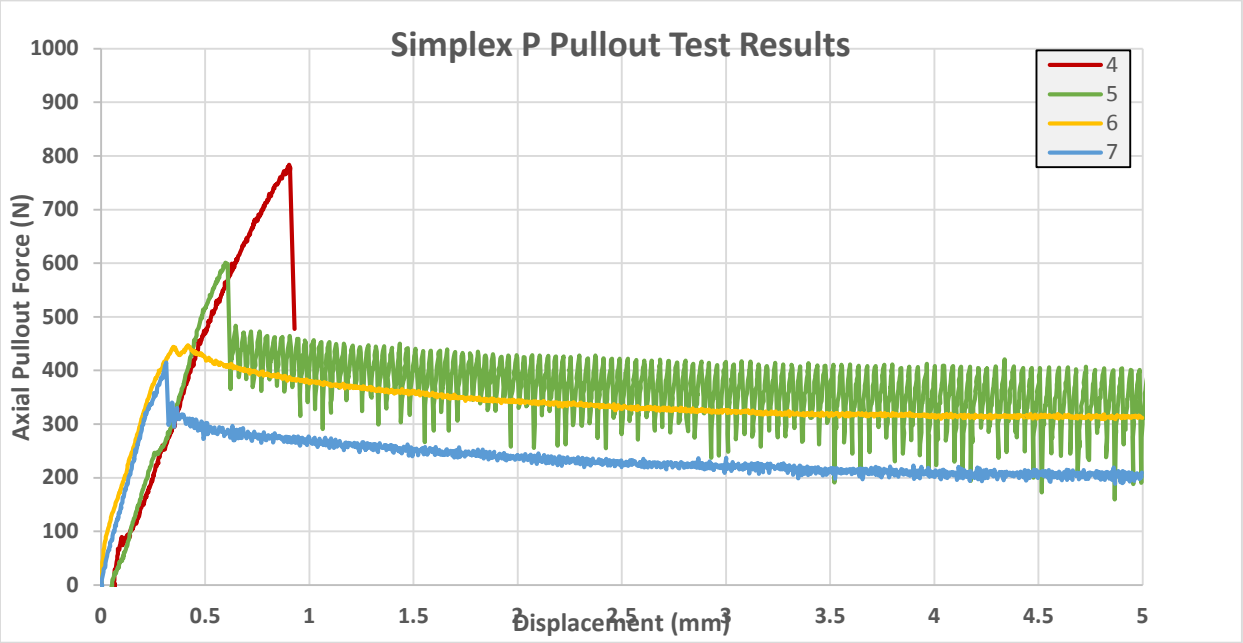


Figure 2. Pull out strengths of cements. A). three silorane samples. B). four Simplex P samples.

Table 2: Pull Out Test Results

Pig ID	Cement	Failure**	Force (N)	Displacement (mm)
1	Silorane	C/B	508	1.3
2	Silorane	R/C***	432	0.95
3	Silorane	C/B	405	
4	Simplex P	R/C***	770	0.9
5	Simplex P	R/C	602	0.6
6	Simplex P	R/C	444	0.34
7	Simplex P	R/C	412	0.3

** R – rod, C – cement, B – bone
*** Estimated location of failure

Results of histological analysis:

To begin to localize position of the titanium rod before cutting, x-rays were taken of the distal femoral diaphysis (Figure 3). After localizing the rod, the sections were cut, cleaned, and polished (Figure 4). The placement of the cement could be more easily visualized showing uneven distribution around the titanium rod. Also, as the commercial cement was white, the silorane cement was a light brownish-grey. H&E staining showed no evidence of an inflammatory reaction in either sample (Figure 5). Pieces or granules of the silorane cement could be visualized by histology as shown in Figure 5, (bottom), whereas, only voids were observed for the commercial cement as this is removed by the xylene step.

Quantitation of white light microscope images of non-stained plastic sections of the cortical bone did not reveal any significant differences between the two samples (Figure 6). The fluorochrome labeling revealed plexiform bone formation in these animals indicating a growing skeleton (Figure 7). To determine if the cements had an effect on this periosteal bone growth, histological quantitation was performed (Figure 8). No significant differences were observed. Next the histological quantitation of endosteal labeling was performed. An increase in both bone formation rate and mineral apposition rate was observed for the silorane cement compared to the commercial bone cement (Figure 9).

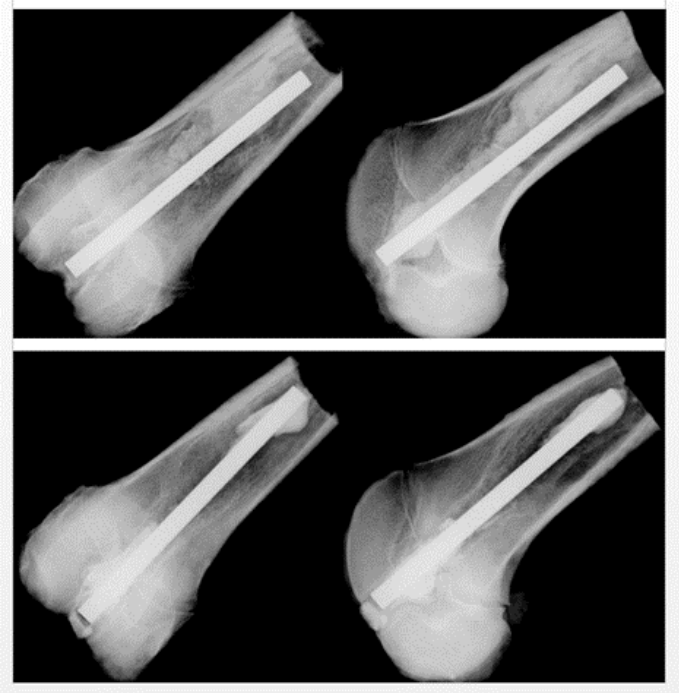


Figure 3. X-rays of two representative samples of the femoral head containing the titanium rod and commercial bone cement (top) and silorane bone cement (bottom). The views are shown ventrally (left) and sagitally (right). Note, that the cement did not always completely cover the rod.

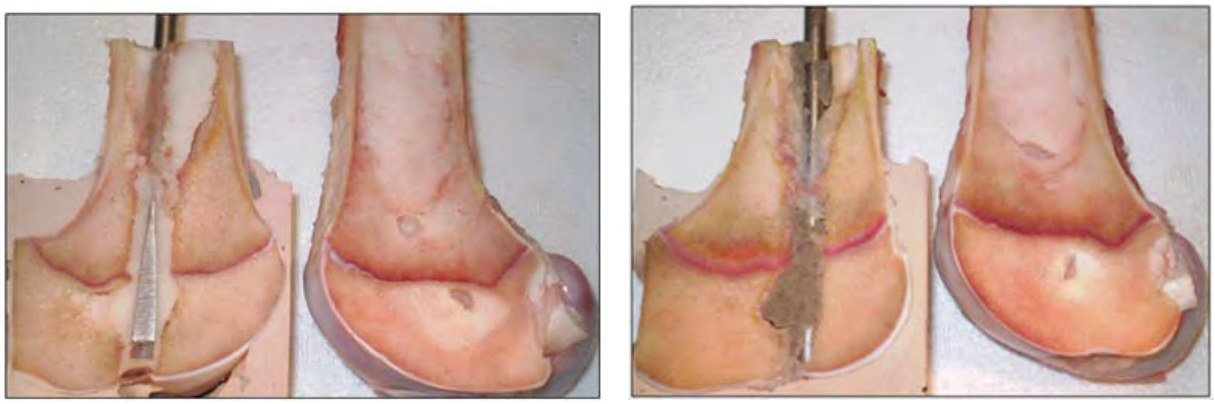


Figure 4: Image showing lateral to sagittal section of femur containing commercial bone cement and its contralateral control (left) and a femur containing silorane bone cement and its contralateral control (right). Note the white color of the commercial bone cement and the pale brownish-grey of the silorane cement. Also note the uneven distribution of cement around the bone. The more intense red color at the growth plate in the silorane containing sample is due to alizarin red. This suggests that greater bone formation is occurring in the growth plate of this animal.

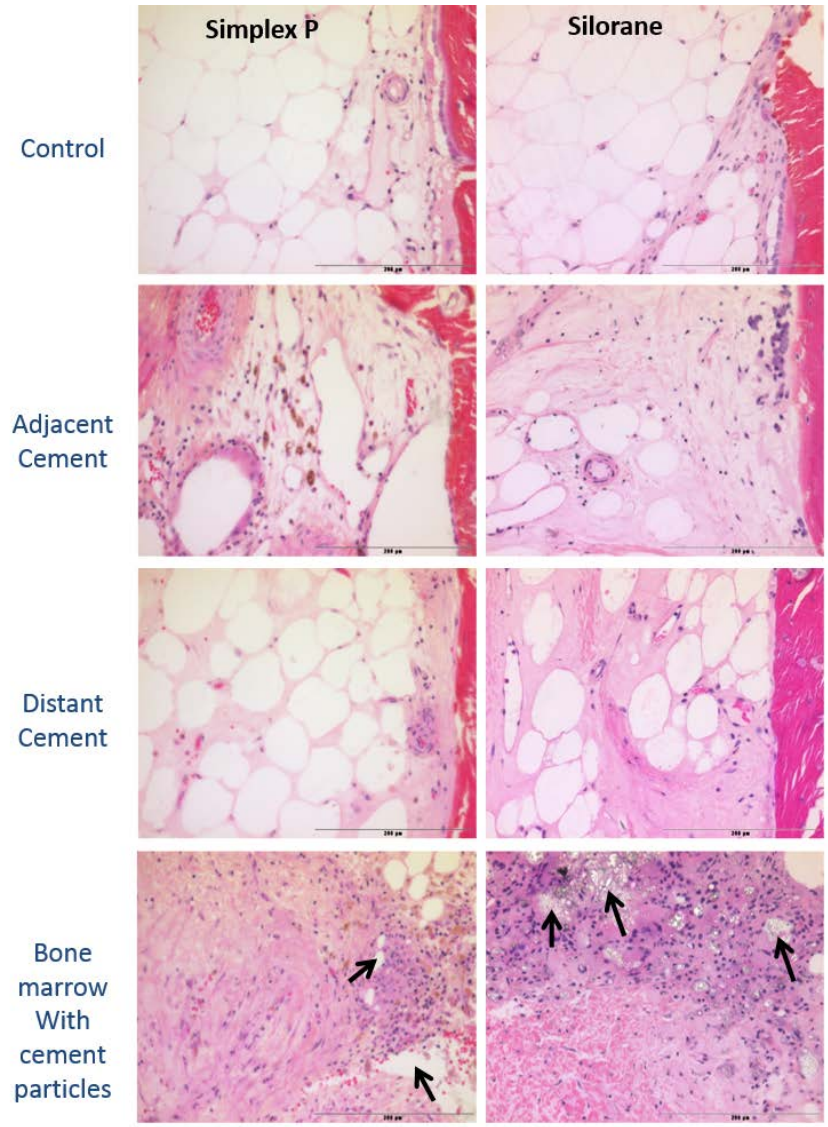


Figure 5. Histological analysis of H & E stained sections. Areas were taken of bone marrow next to cement and adjacent to the cortical bone endosteal surface. Little osteoblast activity is observed. Areas were taken of bone marrow distant from cement and adjacent to the cortical bone endosteal surface. Osteoblastic activity can be observed. All marrow spaces show the highly fatty nature of the swine marrow. No significant differences were observed.

Histological sections of marrow containing cement (bottom row). Granular forms of the silorane cement can be seen (note the light grey material, arrows). The silorane cement is not removed by the xylene step, whereas the Simplex P is removed, leaving voids (arrows). Circular or oval forms are left behind when the Simplex P is removed with the xylene wash.

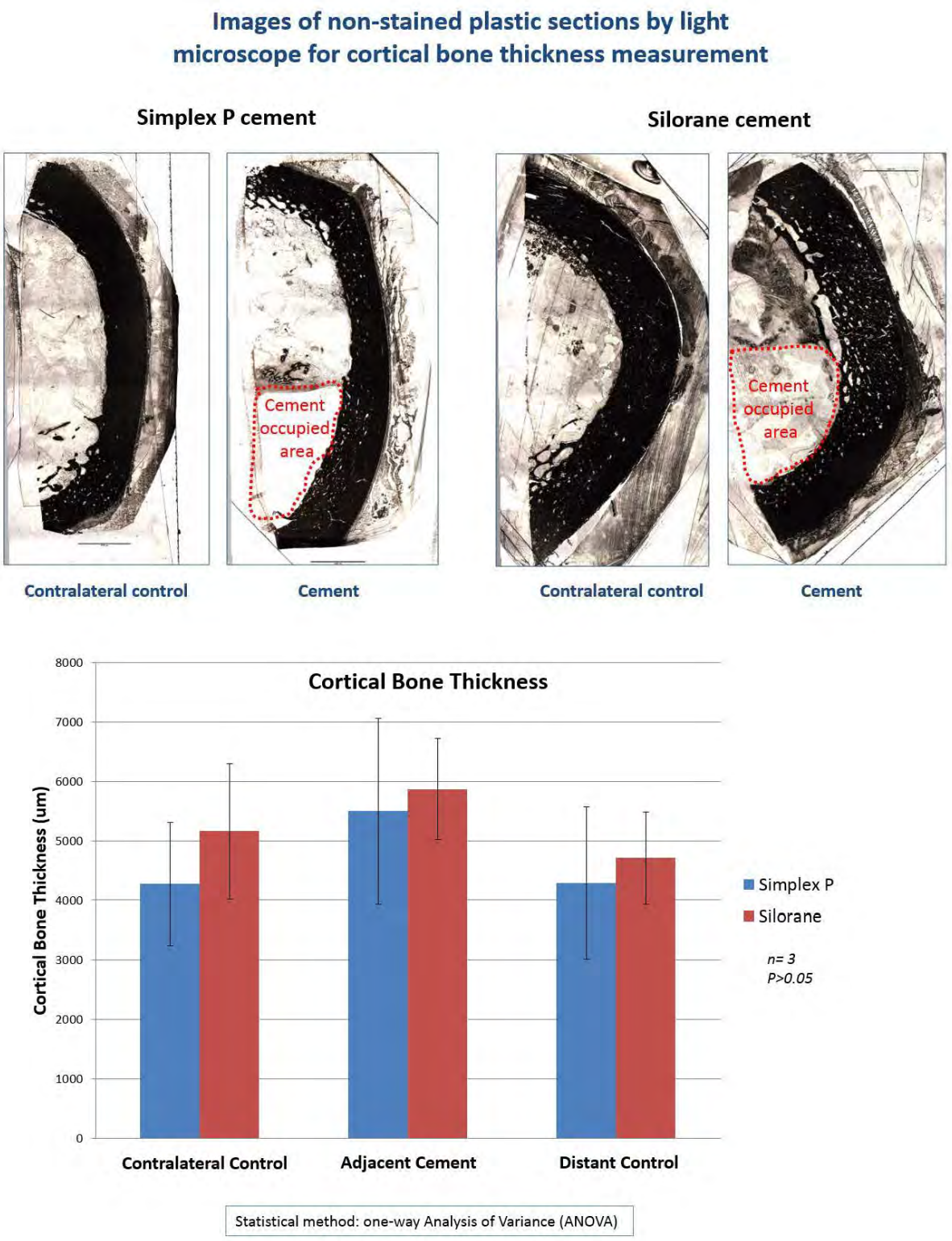


Figure 6. White light microscope images were used to quantitate cortical thickness. No significant differences were observed between cortical bone adjacent to either the Silorane or the Simplex P cements.

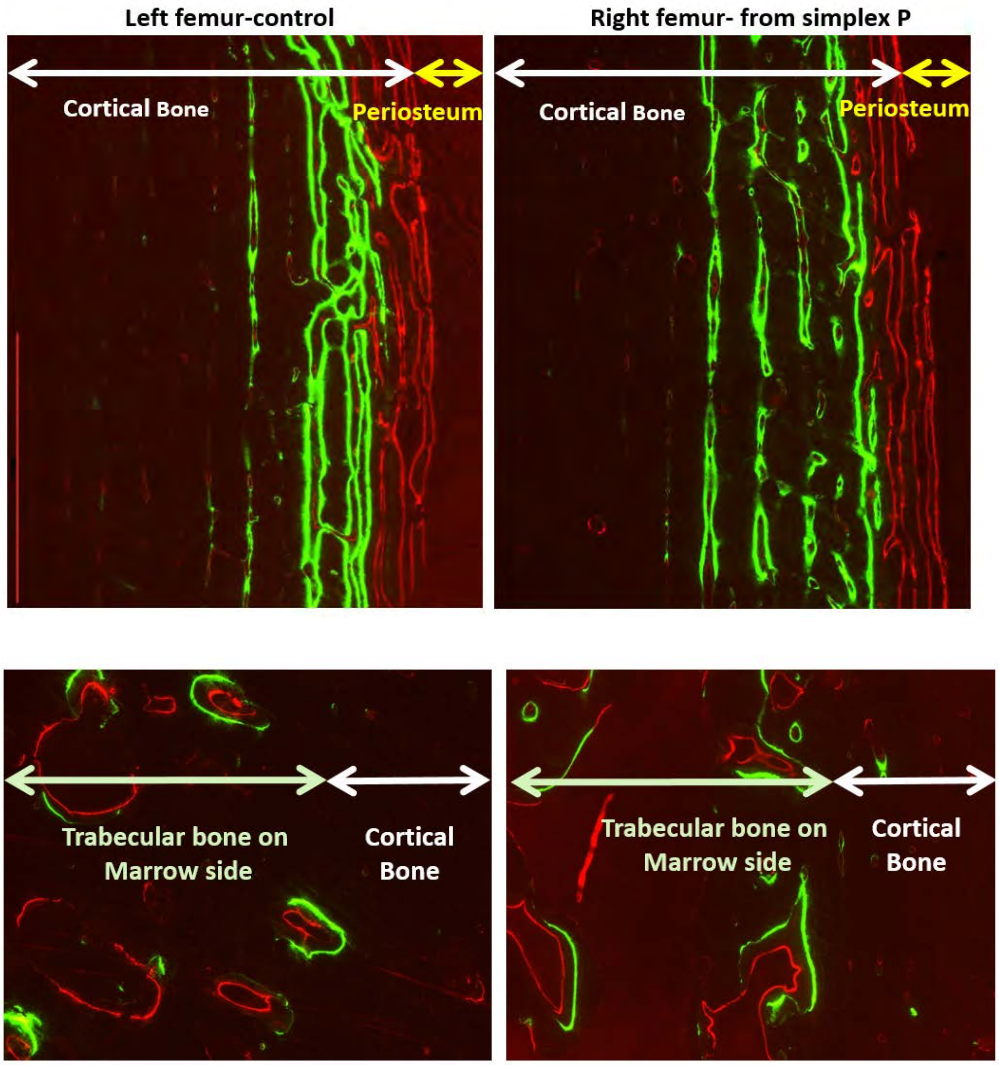


Figure 7. Cross section of midshaft showing Calcein and Alizarin Red fluorochrome labelling of the bone. The animals show plexiform bone formation which is indicative of a growing skeleton. Note the dramatic increase in bone formation on the periosteal surface and the dramatic increase in intracortial remodeling.

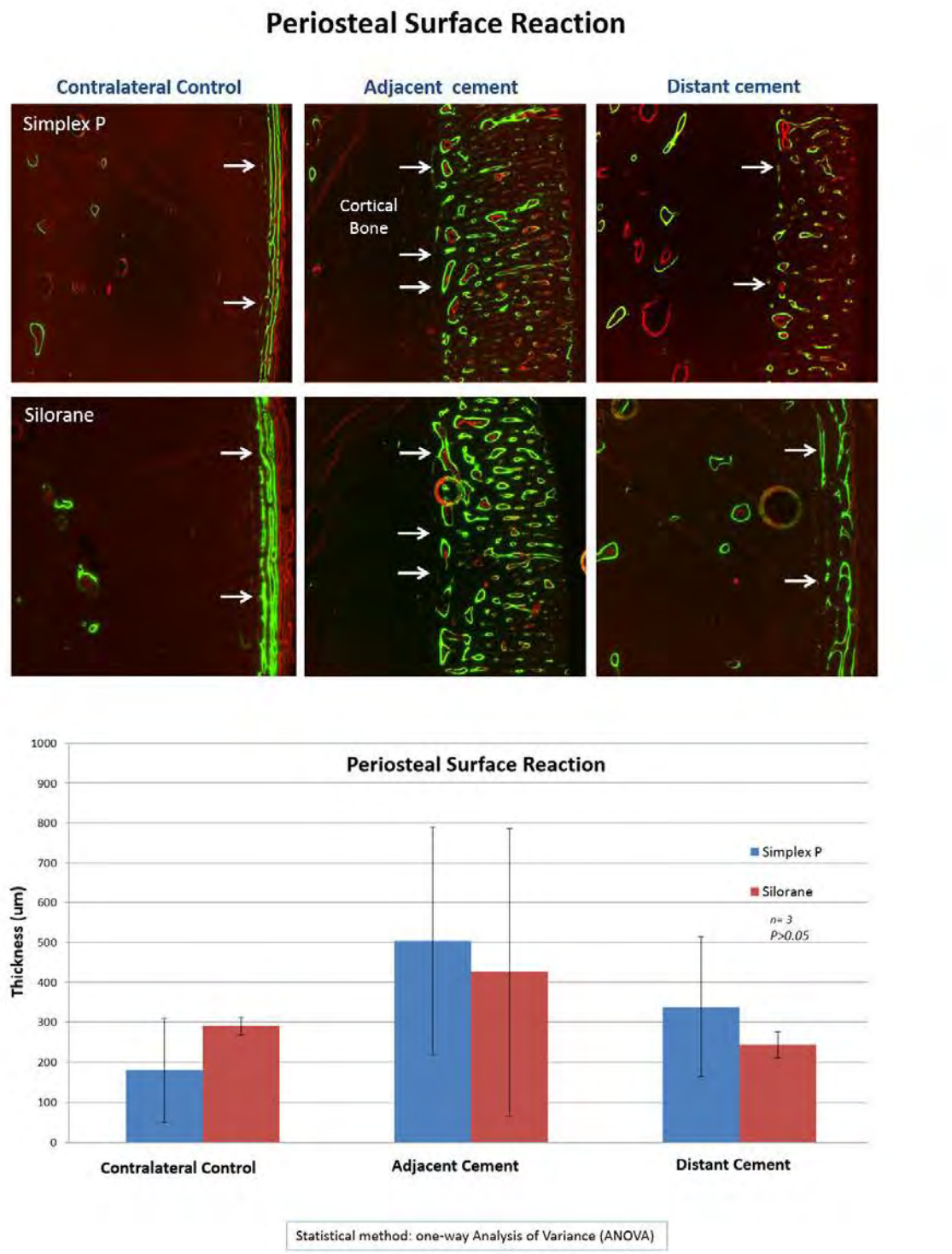
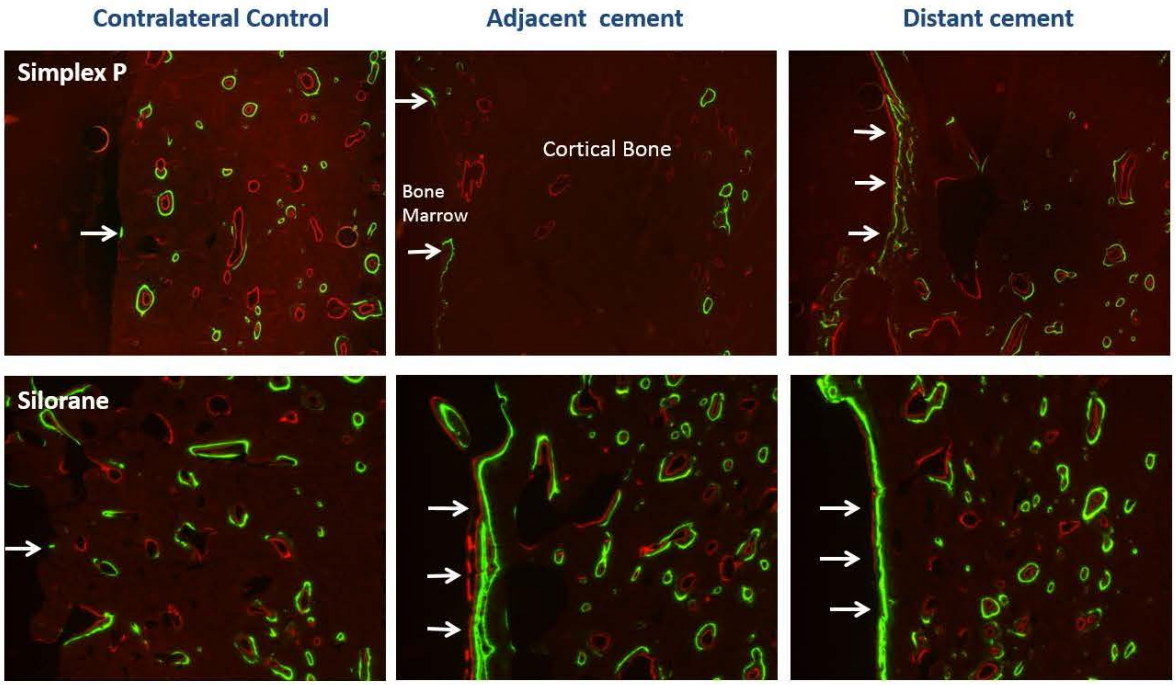


Figure 8. Periosteal surface reaction to the bone cements. The contralateral control is the non-operated femur, adjacent to cement is periosteal bone with cement in contact with the cortical bone, and 'Distant Cement' is cortical bone that is distant from and not in contact with the cement. No significant differences are observed in periosteal width between the commercial or the silorane cement.

Endosteal Surface labels



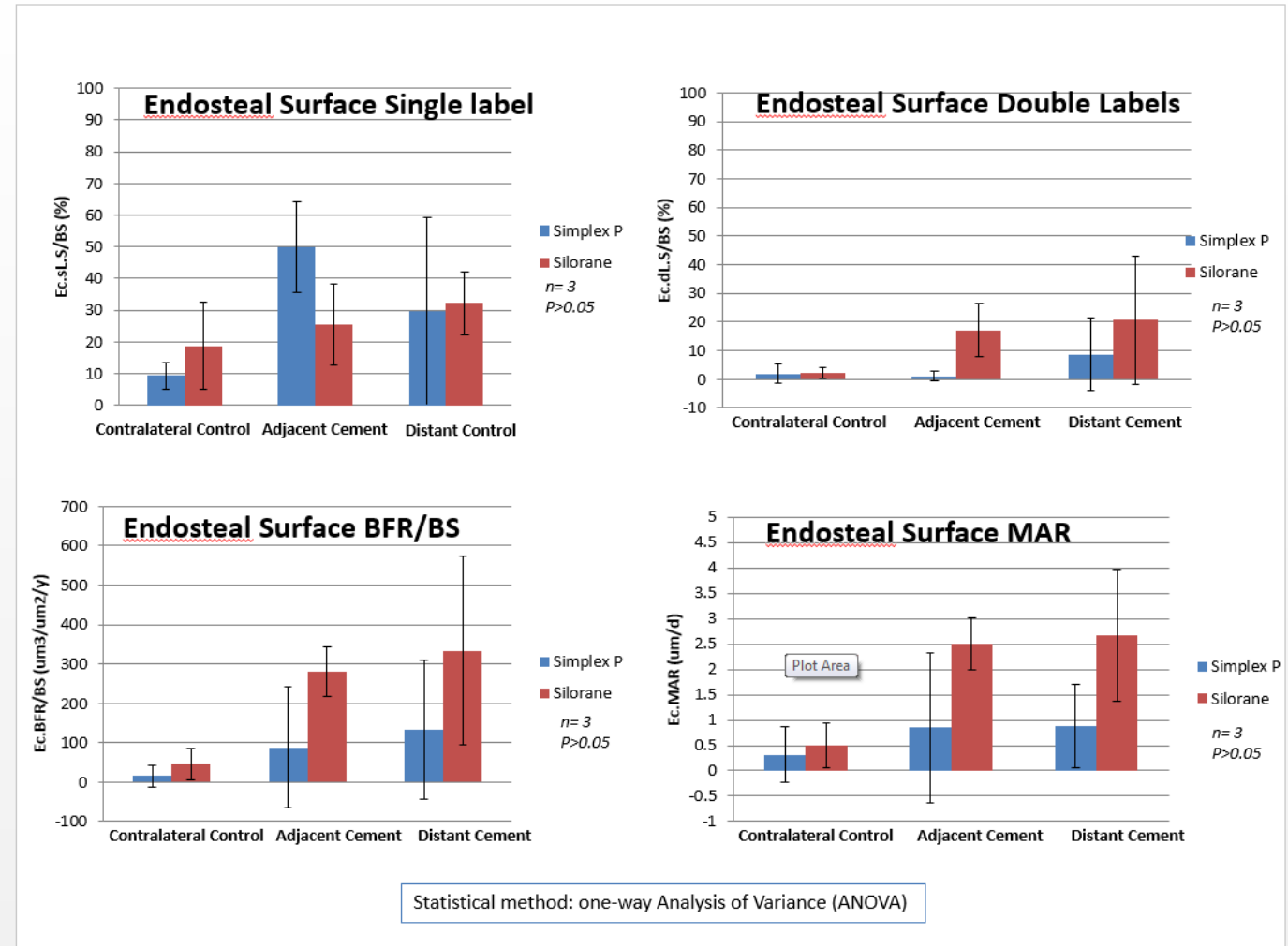


Figure 9. Endosteal parameters of bone from the contralateral non-operated control femur (Contralateral Control), for the endosteal bone adjacent to cement (Adjacent Cement) and from endosteal bone distant to the cement (Distant Cement). The silorane cement showed a greater degree of double label and bone formation rate and mineral apposition rate.

Summary and Discussion:

In summary, a novel bone cement previously shown to be non-toxic, non-shrinking, and non-exothermic, has been tested in a large animal model, the swine titanium rod femoral implant model. Similar to previous studies in rats, the silorane cement and the commercial cement have similar pull out strengths. Eight weeks after surgery, no toxic effects of either cement was observed, no differences in cortical bone width or periosteal bone formation was observed, however, an increase in endosteal bone formation rate and mineralization rate was observed, similar to previous observations using the rat femoral implant model.

Acknowledgments

We would like to thank and acknowledge the assistance of Sherrie Neff, Sara Hansen, Mike Linville, Melissa Samuel, Baylee Beasley, Tricia Meyer, and Adam Lloyd with anesthesia, x-rays, surgeries, sacrifice and histological analysis.

This work was funded by DOD W81XWH-11-1-0805.

Author roles:

DP performed the surgeries, KK oversaw the production of the silorane cement, RW and LH assisted in the preparation of the cements, TS, JR, LFB, MF, EW assisted in the swine surgeries, JR and AX for histological analysis, TI and DX performed the pull-out experiments, LFB oversaw the experiments and wrote the manuscript. All authors have reviewed the contents.

References:

J David Eick, Cielo Barragan-Adjemian, Jennifer Rosser, Jennifer R Melander, Vladimir Dusevich, Rachel A Weiler, Bradley D Miller, Kathleen V Kilway, Mark R Dallas, Lianxiang Bi, Elisabet L Nalvarte, Lynda F Bonewald **Silorane resin supports proliferation, differentiation, and mineralization of MLO-A5 bone cells in vitro and bone formation in vivo**. Journal of Biomedical Materials Research. Part B, Applied biomaterials. 04/2012; 100(3):850-61.

Jennifer R Melander, Rachel A Weiler, Bradley D Miller, Thomas P Schuman, Kathleen V Kilway, Delbert E Day, Mariano Velez, J David Eick **Estimation of properties of a**

photoinitiated silorane-based composite with potential for orthopaedic applications. Journal of Biomedical Materials Research. Part B, Applied biomaterials. 11/2011; 100(1):163-9.

Kilway, K. V.; Bonewald, L. F.; Schuman, T. P. Curators of the University of Missouri, USA) **Biomaterial Compositions,** U.S. Patent US20130210953 A1, 2013.

Lianxiang Bi, Kathleen V. Kilway, Jennifer R. Melander, Rachel A. Weiler, Jennifer Rosser, Anita Xie, Yukiko Kitase, Elizabeth Menuey, Thomas P. Schuman, J. David Eick, and Lynda F. Bonewald **Development of a Novel, Non-Toxic, Non-Exothermic, Osteogenic Bone Cement** Submitted to Nature Materials.

Bone Cement Symposium Program Book

BONE CEMENT SYMPOSIUM

Ewing Marion Kauffman Foundation Conference Center

4801 Rockhill Road

Kansas City, Missouri

Saturday, October 4, 2014

8:00 am—3:00 pm

Supported by Department of Defense (DOD) –
U.S. ARMY Medical Research Acquisition Act (USAMRAA)
Grant # W81XWH-11-1-0805

Bone Cement Symposium
Ewing Marion Kauffman Foundation
4801 Rockhill Road
Saturday, October 4, 2014
PROGRAM

Co-Chair and Moderator

Dr. Tom Schuman

Morning Session:

8:00 AM – 9:00 AM **Breakfast**

9:00 AM – 9:15 AM **Welcome and Introductory Remarks**
Dr. Lynda Bonewald Dr. Kathleen Kilway

9:15 AM – 10:15 AM **Keynote Speaker - Dr. Tim Topoleski**
Professor
Department of Mechanical Engineering
University of Maryland, Baltimore County
Baltimore, Maryland

10:15 AM – 11:15 PM **Keynote Speaker – Dr. David Anderson**
Assistant Professor
Orthopedic Surgery
University of Kansas Medical Center
Kansas City, Kansas

11:15 PM – 12:00 PM **Lunch**

Co-Chair and Moderator

Dr. David Eick

Afternoon Session:

12:00 PM – 12:30 PM **Dr. Suhel Kotwal**
Orthopedic Surgery
Truman Medical Center Hospital Hill
Orthopaedic Surgery Department
Kansas City, Missouri

Bone Cement Symposium
Ewing Marion Kauffman Foundation
4801 Rockhill Road
Saturday, October 4, 2014
PROGRAM

12:30 - 12:45	**Dr. Donna Pacicca** Orthopedic Surgery, Children's Mercy Hospital Associate Professor of Orthopedic Surgery University of Missouri-Kansas City, School of Medicine Adjunct Professor University of Missouri-Kansas City Department of Oral and Craniofacial Sciences, School of Dentistry Kansas City, Missouri
12:45 - 1:00	**Dr. Jonathan R. Dubin** Orthopedic Surgery Truman Medical Center Hospital Hill Assistant Professor Department of Orthopedics University of Missouri-Kansas City, School of Medicine Kansas City, Missouri
1:00 PM – 1:30 PM	**Dr. Kathleen Kilway** Chair and Professor of Chemistry Vice-Chair of Faculty Senate University of Missouri-Kansas City
1:30 PM – 2:00 PM	**Dr. Lynda Bonewald** Vice Chancellor for Translational and Clinical Research Curator's Professor Lee M and William Lefkowitz Professor Director, UMKC Center for the Study of Dental and Musculoskeletal Tissues Director, Mineralized Tissue Research Program School of Dentistry Department of Oral and Craniofacial Sciences University of Missouri-Kansas City

Bone Cement Symposium
Ewing Marion Kauffman Foundation
4801 Rockhill Road
Saturday, October 4, 2014
PROGRAM

2:00 PM – 2:45 PM	**Round Table Discussion**	
	Dr. Lynda Bonewald	**Dr. Kathleen Kilway**
2:45 PM – 3:00 PM	**Conclusion**	
	Dr. Lynda Bonewald	**Dr. Kathleen Kilway**

SYMPOSIUM FACULTY BIOGRAPHIES

Dr. L.D. Timmie Topoleski (Tim), has been a faculty member at UMBC (University of Maryland, Baltimore County) since 1990.

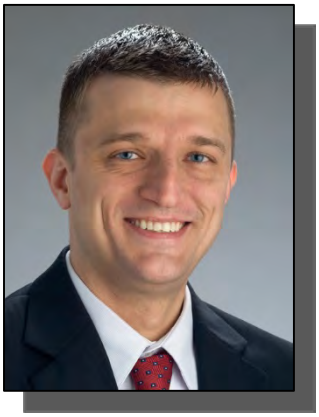
He is currently a Professor in the Department of Mechanical Engineering, and the Director of the UMBC Laboratory for Implantable Materials and Biomechanics.

Dr. Topoleski received his B.S. in the College Scholars Program at Cornell University, and also received his M. Eng. and M.S., both in Mechanical Engineering, at Cornell.

He earned his Ph.D. in Bioengineering from the University of Pennsylvania. At UMBC, Dr. Topoleski teaches classes in solid mechanics and materials, as well as in biomechanics and biomaterials.

He was named the UMBC Presidential Teaching Professor for 2008-2011. His research focusses include failure mechanisms of implantable materials, and structure/function relationships in cardiovascular tissue.

Dr. David W. Anderson is an Assistant Professor in the Department of Orthopedic Surgery and Sports Medicine at the University of Kansas Medical Center. His area of specialty is Adult Reconstruction.



Dr. Anderson received his undergraduate degree in Biology from Creighton University. He went on to get his Master of Science in Cellular and Molecular Biology at the University of Missouri-Kansas City.

He did his residency at the University of Kansas in the Orthopedic Surgery Department.

In 2010 Dr. Anderson received the distinguished honor of winning The Betty and Federico Adler Resident Award for Outstanding Orthopedic Research. Dr. Anderson then did a one year fellowship with the Harvard Combined Orthopaedic Program- Adult Reconstruction at Massachusetts General Hospital.

SYMPOSIUM FACULTY BIOGRAPHIES

(continued)

Dr. Suhel Kotwal, Specialties Musculoskeletal Oncology; Pediatric Joint Reconstructive Surgery; Spine - Scoliosis Surgery.

Dr. Kotwal received his medical education at the University of Bombay, Mumbai, India, Residency, Orthopaedic Surgery, University of Bombay, Fellowship, Adult Reconstructive Surgery, University of Chicago Hospitals, Spine-Scoliosis Surgery, Hospital for Special Surgery, Musculoskeletal Oncology, University of Texas, and MD Anderson Cancer Center, Houston, Texas.

Dr. Donna Pacicca is an attending surgeon at Children's Mercy Hospital in the division of Orthopaedic Surgery, section of Sports Medicine. She is also associate professor of Orthopaedic Surgery at UMKC School of Medicine and adjunct professor of Oral and Craniofacial Sciences at UMKC School of Dentistry.

She received her A.B. in biochemistry from Columbia University and her M.D. from New York University School of Medicine. She then went on to complete a residency in orthopaedic surgery at Albert Einstein/Montefiore Medical Center, and a fellowship in pediatric orthopaedic surgery at Brown University/Hasbro Children's Hospital under Dr. Michael Ehrlich. Prior to joining Children's Mercy, she was an assistant professor at Boston University in Orthopaedic Surgery and Pediatrics. Her clinical interests include sports medicine, pediatric fractures and reconstruction.

SYMPOSIUM FACULTY BIOGRAPHIES

(continued)

Dr. Jonathan R. Dubin specialties include Orthopedic Surgery.

Dr. Dubin is on the medical staff at Truman Medical Center Hospital Hill, Orthopaedic Surgery Department.

Dr. Dubin received his medical education and training from the University of Kansas, School of Medicine, Residency University of Illinois at Chicago, and San Francisco Orthopaedic Residency Program, and Fellowship, University of Minnesota.

Kathleen V. Kilway is a Curators' Teaching Professor and the Chair of the Department of Chemistry at the University of Missouri – Kansas City. She earned her Bachelor of Science in Chemistry from St. Mary's College, and her Master's and Ph.D. in Chemistry from the University of California in San Diego. She has been on the faculty of UMKC since 1996.

Her areas of research are conformational analysis, synthesis of twisted polycyclic aromatic compounds and substituted acenes, molecular assembly of organonitriles and silver salts, synthesis and studies of cyclopropanes, experimental studies of dental-relevant monomers, and development of biomaterals.

The research has been supported by the American Chemical Society Petroleum Research Fund, Department of Defense, National Institutes of Health (General Medicine and Dental and Craniofacial Research), Missouri Life Sciences Research Board, and University of Missouri Research Board; it has been presented at national and international venues.

For several years now, she has been the instructor for Organic Chemistry I and Organic Chemistry II lectures during the on-sequence semesters with as many as 290 students. She has received numerous honors and awards including Teacher of the Year by the School of Pharmacy (2006, 2007, 2010, and 2011), Dean's Outstanding Teaching Award (2000), and the University of Missouri President's Award for Outstanding Teaching (2008).

SYMPOSIUM FACULTY BIOGRAPHIES

(continued)

Dr. Lynda F. Bonewald is a University of Missouri, Kansas City (UMKC) Curators Professor, the Lefkowitz Professor of Oral and Craniofacial Sciences, and Director of the Mineralized Tissue/Bone Biology Research Program at the UMKC School of Dentistry.

She is also the Director of the UMKC Center of Excellence in the Study of Dental and Musculoskeletal Tissues and UMKC Vice Chancellor for Clinical and Translational Research. Dr. Bonewald was educated at the University of Texas, Austin, where she earned a B.A. in Biology and at the Medical University of South Carolina, Charleston, where she received a Ph.D. in Immunology/Microbiology.

She subsequently spent two years at the Veterans Administration Medical Center, Charleston, as a postdoctoral fellow. Prior to joining UMKC, Dr. Bonewald held positions at the University of Texas Health Science Center (UTHSC), San Antonio, with a primary appointment in the Department of Medicine and joint appointments in the departments of Biochemistry, and Cellular and Structural Biology. She is best known for her work in the study of osteocyte biology and function.

The organizers of the Bone Cement Symposium wish to express their gratitude to the following supporters, whose generosity in the form of an educational grant has made this meeting possible:

Department of Defense (DOD) - U.S. ARMY

Medical Research Acquisition Act (USAMRAA)

Grant # W81XWH-11-1-0805

Bone Cement Symposium Announcement

UMKC
UNIVERSITY OF MISSOURI
KANSAS CITY

BONE CEMENT SYMPOSIUM

Ewing Marion Kauffman Foundation
4801 Rockhill Road
Kansas City, Missouri

Saturday, October 4, 2014
8:00 am – 3:00 pm

Supported by Department of Defense (DOD) - U.S. ARMY Medical Research Acquisition Act (USAMRAA)
Grant # W81XWH-11-1-0805

Bone Cement Symposium Round Table Notes

Bone Cement Symposium – Round Table – Notes - October 4, 2014

Name	Discussion Point
Dr. Jonathan Dubin	- drill ability - use as support - Concern for infection - Need to be able to chisel taking it out
	- His interests lay more with the bead-antibiotic application, thus likes a more porous material. - Questions: 1) Can our material be drilled into, so as to use it as a support, but 2) if necessary, can it be removed from the bone canal w/o doing a lot of damage, for revisions?
Dr. Mark Bernhardt	- dealing with infection
	- Must be able to drill a hole through the material
	- Antibiotic nails
	- Fiber wire
	- MRI capability
	- Infection is a concern
	- New applications
	- Material properties consideration
	- He suggests continuing to investigate the material properties. - Questions: 1) Can we make an "antibiotic nail" for local administration in the battlefield; 2) Are 'we' planning to use a cement restrictor/plug, which is placed at the distal end of the cement; 3) Are we going to "pressurize" the cement, once it is in the canal, but before the rod is inserted? - He considers the swine to be a pivotal comparison to a human situation, recommends various monitoring of vitals during anesthesia/surgery if possible. I.e., heart monitor, echo - I'm not sure if I understood this correctly…pressurization could lead to embolism…I think the cement restrictor lessens this possibility??
Dr. Donna Pacicca	- Bad stuff
	- Materials application
	- Good for children
	- Broad area – several possibilities of application
	- Trauma
	- Spine
	- pediatrics
	- Pediatrics – off label – more looking at this - toxicity

	Spinal cord effect
	- Suggests to keep broad applications of the cement use in mind i.e., use in pediatrics, spine, around spinal cord, total joints, effects on growth plate?
Dr. Lynda Bonewald	- No toxic effect is the hope with the bone cement mixing to date
	- Swine study - expensive
	- There is a learning curve
	- Objective do not harm
Dr. David Anderson	- Pre molded spacers
	- shelf life
	- spacer
	- biomedical
	- curing time
	- dual therapy or triple therapy
	- cement /silorane
	- mold with silorane
	- Anderson's idea – pre mold last 2 -3 months
	- Interested in antibiotic application, and as pre-made/molded spacer, that could last 2-3 months in vivo; what is the shelf life? - Use as Dual antibiotic therapy: will the PMMA bind to a pre-made silorane spacer? - Fungal agents in pre-made porous spacers - Suggests gathering and reporting data numbers regarding bacterial & fungal bone infections to support our future applications -
Dr. Liang Chen	- Chemistry point of view
	- Kilway answered
	- Shelf life a concern
	- Methatricylite (not sure how to spell)
	- Had a chemistry question….I think Rachel understood this one. -
Drs. Bernhardt, Eick, Kilway, and Bonewald	- Made points on this question from Dr. Chen - Brown bottle (light) - Silorane different reaction - On the market – commercial toxic stuff - Our silorane is non toxic - Pigs – Pacicca/Bonewald practice on pig bones
	- Control with pigs - anesthesiologist Compare effects echocardiogram - Anderson talked about a land mark study on - echo chamber - Measure breathing

	- Heart rate - Probe going in vein - CT - MRI - Inflammation
KU Resident	- Drains
	- Staph infection study
Dr. Bonewald	- Pig and rat study - Blood samples - Not ready anti - Invitro first - MRSA - MRI Global – dog facility - Larry Suva – colleague at Arkansas – rabbit module - Collaborations with Arkansas - Rats resistant to infection - Pigs resistant to infection
Dr. David Eick	- Talking about difference Bio film – protein film wets surface of the cement
	- salvia
	- Bio film more bacteria generation
	- Dr. Bonewald and Kilway – no infection
Other comments	- Does a bacterial biofilm form on the silorane? - Did we see any bone infections in our in vivo experiments? No - Dealing with FDA: - - Dr. Tim Topoleski- Suggests we are going to need clinical data before considered for FDA approval. - - Dr. Dubin- Suggest looking into “humanitarian device exception/exemption”- if our product functions as limb sparing/saving. -
Other comments - Drs. Dubin, Topoleski, Anderson, Bonewald, Kilway, Bernhardt, Chen	- Dr. Dubin asked about the timeline, ready to use - Dr. Bonewald talked about the dependence on the FDA (next year) - The argument is the silorane is used in dental composites currently. - 501 - Dr. Tim Topoleski – stated clinical data and human trials will be required for FDA approval - Suggested talking to FDA - Dr. Dubin suggested give the numbers or statistics on how the silorane is a limb saving/life saving measure. Talk to Truman and KU about statistics, fungal infections using current practices, give real numbers. - Anti-fungal testing - FDA suggestions are great idea’s - Eradicate infection

	- Clinical application to cure and reduce bone infections. -
Interesting Talk Points	- Interesting Talk Points: - Dubin: use as spacer to fill dead space to prevent soft tissue ingrowth; Masqulet method- can we induce membrane growth around our cement (4-6wk), creating a 'pouch', which can later be filled with grafting material? - - Anderson: use of cement plug/restrictor, pulsatile lavage of the canal until you get clear fluid, pressurization of cement into the pores/trabeculae of bone; use of amikacin in spacers for mycobacterial infections; need fungal applications; prolonged elution > 2-3wks; consider revisional ease. - Kotwal: use of cement restrictor to decrease intramedullary pressure; keeping the product cool to increase the working time.

Fast Track Award

June 16, 2014

Lynda Bonewald
Curator's Professor
UMKC School of Dentistry
650 East 25th Street
Kansas City, MO 64108

RE: UM Intellectual Property Fast Track Initiative funding proposal entitled
Curing Bone Infection While Stabilizing Implants
Funded Period: 12 months; Project Start Date: 7/1/2014
Amount: $50,000
Award No: FastTrack-14002K

Dear Dr. Bonewald:

It is a pleasure to inform you that your UM Intellectual Property Fast Track Initiative FY14 funding proposal referenced above has been approved for funding.

Please note that you must meet all campus compliance requirements relative to administration of awards. **Acceptance of this award constitutes a commitment on your part to adhere to the following guidelines:**

1. Funds provided to you will reside in an Operating Fund.
2. Expenditure of the funds will require the use of a unique Program Code to allow the funds to be tracked for reporting.
3. You will provide a financial report no later than ninety (90) days after completion of the funded period. Any funds remaining at the end of your project must be returned to the Office of Academic Affairs.
4. You will provide a technical report within ninety (90) days after completion of the funded period.
5. You will provide an Award Impact Report (in the form attached hereto) one (1) year following completion of the Funded Period.

The above reports are to be sent to the attention of Ashley Wilson in the Office of Academic Affairs, with a copy to your campus Research Officer.

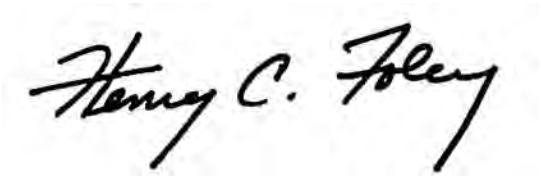
Approval of your project expresses confidence that your work will enhance the intellectual property position of the technology, improve its commercialization potential, and contribute to the university's economic development mission for the state of Missouri. If, for some reason, you are unable to accept this award, please notify me as soon as possible.

To accept this award, please ask your fiscal person to contact Ashley to discuss where the award money should be transferred to. The chartfield used to expend the funds must be the Operating Fund (fund 0000) and all expenditures must use a Program Code in order to facilitate reporting purposes. Ashley Wilson's contact information is as follows:

Ashley Wilson
Office of Academic Affairs
309 University Hall
Columbia, MO 65211-2015

Questions related to this award should be directed to Ashley Wilson. I look forward to learning the results of this project.

Sincerely,



Henry C. Foley, Ph.D.
Executive Vice President of Academic Affairs

University of Missouri
Intellectual Property Fast Track Initiative

Award Impact Report

Date:

Award Number:	FastTrack-14002K
Project Title:	Curing Bone Infection While Stabilizing Implants
Project End Date:	7/1/15
Award Amount:	

PI Name:	Lynda Bonewald
Title:	Curator's Professor
Campus:	Kansas City
Department:	
Address:	

As a condition of grant acceptance, you agreed to complete a report one year after termination of your project. Please complete this form and mail, or email, a copy to Ashley Wilson, at the below address, with a copy to your campus Research Officer.

Ashley Wilson
Office of Academic Affairs
309 University Hall
Columbia, MO 65211-2015
Ph: (573) 882-1714
Email: wilsonak@umsystem.edu

Fast Track Funding Impact

The following questions are meant to assess the direct impact of the UM Intellectual Property Fast Track Initiative funding on furthering the technology and moving it towards commercialization. If needed, use additional sheets.

A. Intellectual Property:

1. Discuss how the Fast Track funding impacted any pre-existing intellectual property or resulted in the creation of new intellectual property.

2. Discuss the activities you've had with the campus technology transfer office in relation to the intellectual property (i.e. disclosure of invention, filing of patent applications, etc.) and provide relevant details, such as disclosure number, patent application number, and filing dates.

B. Commercialization:

1. Discuss how the Fast Track funding assisted in efforts to commercialize the technology, or improved the potential for commercialization, as the case may be.

2. What activities have taken place to commercialize the technology (i.e. Have companies been contacted to discuss licensing and, if so, what has been the outcome? Has a startup company been created around the technology? Has an option or license agreement been entered into, or are negotiations underway?)

3. If a startup company has been created, provide the company name and creation date, and discuss any efforts made to obtain financing.

C. Additional Funding:

1. Discuss how the Fast Track funding improved the ability to obtain future funding for the technology.

2. Provide information on any grant proposals submitted that relate directly to this funded project. Provide the name of the agency, or industrial partner, and indicate whether the proposals were funded (F), not funded (NF), or pending (P).

3. If a proposal from question C.2. above was funded, provide the grant number and the amount of the award.

D. Publications: List, and provide details, of any peer reviewed papers, poster presentations, etc. that relate directly to the funded project.

E. Additional Comments: Provide any general comments you have regarding the UM Intellectual Property Fast Track Initiative funding program (i.e. benefits, ways to improve, etc.).