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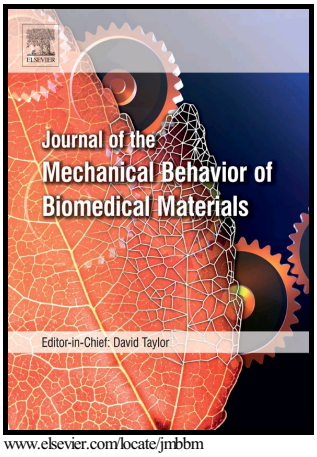


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# Vancomycin elution, activity and impact on mechanical properties when added to orthopedic bone cement

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**Abstract**

Infection incidence for total hip and knee arthroplasty (THA and TKA, respectively) is between 0.2 and 5% and results in approximately 100,000 device failures per year in the United States. Treatment requires prolonged systemic antibiotic therapy with additional surgical revisions. As a prophylactic measure against infection, antibiotics can be incorporated into bone cement during THA and TKA to provide drug administration at the implant site. Antibiotics in bone cement are only effective if they can elute out of the cement at a concentration that is active against common organisms. There is evidence that added antibiotics may affect the cement's mechanical properties, especially at higher dosages.

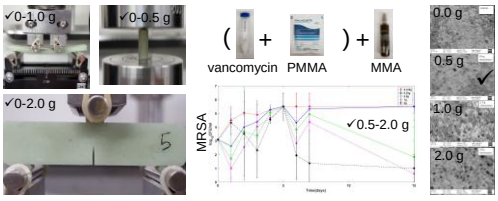
The purposes of this investigation were to (i) determine the mechanical properties of a commercially available bone cement with the addition of vancomycin, (ii) determine the release characteristics of vancomycin added to bone cement, and (iii) evaluate eluted vancomycin efficacy at eliminating some of the most common causative orthopedic implant pathogens.

Palacos bone cement was impregnated with incrementally larger clinically relevant weight percentages of vancomycin. Vancomycin is a treatment standard for invasive gram-positive infections, and Palacos cement is one of the most commonly used bone cements. After 21 days of curing in PBS, added masses of vancomycin greater than 0.5 g per 40.0 g cement packet decreased the cement's compressive yield strength to below ISO standard. The addition of vancomycin reduced the bone cement's mechanical properties in compression more than in bending. Vancomycin eluted from Palacos with a steady rise in eluted volume up to 8 days, after which non-therapeutic elution concentrations were observed up to a 60-day end point. The eluted concentration from samples with greater than 0.25 g vancomycin per Palacos packet was sufficient to eliminate a $10^3$ colony forming unit per mL (CFU/mL) initial inoculum of *S. aureus*, including methicillin-resistant *S. aureus* (MRSA). However, none of the tested dosages were able to fully clear a $10^3$ CFU/mL initial inoculum of a known high biofilm producing strain of *S. epidermidis*.

When used for infection prophylaxis at the time of THA and TKA, the findings of this study do not support the addition of more than 0.5 g vancomycin to a 40 g packet of Palacos cement due to a reduction in compression yield strength below ISO standards. Vancomycin doses up to 0.5 g were shown to elute from the bone cement matrix and are effective at treating bacterial infections of $10^3$ CFU/mL in bacterial strains of *S. aureus*, but may have limited effect against high-biofilm producing strains including *S. epidermidis*.



Graphical abtract



## 1. Introduction

Total joint replacement is an effective and common procedure that continues to increase in prevalence (Fu et al., 2017; Maradit Kremers et al., 2015). However, infection rates for orthopedic implants range from 0.2% to 5%, resulting in a burden of 100,000 infections per year in the United States alone (Darouiche, 2004). Prosthetic infections with bacterial colonization are particularly problematic to treat due to the of the lack of early recognition of infection and propensity for biofilm formation on implant surfaces. Bacteria within biofilms adhere to the surface of the device and produce an external polysaccharide matrix (glycocalyx) structure (Parra-Ruiz et al., 2010; Van Praagh et al., 2011). Antimicrobial susceptibilities of bacteria within biofilms can be reduced by 10 to 1,000 fold compared to those in a non-adherent planktonic state (Ceri et al., 1999) . As a result, device failure following bacterial contamination with subsequent infection is common and requires prolonged systemic antibiotic therapy followed by device removal and surgical revisions.

The most common culprits of total hip and knee arthroplasty (THA and TKA, respectively[1]) infections include *Staphylococcus aureus* and *Staphylococcus epidermidis*, constituents of the normal skin flora (Fernandez-Hidalgo et al., 2006; Mah and O'Toole, 2001; Rupp and Archer, 1994). *Staphylococcus aureus* is now an endemic pathogen in most hospital settings and is responsible for one of the most common types of nosocomial disease and device-related infections (Brescó et al., 2017; Morgenstern et al., 2016; Rosenthal et al., 2006). Methicillin-resistant *S. aureus* (MRSA) account for nearly half of *S. aureus* infections, which limit potential treatment options (Klein et al., 2017). *S. epidermidis*, while not a virulent pathogen, can be equally antibiotic resistant and is attune to site colonization and vigorous biofilm formation (Brescó et al., 2017).

In addition to the administration of pre-operative systemic antibiotics, bone cement used at the time of THA and TKA can be impregnated with antibiotics to target these pathogens and provide local implant site drug administration while avoiding systemic antibiotic toxicity. To provide prophylactic efficacy, antibiotics added to bone cement must have proven antimicrobial activity against organisms commonly associated with THA and TKA infection and must elute from bone cement above organism minimal inhibitory concentration (MIC). There is evidence that antibiotics may affect the cement's mechanical properties, especially at higher dosages (Lautenschlager et al., 1976; Pelletier et al., 2009; Slane et al., 2014A, 2015; Slane et al., 2014B).



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*Abbreviations:* $a_c$ crack length in $K_{Ic}$ specimen; $a$ distance between inner and outer bend test supports; $b$ specimen thickness; $d$ diameter of compression specimen; $E_c$ compressive modulus; $E_f$ flexural modulus; $F_{max}$ maximum force; $F_Y$ force at 2% yield; $\Delta F/\Delta D$ slope of force-deflection data; $h$ height of compression and bend specimen; HPLC high performance liquid chromatography; $K_{Ic}$ mode I fracture toughness; $L$ distance between lower bend test supports; MIC minimal inhibitory concentration; MRSA Methicillin-resistant *S. aureus*; $P$ maximum load in $K_{Ic}$ test; PMMA poly(methyl methacrylate); THA total hip arthroplasty; TKA total knee arthroplasty; $S$ lower span length in $K_{Ic}$ test supports; $S_f$ flexural strength; $S_Y$ compressive yield strength; $W$ specimen width in $K_{Ic}$ specimen; $x$ location of measured flexion.

The relatively hydrophobic nature of bone cement limits the amount of antibiotic that can be released and typically only 10% of the total incorporated drug elutes from the cement. Therefore, large quantities of antibiotic, as much as 2.0 g per 40 g packet of poly(methyl methacrylate) (PMMA) polymer, in the case of active infections, can be used in bone cements in order to provide high local antibiotic concentrations. Previous work has shown poragens, such as xylitol, increase antibiotic elution (Josh A. Slane et al., 2014), but this has not been used in the clinical setting.

This study aimed to (i) determine the mechanical properties of a commercially available bone cement with the addition of vancomycin, (ii) determine the release characteristics of vancomycin impregnated in bone cement, and (iii) evaluate eluted vancomycin efficacy at eliminating some of the most common causative orthopedic implant pathogens.

## 2. Materials and Methods

### 2.1 Materials

Commercially available bone cement was purchased for all testing (Palacos® R, Heraeus Medical GmbH, Wehrheim, Germany). Each packet of Palacos® R cement contains a 40 g radiopaque polymer powder and a 20 mL ampule with liquid monomer. Upon mixing, a polymerization process occurs between the monomer and polymer powder. The radiopaque polymer consists of 33.8 g PMMA, 5.9 g zirconium dioxide, 0.3 g hydrous benzoyl peroxide, and trace amounts of chlorophyll VIII as a green colorant. The liquid monomer contains 18.4 g methyl methacrylate and 0.4 g N,N-dimethyl-p-toluidine as well as trace chlorophyll VIII and hydroquinone. Palacos was used because it is one of the two most commonly used cements, along with Simplex®. Palacos is one of the most commonly used bone cements for THA and TKA in the United States and Europe (Kärrholm et al., 2016; Spierings, 2007).

Analytical grade vancomycin was purchased from Sigma-Aldrich (St. Louis, MO, USA). Vancomycin was chosen for study as it is a current standard treatment for methicillin-resistant *S. aureus* and *S. epidermidis* commonly found in orthopedic infections (Bistolfi et al., 2011).

### 2.2 Sample Preparation

All cement packets were stored at 22°C ± 1°C prior to mixing. Cement mixing was performed using the Zimmer Compact Vacuum Cement Mixing System® with vacuum pressure of -50 – 100mbar. All other mixing techniques were performed as previously described (Slane et al., 2015). There were six different experimental groups: Control (Palacos® R Cement), and five antibiotic loaded groups with 0.125 g, 0.25 g, 0.50 g, 1.0 g, and 2.0 g of vancomycin powder added to the polymer powder before mixing with the liquid monomer. Four different test samples were prepared in open molds: drug elution disks (6 mm diameter x 4.5 mm height), fracture toughness beams (44 mm x 10 mm x 5 mm with crack length between 4.5 mm and 5.5 mm), compression cylinders (6 mm diameter x 12 mm height), and four-point bending samples (75 mm x 10 mm x 3.3 mm). All dimensional tolerances were ±0.1 mm. Cracks for fracture samples were created using a wet-cutting method with a Buehler® IsoMet™ (Lake Bluff, IL, USA) low speed saw resulting in a crack width of 0.37 mm.

Samples for mechanical testing were handled and analyzed separately from those used in antibiotic activity or elution testing. Drug elution cements were stored in -20°C freezer until use for drug elution or activity tests while all mechanical testing cements were wet cured in a 1x PBS for 21 days at

22°C ± 1°C before testing (Slane et al., 2014). All subsequent mechanical testing was performed in open air at room temperature.

**2.3 Mechanical testing**

Quasi-static mechanical and fracture toughness testing were performed using an electromechanical materials testing frame (Criterion C43.104, MTS Systems, Eden Prairie, MN) with force and displacement data recorded at 100 Hz. Compression and four-point flexural testing were conducted in accordance with the International Organization for Standardization (ISO, Geneva) ("ISO 5833:2002 Implants for surgery - Acrylic resin cements," 2002) with the only modification being the addition of the wet-curing process. Fracture testing and fracture toughness calculations were performed as previously described (Slane et al., 2014A). The only change was a 32 lower span mm from 40 mm in the previous study for fracture testing to support the sample during testing better. Displacement rate was 5 mm/min for four-point bending and compression test and 10 mm/min for fracture toughness tests. A minimum of six samples per group were used for all four-point bending and fracture tests, and a minimum of ten samples per group were used for compression testing.

Compressive yield strength, $S_Y$ in MPa, was calculated using the 2% offset method, as described in ISO 5833. The compressive modulus, $E_c$ in MPa, was determined from taking as a slope from a linear range in which the stress increases in proportion to the strain. The force and displacement data from the compression testing and Equations, 1 and 2, respectively:

$$S_Y = \frac{4\,F_Y}{\pi d^2} \quad (1)$$

$$E_c = \left(\frac{4h}{\pi d^2}\right)\left(\frac{\Delta F}{\Delta D}\right) \quad (2)$$

In equation 1, $F_Y$ (in N) is the applied load at yield, $d$ (in mm) is the diameter of the compression test cylinder. For equation 2, $h$ (in mm) is the height of the compression test cylinder, and $\Delta F/\Delta D$ (in N/mm) is the slope of the linear region of the force displacement data.

Flexural strength, $S_f$ in MPa, and bending modulus, $E_f$ in MPa, were calculated from the maximum force (in N)-displacement (in mm) data from four-point bend testing and the following Equations, 3 and 4, respectively:

$$S_f = \frac{3F_{max}a}{bh^2} \quad (3)$$

$$E_f = \left(\frac{a\left(3Lx-3x^2-a^2\right)}{bh^3}\right) * \left(\frac{\Delta F}{\Delta D}\right) \quad (4)$$

In equation 3, $a$ (in mm) is the distance between the inner and outer four-point bending supports, $F_{max}$ (in N) is the applied load at failure, $b$ (in mm) is the sample thickness, and $h$ (in mm) is the sample height. For equation 4, $L$ (in mm) is the lower support distance, and $x$ (in mm) is the position of deflection measurement. In this study, displacement was measured at the position of load application; therefore, $x = a$.

The fracture surfaces of four-point bending samples were investigated with scanning electron microscopy (SEM). Samples were cut 5 mm from fracture surface and mounted on aluminum stubs covered with carbon tape. A thin layer of gold was deposited on the sample surfaces for 35 s for 45 mA.

Images were obtained with a LEO DSM 1530 field emission SEM (Zeiss-LEO, Oberkochen, Germany) using an acceleration voltage of 3 kV and a 4.9 to 7.9 mm working distance.

Fracture toughness testing was performed using the single-edge notched beam method as previously described (Slane et al., 2014). The mode I plane strain fracture toughness, $K_{Ic}$ in Pa $m^{1/2}$, was calculated with Equation 5 (ASTM E399-08, Hasenwinkel et al., 2002):

$$K_{Ic} = \frac{3PS}{2bW^{3/2}} * f(x) \quad (5)$$

Where P is the maximum force (in N); S (in m) is the lower span length; b (in m) is the sample thickness, W (in m) is the sample width; and, f(x) is a factor defined by Equation 6 with crack length, $a_c$ (in m):

$$f(x) = 1.93\left(\frac{a_c}{W}\right)^{1/2} - 3.07\left(\frac{a_c}{W}\right)^{3/2} + 14.53\left(\frac{a_c}{W}\right)^{5/2} - 25.11\left(\frac{a_c}{W}\right)^{7/2} + 25.80\left(\frac{a_c}{W}\right)^{9/2} \quad (6)$$

According to ISO 5833 standard for acrylic bone cement, the flexural modulus, flexural strength and compressive yield strength must be above 1800 MPa, 50 MPa, and 70 MPa, respectively ("ISO 5833:2002 Implants for surgery - Acrylic resin cements," 2002).

**2.4 Release characteristic testing**

Five cylindrical samples (6 mm diameter × 4.5 mm height) from each of the six experimental groups was submerged in 5 mL of sterile PBS and placed in an incubator shaker operating at 37°C and 100 rpm. At time intervals of 1, 2, 4, 8, 10, 15, 25 and 45, and 60 days, 1.5 mL of the PBS was aspirated off and the samples were placed into 5 mL of fresh PBS. The aspirated fluid was then stored in cryotubes at −20°C until time of analysis. Vancomycin present in the collected PBS was determined using high performance liquid chromatography (HPLC) with a $C_{18}$ column as previously described (Gu et al., 2015). Each sample was tested in triplicate. Ten microliters of the sample was developed isocratically with 50 mM potassium phosphate buffer (pH 6.8) and acetonitrile (17:3) at a flow rate of 0.5 mL/min. Absorbance was monitored at 210 nm and peak intensity was used to correlate concentrations according a vancomycin standard curve. The validity of this vancomycin assay has been previously confirmed (Berti et al., 2015).

**2.5 Antimicrobial activity testing**

Three cylindrical samples (6 mm diameter x 4.5 mm height) were sterilized by ethylene oxide gas and then submerged in 3.4 mL of tryptic soy broth (TSB; Becton Dickenson, Franklin Lakes, NJ) inoculated with bacteria for each test condition. Four strains were evaluated: Methicillin-resistant Staphylococcus aureus (MRSA) n315 with a vancomycin minimum inhibitory concentration (MIC) of 0.5-1 mg/L (Kuroda et al., 2001), ATCC MRSA 33591 (vancomycin MIC 2 mg/L), ATCC *S. aureus* 29213 (vancomycin MIC 0.5 mg/L), and ATCC *S. epidermidis* 35984, a prototypical high biofilm-producing strain (vancomycin MIC 1 mg/L ) (Dunne et al., 1993).

To understand the bacterial inhibition properties of vancomycin released from bone cements, a simulated bacterial contamination model was used. The cement samples were submerged in 3.4 mL of broth and exposed to low inoculum (1000 CFU/mL) of bacteria consistent with colonization or contamination. MRSA n315 was also tested at $10^6$ CFU/mL to determine the effect of eluted antibiotic against bacterial inoculum consistent with an infection. Samples of the tryptic soy broth were taken at

inoculation, daily for 7 days, and again at 14 days and serially diluted on Mueller Hinton II agar plates (Sigma-Aldrich, St. Louis, MO, USA) for bacterial enumeration. Agar plates were incubated for 18-24 hours and bacterial colonies were then quantified. The number of colony forming units per milliliter (CFU/mL) was enumerated to quantify the ability of eluted antibiotic from the cement to inhibit or kill the bacteria in culture. The limit of bacterial detection with this method is 10 CFU/mL. All testing was performed in triplicate.

### 2.6 Statistical analysis

All mechanical testing results were statistically analyzed using Minitab 18 (Minitab Inc., State College, PA). A p-value of <0.05 was considered statistically significant. Kolmogorov-Smirnov method was employed to determine if the data followed a normal distribution. Kruskal-Wallis tests with post hoc Mann-Whitney U tests were conducted to test for difference in mechanical properties between the control group and the groups with vancomycin loading. Wilcoxon Signed Rank tests were performed to test strengths against the minimum ISO requirement. Linear regression of the compressive yield strength data versus vancomycin mass was performed.

## 3. Results

### 3.1 Mechanical Properties

All sample groups exceeded the minimum required flexural modulus required by ISO 5833 **(Fig 1)**. The control's median flexural modulus was 2190 ± 164 MPa. For flexural strength, the control (median 55.4± 3.53 MPa) was statistically greater than the ISO minimum requirement (50 MPa) and all groups except the 2.0 g vancomycin were statistically equivalent to the controls. Flexural strength for the 2.0 g vancomycin group was significantly lower than the control and not greater than the ISO minimum requirement **(Fig. 2)**.

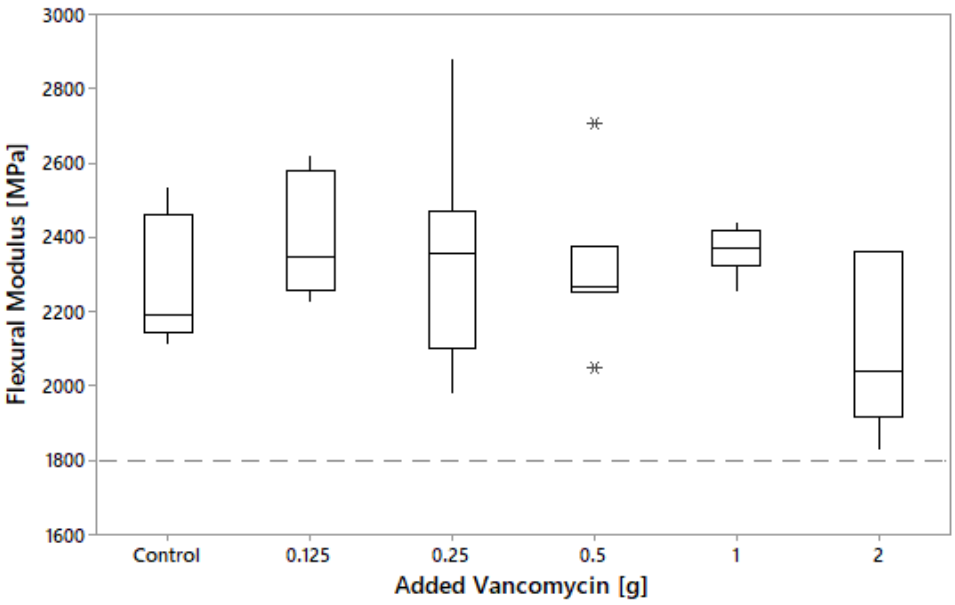


**Figure 1:** Flexural modulus versus amount of vancomycin. The * represents outliers in the data set. Dashed line at 1800 MPa indicates ISO 5833 standard for minimum flexural modulus, which was

significantly exceeded by all test groups. Graph shows median, first and third quartiles, and the lowest/highest datum within 1.5 interquartile lower/higher range.

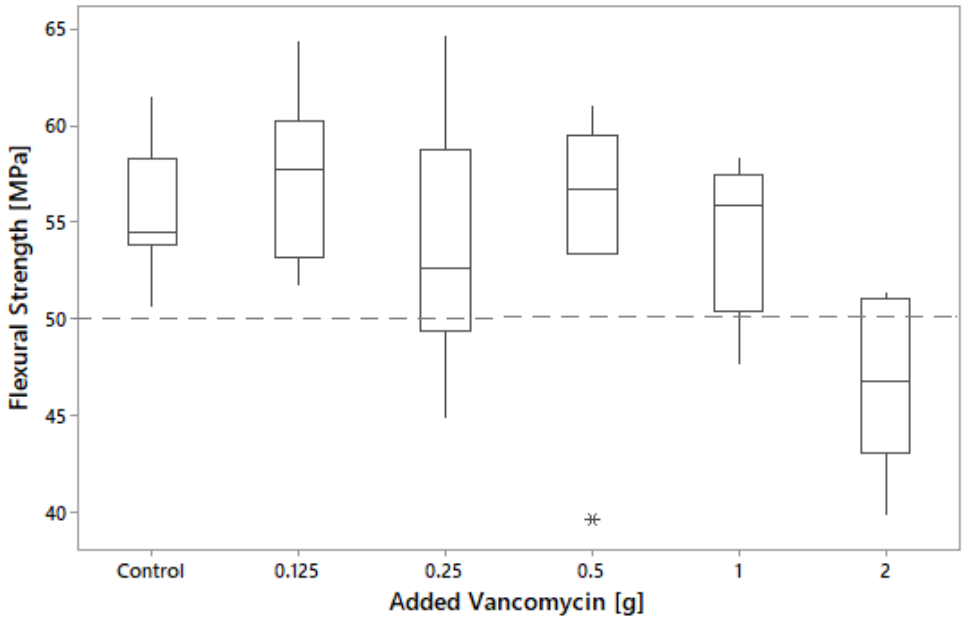


**Figure 2:** Flexural strength versus amount of vancomycin. The * represents one outlier in the data set. Dashed line at 50MPa indicates ISO 5833 standard for minimum flexural strength. Flexural strength for the 2.0 g vancomycin group was significantly lower than the control (median 55.4 ± 3.53 MPa) and not greater than the 50 MPa ISO minimum requirement. Graph shows median, first and third quartiles, and the lowest/highest datum within 1.5 interquartile lower/higher range.

Compression test results found the compressive modulus ($R^2$ = 0.945, $E_c$ = -9.40*$mass_{vanco}$ + 79.6) and strength ($R^2$ = 0.814, $S_c$ = -279*$mass_{vanco}$ + 1470) decreased with additional vancomycin. Compressive modulus for treatment groups 0.25 g – 2.0 g added vancomycin were significantly lower than the control (median 1560 ± 207 MPa). Compressive yield strength of all treatment groups was significantly lower than the control (median 82.7 ± 63.5 MPa). Three treatment groups, 0.125 g - 0.50 g added vancomycin, and the control, were significantly greater than the 70 MPa ISO minimum requirement for compressive yield strength **(Figs. 3 and 4)**. Fracture toughness was not affected by the amount of vancomycin added to bone cements. All treatment groups were statistically equivalent to the control (median 2.69 ± 0.091 MPa $mm^{1/2}$) **(Fig. 5)**.

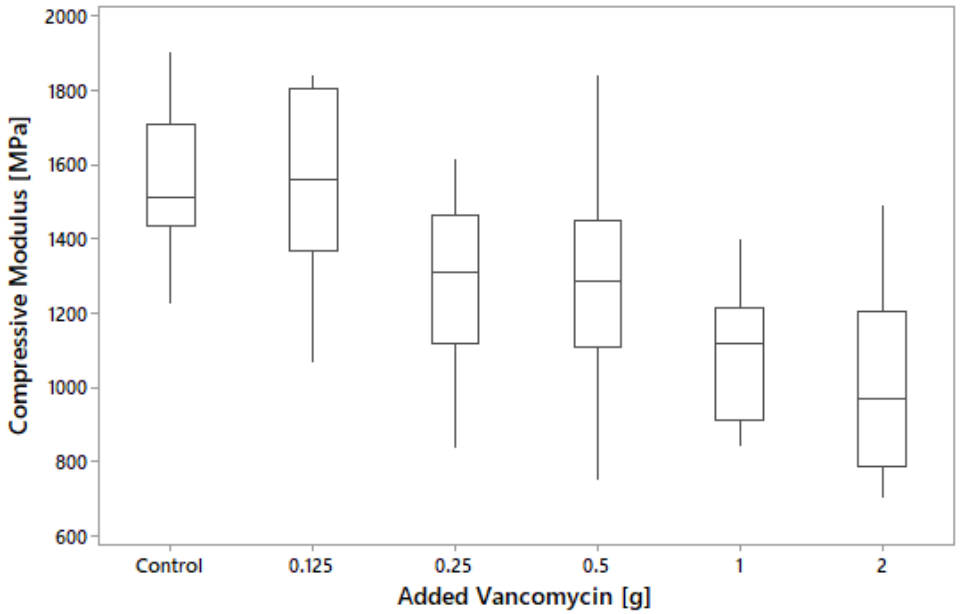


**Figure 3:** Compressive modulus versus amount of vancomycin. Data showed a decreasing trend in compressive modulus with amount of vancomycin ($R^2$ = 0.814, $S_{yc}$ = -279*($mass_{vanco}$) + 1470). Graph shows median, first and third quartiles, and the lowest/highest datum within 1.5 interquartile lower/higher range.

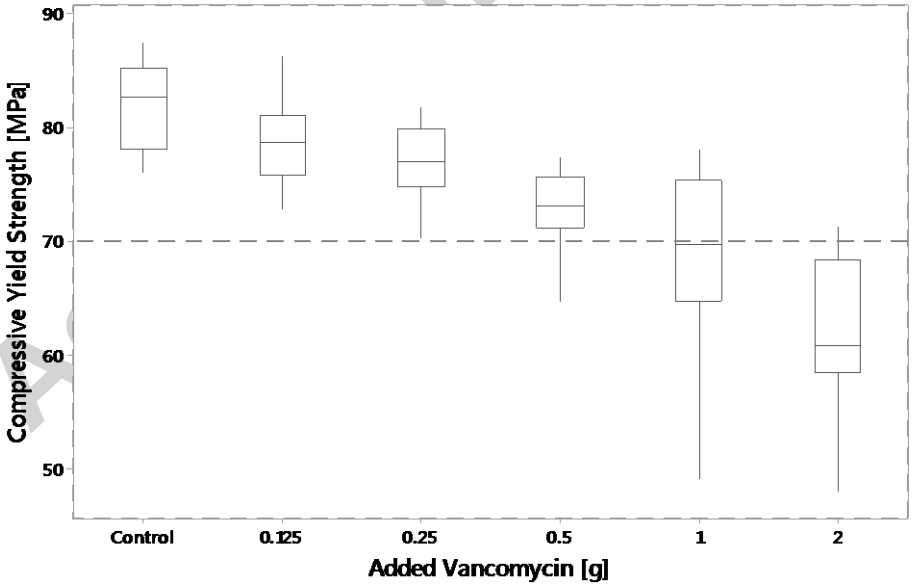


**Figure 4:** Compressive yield strength versus amount of vancomycin. Dashed line at 70 MPa indicates ISO minimum requirement for compressive yield strength. Data showed a decreasing trend in compressive yield strength with amount of vancomycin ($R^2$ = 0.945, $E_c$ = -9.40*$mass_{vanco}$ + 79.6). Graph shows median, first and third quartiles, and the lowest/highest datum within 1.5 interquartile lower/higher range.

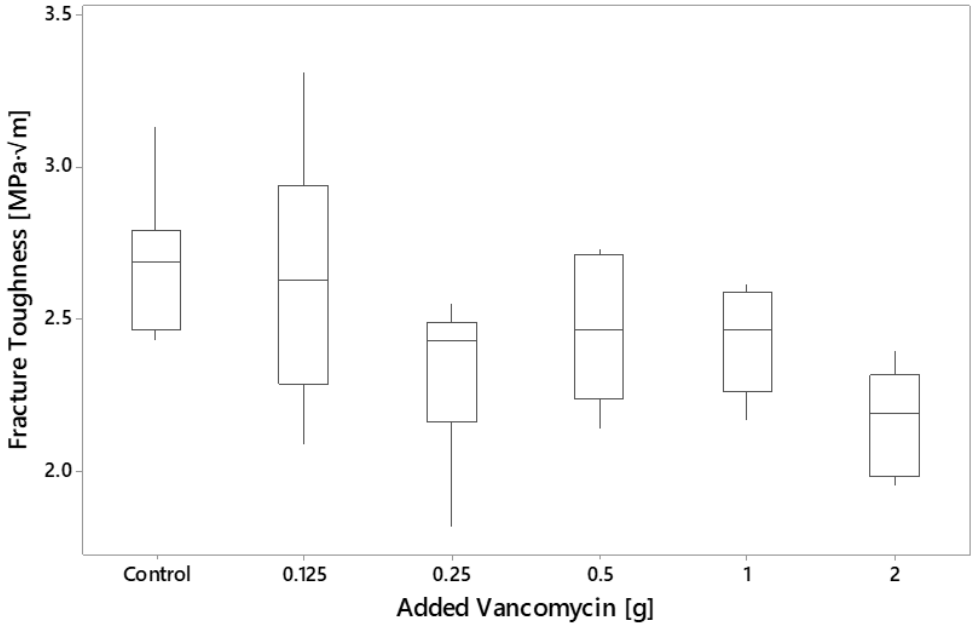


**Figure 5:** Fracture toughness versus amount of vancomycin. All treatment groups were statistically not different than the control (median 2.69 ± 0.091 MPa $mm^{1/2}$). Graph shows median, first and third quartiles, and the lowest/highest datum within 1.5 interquartile lower/higher range.

### 3.2 Vancomycin Elution

Over the entire 60-day period, a total of 1.9-9.7% of vancomycin eluted from the cement. Vancomycin elution was bimodal, with two major inflection points, immediately at day 1, and again between days 8-10 **(Fig. 6)**. For the no antibiotic control up to the 1.0 g vancomycin dose, at least 99% of the total antibiotic eluted over the entire 60-day period occurred in the first 8 days. For the 2.0 g samples, 97% of the total eluted was released by day 8. No significant amounts of vancomycin were released after 8 days in all the samples. The 1.0 g samples released the most vancomycin per disk (average of 0.105 +/- 0.00002 mg) but was statistically equivalent to the 2.0 g samples. The 0.125 g samples released the highest percentage of total added vancomycin at 9.66%.

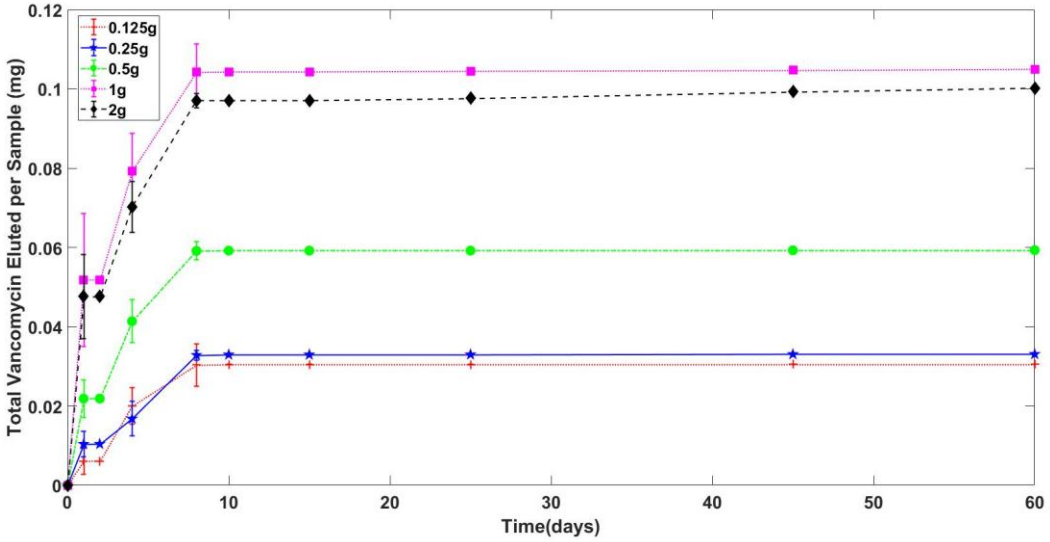


**Figure 6:** Vancomycin elution (error bars show ± 1 standard deviation) from Palacos cement disks in PBS. 97% - 99% of 60-day elution occured in the first eight days.

### 3.3 Vancomycin Activity

The antimicrobial activity of vancomycin eluted from bone cement was similar between the three *S. aureus* isolates; however, it was less effective against ATCC *S. epidermidis* 35984, the prototypical high biofilm producing strain. For the *S. aureus* strains, loading bone cement with 0.5 g or more vancomycin per 40 g packet PMMA polymer cleared the bacterial inoculum below the limit of detection (10 CFU/mL) within 1-2 days (**Fig 7**. For the *S. aureus* strains, loading bone cement with 0.5 g or more vancomycin per 40 g packet PMMA polymer cleared the bacterial inoculum below the limit of detection (10 CFU/mL) within 1-2 days (**Fig 7**). The higher doses of 1.0 and 2.0 g vancomycin per 40 g packet PMMA polymer resulted in undetectable bacteria at the last time point only (15 days). Palacos loaded with 0.5 g and 1.0 g vancomycin per 40 g packet PMMA reduced the bacterial concentration below the initial inoculum ($10^3$ CFU/mL) after 15 days; however, detectable amounts of bacteria were present at all assay time points. (**Fig 8**).

In order to investigate higher bacterial inoculum, MRSA n315 was also tested at initial concentration of $10^6$ CFU/mL. No tested vancomycin dose was able to completely eliminate the $10^6$ CFU/mL inoculum of MRSA n315 at 7 days. Doses of 0.5 g and larger initially demonstrated killing by day 2, but all regrew to at least 10 CFU/mL by day 3. However, the largest dose tested, 2.0 g, did reduce the bacteria below the limit of detection at the 14-day time point. These data indicate that vancomycin added to Palacos bone cement would likely not be effective at treating an active infection without additional systemic antibiotic use and implant removal.

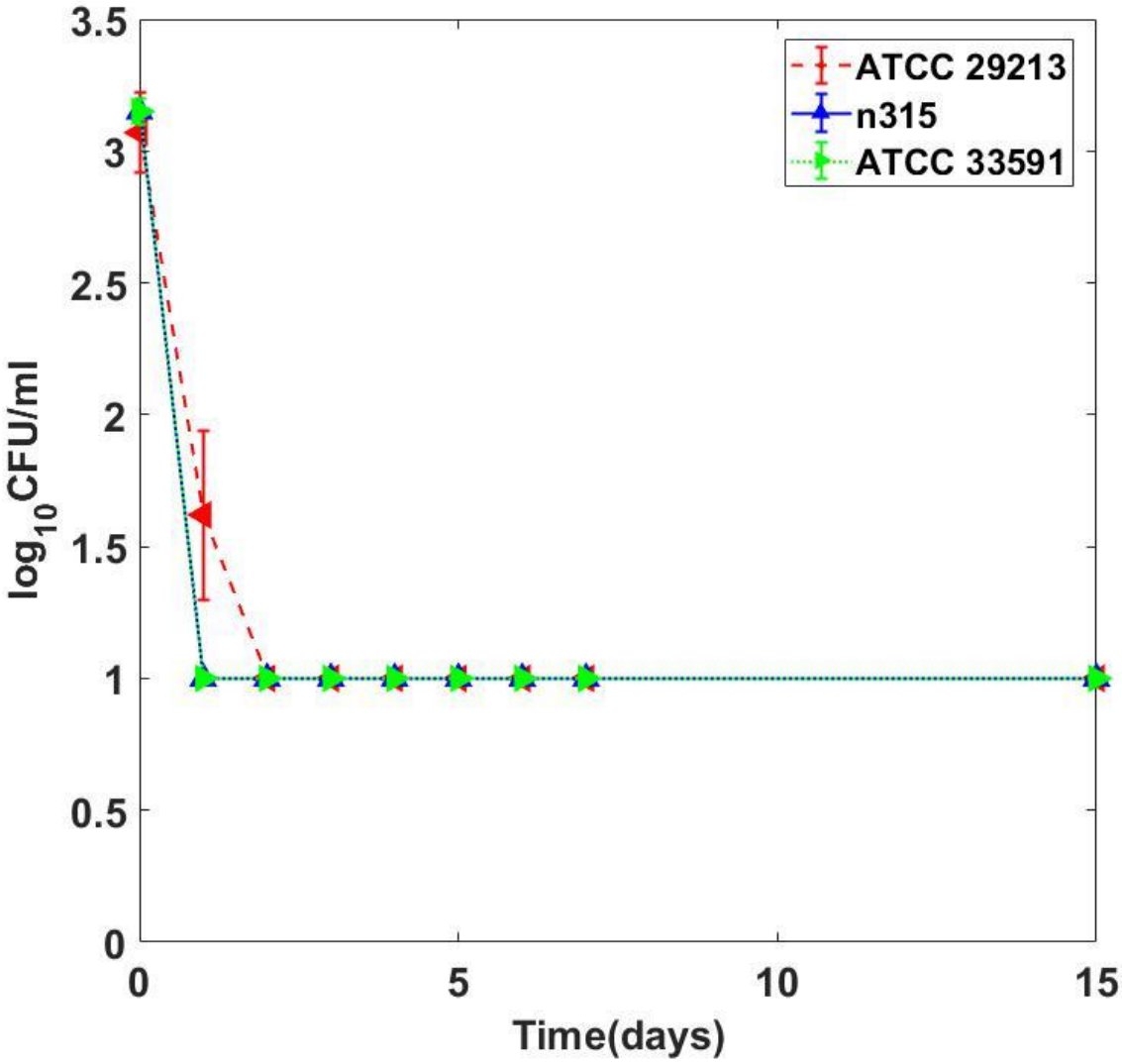


**Figure 7:** Antimicrobial activity of eluted vancomycin (0.5 g) for three *S. aureus* strains. All bacterial colonies reduced below limit of detection by day 2 and no re-growth was observed. Error bars show ± 1 standard deviation.

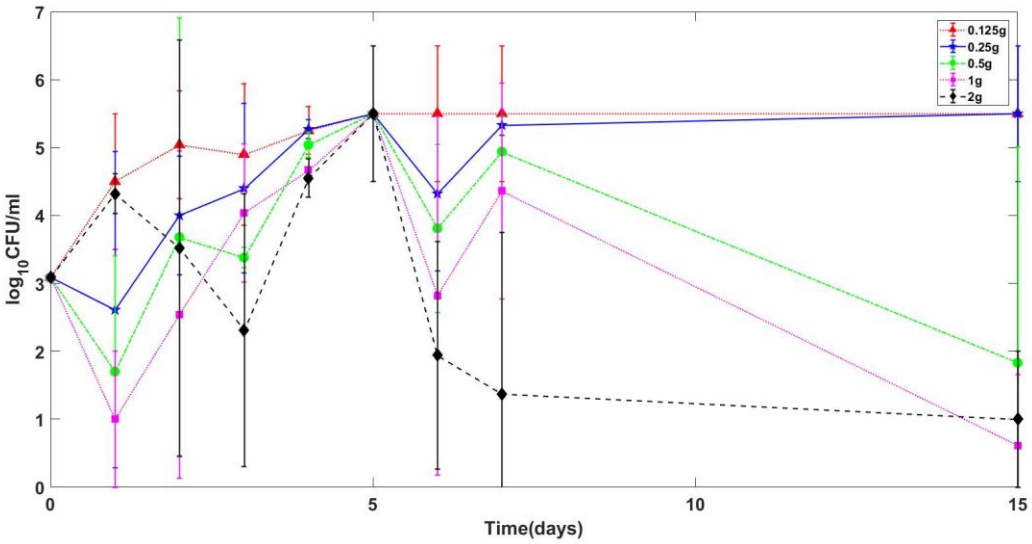


**Figure 8** Antimicrobial activity of eluted vancomycin (0.125 g – 2.0 g) for *S. epidermidis 35984,* a high *in vitro* biofilm producer. Error bars show ± 1 standard deviation.

### 3.4 Scanning Electron Microscope (SEM) Imaging

The fractured surfaces of four-point bending samples showed an increasing number of voids in the cement matrix with an increase in amount of added vancomycin. The pores are approximately 50-200 microns in diameter.

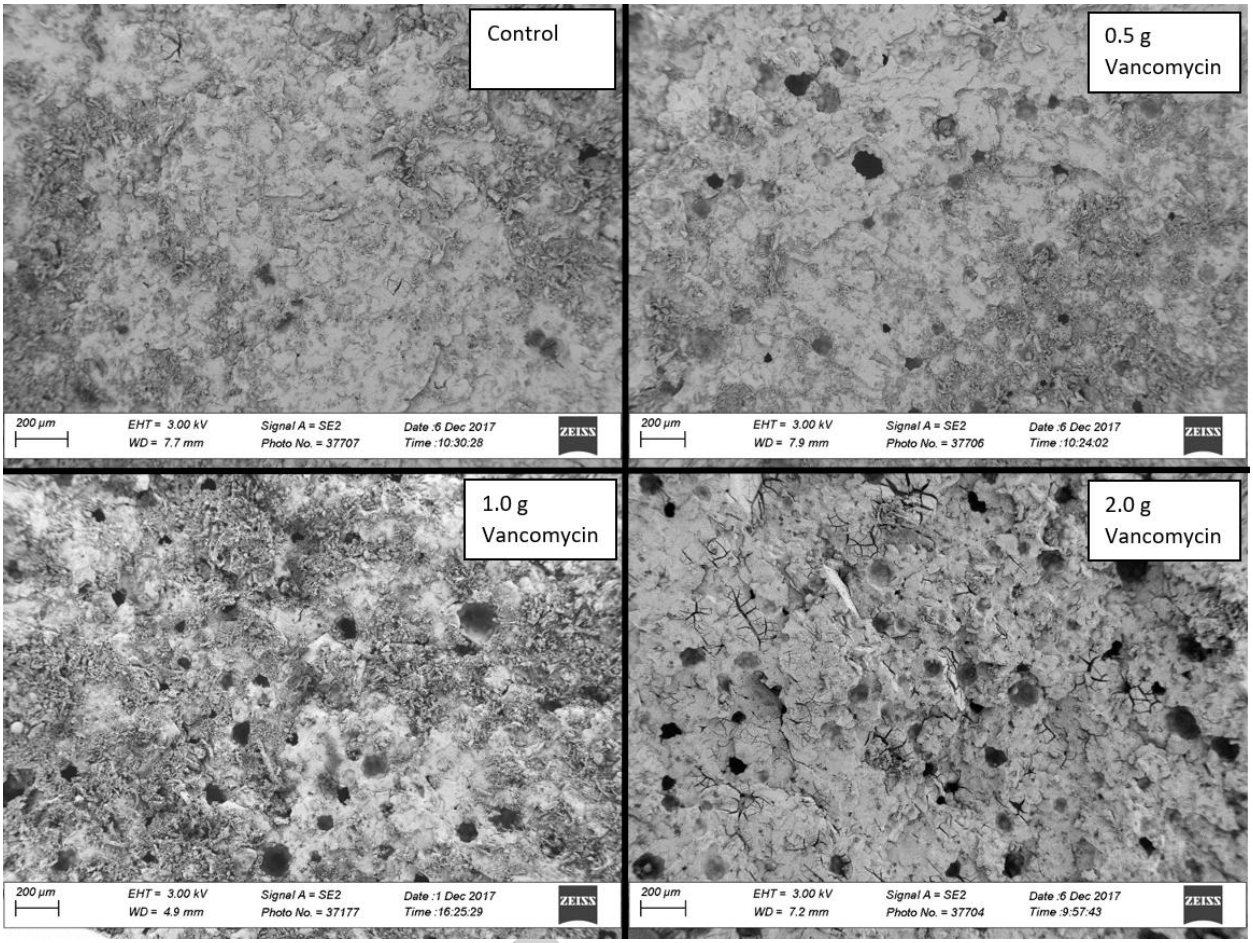


**Figure 9:** SEM images of bone cement fracture surfaces from four-point bend specimens with increasing amounts of added vancomycin from control to 2.0 g. Porosity increased with increasing amount of vancomycin.

## 4 Discussion

The addition of vancomycin powder into Palacos polymer powder prior to the polymerization process resulted in changes to the cement's mechanical properties. However, not all properties were affected equally. Fracture toughness was relatively un-affected while compressive modulus and compressive yield strength showed a linear decrease with increasing amounts of vancomycin. This investigation found adding vancomycin powder to Palacos bone cement affected the cement's compressive properties more strongly than it's bending properties. The compressive modulus was lower than the flexural modulus demonstrating the cement did not behave as a linear-elastic material. These results are consistent with previously published findings in Palacos cement (Slane et al., 2014A; Slane et al., 2014B) and likely due to the effect of the pores (Barralet et al., 2002). The compression test force-deflection data did not demonstrate a toe region associated with reduced stiffness due to end conditions. In addition, wet curing has been shown to decrease the flexural modulus of plain bone cement by 44% and compressive modulus by 30% compared to dry test conditions (Puska et al., 2003).

All mechanical properties of the controls were similar to previously published results for 21-day wet-cured samples of Palacos cement. The greatest deviation was an approximately 10% lower

compressive modulus and compressive yield strength (Slane et al., 2014A; Slane et al., 2014B). Mechanical property trends with increasing amounts of added vancomycin were similar to those found with the addition of xylitol (a poragen additive) to Palacos (Slane et al., 2014B). The mechanical properties of Palacos investigated in this study with added vancomycin and 21-day wet curing have not been previously published. However, other studies have also found mechanical strength decreases with length of wet curing time (Lautenschlager et al., 1976; Lee et al., 2016). A review article of bone cement studies found mechanical properties of Palacos were unaffected or degraded by the addition of antibiotic depending on the testing environment, wet curing process, and amount of added antibiotic (Lewis, 2009).

The SEM images showed more and larger pores in the samples with higher levels of added vancomycin. These pores may have been due to vancomycin that eluted from the cement matrix over the 21-day curing process. The pores were approximately 50-200 microns in diameter and may be responsible for the degradation of the cement's compressive yield strength. Since bending puts a sample in both compression and tension the pores may have a different effect on the mechanical properties in bending. Regardless of how the pores were generated, through the mixing process or antibiotic elution, pores in bone cement are thought to be a major contributor to bone cement failure (De Santis et al., 2003; Qian et al., 2005). Generally, pores result in a loss of mechanical strength of bone cement due to a reduction in load carrying cross sectional area, and the tendency of pores to cluster may also lead to stress concentrations (Lewis and Janna, 2003).

The majority of the antibiotic elution from the cement disks over the 60-day test period occurred in the first 8 days, consistent with previously published works (Lee et al., 2016). In the bactericidal assay, if the culture was not reduced to below detection within 8 days, the antibiotic loaded cement had little further effect. The elution data closely match the activity data observed indicating vancomycin activity was stable in the bone cement. This elution profile is suited for clinical use since the maximum elution occurs during the critical first week after surgery and would effectively eliminate *S. aureus* contamination that may inadvertently occur during the surgical procedure. This investigation indicated that vancomycin released from bone cement appears to have limited effect on *S. epidermidis*; however, additional S. epidermidis strains should be evaluated to confirm this finding. The drop in antibiotic levels after day 8 could limit unnecessary antibiotic exposure to the surgical site. Based on our results, vancomycin doses of 0.5 - 1.0 g per packet of Palacos bone cement produced optimal prophylactic antibiotic activity against *S. aureus.*

Several limitations to this study are noted. Only one type of bone cement was used (Palacos) and other cements may produce different results. Palacos was chosen due to its commercial availability and popularity with surgeons in North America and Europe (Kärrholm et al., 2016; Spierings, 2007), as well as at our own institution. The samples were all produced by hand. Because all vancomycin impregnated Palacos samples were produced by a single individual following clinical bone cement mixing methods, this could have introduced subtle systemic biases or sample variations affecting antibiotic elution or antibiotic cement mechanical properties may have been present.

Future work should be performed to test other vancomycin similar glycopeptide antibiotics to increase the number of treatment options available and reduce the risk of developing antibiotic resistant bacteria. Different cements should be tested to determine if the results vary based on the type of cement used. More investigation should also be performed to determine effective methods of

eliminating *S. epidermidis* or other high biofilm producing strains, as bacteria in this state have increased resistance to most antibiotics.

## 5 Conclusion

Activity testing showed that 1.0 g of added vancomycin was optimal for drug release from wet-cured cement. However, Palacos compressive yield strength may be compromised with 1.0 g added vancomycin or greater per packet of PMMA. While lower in total vancomycin release, adding 0.5 g vancomycin was equivalent to the 1.0 g samples in activity, showing similar efficacy at eliminating $10^3$ CFU/mL initial inoculum of bacterial that are not high biofilm-producers. None of the tested vancomycin loads could eliminate a $10^3$ CFU/mL inoculum of a biofilm producing bacteria within 7 days.

The cement's mechanical properties were affected by adding vancomycin. However, up to 0.5 g added to a packet of Palacos bone cement produced a mixture that met all ISO standards even after 21 days of wet curing in PBS. Adding 1.0 g of vancomycin produced a cement that was below the ISO standard for compressive yield strength and 2.0 g added vancomycin resulted in cement below the ISO standard for both compressive yield strength and flexural strength. These data lead to the conclusion that 0.5 g vancomycin per packet of Palacos cement provided the maximal amount of antibiotic activity while meeting ISO mechanical property standards.

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Declarations of interest

none



## References

ASTM International, 2008. Standard test method for plain-strain fracture toughness $K_{Ic}$ of metallic materials. Specification E399-08. In: ASTM Standards. Philadelphia: American Society of Testing and Materials.

Barralet, J.E., Gaunt, T., Wright, A.J., Gibson, I.R., Knowles, J.C., 2002. Effect of Porosity Reduction by Compaction on Compressive Strength and Microstructure of Calcium Phosphate Cement. J Biomed Mater Res (Appl Biomater) 63, 1–9. https://doi.org/10.1002/jbm.0000

Berti, A.D., Hutson, P.R., Schulz, L.T., Webb, A.P., Rose, W.E., 2015. Compatibility of cefepime and vancomycin during simulated Y-site administration of prolonged infusion. Am. J. Health. Syst. Pharm. 72, 390–5. https://doi.org/10.2146/ajhp140369

Bistolfi, A., Massazza, G., Verné, E., Massè, A., Deledda, D., Ferraris, S., Miola, M., Galetto, F., Crova, M., 2011. Antibiotic-Loaded Cement in Orthopedic Surgery: A Review. ISRN Orthop. https://doi.org/10.5402/2011/290851

Brescó, M.S., Harris, L.G., Thompson, K., Stanic, B., Morgenstern, M., O'Mahony, L., Richards, R.G., Moriarty, T.F., 2017. Pathogenic mechanisms and host interactions in Staphylococcus epidermidis device-related infection. Front. Microbiol. https://doi.org/10.3389/fmicb.2017.01401

Ceri, H., Olson, M.E., Stremick, C., Read, R.R., Morck, D., Buret, A., 1999. The Calgary Biofilm Device: New technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J. Clin. Microbiol. 37, 1771–1776.

Darouiche, R.O., 2004. Treatment of Infections Associated with Surgical Implants. N. Engl. J. Med. 350, 1422–1429. https://doi.org/10.1056/NEJMra035415

De Santis, R., Mollica, F., Ambrosio, L., Nicolais, L., Ronca, D., 2003. Dynamic mechanical behavior of PMMA based bone cements in wet environment. J. Mater. Sci. Mater. Med. https://doi.org/10.1023/A:1024014822277

Dunne, W.M., Mason, E.O., Kaplan, S.L., 1993. Diffusion of rifampin and vancomycin through a Staphylococcus epidermidis biofilm. Antimicrob. Agents Chemother. 37, 2522–2526. https://doi.org/10.1128/AAC.37.12.2522

Fernandez-Hidalgo, N., Almirante, B., Calleja, R., Ruiz, I., Planes, A.M., Rodriguez, D., Pigrau, C., Pahissa, A., 2006. Antibiotic-lock therapy for long-term intravascular catheter-related bacteraemia: Results of an open, non-comparative study. J. Antimicrob. Chemother. 57, 1172–1180. https://doi.org/10.1093/jac/dkl103

Fu, S., Wang, C., Yang, R., Wu, F.L., Hsiao, F., 2017. Bisphosphonate Use and the Risk of Undergoing Total Knee Arthroplasty in Osteoporotic Patients with Osteoarthritis. J. Bone Jt. Surg. 99, 938–946. https://doi.org/10.2106/JBJS.16.00385

Gu, Z., Wong, A., Raquinio, E., Nguyen, A., 2015. Stability of Reconstituted Telavancin Drug Product in Frozen Intravenous Bags. Hosp. Pharm. 50, 609–14. https://doi.org/10.1310/hpj5007-609

Hasenwinkel, J.M., Lautenschlager, E.P., Wixson, R.L., Gilbert, J.L., 2002. Effect of initiation chemistry on the fracture toughness, fatigue, strength, and residual monomer content of a novel high-viscosity, two-solution acrylic bone cement. J. Biomed. Mater. Res. 59, 411–421. https://doi.org/10.1002/jbm.1257

ISO 5833:2002 Implants for surgery - Acrylic resin cements, 2002.

Kärrholm, J., Lindahl, H., Malchau, H., Mohaddes, M., Nemes, S., Rogmark, C., Rolfson, O., 2016. The Swedish Hip Arthroplasty Register Annual Report 2016. https://doi.org/DOI: 10.18158/SJy6jKyrM

Klein, E.Y., Mojica, N., Jiang, W., Cosgrove, S.E., Septimus, E., Morgan, D.J., Laxminarayan, R., 2017. Trends in Methicillin-Resistant Staphylococcus aureus Hospitalizations in the United States, 2010-2014. Clin. Infect. Dis. 65, 1921–1923. https://doi.org/10.1093/cid/cix640

Kuroda, M., Ohta, T., Uchiyama, I., Baba, T., Yuzawa, H., Kobayashi, I., Cui, L., Oguchi, A., Aoki, K., Nagai, Y., Lian, J., Ito, T., Kanamori, M., Matsumaru, H., Maruyama, A., Murakami, H., Hosoyama, A., Mizutani-Ui, Y., Takahashi, N.K., Sawano, T., Inoue, R., Kaito, C., Sekimizu, K., Hirakawa, H., Kuhara, S., Goto, S., Yabuzaki, J., Kanehisa, M., Yamashita, A., Oshima, K., Furuya, K., Yoshino, C., Shiba, T., Hattori, M., Ogasawara, N., Hayashi, H., Hiramatsu, K., 2001. Whole genome sequencing of meticillin-resistant Staphylococcus aureus. Lancet (London, England) 357, 1225–40. https://doi.org/10.1016/S0140-6736(00)04403-2

Lautenschlager, E.P., Jacobs, J.J., Marshall, G.W., Meyer, P.R., 1976. Mechanical properties of bone cements containing large doses of antibiotic powders. J. Biomed. Mater. Res. 10, 929–38. https://doi.org/10.1002/jbm.820100610

Lee, S.-H., Tai, C.-L., Chen, S.-Y., Chang, C.-H., Chang, Y.-H., Hsieh, P.-H., 2016. Elution and Mechanical Strength of Vancomycin-Loaded Bone Cement: In Vitro Study of the Influence of Brand Combination. PLoS One 11, e0166545. https://doi.org/10.1371/journal.pone.0166545

Lewis, G., 2009. Properties of antibiotic-loaded acrylic bone cements for use in cemented arthroplasties: A state-of-the-art review. J. Biomed. Mater. Res. - Part B Appl. Biomater. https://doi.org/10.1002/jbm.b.31220

Lewis, G., Janna, S., 2003. Effect of test specimen cross-sectional shape on the in vitro fatigue life of acrylic bone cement. Biomaterials 24, 4315–4321. https://doi.org/10.1016/S0142-9612(03)00316-

Mah, T.F.C., O'Toole, G.A., 2001. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol. https://doi.org/10.1016/S0966-842X(00)01913-2

Maradit Kremers, H., Larson, D.R., Crowson, C.S., Kremers, W.K., Washington, R.E., Steiner, C.A., Jiranek, W.A., Berry, D.J., 2015. Prevalence of Total Hip and Knee Replacement in the United States. J. Bone Jt. Surgery-American Vol. 97, 1386–1397. https://doi.org/10.2106/JBJS.N.01141

Morgenstern, M., Erichsen, C., Hackl, S., Mily, J., Militz, M., Friederichs, J., Hungerer, S., Bühren, V., Moriarty, T.F., Post, V., Richards, R.G., Kates, S.L., 2016. Antibiotic resistance of commensal Staphylococcus aureus and coagulase-negative staphylococci in an international cohort of surgeons: A prospective point-prevalence study. PLoS One. https://doi.org/10.1371/journal.pone.0148437

Parra-Ruiz, J., Vidaillac, C., Rose, W.E., Rybak, M.J., 2010. Activities of high-dose daptomycin, vancomycin, and moxifloxacin alone or in combination with clarithromycin or rifampin in a novel in vitro model of Staphylococcus aureus biofilm. Antimicrob. Agents Chemother. 54, 4329–34. https://doi.org/10.1128/AAC.00455-10

Pelletier, M.H., Malisano, L., Smitham, P.J., Okamoto, K., Walsh, W.R., 2009. The Compressive Properties of Bone Cements Containing Large Doses of Antibiotics. J. Arthroplasty 24, 454–460. https://doi.org/10.1016/J.ARTH.2007.10.023

Puska, M.A., Kokkari, A.K., Närhi, T.O., Vallittu, P.K., 2003. Mechanical properties of oligomer-modified acrylic bone cement. Biomaterials. https://doi.org/10.1016/S0142-9612(02)00354-X

Qian, L., Li, M., Zhou, Z., Yang, H., Shi, X., 2005. Comparison of nano-indentation hardness to microhardness. Surf. Coatings Technol. https://doi.org/10.1016/j.surfcoat.2004.07.108

Rosenthal, V.D., Maki, D.G., Salomao, R., Moreno, C.Á., Mehta, Y., Higuera, F., Cuellar, L.E., Arikan, Ö.A., Abouqal, R., Leblebicioglu, H., 2006. Device-Associated Nosocomial Infections in 55 Intensive Care Units of 8 Developing Countries. Ann. Intern. Med. 145, 582. https://doi.org/10.7326/0003-4819-145-8-200610170-00007

Rupp, M.E., Archer, G.L., 1994. Coagulase-negative staphylococci: pathogens associated with medical progress. Clin. Infect. Dis. 19, 231–245. https://doi.org/10.1093/clinids/19.2.231

Slane, J., Vivanco, J., Meyer, J., Ploeg, H.L., Squire, M., 2014A. Modification of acrylic bone cement with mesoporous silica nanoparticles: Effects on mechanical, fatigue and absorption properties. J. Mech. Behav. Biomed. Mater. https://doi.org/10.1016/j.jmbbm.2013.10.008

Slane, J., Vivanco, J., Rose, W., Ploeg, H.L., Squire, M., 2015. Mechanical, material, and antimicrobial properties of acrylic bone cement impregnated with silver nanoparticles. Mater. Sci. Eng. C. https://doi.org/10.1016/j.msec.2014.11.068

Slane, J.A., Vivanco, J.F., Rose, W.E., Squire, M.W., Ploeg, H.L., 2014B. The influence of low concentrations of a water soluble poragen on the material properties, antibiotic release, and biofilm inhibition of an acrylic bone cement. Mater. Sci. Eng. C 42, 168–176. https://doi.org/10.1016/j.msec.2014.05.026

Spierings, P., 2007. Bone Cements-are they different? in: Walenkamp Local Antibiotics in Arthroplasty: State of the Art from an Interdisciplinary Point of View. Thieme.

Van Praagh, A.D.G., Li, T., Zhang, S., Arya, A., Chen, L., Zhang, X.-X., Bertolami, S., Mortin, L.I., 2011. Daptomycin antibiotic lock therapy in a rat model of staphylococcal central venous catheter biofilm infections. Antimicrob. Agents Chemother. 55, 4081–9. https://doi.org/10.1128/AAC.00147-11

**Highlights**

- Bone cement with antibiotic is a prophylactic measure against infection
- Mechanical properties of Palacos with upto 0.5g of vancomycin met ISO requirements
- Maximum elution occured during the critical first week after surgery
- Palacos with more than 0.25g vancomycin killed infections of bacteria not high in biofilm production
- 0.5 g of vancomycin can be safely added to Palacos and kills representative infections of bacteria