



■ BIOMATERIALS

Elution of rifampin and vancomycin from a weight-bearing silorane-based bone cement

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Aims

Poly(methyl methacrylate) (PMMA)-based bone cements are the industry standard in orthopaedics. PMMA cement has inherent disadvantages, which has led to the development and evaluation of a novel silorane-based biomaterial (SBB) for use as an orthopaedic cement. In this study we test both elution and mechanical properties of both PMMA and SBB, with and without antibiotic loading.

Methods

For each cement (PMMA or SBB), three formulations were prepared (rifampin-added, vancomycin-added, and control) and made into pellets (6 mm × 12 mm) for testing. Antibiotic elution into phosphate-buffered saline was measured over 14 days. Compressive strength and modulus of all cement pellets were tested over 14 days.

Results

The SBB cement was able to deliver rifampin over 14 days, while PMMA was unable to do so. SBB released more vancomycin overall than did PMMA. The mechanical properties of PMMA were significantly reduced upon rifampin incorporation, while there was no effect to the SBB cement. Vancomycin incorporation had no effect on the strength of either cement.

Conclusion

SBB was found to be superior in terms of rifampin and vancomycin elution. Additionally, the incorporation of these antibiotics into SBB did not reduce the strength of the resultant SBB cement composite whereas rifampin substantially attenuates the strength of PMMA. Thus, SBB emerges as a potential weight-bearing alternative to PMMA for the local delivery of antibiotics.

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Article focus

- Investigating the ability of PMMA and Silorane-based cements to successfully incorporate and elute orthopaedic-relevant antibiotics.
- Elucidating weight-bearing applicability of a novel SBB cement.

Key messages

- SBB cement is better able to release both rifampin and vancomycin than PMMA in vitro.
- PMMA loaded with rifampin shows both attenuated elution and compressive strength, while SBB is unaffected.

Strengths and limitations

- Our study was the first reported investigation of antimicrobial-laden Silorane-based biomaterial as a potential candidate for use as an orthopaedic cement where PMMA-incompatible antibiotics are not an option.
- All tests were performed in vitro, therefore elution and compressive strengths may vary in vivo.

Introduction

Poly(methyl methacrylate) (PMMA) is frequently used in conjunction with arthroplasty for implant anchoring and as

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Table 1. Experimental group compositions and additives.

Group name	Cement type	Antibiotic included	Antibiotic (w/w%)	Antibiotic (v/v%)	Pellet density (g/mm ³)
PMMA CON	PMMA	None	N/A	N/A	1.33 × 10 ⁻³
PMMA RIF	PMMA	Rifampin	2.43	3.16	1.30 × 10 ⁻³
PMMA VANC	PMMA	Vancomycin	2.43	3.16	1.30 × 10 ⁻³
SIL CON	SBB	None	N/A	N/A	1.70 × 10 ⁻³
SIL RIF	SBB	Rifampin	1.89	3.14	1.66 × 10 ⁻³
SIL VANC	SBB	Vancomycin	1.89	3.14	1.66 × 10 ⁻³

CON, control; N/A, not available; PMMA, poly(methyl methacrylate); RIF, rifampin; SIL, silorane; VANC, vancomycin.

antibiotic-loaded cement (ALBC) spacers for use in the treatment of periprosthetic joint infection (PJI).¹ However, PMMA bone cements and spacers offer prime surfaces for bacterial attachment and biofilm formation. Biofilm formation has been implicated as a primary contributor to antimicrobial resistance and increased difficulty in treating many orthopaedic-related infections.²⁻⁶ Antibiotics are often prophylactically and therapeutically added to bone cement to help combat and prevent infections such as PJI. Antibiotic elution from ALBC provides a high local dose of drug required to eradicate some infections which cannot otherwise be cleared by systemically administered antibiotics alone.⁶⁻⁸

Rifampin is an antibiotic widely used systemically as part of many orthopaedic infection treatment regimens and has been shown to be superior to other antibiotics in eradicating biofilms.^{4,9} Despite the potential advantages of delivering rifampin locally via PMMA, researchers and surgeons have noted an incompatibility between the two materials.^{10,11} A recent study found that rifampin acts as a radical scavenger during PMMA's radical polymerization.¹² The hydroquinone moiety behaves as a radical trap and inhibits cement polymerization, resulting in the cement having a prolonged curing time, reduced mechanical strength, and increased release of unreacted MMA monomer.

Although initially developed for use in dentistry, silorane-based cements have been recognized for their potential applications in orthopaedics.^{13,14} We have developed a silorane-based biomaterial (SBB) intended for use as an orthopaedic cement, suitable for weight-bearing applications, local delivery of antibiotics, grafting for bone lesions, voids or defects, and augmentation of various implants.¹⁵ Unlike the silorane-based dental cements, which require ultraviolet (UV) light for polymerization, this SBB uses a dual-cure initiation system composed of both a light photoinitiation and chemical catalysts. The latter is Lamoreaux's catalyst (LMC), which allows the formulation to polymerize in ambient light. SBB has the following properties: a non-exothermic polymerization without the use of UV light, contains a radiopaque filler, and is composed of non-toxic monomers already Food and Drug Administration (FDA)-approved for oral applications.¹³ Moreover, these silorane-based resins have the potential to enhance bone cell mineralization and exhibit significantly less shrinkage than PMMA upon curing.^{16,17}

While PMMA bone cements remain the 'go-to' weight-bearing local drug delivery vehicle in orthopaedics, their inherent properties make them ill-suited to deliver a variety of desired drugs. Our novel weight-bearing, silorane-based cement is a potential alternative for delivery of these PMMA-sensitive drugs. This study tested the ability of the silorane-based cement to incorporate and deliver antibiotics over the course of 14 days. Specifically, we focused on the incorporation of rifampin, which has been shown to be incompatible in PMMA-based cements, while using vancomycin as a control. We hypothesized that the incorporation of either rifampin or vancomycin into SBB would not alter SBB's mechanical properties compared to non-loaded controls. Furthermore, we hypothesized that the amount of these antibiotics eluted from SBB would be greater and more enduring than that from PMMA.

Methods

SBB preparation. The silorane-based material used in this study is an epoxy primarily made of CYGEP and PHEPSI monomers combined with fillers and initiators. CYGEP and PHEPSI were prepared as previously described.¹⁶ Proton nuclear magnetic resonance (¹H NMR) spectroscopy was used to ensure that the monomer purity was > 95.8%. The monomers, CYGEP and PHEPSI, were combined in a 1:1 ratio by weight and mixed for 15 minutes. This combination (SilMix) was then dried as described by Ranaweera et al.¹⁸ A photoinitiation system composed of *p*-(octyloxyphenyl)phenyliodonium hexafluoroantimonate (1.19 wt%), camphorquinone (0.40 wt%), and ethyl *p*-dimethylaminobenzoate (0.06 wt%) was combined with dried SilMix and mixed for 20 minutes. A DY5-1TOSU modified filler, comprised mainly of SiO₃, was then added to the light cured SilMix (LCSM) material and mixed for an additional 20 minutes. At the time of use, polymerization was initiated by adding Lamoreaux's catalyst (LMC) to the SilMix to form the complete SBB mixture, thus avoiding reliance on light initiation and making the SBB suitable for use in orthopaedics. No UV light exposure was required.

PMMA, additives, and experimental groups. Depuy SmartSet MV (Depuy, USA) bone cement was used for all PMMA groups, and both medical grade rifampin and vancomycin were obtained from Fagron Inc, through Sigma-Aldrich (USA). Six cement formulations were prepared

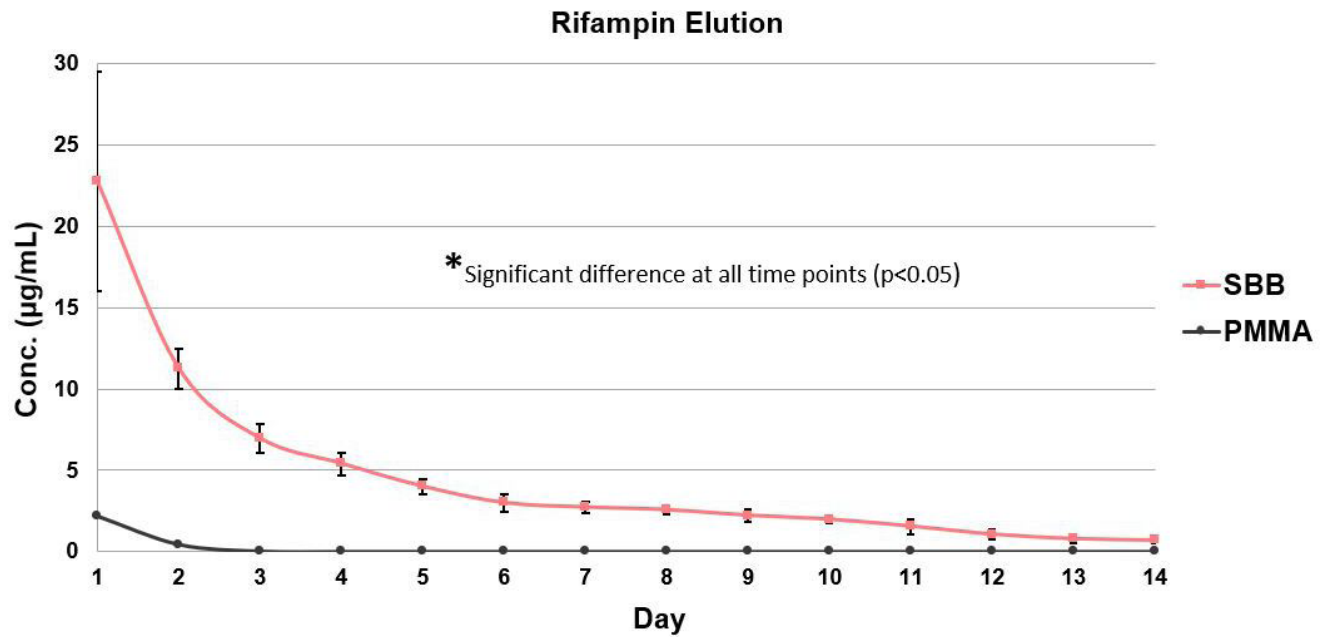


Fig. 1

Daily mean elution concentrations of rifampin from both silorane-based biomaterial (SBB) and poly(methyl methacrylate) (PMMA). Error bars indicate \pm one standard deviation from the mean ($n = 10$). A significant difference was detected between the groups at every timepoint ($p < 0.05$).

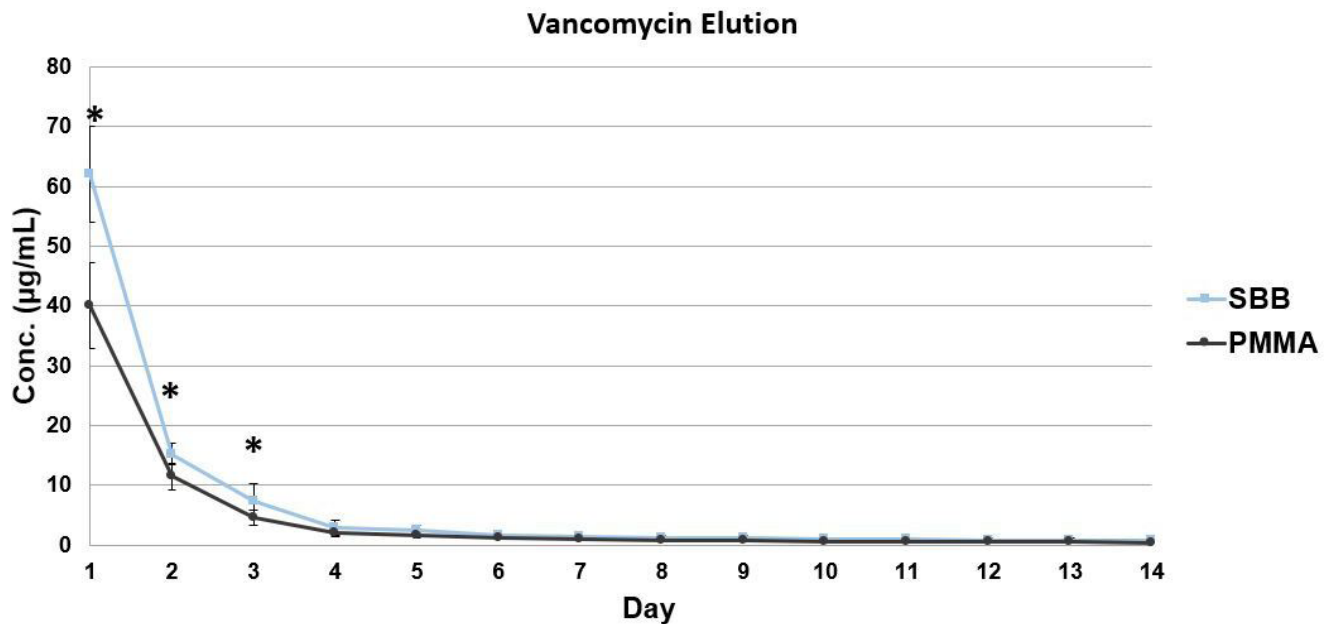


Fig. 2

Daily mean elution concentrations of vancomycin from both silorane-based biomaterial (SBB) and poly(methyl methacrylate) (PMMA). Error bars indicate \pm one standard deviation from the mean ($n = 10$). An asterisk denotes a significant difference between groups ($p < 0.05$).

for this study (Table I), with the dosing of the antibiotic incorporated groups representing a low prophylactic dose commonly added to PMMA (1 g antibiotic/40 g dry component cement kit). Three PMMA groups were formulated: PMMA control (PMMA CON), PMMA with rifampin (PMMA RIF), and PMMA with vancomycin (PMMA VANC). Three silorane groups were prepared: SBB control

(SIL CON), SBB rifampin (SIL RIF), and SBB vancomycin (SIL VANC). Antibiotic incorporation was adjusted to be equivalent by volume as seen in Table I. This meant that the weight of antibiotic added to each type of cement varied due to PMMA and SBB having different densities. All groups were formed from a single mixing event to avoid batch-to-batch variability.

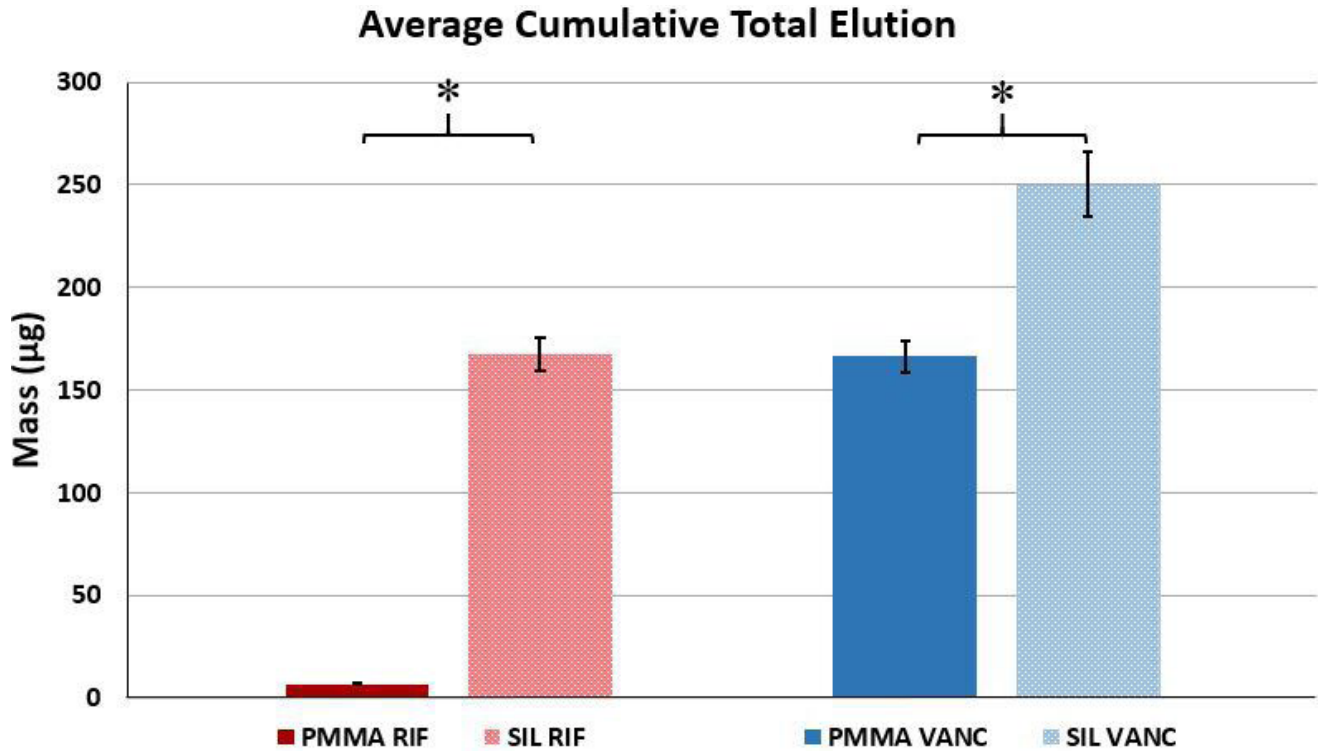


Fig. 3

Cumulative antibiotic elution mass for all groups by pellet, averaged over 14 days. An asterisk indicates a significant difference between groups ($p < 0.001$; $n = 10$). Error bars indicate \pm one standard deviation from the mean. PMMA, poly(methyl methacrylate); RIF, rifampin; SIL, silorane; VANC, vancomycin.

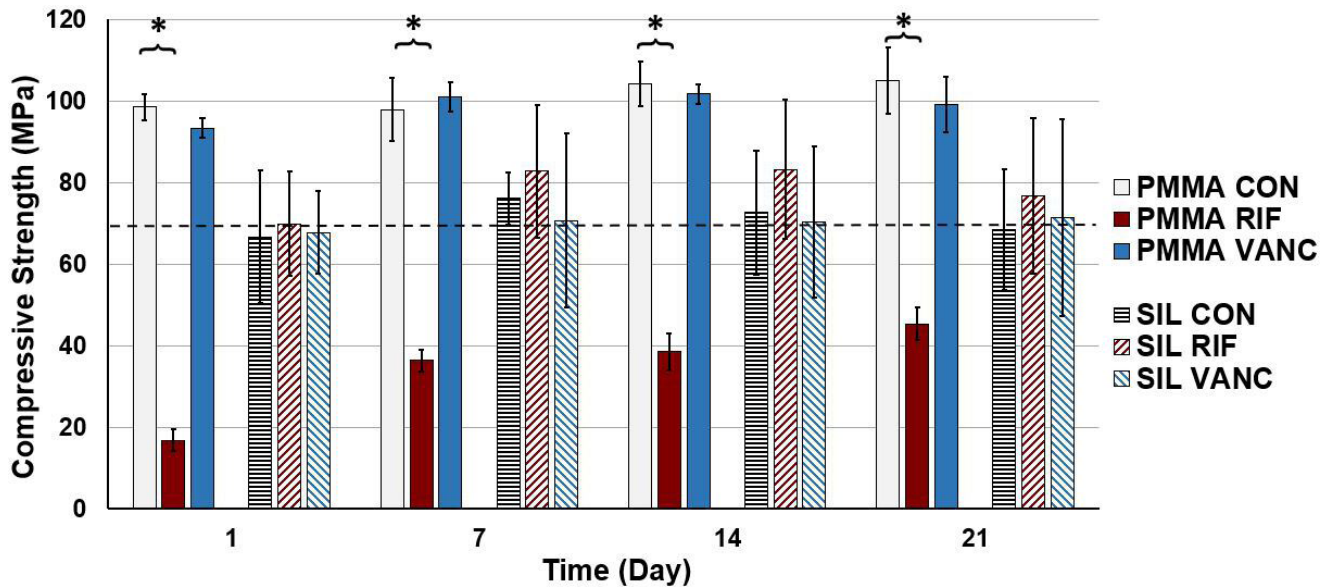


Fig. 4

Compressive strengths of all cement composites over the course of 21 days. Dashed line signifies 70 MPa threshold as defined by the International Organization for Standardization (ISO).²¹ Asterisk (*) denotes significant difference between groups ($p < 0.05$). Error bars indicate \pm one standard deviation from the mean ($n = 10$). CON, control; PMMA, polymethyl methacrylate; RIF, rifampin; SIL, silorane; VANC, vancomycin.

Cement pellet preparation. PMMA groups were prepared by mixing the dry components (40 g) and the respective antibiotic in a DePuy surgical mixing bowl until visual homogeneity was observed. Afterwards, the liquid

component was added to begin polymerization, and the cement was mixed at 1 Hz for 1.5 minutes. The SBB groups were formulated by hand-mixing the respective antibiotic with the unpolymerized SBB using lab grade

polyethylene bowls until it appeared uniform upon visual inspection. Polymerization was then initiated by adding LMC dropwise via a syringe and hand-mixing for approximately 45 seconds. The final composition of antibiotic-incorporated SBB was LCSM (33.94%), LMC (0.40%), DY5-1TOSU filler (63.77%), and antibiotic (1.89%).

Once the cements began curing, they were placed into custom polytetrafluoroethylene (PTFE) moulds and allowed to cure for one hour at 37°C. The moulds produced cylindrical specimens with dimensions of 6 mm × 12 mm, as per ASTM F451.¹⁹ All cement pellets were removed from the moulds and inspected for significant defects. Defective samples were discarded, and the remainder were massed to assess density.

Elution testing. Ten specimens from each antibiotic-containing group were placed separately into 15 ml polypropylene tubes to which a 2.5 ml aliquot of phosphate-buffered saline (PBS, pH = 7.4; Ricca Chemical, USA) was added, allowing enough volume to cover the entirety of the pellets. All tubes were stored in an incubator at 37°C. For all cement sample groups, the antibiotic-PBS eluent was collected every 24 hours for 14 days. The residual PBS solution remaining in the tubes was discarded and replenished with a fresh aliquot of PBS (2.5 ml). All elution analyses were performed using a Shimadzu 10 series HPLC (Shimadzu, Japan) with a Hypersil GOLD C18 column (5 µm, 250 mm × 4.6 mm; Thermo Fisher Scientific, USA). The rifampin concentration of each eluate was elucidated via isocratic elution with a mobile phase consisting of acetonitrile—methanol—75 mM monobasic sodium phosphate—1 M citric acid (composition ratio, 30/28/38/4, v/v/v/v%). The rifampin detection wavelength was set at 254 nm. The vancomycin concentration of each eluate was elucidated via isocratic elution with a mobile phase consisting of HPLC-grade water—acetonitrile—100 mM ammonium acetate (composition ratio, 78/12/10 v/v/v%), and adjusted to a pH of 5.4 with glacial acetic acid (Thermo Fisher Scientific).²⁰ The vancomycin detection wavelength was set to 214 nm. Both the daily elution concentrations and total cumulative release of these antibiotics from both cements were recorded.

Compression testing. The compressive properties of samples from each group were determined using an MTS Mini Bionix 858 loadframe with MTS Series 793 Control Software (MTS Systems Corporation, USA). Ten dry samples from each group were tested 24 hours post-curing, designated as “Day 1”. After this, ten samples from each group were tested at 7, 14, and 21 days after soaking in PBS (2.5 ml) at 37°C with daily fluid change out. Compression testing was performed at a loading rate of 20 mm/minute to either 40% strain or fracture, whichever occurred first. Data were collected at 20 Hz. Fracture was considered to have occurred when the load fell to below 90% of its peak value. The compressive strength of the pellets was defined as the failure load divided by the cross-sectional area of the pellets. The failure load was taken as either the load at the 2.0% offset from the elastic

section of the stress-strain curve, the ultimate yield load, or the load at fracture, whichever occurred first.¹⁹ Young’s modulus was defined as the slope of the elastic section of the material’s stress-strain curve.

Statistical analysis. A sample size of ten was used for all elution and strength tests per timepoint. All data were visually inspected for normality using a normal Q-Q plot. Paired *t*-tests were employed to compare both the daily and cumulative mean elution concentrations between the PMMA and SBB cements. To determine the mechanical strength deviation of antibiotic-laden cements from their respective controls, a single-factor analysis of variance (ANOVA) was used. All statistical calculations were performed using SPSS software (SPSS, USA).

Results

Antibiotic elution. The results of the rifampin elution from both PMMA and SBB cements are depicted in Figure 1. SIL RIF released significantly higher concentrations of rifampin than PMMA RIF over the course of 14 days ($p < 0.001$). The largest difference between the two cements was observed on Day 1 where the SIL RIF elution was 959% greater than that from PMMA RIF ($p < 0.001$). Furthermore, PMMA RIF did not release any detectable amounts of rifampin after Day 2. The daily elution concentration of vancomycin from both PMMA VANC and SIL VANC cements are depicted in Figure 2. SIL VANC samples released higher concentrations of vancomycin than PMMA VANC through Day 3 ($p = 0.009$). Specifically, on Day 1, a 55.1% increase of vancomycin elution was observed for SIL VANC ($p < 0.001$). The 14-day cumulative mean of eluent mass for each antibiotic-loaded cement composite is displayed in Figure 3. The cumulative mean mass was calculated as the total concentration mean of the elution multiplied by elution volume over the 14 days per group. SBB samples eluted 2,450% more rifampin ($p < 0.001$) and 50.6% more vancomycin ($p < 0.001$) than PMMA samples in terms of cumulative antibiotic mass.

Compressive strength. The mechanical strength and moduli of all cement composite groups are displayed in Figures 4 and 5, respectively. When comparing the effects of antibiotic incorporation into PMMA, we found that rifampin significantly reduces both the compressive strength and modulus of the resulting composite ($p < 0.001$). In contrast, the incorporation of rifampin into SBB had no significant effect on either the compressive strength or modulus of the resultant composite ($p < 0.05$). There was no difference between either the compressive strengths or moduli of PMMA VANC and PMMA CON ($p < 0.05$). For all the vancomycin-containing specimens, no effect on the compressive strength was observed ($p = 0.782$). However, the compressive modulus of the SIL VANC group was found to be significantly different from the SBB control ($p = 0.006$).

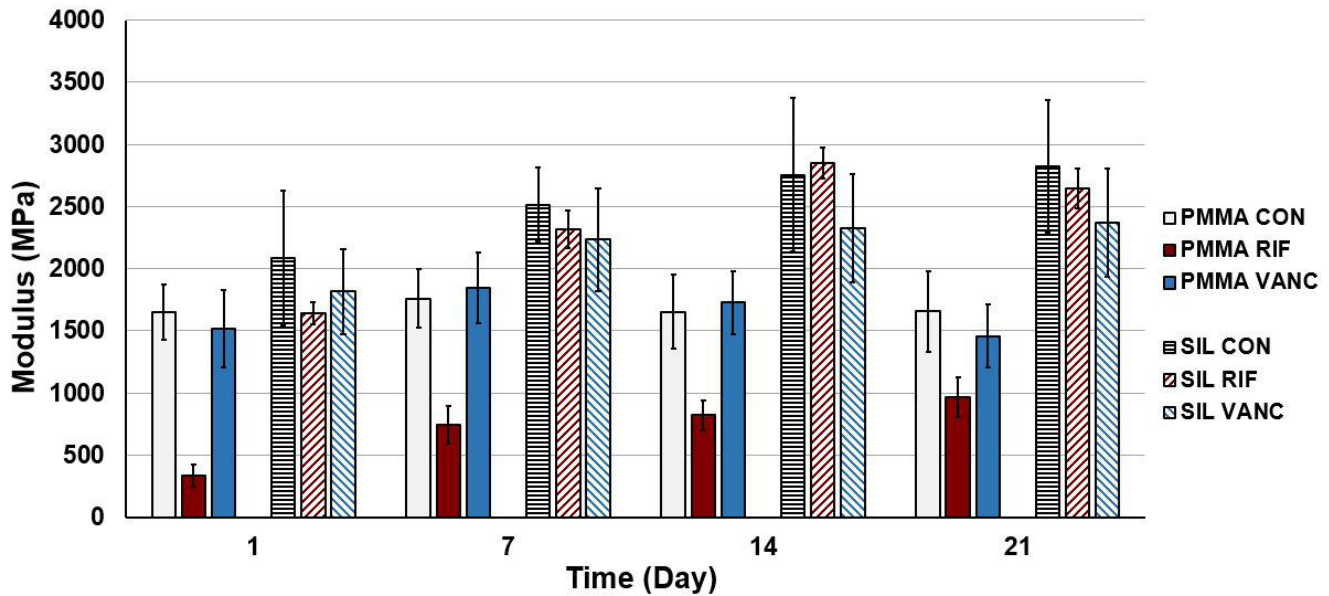


Fig. 5

Moduli of all cement composites over the course of 21 days. Error bars indicate \pm one standard deviation from the mean ($n = 10$). CON, control; PMMA, polymethyl methacrylate; RIF, rifampin; SIL, silorane; VANC, vancomycin.

Discussion

While PMMA bone cements currently remain the weight-bearing standard in orthopaedics, these materials are far from ideal. Specific deleterious properties of PMMA have been implicated as factors for aseptic loosening and failure of arthroplasty surgeries. Thermal-induced osteonecrosis at the cement-bone interface secondary to exothermic PMMA polymerization may weaken implant fixation and lead to loosening.^{21,22} In addition, several authors have reported that a thick fibrous membrane is formed at the PMMA cement-bone interface due to the release of toxic methyl methacrylate (MMA) monomers generating an inflammatory response and osteolysis.^{7,21,23,24} MMA toxicity has been shown to result in monomer-mediated bone damage and induce apoptosis in osteoblasts.^{22,25} While most of the MMA monomer is consumed during curing, there is the potential for large quantities of MMA to be released when polymerization is inhibited. This phenomenon occurs with rifampin-laden PMMA cement. In fact, the incorporation of rifampin into PMMA cement leads to a 500% increase in leaching of MMA over 24 hours, potentially further exacerbating these effects.¹² Due to the aforementioned limitations, alternatives to PMMA, such as calcium-based ceramics, have been evaluated for use in spine and other orthopaedic applications. While these alternative cements avoid the cytotoxicity associated with PMMA and offer other benefits such as bioabsorbability and osteoconductive properties, they are associated with other disadvantages including decreased biomechanical strength, poor handling characteristics, and prolonged curing times. These drawbacks have limited their routine use,^{26–28} therefore the

need remains for a less toxic yet mechanically comparable biomaterial than PMMA.

The current study is not without several limitations. In our study, specimens were soaked in PBS for determination of elution properties and changes in mechanical strength over time. It should be noted that a simulated body fluid may yield elution results that better predict actual elution rates in vivo. Secondly, PMMA groups were mixed with a commercially available mixing system, whereas SBB groups were hand-mixed as no system was available. This difference may result in slight variation in antibiotic elution between the two materials. Furthermore, the mechanical strength and modulus were only determined by axial loading. The effect of antibiotic incorporation may have seen greater effects in other modes of testing. Future testing should be expanded to include additional loading methods such as three-point bending and torsion.

While vancomycin has been utilized for local delivery from PMMA for years, rifampin local delivery remains largely unassessed. Our first objective was to assess the release of these antibiotics from SBB as compared to PMMA. In order to evaluate this claim, vancomycin and rifampin were incorporated into both SBB and PMMA. The former showed superior elution of both vancomycin and rifampin, both in terms of daily concentrations and cumulative total elution. Conceivably, the most important result is the ability of SBB to deliver rifampin continuously over 14 days, whereas PMMA was shown to be incapable of doing so. The inability of a low-dose rifampin-incorporated PMMA cement to produce sustained elution has been remarked upon previously by other researchers. Han et al²⁹ found that PMMA loaded

with low doses (up to 2 g) of rifampin was only able to elute detectable amounts of rifampin during the first 24 hours. The mechanism for rifampin inhibition of PMMA polymerization has been proposed, but the mechanism leading to attenuated elution is not yet defined.¹² We have previously speculated that rifampin may become chemically bound to polymerizing PMMA during its radical scavenging process, leading to low elution. Therefore, much of the incorporated drug would be bound to the PMMA matrix and unable to escape. Further investigation will be required to discern this mechanism and to further verify the safety and activity of the rifampin and byproducts delivered after incorporation into PMMA and SBB cements.

Regarding the mechanical properties of the cement composites over time, only a small deviation was seen in SBB samples upon antibiotic incorporation. This could be explained by the relatively low 'prophylactic' dose of antibiotics incorporated or chemical inertness between them and the SBB cement itself. On the other hand, rifampin incorporation into PMMA drastically reduced both the compressive strength and modulus of the cement. In fact, the reduction of the compressive strength in PMMA from rifampin incorporation was well below the ISO 5833 standard.³⁰ This result suggests that the use of rifampin-loaded PMMA in weight-bearing applications is contraindicated.

This study demonstrates the potential for a novel silorane-based cement to act as a carrier for local antibiotic delivery, specifically when a PMMA-incompatible antibiotic, such as rifampin, is desired. In this regard, we have found SBB to be superior to PMMA in terms of rifampin and vancomycin elution. Furthermore, this study demonstrated that the incorporation of these antibiotics into SBB did not significantly alter the compressive strength of the cement. The ability to locally deliver rifampin from a mechanically sound bone cement provides a potential breakthrough in preventing and treating orthopaedic infections, particularly when used in conjunction with a highly invasive joint arthroplasty or revision surgery.^{2,11} Silorane-based cements loaded with rifampin and vancomycin, or an appropriate alternative for co-delivery, present an excellent option in the treatment of PJI and as prophylactic antibiotic-loaded cement in primary and revision arthroplasty. Given that rifampin is highly effective against bacterial biofilms, SBB's compatibility with rifampin may prove valuable in local microbial control. Rifampin is generally used adjunctively along with another suitable antibiotic due to its tendency to induce antimicrobial resistance. Local rifampin co-delivery could circumvent the number of cases requiring sustained high concentrations of systemically administered antibiotics which is routine upon biofilm detection in patients. In addition to the previously demonstrated advantages of this silorane-based biomaterial's low toxicity, immunogenic profiles, and favourable biocompatibility, its use in arthroplasty may result in positive patient outcomes with

a potential reduction in adverse effects related to cement-tissue interactions implicated with current PMMA-based cements.⁷ In summary, we have shown SBB to be superior to PMMA as a delivery vehicle for both rifampin and vancomycin.

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- G. A. Funk: Conceptualized and designed the study, Collected and analyzed the data, Drafted and revised the article.
- E. M. Menuy: Synthesized the Silorane-Based Biomaterial, Collected the data, Revised the article.
- W. P. Ensminger: Collected the data, Revised the article.
- K. V. Kilway: Developed the Silorane-Based Biomaterial, Conceptualized and designed the study, Analysed and interpreted the data, Revised the article.
- T. E. McIff: Conceptualized and designed the study, Analysed and interpreted the data, Revised the article, Provided final approval.

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