ORTHOPAEDIC BONE CEMENT: EXPERIMENTAL AND COMPUTATIONAL ANALYSES

By

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

ABSTRACT

The use of antibiotic-loaded bone cement is an established method in the management of periprosthetic hip and knee joint infection. Despite inconsistencies among published studies, mechanical failure and infection are leading causes of failure in early revisions (15.7% and 8.2% for hip; 21% and 7.9% for knee). This study evaluates the effect of antibiotics on mechanical properties, antibacterial properties, and release characteristic when incorporated into the bone cement. These data are needed as a benchmark for the next generation of new antibiotic loaded bone cements and also can be used to develop new formulations.

Chapter 3 (Manuscript #1) investigated the mechanical properties of a commercially available bone cement with the addition of vancomycin, to determine the release characteristics and efficacy at eliminating the orthopedic implant pathogens. Palacos® R was loaded with incrementally larger clinically relevant weight percentages of vancomycin. The addition of vancomycin reduced the bone cement's mechanical properties. Also, vancomycin eluted from Palacos® R 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® R 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).

Chapter 4 (Manuscript #2) investigated Simplex™ P, a commercially available bone cement with vancomycin. Vancomycin at five different loading masses (0.125, 0.25, 0.5, 1.0 and 2.0 g) was added to 40 grams of Simplex™ P. Addition of vancomycin affected the mechanical properties and antimicrobial activity with significant differences from controls. Flexural and compression mechanical properties were compromised with added vancomycin. The flexural

strength of samples with added vancomycin of 0.5 g and greater were not greater than ISO 5833 minimum requirements. 2.0 g of vancomycin added to bone cement was able to eliminate completely the four bacterial strains tested. 2.0 g of vancomycin also showed the highest mass elution from the cement over a 60-day period. Given the reduced flexural strength in samples with 0.5 g and greater of added vancomycin and the inability of vancomycin in amounts less than 2.0 g to eliminate bacteria, this study did not find an ideal amount of vancomycin added to Simplex™ P that meets both strength and antibacterial requirements.

Chapter 5 (Manuscript #3) evaluated telavancin elution, stability, and antimicrobial activity when incorporated into the commercial bone cements Palacos® R and Simplex™ P. Telavancin at five loading volumes (0.3 vol%, 0.6 vol%, 1.2 vol%, 2.4 vol%, and 4.8 vol%). was added to the two cements. The release characteristics of telavancin were recorded for 60 days to estimate the elution profiles. The efficacy of eluted telavancin for eliminating four common implant pathogens was determined. Mechanical testing was also performed. Telavancin affected the elution, antimicrobial activity, and mechanical properties in a dose-dependent manner. Telavancin added to Palacos® R at 4.8 vol% was able to eliminate MSSA, MRSA, and *S. epidermidis*, while the same concentration in Simplex™ P failed to kill all tested strains. Telavancin also exhibited better elution in the former case over a 60-day period. Both cements showed reduced flexural and compression properties with added telavancin. Telavancin loaded to Palacos® R achieved better efficacy than in Simplex™ P. Under microscopic examination the two cements showed different numbers and size of pores, potentially affecting strength. Telavancin loaded in Palacos® R is a promising prophylactic option. However, the feasibility of using telavancin as an alternative to traditional antibiotics in acrylic bone cement requires further consideration due to the unsustained telavancin release and reduced strength.

Chapter 6 (Manuscript #4) was a computational investigation of the effect of porosity and its distribution on bone cement fracture toughness. The effect of pores was analyzed using the extended finite element method (X-FEM) crack propagation simulation method with different sizes of pores and locations. Predicted force-displacement behavior and fracture toughness were compared to experimental results. Crack growth and propagation are affected by porosity parameters, for example, pore size an independent parameter and dependent parameters such as pore-pore and pore-crack interactions. These dependent porosity parameters are primarily affected by pore size and pore location. The experimental and simulation results of the current study contribute to a better understanding of the effect of porosity on bone cement fracture toughness.

DOCUMENT ORGANIZATION

This dissertation consists of nine chapters. Chapter 1 introduces the reader to the concept of joint arthroplasty, joint implant and bone cement. Chapter 2 describes the four overall objectives of enclosed work. Chapters 3, 4, 5 and 6 contain four separate manuscripts to address the objectives of this dissertation, following the format of chapters that were published or submitted. Chapter 7 provides a summary of all findings of the four studies and presents discussion and conclusions, followed by references in Chapter 8. Appendices in Chapter 9 are included at the end of the thesis that includes all raw data obtained from experimental testing. The four chapters intended for publication are as follows:

- Chapter 3 describes the influence of vancomycin on the mechanical properties, antibiotic release, and antibacterial properties of Palacos® R. This chapter was published in the *Journal of the Mechanical Behavior of Biomedical Materials*.
- Chapter 4 is a continuation of the work introduced in Chapter 3, which has been expanded to examine vancomycin in a different bone cement. In this work, a different viscosity cement, Simplex™ P, was used. This chapter was also published in the *Journal of the Mechanical Behavior of Biomedical Materials*.
- Chapter 5 investigates the feasibility of using telavancin as an alternative to traditional antibiotics in Palacos® R and Simplex™ P. The impact of telavancin on the cement's mechanical properties, antibiotic elution, and antibacterial properties were assessed. This chapter has been submitted for publication to the *Clinical Orthopaedics and Related Research*.
- Chapter 6 describes how the size and distribution of pores can affect the fracture toughness of antibiotic-loaded bone cement using extended finite element method (X-

FEM). Crack initiation and propagation were also assessed using this computational method. The XFEM results were evaluated against experimental results. This work is intended to be submitted for publication to the *Journal of Biomechanics*.

I. INTRODUCTION

Total joint replacement (TJR) is a life-enhancing procedure for millions of people worldwide each year. Successful joint replacement provides pain relief, restores function and independence, and improves patient quality of life. While already a frequently performed procedure, the incidence of prosthesis implantation is expected to continue to rise. In the United States alone, there were 443,000 total hip and 680,000 total knee arthroplasties performed in between 2012 – 2017. [1] The numbers are projected to reach 572,000 and 3.48 million by 2030 for hips and knees, respectively. [2, 3, 4]

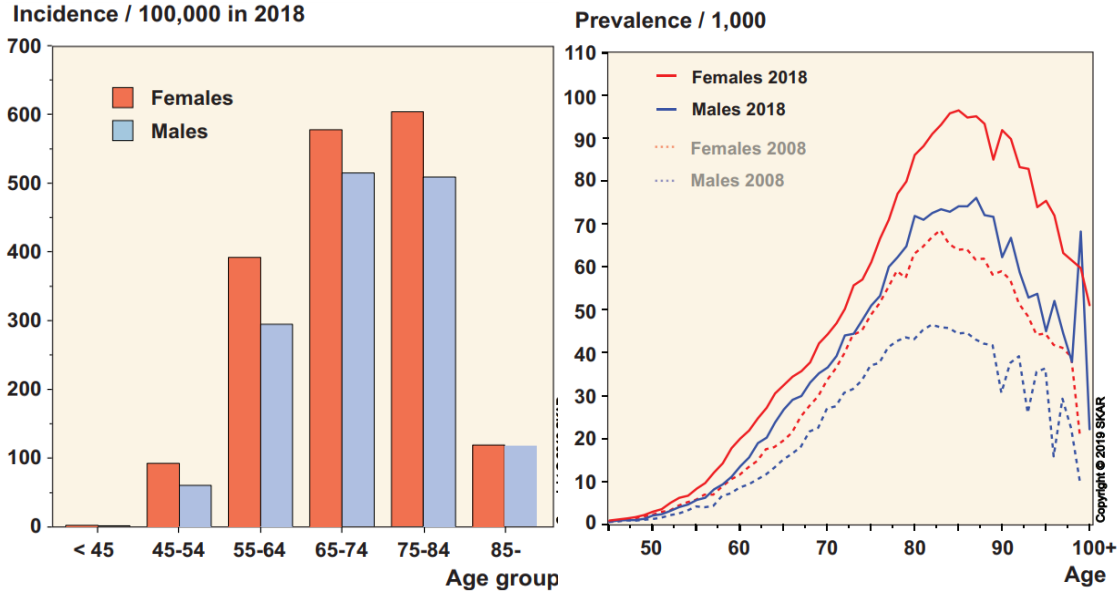

Figure I-1. Incidence of primary knee arthroplasty in 2018 per 100,000 inhabitants (males and females) in the different age group (left). The prevalence of knee arthroplasty in 2008 and 2018. One of fourteen elderly women has a knee arthrolasty (right). [5]

The Figure I-1 shows the incidence of total knee arthroplasty (TKA) among different age groups in Sweden during 2018. It is highest in the group of those 65-84 years of age. At this age, knee arthroplasty is 7 times more common than among those 45-54 years old and 5 times more common than among those 85 year or older. In 2018, as well as 2017, women were more highly represented in all the age groups. For both men and women in 2018, the prevalence peaks around

80 – 85 years of age at which almost 10% of the women and almost 8% of the men had at least one knee arthroplasty. Comparing the prevalence in 2018 with that in 2008, it can be seen that the number of patients has increased in all age groups as shown in Figure I-1. The fact that a large proportion of the older population have knee-, hip- or other types of joint implants, will probably result in an increase need for revisions in the future as well as an increased risk of periprosthetic fractures when such patients are exposed to trauma.

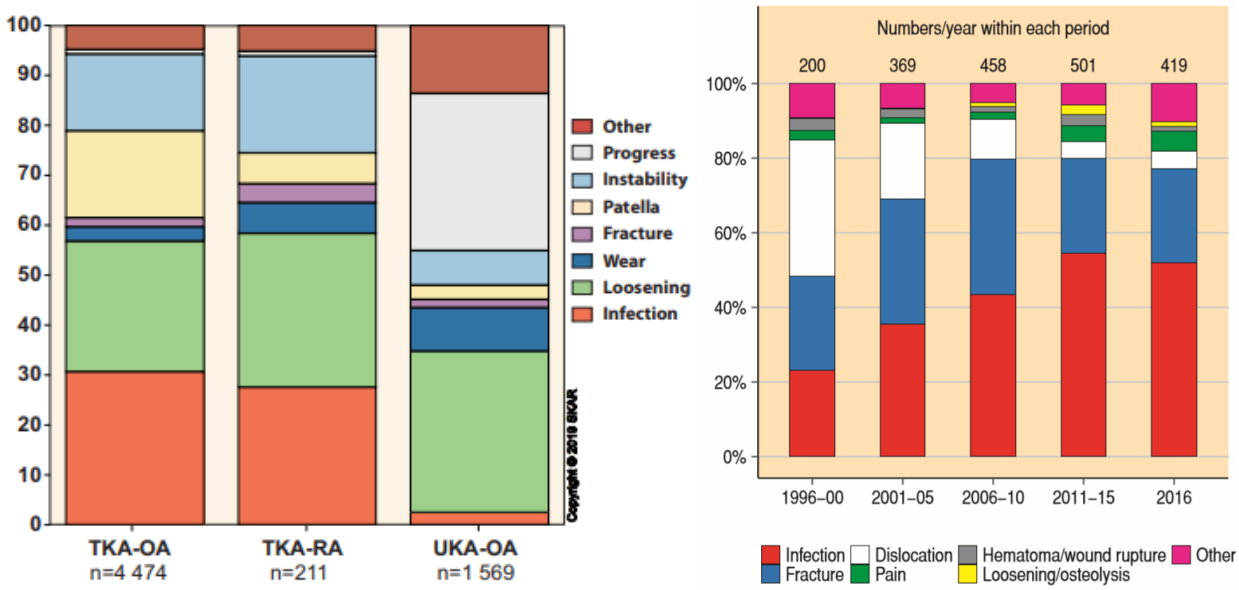

Figure I-2. Distribution (%) of indications for revision of total knee arthroplasty (TKA) and unicompartmental knee arthroplasty (UKA) during 2008 – 2018 (left). The most common reasons for reoperation for total hip arthroplasty (THA) in which the implant is left untouched during the period 1996 – 2016 (right). [5,6]

During 2008 – 2017, 6,701 first time revisions were performed as shown in Figure I-2. The main reason for revision of TKA and total hip arthroplasty (THA) was infection. However, the second most common cause for revision depended on type of TJR. Loosening was the second most common reason for TKA and periprosthetic bone fracture for THA. In 34.9 % of cases, the bone fracture was localized in the prosthesis length and in 54.8% of the cases, the localization

was distal to the tip of the prosthesis. In other cases, it had mostly to do with trochanteric fractures (2.9%).

1. Hip and Knee Arthroplasty

1.1 Hip Arthroplasty

The hip is one of the body's largest joints. It is a ball-and-socket joint. The socket is formed by the acetabulum, which is part of the large pelvic bone. The ball is the femoral head, which is the upper end of the femur (thighbone). The bone surfaces of the ball and socket are covered with articular cartilage, a smooth tissue that cushions the ends of the bones and enables them to move easily. A thin tissue called synovial membrane surrounds the hip joint. In a healthy hip, this membrane makes a small amount of fluid that lubricates the cartilage and eliminates almost all friction during hip movement. Bands of tissue called ligaments (the hip capsule) connect the ball to the socket and provide stability against dislocation to the joint.[7]

The most common cause of chronic hip pain and disability is arthritis. Osteoarthritis, rheumatoid arthritis, and traumatic arthritis are the most common forms of this disease. Osteoarthritis is an age-related "wear and tear" type of arthritis. It usually occurs in people 50 years of age and older and often in individuals with a family history of arthritis. The cartilage cushioning the bones of the hip wears away. The bones then rub against each other, causing hip pain and stiffness. Osteoarthritis may also be caused or accelerated by subtle irregularities in how the hip developed in childhood. Rheumatoid arthritis is the most common type of a group of disorders termed "inflammatory arthritis". Rheumatoid arthritis is an autoimmune disease in which the synovial membrane becomes inflamed and thickened. This chronic inflammation can damage the cartilage, leading to pain and stiffness. Avascular necrosis

may result from an injury to the hip, such as a dislocation or fracture, resulting in limited blood supply to the femoral head. This is called osteonecrosis. The lack of blood may cause the surface of the bone to collapse, and arthritis will result. Some infants and children also have hip problems. Even though the problems are successfully treated during childhood, they may still cause arthritis later in life. This happens because the hip may not grow normally, and the joint surfaces are affected.[8]

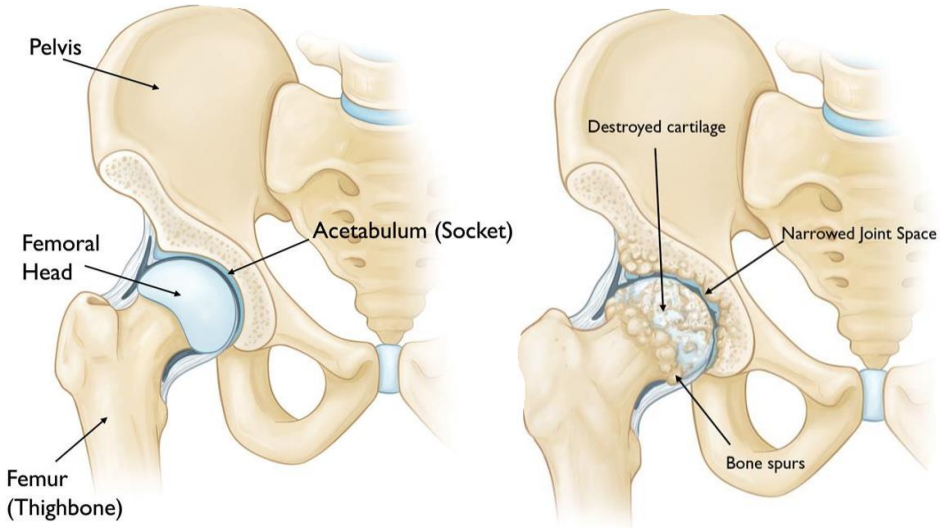

Figure I-3. Normal hip anatomy and hip osteoarthritis. The smooth articular cartilage wears away and becomes frayed and rough.[9]

1.2 Knee Arthroplasty

The knee is the largest joint in the body and having healthy knees is required to perform most everyday activities without pain and limitation. As shown in Figure I-4, the knee is made up of the lower end of the thighbone (femur), the upper end of the shinbone (tibia), and the kneecap (patella). The ends of these three bones where they touch are covered with articular cartilage, a smooth substance that protects the bones and enables them to move easily. The menisci are located between the femur and tibia. These C-shaped wedges act as "shock absorbers" that cushion the joint. Large ligaments hold the femur and

tibia together and provide stability against dislocation. The muscles crossing the knee joint give the knee strength. All remaining surfaces of the knee are covered by a thin lining called the synovial membrane. This membrane releases a fluid that lubricates the cartilage, reducing friction to nearly zero in a healthy knee. Normally, all of these components work in harmony. But disease or injury can disrupt this harmony, resulting in pain, muscle weakness, and reduced function.

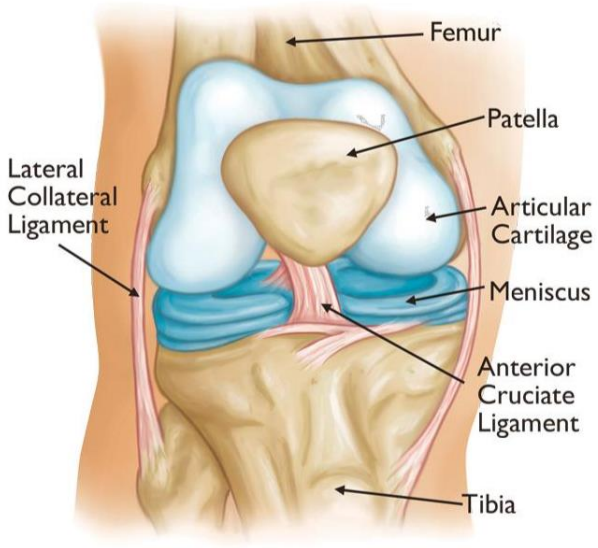

Figure I-4. Normal knee anatomy. In a healthy knee, these structure work together to ensure smooth, natural function and movement.[10]

The most common cause of chronic knee pain and disability is arthritis. Although there are many types of arthritis, most knee pain is caused by just three types: osteoarthritis, rheumatoid arthritis, and post-traumatic arthritis. These types of arthritis occur in a similar way to the arthritis in a hip. The most common symptom of joint osteoarthritis and rheumatoid arthritis is pain around the joint. The pain develops slowly and worsens over time, although rapid onset is also possible. Pain and stiffness may be worse in the morning, or after sitting or resting for a while. Over time, painful symptoms may occur more frequently, including during rest or at night. Post-traumatic arthritis can follow a serious knee injury. Fractures of the bones

surrounding the knee or tears of the knee ligaments may damage the articular cartilage over time, causing knee pain and limiting knee function.

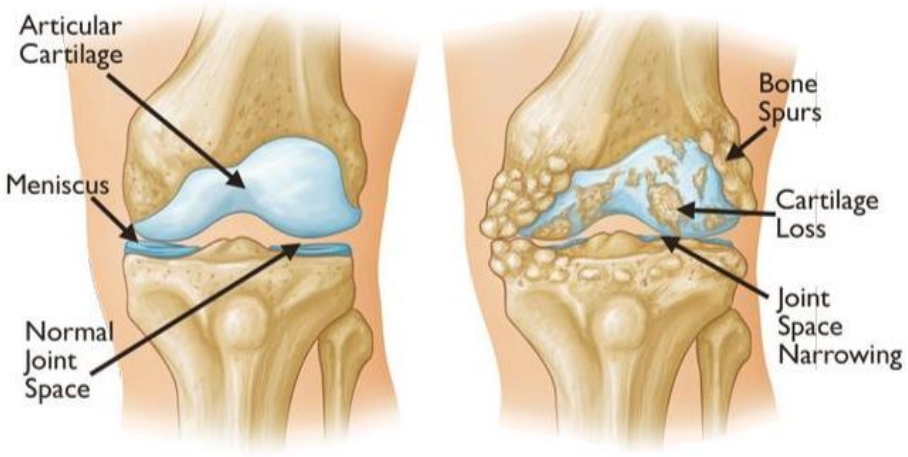

Figure I-5. Osteoarthritis often results in bone rubbing on bone. Bone spurs are a common feature of this form of arthritis.[10]

2. Hip and Knee Implants

2.1 Hip Implant

If the hip joint has been damaged by arthritis, a fracture, or other conditions, common activities such as walking or getting in and out of a chair may be painful and difficult. The hip may be stiff, and it may be hard to put on shoes and socks. Hip replacement surgery is a safe and effective procedure that can relieve the pain, increase motion, and help patients get back to enjoying normal, everyday activities.

First performed in 1960, hip replacement surgery is one of the most successful operations in all of medicine. Since 1960, improvements in joint replacement surgical techniques and technology have greatly increased the effectiveness of total hip replacement. According to the Agency for Healthcare Research and Quality, more than 300,000 total hip replacements are performed each year in the United States.[9] In a total hip replacement

surgery (also called total hip arthroplasty), the damaged bone and cartilage is removed and replaced with prosthetic components. The Figure I-6 shows the hip implant components. The damaged femoral head is removed and replaced with a metal stem that is placed into the hollowed-out center of the femur. The femoral stem may be either cemented or press fit (cementless) into the bone. A metal or ceramic ball is placed on the upper part of the stem. This ball replaces the damaged femoral head that was removed. The damaged cartilage surface of the socket (acetabulum) is removed and replaced with a metal socket. Screws or cement are sometimes used to hold the socket in place. A plastic, ceramic, or metal spacer is inserted between the new ball and the socket to allow for a smooth gliding surface.

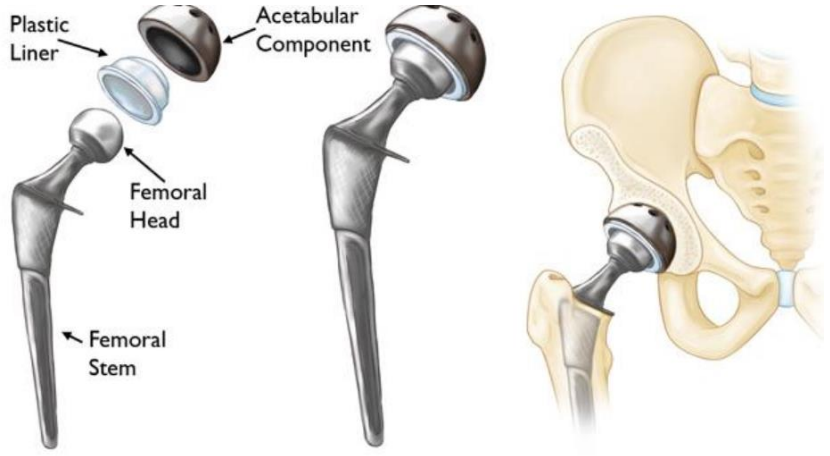

Figure I-6. (Left) The individual components of a total hip replacement. (Center) The components merged into an implant. (Right) The implant as it fits into the hip.[11]

2.2 Knee Implant

If the knee joint is severely damaged by arthritis or injury, it may be hard to perform simple activities, such as walking or climbing stairs. Patients with arthritis may begin to feel pain while sitting or lying down. Knee replacement surgery is a safe and effective procedure to relieve pain, correct leg deformity, and help resume normal activities.

Knee replacement surgery was first performed in 1968. Since then, improvements in surgical materials and techniques have greatly increased its effectiveness. Total knee replacements are one of the most successful procedures in all of medicine. According to the Agency for Healthcare Research and Quality, more than 600,000 knee replacements are performed each year in the United States. [12] A knee replacement (also called knee arthroplasty) might be more accurately termed a knee "resurfacing" because only the surface of the bones is actually replaced. The Figure I-7 shows the individual components of implants. The damaged cartilage surfaces at the ends of the femur and tibia are removed along with a small amount of underlying bone. The removed cartilage and bone are then replaced with metal components that recreate the surface of the joint. These metal parts may be cemented or press-fit into the bone. The undersurface of the patella (kneecap) is cut and resurfaced with a plastic button. Some surgeons do not resurface the patella, depending upon the case. As a final step, a medical-grade plastic spacer is inserted between the metal components to create a smooth gliding surface.

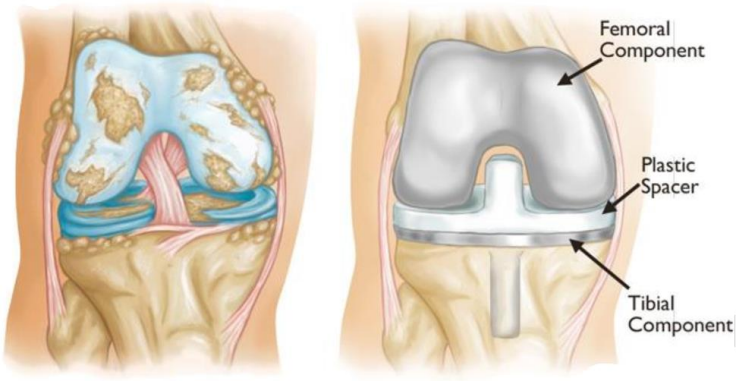

Figure I-7. (Left) Severe osteoarthritis. (Right) The arthritic cartilage and underlying bone has been removed and resurfaced with metal implants on the femur and tibia. A plastic spacer has been placed in between the implants.[13]

2.3 Implant Fixation

If a patient suffering from chronic joint pain due to arthritis opts to have a joint replacement surgery, the orthopedic surgeon will replace the existing joint surfaces with artificial joint prostheses. These prostheses, or prosthetic components, must be fixed to the patient's natural bone. How this fixation is achieved depends on the type of prosthesis used. There are two different fixation methods. A cemented joint prosthesis uses fast-polymerizing bone cement to help affix it to the bone.
A cementless joint prosthesis, sometimes called a press-fit prosthesis, is specially textured to allow the bone to grow onto it and adhere to it over time. Before a knee replacement, hip replacement, shoulder replacement, or other joint replacement surgery, the surgeon will talk to the patient and decide whether to use cemented prostheses, cementless prostheses, or a combination of the two. The type of components used may depend on the patient's physiology, the type of surgery being done, and the surgeon's preference (Table I-1).

Table I-1. Typical indications for uncemented or cemented hip implant. [14]

Uncemented	Cemented
Long life expectancy	Short life expectancy
Young	Pathological femoral neck fracture
Good bone geometry	Problematic socket and shaft geometry
No osteoporosis	Osteoporosis
Good bone quality	Previous press-fit failure (revision)

2.3.1 Cemented Fixation

A cemented prosthesis is designed to have a layer of bone cement, typically an acrylic polymer called polymethylmethacrylate (PMMA), between the patient's natural bone and the prosthetic joint component as shown in Figure I-8.

1. Bone cement allows a surgeon to affix prosthetic joint components to bone that is slightly porous from osteoporosis.
2. Cemented fixation is more suitable for obese patients.
3. Cemented fixation has a three times lower risk for intra-operative femoral bone fractures.
4. Cemented fixation is less expensive than cementless fixation.
5. Cemented fixation has a better outcome in case of dysplastic hip in the risk of failure.
6. A small amount of antibiotics can be added to the bone cement, helping to decrease the risk of post-surgical infection.
7. The bone cement sets within 10 minutes of application, so the surgeon and patient can be confident the prosthetic is firmly in place.

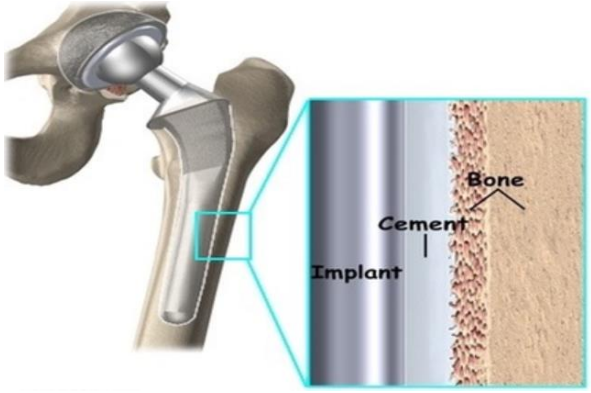

Figure I-8. There are a few advantages to using bone cement in joint replacement surgeries [15]:

The drawback to using bone cement is that it may degrade over time and bits of cement can break off, potentially causing problems [15]:

1. A breakdown of the cement can cause the artificial joint to come loose, which may prompt the need for another joint replacement surgery (revision surgery).
2. The cement debris can irritate the surrounding soft tissue and cause inflammation.
3. The cement debris can cause third body wear in the TJR.

4. While rare, the cement can enter the bloodstream and end up in the lungs, a condition that can be life-threatening. This risk is greatest for people who undergo spinal surgeries.

Exactly how often these complications occur following specific types of joint replacement surgeries is difficult to quantify. Not all patients with bone cement debris experience symptoms. Bits of cement debris can be removed arthroscopically to alleviate or prevent symptoms. In rare cases, patients have an allergic reaction to the bone cement and must undergo a second surgery to remove the cement and prostheses. [16]

2.3.2 Uncemented Fixation

A cementless prosthesis, also called a press-fit prosthesis, has a rough surface or porous coating that encourages the natural bone to grow onto it as shown in Figure I-9. New bone growth will span only 1 or 2 mm, so the surgeon must use special tools to shape the natural bone to fit snugly with the prosthesis. Some prosthetic components have screws or pegs that help hold the bone and prostheses in place until new bone growth can create a more secure attachment. For example, during some shoulder replacement surgeries, the new shoulder socket is backed with short pegs that fit into the patient's natural bone, thereby helping to stabilize the new prosthesis and the patient's scapula (shoulder blade). A number of surgeons prefer cementless components because [15]:

1. The cementless components offer a better long-term bond between the prostheses and bones. Therefore, the revision rate is lower than cemented TKA within ten years after operation.
2. Osteolysis from cement debris is avoided.
3. The risk for implant loosening is low. It has been implied that once stable fixation occurs in the cementless implant, it does not deteriorate with time.
4. The incidence of fat embolism during the operation is much lower.

5. During the process of cement polymerization, Bone Cement Implantation Syndrome (BCIS) may develop. The uncemented fixation is free of this complication.
6. Cementless components eliminate worry about the potential breakdown of cement.

However, the downsides to cementless prostheses are that [15]:

1. Press-fit prostheses require healthy bones. Patients with low bone density due to osteoporosis are contraindicated for these components.
2. It can take up to three months for bone to grow into a new joint component.

Because it takes time for the natural bone to fully adhere to the new joint components, experts debate whether or not patients should postpone putting their full weight on new joints. This limits and delays a patient's return to activities of daily living, a requirement for health and wellness.

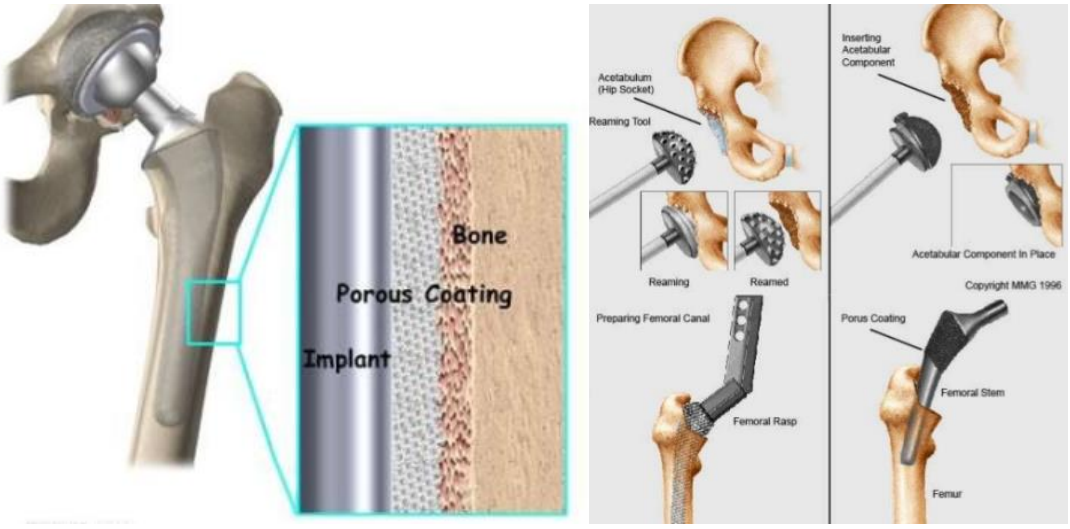

Figure I-9. Cementless hip replacement.[15]

3. Bone Cement

3.1 Historical Perspective of Bone Cement

Themistokles Gluck (1870), led the way in his development of a hip implant fixation. He produced an ivory ball and socket joint that he fixed to the bone with nickel plated screws. He also used a mixture of plaster and colophony. In 1902, German chemist Otto Rohm first described polymethylmethacrylate (PMMA), which was commonly known by its trade names of Plexiglas and Perspex. Industrial-size chemical synthesis of methylmethacrylate (MMA) was achieved in the 1920s in the laboratories of Rohm and Hass and the first biomedical application of PMMA was the fabrication of dentures. In the 1930s it was discovered that the mixing of MMA monomer and benzoyl peroxide initiator with pre-polymerized PMMA powder resulted in the formation of a dough-like material which could slowly harden into a glassy polymer. This two-component polymer was initially used to close cranial defects. [17,18] Due to its inherent transparency, strength, and stability in the early 1930s, PMMA was widely used by a variety of industries. The era of modern PMMA bone cements comes from the patent by Degussa and Kuzler (1943), who had described the mechanism of polymerization of methyl methacrylate (MMA) at room temperature ('cold-curing') if a co-initiator, such as a teriary aromatic amine, is added [19]. This cold-curing PMMA became a widely used material in the dental industry where it saw considerable use as a cement to fix metal dental post crowns into the root canal.

In the late 1950s and early 1960s, English surgeon John Charnley used bone cement in total hip arthroplasty. He used cold cured PMMA to attach an acrylic cup to the acetabulum and to seat a metallic femoral prosthesis. This was a significant milestone in the advancement of orthopedic surgical procedures. Charnley was the first to realize that PMMA could easily be used to fill the intramedullary canal and interdigitate with the patient's bone, thus allowing for

immediate implant fixation and stress transfer between the implant and bone. Charnely investigated a wide variety of dental acrylic cements and was focused on issues relating to residual monomer content, toxicity, and the static/dynamic mechanical properties. [17,18]

In the 1970's, the U.S Food and Drug Administration (FDA) approved bone cement for use in hip and knee prosthetic fixation. Since then, while bone cement has become widely used for fixation of protheses to living bone, the trends of bone cement usage have evolved including the addition of antibiotics.

3.2 Chemical Properties of Bone Cement

3.2.1 Chemical composition of bone cement

The MMA monomer consists of two carbon atoms. One carbon atom bound to two hydrogen atoms and the other attached to methyl and acrylic groups through a covalent bond. The PMMA polymer is produced by polymerization of MMA. Hardened acrylic bone cement consists of linear, uncross-linked PMMA macromolecules of various lengths ranging from few tens to millions grams per mole. Acrylic bone cements consist of two primary components, a powder consisting of copolymers based on PMMA normally included in a 40 g package, and MMA liquid monomer in a 20 mL ampoule, mixed at ratio of 2:1 by weight to form cement (**Table *I-2***).

Table I-2. Commercial constituents of bone cement

Constituent	**Role**
Powder components	
Polymer	Polymethylmethacrylate
Co-polymer (e.g. MA-MMA)	Alter physical properties of the cement
Barium sulphate or Zirconium dioxide	Radio-opacifiers
Antibiotics*	Antimicrobial prophylaxis
Dye (e.g. chlorophyll)	Distinguish cement from bone
Liquid components	
Monomer	Methylmethacrylate monomer

N,N-dimethyl-p-toluidine (DMPT)	Initiates cold curing of polymer
Benzoyl peroxide	Reacts with DMPT to catalyze polymerization
Hydroquinone	Stabilizer preventing premature polymerization
Dye (e.g. Chlorophyll)	Distinguish cement from bone

The powder is a variable component in composition of bone cements from several brands, contributing to differences in properties (Figure I-10). The powder component mainly consists of prepolymerized PMMA beads with a diameter of 10 to 150 µm, contributing to 85% to 99% of the powder. The prepolymerized beads of different bone cements contain copolymers of MMA with styrene, methyl acrylate, or butyl methacrylate comonomers. The remaining components contain a radiopacifier, either barium sulfate (BaSO4) or zirconium dioxide (ZrO2), as well as an initiator, benzoyl peroxide. The MMA monomer is able to self-polymerize under exposure to heat and light, although polymerization is very slow. Therefore, dibenzoyl peroxide (BPO) plays a role as reaction initiator in powder form. The other initiator, radiopacifier, and antibiotic powders are also included in the powder component. [20]

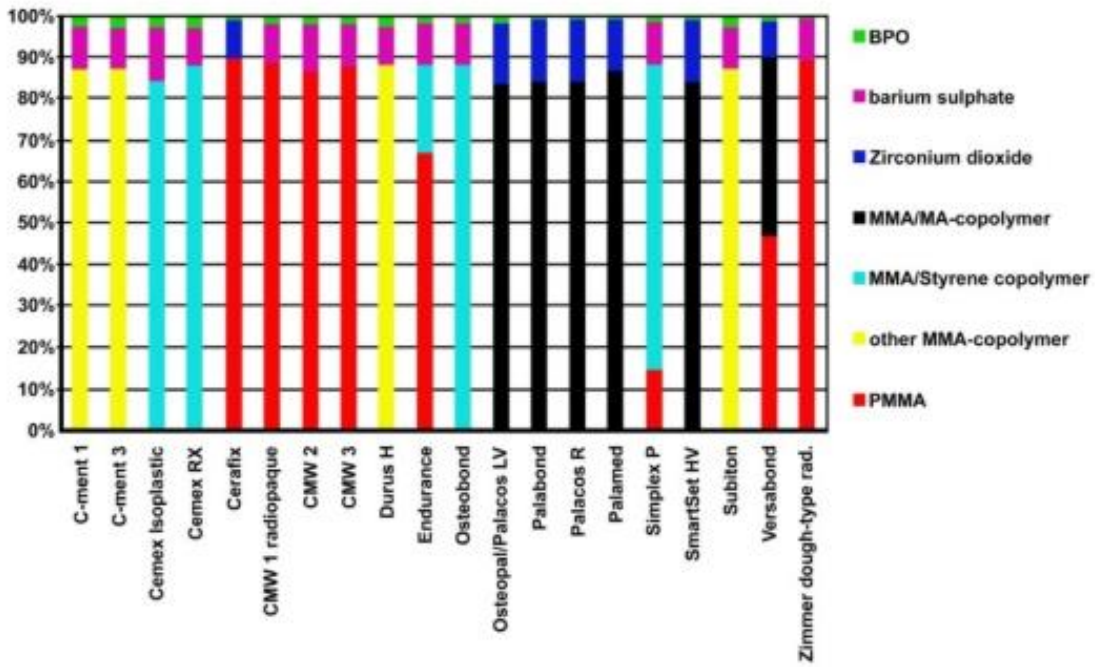

Figure I-10. The composition of the powder of several PMMA bone cements on the market. [21]

The monomer, a transparent liquid with a unique odor, is packaged in 20 mL ampules. 97% to 99% of this liquid consists of MMA. N,N-dimethyl-para-toluidine (DMPT) makes up 0.4% to 2.8% by weight and acts as an accelerator to speed up the polymerization and setting of the cement. Since MMA can spontaneously polymerize during storage, addition of trace amounts of a stabilizer, usually hydroquinone (15 to 75 ppm), stabilizes and prevents premature polymerization of monomers. The MMA is polymerized by the mechanism of free radical polymerization, which consists of three steps: initiation, propagation, and termination. [20]

3.2.2 Polymerization

The DMPT in the liquid component starts decomposing BPO from the powder when the two components are mixed (Figure I-11). The polymerization consists of three steps: initiation, propagation, and termination. The initiation step involves decomposition of BPO monomer into radicals at room temperature. The second step of the free radical polymerization is chain propagation in which the benzoyl radical reacts with the MMA monomer. The free radical attacks one of the double bonds of the MMA monomer. One electron of the double bond pairs up with the electron of the free radical to form a bond between the oxygen of the benzoyl free radical and one of the carbon atoms of the MMA monomer while the second electron of the double bond shifts to the other carbon atom, which then turns into a free radical. This free radical then attacks another MMA monomer and the chain propagates until a PMMA of relatively high molecular weight is achieved. Finally, chain termination can be achieved by chain coupling. [20]

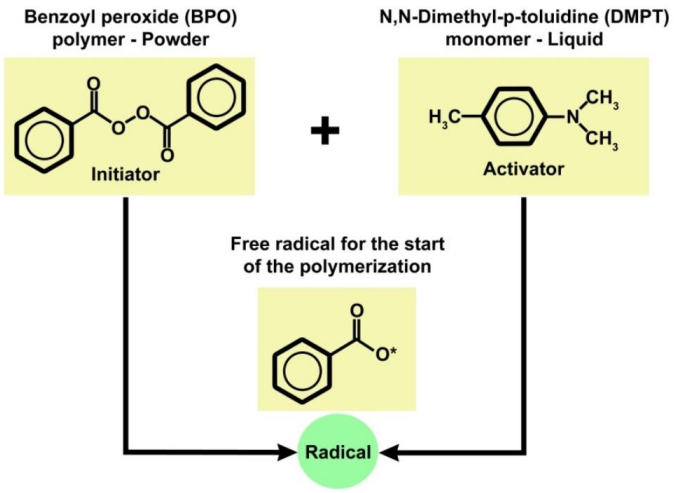

Figure I-11. The initiation of the polymerization of MMA: BPO from the powder and DMPT from the liquid react to form radicals, starting the curing of bone cements.

There are several reasons for using a two-component bone cement instead of simply polymerizing pure MMA monomer. The polymerization of MMA monomer is too slow and can take several hours or days, depending on the type and amount of reaction initiator used. Pure MMA monomer also has a very low viscosity and can easily diffuse into the blood stream, which can lead to cardiorespiratory and vascular complications. Furthermore, the heat of polymerization can easily increase the temperature of the cement to over 100°C (boiling point for MMA = 100.3°C), which could lead to boiling of the volatile MMA monomer. The use of less monomer and the presence of prepolymerized PMMA beads in the powder decreases the number of polymerization reaction and hence, the amount of released heat and assists in heat dissipation, decreasing the overall temperature. In vitro tests have shown polymerization heats of ≥ 80 ℃ for several common antibiotic cement (Palacos G, CMW G and Antibiotic Simplex). These temperatures are not associated with inactivation of antibiotics commonly commercially mixed in cement such as gentamicin and tobramycin.[22] Other antibiotics destined to be mixed with the cement must be chemically and thermally stable. [23]

3.3. Processing and Handling of Bone Cement

The handling characteristics and setting times of acrylic cements are of great importance for orthopedic surgeons. The handling of bone cements can be described by four different phases, such as the mixing phase, the waiting phase, the working phase, and the hardening phase.

The mixing phase (up to 1 minute) is the period during which the powder and the liquid are homogenized thoroughly. The powder and the liquid can be mixed manually by using a bowl and a spatula or by a special mixing system, applying vacuum to avoid the formation of voids. The waiting phase (up to several minutes, according to the type of cement and the handling temperature) is the period to reach a non-sticky state of the cement. During the waiting period swelling of the beads occurs and allows the polymerization to proceed, leading to an increase in viscosity. At this stage, the cement turns into a sticky dough.

The working phase (2–4 minutes, according to the type of cement and the handling temperature) is the period during which the surgeon can inject the cement and insert the prosthesis. The working period begins when the cement is no longer sticky but of sufficiently low viscosity to permit the surgeon to easily apply the cement into the prepared place. During this period, the chain propagation continues, along with an increase in viscosity. The viscosity of the cement must be carefully assessed before inserting the cement because with a very low viscosity the cement would not be able to withstand the blood pressure. This would result in blood lamination in the cement, which can weaken the cement. The heat produced during this period, results in thermal expansion of the cement. On the other hand, there is a volumetric shrinkage of the cement as the MMA monomer converts into the denser PMMA polymer.

The hardening phase (1–2 minutes) is the period of the final setting process and the development of the polymerization heat. The temperature of the cement continues to elevate during this period and then slowly decreases to body temperatures. During this period, the

cement undergoes volumetric shrinkage along with thermal shrinkage as the cement cools down to body temperature. While the manufacturer can determine the hardening period length using in vitro measurements at a controlled temperature and humidity in a laboratory environment, it is difficult to predict the hardening period in vivo, with accuracy due to variations in the ambient environment in the operating room, the body temperature, and thickness of the cement mantle, all of which can alter the setting times of the cement. Several factors, such as the type of mixing method used, the viscosity of the cement, the precooling of the monomer and/or powder, the preheating of the powder component, and the preheating of the prosthesis, can also significantly alter the times of some of the handling phases. Thus it is important for the surgeon to know each of the factors that can alter the duration of each phase.

In the standards ASTM F 451, there are two further time parameters defined: the doughing time and the setting time. The doughing time ends by the beginning of the working phase, and it is determined by recording from the start time of mixing until the mixture is able to separate cleanly from a gloved finger. The second time parameter is the setting time, which is defined as the time to reach a temperature midway between ambient and maximum. The end of setting time marks the final hardening of the bone cement.[1–3]

3.4 Mixing Techniques

The method of mixing is considered one of the most important factors in determining the size of flaws. Historically, three methods of mixing of cement have been employed: (a) hand mixing in air; (b) hand mixing followed by centrifugation; and (c) hand mixing in an evacuated mixing device, commonly known as “vacuum mixing.” [24–26] Hand mixing involves mixing the liquid and powder components in an open bowl using a spatula at a speed of 1 to 2 Hz for a period of duration of approximately 2 minutes. The hand-mixing method can introduce a

porosity of 7% or higher. [26] It has been confirmed that excessive mixing can lead to increased porosity. By decreasing the number of beats and waiting for a short duration after wetting the powder component with the monomer the porosity of cement can be reduced to approximately 5%. [27] Centrifugation is another method to eliminate pores. In this method, the liquid and powder components are initially hand mixed and then placed in a tube and subjected to centrifugation at a speed of 2300 to 4000 rpm for a duration of 0.5 to 3 minutes. With this method the total porosity decreases to 1% or less, which is significantly lower than the porosity observed in hand mixing. [11, 13] It is obvious that for centrifugation to be effective, the viscosity of the cement must be relatively low, allowing the air bubbles to flow to the surface of the cement under the centrifugal force. One way to assist centrifugation is to chill the MMA monomer prior to mixing. [28] One potential disadvantage of the centrifugation mixing technique is that it can lead to an inhomogeneous distribution of radiopacifier particles in the centrifuged cement, due to the difference in density of radiopacifier particles and PMMA and MMA monomer.

The third type of mixing technique is vacuum mixing, in which the two components of bone cements are placed in a mixing bowl and are mixed after subjecting the bowl to vacuum conditions. The vacuum mixing devices have proven to substantially decrease porosity in cements to less than 1% and consequently to increase their fatigue properties. Another major reason for using vacuum mixing is that MMA monomer is contained within the mixing bowl, which limits exposure to its vapors. Toxicology information obtained from materials safety data sheets (MSDSs) show that MMA monomer is harmful if inhaled, swallowed, or absorbed through the skin. [26] It must be noted that this reduction in the porosity of cement mantle with vacuum-mixing, is not always the case. Messick et al. found that vacuum-mixed cement does not result in overall lower porosity of the cement, but the distribution of porosity may be different

when compared with that of hand-mixed cement. It has also been demonstrated that very high vacuum levels can be associated with the presence of cracks in the cement. [29]

Antibiotics are mixed with 40 g of bone cement polymer prior to the addition of the monomer component and mixed by hand-shaking for 1 min in a syringe and then liquid monomer is added into the antibiotic cement powder. Another method is liquid mixing. Hsieh et al. used this method to mix an antibiotic with the bone cement. 2 g of antibiotic powder is added in 12 ml of the of the distilled water and vortexed for 30 seconds before mixing with bone cement. [30]

3.5 Cement Morphology

The composition of hardened cement consists of prepolymerized beads of PMMA or their copolymers fused with the polymerized MMA monomer, in addition to radiopacifiers and additives, such as powders of antibiotics, as well as pores or voids and residual initiator. These parts of a cement composition act as flaws. Larger particle sizes result in greater pore sizes and pore depths on the construct surface.[31] Other causes of flaws are: (a) air dissolved within the powder particles; (b) air entrapment during mixing of powder and liquid monomer; (c) incomplete fusion of prepolymerized PMMA beads with the setting MMA; (d) evaporation of the volatile monomer due to the heat of reaction during setting; (e) air entrapment during transfer of the dough to the gun; and (f) air entrapment during introduction of cement into the medullary canal.

The major problem associated with the presence of flaws is that when a critical flaw size is achieved, the flaws act as sites of stress concentration, leading to weakening of the cement. But if the critical flaw size is not reached, the pores blunt cracks in the cement upon its fracture. In other words, the cracks deviate from their path when they encounter flaws in their path

associated with pores and radiopacifier particles. The Griffith crack criterion assumes that there exists a critical flaw size unique to each material above which its fracture strength is compromised. [32] For PMMA, the critical flaw size is 70 μm. Thus, if the pores are smaller than the critical flaw size for PMMA, the porosity will not compromise the fracture strength of bone cement. It is generally well known that hardened bone cement contains macropores (pore diameter greater than 1 mm) and micropores (pore diameter 0.01-1.0 mm). Based on the Griffith theory, elimination of the macropores would be more important than elimination of the micropores, especially the pores much smaller than 70 μm in diameter.

Another source of flaws that can be potential sites of high stress concentration comes from challenges on using radiopacifier powder. A radiopacifier powder, usually barium sulfate or zirconium oxide, consists of particles with a broad range of sizes, from approximately 0.2 to 2 μm in diameter. Zirconium oxide is a harder material than barium sulfate. Thus, if there is implant loosening, there is a risk of third body abrasive wear in the bearing surface of the joint replacement. Barium sulfate is generally insoluble, but there are concerns about toxicity of barium ions. Poor spread of radiopacifier particles in the region between the prepolymerized cement beads can affect both crack initiation and crack propagation, especially if they are larger than the critical flaw size for PMMA. The radiopacifier particles do not bond with PMMA and instead reside within pores, which are larger than these particles due to cement shrinkage.

3.6 Mechanical and Physical Properties

Mechanical properties of acrylic bone cement are important factors to determine cement stability and strength *in vivo*. The main function of acrylic bone cement is to provide a stable fixation at the implant site. The cement must endure considerable cyclic stress to guarantee long-term fixation of the implant. The cement layer has the effect of an elastic buffer between

prosthesis and bone. Because of its close adaptation to bone and its viscoelastic properties, it can reduce the stress concentration. The mechanism of loading is especially complex for hip arthroplasty. The total load affecting bone cement is a mixture of compressive loading combined with bending, tension, shear, and torsion. It has been extremely difficult to simulate this complex situation. Two mechanical tests have been introduced into ISO 5833 for testing acrylic bone cements. These tests are the compression and the four-point bending test for the determination of the flexural modulus, flexural strength, compressive modulus, and compressive yield strength. The standard ASTM F 451 includes the compression test only. Generally, there are two different fundamental measuring principles to determine mechanical properties of bone cements: applying static (also called quasi-static) stress and dynamic stresses. Static tests are destructive tests with uniaxial single loading, increasing until failure, in contrast to dynamic tests that involve cyclic loading.

3.6.1 *Flexural and Compressive Properties*

The compression and bending test are destructive tests with a uniaxial single loading, increasing until failure. The compression test, according to ISO 5833 and ASTM F 451, is a static method in which the compression strength is defined as the maximum strength that a material can withstand before failure in compression. The minimum requirement for compression strength in both standards is 70 MPa. [33,34] The four-point bending test, according to ISO 5833, is also a static method for determining the flexural modulus and strength. The minimum requirement for the flexural modulus is 1800 MPa and 50 MPa for flexural strength. All commercial cements must fulfill these mechanical property requirements. Flexural modulus represents the ratio of stress to corresponding strain of the material within the elastic region characterizing the relative stiffness of the material in bending. Stiff materials have a high

modulus; and compliant materials have a low modulus. Stress and strain are proportional within the elastic region and if load is released, the material recovers its initial dimensions. The elastic region is limited by a stress limit, which is called “proportional limit”. When an applied force results in a stress that exceeds this limit, the material might not recover its initial shape. The modulus of elasticity of bone cement is two orders of magnitude lower than the modulus of the high strength steel prosthesis and one order of magnitude lower than the modulus of cortical bone. The compressive yield strength or ultimate strength of bone cement is usually higher than the flexural strength and that is higher than the tensile strength. This means tensile loading may be a higher risk factor for failure than compressive loading. Complex combinations of different loading types are also relevant. From physical point of view, bending is a combination of compressive and tensile loading; therefore, the bending test is a relevant test. [35]

3.6.2 *Tensile Properties*

Bone cement is susceptible to fracture that might result from tensile loading although bone cement has a high compressive strength. Tensile testing is carried out according to ISO 527-1 or ASTM 638. These standards describe a static test method applicable for all polymer materials. The flat tapered (“dog bone”-shaped) specimens are tested under the uniaxial tensile loading. The ultimate tensile strength is defined as the maximum stress that a material can withstand before failure in tension. Harper and Bonefield investigated ten different commercial bone cements without additives. [36] They found tensile strength was approximately 50 – 60 MPa and there were no significant differences between the tested cements.

3.6.3 *Shear Properties and Implant-Cement Interface Properties*

One further static test applied with bone cements is the shear strength test according to ASTM D732. Failure of the cemented hip stems is typically initiated by debonding of the stem-

cement interface. This debonding may occur as a result of excessive shear or tensile stresses at the interface or combination of these two loading types. The interface static shear strength is influenced by surface roughness, cement type, and porosity. Surface finish has the greatest effect on the interface strength. Increasing the surface roughness increases the interface shear strength. Cement type and porosity have a minor influence on the static interface strength. [37]

3.6.4 *Fracture Toughness*

Test methods to determine the fracture properties and impact strength are described in ASTM E399/ISO 13586. There is *ex vivo* evidence to infer that cement containing discontinuities is the weak-link in cemented hip implants. The initiation of long-term aseptic loosening begins with this weak-link.[38] Pores are well known to make the cement prone to fracture, thus threatening the life of the prothesis. The fracture toughness of the cement is clearly an important property reflecting on the reliability and defect tolerance of the cement. Cement with a crack loaded in tension or three-point bending cracks predominantly open in mode I and produces linear elastic conditions. The fracture toughness is obtained *in vitro* as the critical value (plain strain) of the mode I stress intensity factor (K_{IC}). K_{IC} is markedly affected by porosity. This is because K_{IC} of a nonhomogeneous material is a function of both materials and structure, unlike homogeneous materials.

3.6.5 *Fatigue Properties*

Bone cement must be able to withstand repetitive loading to ensure long-term survival of the cement in the human body. The vast majority of normal daily activities such as walking and running involve subjecting bone cement to many cyclic loading patterns. Fatigue testing is a dynamic test and is executed with sinusoidal cyclic loading under stress or strain control. The tests are continued until failure or until run out. The run-out limit is a predetermined number of

cycles at which the testing on a specimen is stopped. One method is equivalent to tensile testing according to ISO 527. Again, the test run is performed with a sinusoidal cyclic with a stress ratio of 30MPa (max)/0.03MPa(min) until failure or until run-out. Another method is according to ASTM F2118. The specimens are subjected to fully reversed compressive and tensile loading in a sinusoidal cyclic manner. Again, the tests are continued until failure or until the run-out limit is reached. Most fatigue tests run at a specified frequency, e.g. 5 Hz, in phosphate buffered solution (PBS) at 37℃. [21] Four-point bending is the preferred method for fatigue testing. Bone cements have different fatigue behavior if tested dry or in aqueous solution. The tests in air at room temperature result in considerably higher fatigue strength than tests in aqueous solution.[21] The tests should be performed in an appropriate solution, such as simulated body fluid or Ringer's solution at 37°C for the body environment.

3.6.6 *Viscoelastic Properties*

Bone cements exhibit a combination of elastic and viscous behavior called viscoelasticity. When a polymer is subjected to a constant load, the resulting deformation can be divided into two parts: the immediate elastic deformation and continuous deformation. The immediate elastic deformation happens instantaneously by applying load. It is a recoverable deformation and independent of time. Following this rapid deformation, there is a delayed continuous deformation. One part of this deformation is recoverable in time after releasing load. This part is called delayed elastic deformation or primary creep. The second part of this continuous deformation is a non-recoverable permanent deformation called secondary creep.

Test methods are described in the standard ASTM D 2990 to measure creep. The test specimen is subjected to tensile, compressive, or bending loading. In each method the change in deformation of the specimen is measured and divided by the initial deformation for the

calculation of creep. All plastic materials including PMMA bone cement show creep behavior. The degree of creep depends on several factors, such as composition of the material, temperature, humidity, load type, and duration of time under load. Lee et al. proposed that creep of PMMA bone cement may contribute to loosening of cemented total joint replacements. [39] However, the long-term prosthesis subsidence rates caused by creep of PMMA bone cement are very small. [39]

3.7 Finite Element Method

The Finite element method (FEM) is a mathematical technique which allows stress and strains of complex, irregular shapes with varying internal properties to be modeled and simulated.[40] This makes it ideal to analyze the strength of antibiotic loaded bone cement (ALBC) which may be reduced from by increased porosity. The fracture toughness of ALBC after conditioning in saline solution is reduced due to the increased porosity [41,42]; however, others have found pores can also result in fracture toughening.[43,44] It is challenging to experimentally measure damage parameters locally and to distinguish how the fracture resistance is affected by local material or structural alterations. To understand the fracture toughness reduction or the toughening mechanism of bone cement, analyzing crack initiation and propagation is necessitated. A promising approach for this analysis is FEM.

There are two types of theoretical models for composite materials for analyzing crack propagation at an interface. The first is a strength-based model, as introduced by Cook and Gordon [45], where the difference in strength between interface and substrate (e.g. osteon in the case of cortical bone) dictates whether or not the crack will be deflected by the interface. The other method is an energy-based model where the crack path is determined by the maximum energy released by comparing different possible crack paths. [46] These ideas are combined in

cohesive damage models that account for both strength and energy when modeling crack propagation. Parmigiani and Thouless used cohesive finite elements to show that both strength and energy can be important for the crack trajectory at an interface. [47] In the biomechanics field, Mischiniski and Ural modeled crack propagation in cortical bone by outlining two possible crack paths with cohesive elements: one penetrating the osteon and the other deflecting along the cement line. They concluded that low cement line strength was the most critical factor for promoting crack deflection. [48]

Another option for modeling crack propagation is the extended finite element method (XFEM) which has the primary benefit of not requiring a predefined crack path. [49,50] In the biomechanics field, a handful of XFEM models have been used to model crack propagation in 2D at the microscale in cortical bone, and the maximum principal strain (MAXPE) criterion has been commonly used to model damage initiation with cohesive cracks.[51–54] However, the MAXPE criterion cannot be used to model realistic crack trajectories around osteons, as it always predicts crack penetration of the cement line interface.[55] Extended finite element method has not yet been applied to investigate crack propagation in porous bone cements, for example in antibiotic loaded bone cements.

3.8 Antibiotic Loaded Bone Cement

Bucholz and Engelbrecht were the first to add gentamicin antibiotic to a bone cement.[56] Bone cement loaded with antibiotic is widely available as a treatment of antimicrobial prophylaxis in primary arthroplasty. It was shown later that other kinds of antibiotics such as oxacillin, cefazolin and gentamicin are all stable in PMMA bone cement. The largest release of antibiotics occurred in the first 24 hours, but bactericidal concentrations were measured up to 21

days after implantation. Bone cement without any antibiotics had no bacteriostatic effect on *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* organisms.[57]

Bone cement can play a role as matrix for the local application of antibiotics. The use of bone cements has great advantages compared to a systemic antibiotic therapy because of the high local concentration of antibiotic surrounding the implant. The implant is especially sensitive to bacterial contamination on its surface, because the microorganisms may proliferate on the surface creating a biofilm resistant to antibiotics. As the bacteria rapidly generate a protective mucus layer and keep an inactive state with low sensitivity to antibiotics, a local antibiotic treatment is important. Pharmacokinetics of the antibiotic release from the matrix is clinically important. The local antibiotic concentrations reached at the infected site must be clearly greater than the minimal inhibitory concentration and the minimal bactericidal concentration for the organisms. However, not all antibiotics are suitable for addition into bone cements. The following bacteriologic, physical, and chemical factors should be considered in the choice of an antibiotic:

1. good release from bone cement;
2. chemical and thermal stability;
3. good solubility in water;
4. good antibacterial effect in low concentration; and,
5. broad antibacterial spectra, including gram-positive and gram-negative organisms.

In the early 1970s, gentamicin was the favorite antibiotic among surgeons and the most common additive for bone cements because of a wide range of spectrum for bacterial activity, thermal stability, and high water solubility.[20] The diffusion rate of the antibiotics depends on several factors, such as the chemical composition of the cement, the surface area at the cement-

bone interface, and cement handling. For example, a study by Perry and co-workers found that Palacos® R containing gentamicin tended to elute more rapidly than that of Simplex™ P. In addition, vacuum mixing, which decreases the porosity in the bone cement, can also alter the kinetics of the elution of antibiotics and was shown to decrease their rate of elution by 50%. [58]

Penner et al. investigated the release of vancomycin and tobramycin from bone cement separately or combined in non-vacuum preparations. They observed that the combined use of the two antibiotics led to an increased elution of both antibiotics from the bone cement. Baleani et al. also showed that the presence of meropenem broadened the antibacterial spectrum and enhanced the elution amount of vancomycin from the bone cement. [59]

Many researchers also investigated the effects of addition of liquid gentamicin to bone cement because gentamicin powder is expensive. The liquid gentamicin, a much less costly antibiotic (1/20 the price of tobramycin) with a broad antimicrobial spectrum, is widely available throughout the world, but the addition of liquid gentamicin reduces the mechanical properties of the bone cement. [60] Hsieh PH et al. studied the use of liquid gentamicin, alone and in combination with vancomycin, incorporated into acrylic bone cement as a potential treatment of complex orthopedic infections. They evaluated the bone cement loaded with liquid gentamicin, vancomycin powder, and the combination of both antibiotics for the elution properties, bioactivity, and compressive strength and porosity. The eluted amount of vancomycin was enhanced by 146% with the addition of gentamicin liquid, and gentamicin elution was also increased by 45% when combined with vancomycin. Bioassays confirmed the bactericidal activity of the released antibiotics. The addition of liquid gentamicin increased porosity, whereas adding vancomycin did not. Compressive strength decreased by 13%, 37%, and 45% in specimens containing vancomycin, liquid gentamicin, and both antibiotics, respectively. Despite

the inferior mechanical properties, the temporary nature of cement beads and spacers makes the liquid gentamicin-vancomycin mixture potentially more cost effective in bone cement to treat musculoskeletal infections. [30]

Other antibiotics such as cefazolin, ciprofloxacin, linezolid, levofloxacin and rifampin have been also tested for their elution and bactericidal activities. Results found that these antibiotics may be suitable for incorporation into polymethymethacrylate for management of orthopedic infections. [61] However, not all antibiotics are suitable for adding to bone cement. Goss et al. have shown that amphotericin B does not elute from bone cement, although the mechanical strength was increased by forming covalent crosslinks in the PMMA matrix. [62].

Antibiotic premixed into the cement by the manufacturer can be advantageous because the addition of powder by hand-mixing can lead to agglomeration and a decrease in the mechanical strength of the cement due to interference with the early stages of polymerization of the MMA monomer. The amount of antibiotic powder required for a therapeutic level of elution is approximately 0.5 to 2 g in a standard 40 g package of pre-polymerized PMMA powder. An excess amount of added antibiotic may result in defects or flaws in bone cement. A reason for this is that the undissolved antibiotics agglomerate and exceed the critical flaw size for bone cement. However, doses of 2 g of well-dispersed antibiotic powder may not have any adverse effect on the mechanical properties of bone cement if the size of the inclusions remains below the critical flaw size for bone cement.

II. OBEJCTIVES

Study Objectives

The overall goal of this work was to use two different antibiotics, vancomycin and telavancin, to enhance the properties of acrylic bone cements, with particular emphasis placed on improving the mechanical properties, cumulative antibiotic elution, and antimicrobial properties. For all testing conducted in this thesis, commercially available cements, Palacos® R and Simplex™ P, were used. These cements are the most widely used in North America and Europe and were thus deemed an appropriate model to study. To this end, three primary objectives are identified along with several specific aims for each.

Objective 1 – Evaluate the antibiotic release, antibacterial properties and mechanical properties of bone cements when traditional antibiotic, vancomycin, was incorporated into the bone cements Palacos® R and Simplex™ P.

- Quantify the impact of vancomycinon the flexural, compressive and fracture toughness properties of Palacos® R and Simplex™ P.
- Evaluate the cumulative vancomycin release from Palacos® R and S Simplex™ P.
- Investigate the antimicrobial/antibiofilm activity of Palacos® R and Simplex™ P.

Objective 2 – Determine the feasibility of using telavancin as an alternative to traditional antibiotics in acrylic bone cement.

- Determine the impact of telavancin on the mechanical properties of Palacos® R and Simplex™ P.
- Measure the cumulative telavancin elution from Palacos® R and Simplex™ P.
- Evaluate the antimicrobial/antibiofilm activity of Palacos® R and Simplex™ P.

Objective 3 – Assess the impact of porosity on the mechanical properties of bone cement using experimental and computational methods.

- Investigate the sizes of pores and their distribution on bone cements using scanning electron microscopy (SEM) and micro computed tomography (μ-CT).
- Analyze the effect of pores using the X-FEM crack propagation simulation method with different sizes of pores and locations.
- Compare predicted force-displacement behavior and fracture toughness with the experimental results of Objective 2.

III. MANUSCRIPT #1

Vancomycin Elution, Activity and Impact on Mechanical Properties when Added to Orthopedic Bone Cement

Published at Journal of the Mechanical Behavior of Biomedical Materials

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® R was impregnated with incrementally larger clinically relevant weight percentages of vancomycin. Vancomycin is a treatment standard for invasive gram-positive infections, and Palacos® R 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® R 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® R 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® R 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*

Introduction

Total joint replacement is an effective and common procedure that continues to increase in prevalence. [63,64] 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. [65] 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. [66,67] Antimicrobial susceptibilities of bacteria within biofilms can be reduced by 10 to 1,000 fold compared to those in a non-adherent planktonic state. [68] 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. [69–71] *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. [72–74] Methicillin-resistant *S. aureus* (MRSA) account for nearly half of *S. aureus* infections, which limit potential treatment options. [75] *S. epidermidis*, while not a virulent pathogen, can be equally antibiotic resistant and is attune to site colonization and vigorous biofilm formation.[72]

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

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Abbreviations: a_c crack length; a distance between inner and outer supports; b specimen width; 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 specimen; HPLC high performance liquid chromatography; K_{IC} mode I fracture toughness; L distance between lower supports; MIC minimal inhibitory concentration; MRSA Methicillin-resistant *S. aureus*; PMMA poly(methyl methacrylate); THA total hip arthroplasty; TKA total knee arthroplasty; S_f flexural strength; S_Y compressive yield strength; x location of measured displacement.

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. [76–80]

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 [80], 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

Materials and Methods

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® R was used because it is one of the two most commonly used cements,

along with Simplex™ P. Palacos® R is one of the most commonly used bone cements for THA and TKA in the United States and Europe. [6,81]

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. [82]

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. [78] 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. [80] All subsequent mechanical testing was performed in open air at room temperature.

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) [33] with the only modification being the addition of the wet-curing process. Fracture testing and fracture toughness calculations were performed as previously described. [80] 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:

$$S_Y = \frac{4F_Y}{\pi d^2} \tag{1}$$

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

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:

$$S_f = \frac{3F_{max}a}{bh^2} \tag{3}$$

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

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 width, 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 [80]. The mode I plane strain fracture toughness, K_{IC} in MPa/m$^{1/2}$, was calculated with Equation 5 [83,84]:

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

Where f(x) is a dimensionless factor defined by Equation 6 with crack length, a_c:

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

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.[33]

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. [85] 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. [86]

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 [87], 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).[88]

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.

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.

Results

Mechanical Properties

All sample groups exceeded the minimum required flexural modulus required by ISO 5833. [33] 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 not statistically different from the controls. Flexural strength for the 2.0 g vancomycin group was significantly lower than that of the control and not greater than the ISO minimum requirement (Figure III-2).

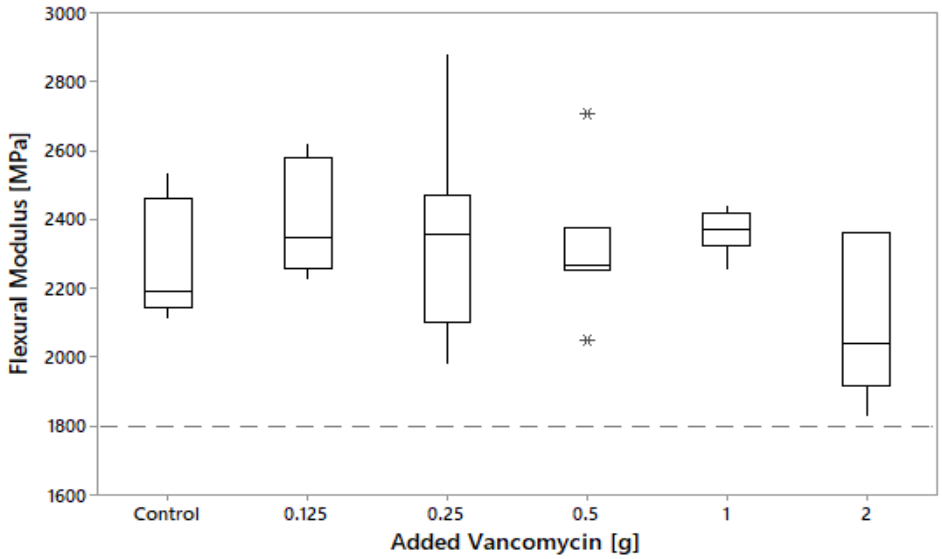


Figure III-1: Flexural modulus versus amount of vancomycin. The * represents outliers in the data set. Dashed line at 1800 MPa indicates ISO 5833 standard for minimum flexural modulus, which was significantly exceeded by all test groups. Graph shows median, first and third quartiles, and the lowest/highest datum within 1.5 interquartile lower/higher range.

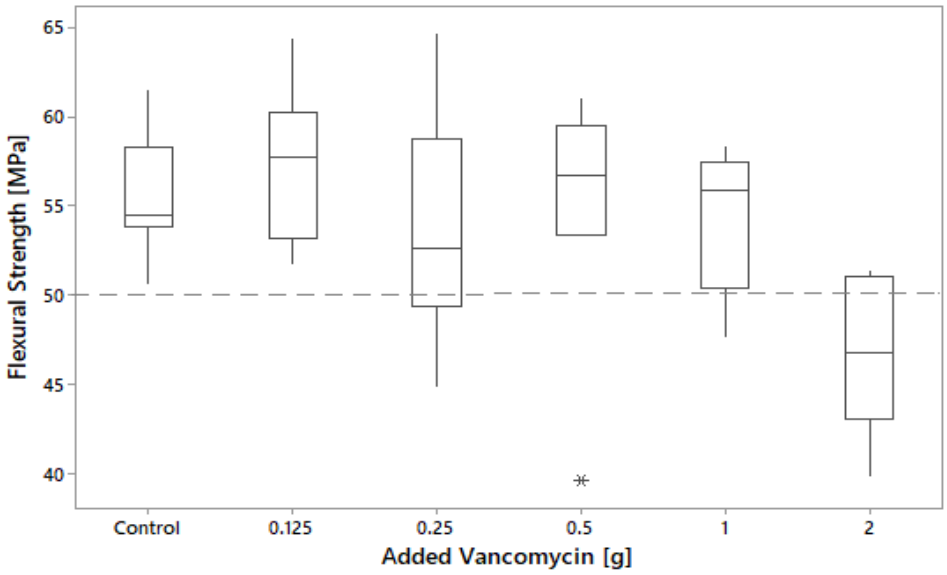


Figure III-2: Flexural strength versus amount of vancomycin. The * represents one outlier in the data set. Dashed line at 50MPa indicates ISO 5833 standard for minimum flexural strength. Flexural strength for the 2.0 g vancomycin group was significantly lower than the control (median 55.4 ± 3.53 MPa) 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 (Figure III-3 and Figure III-4). There is a visual trend that fracture toughness decreased with increasing amounts of vancomycin, but these were not significant due to sample-to-sample variability. All treatment groups were not statistically different from the control (median 2.23 ± 0.613 MPa/$m^{1/2}$) (Figure III-5).

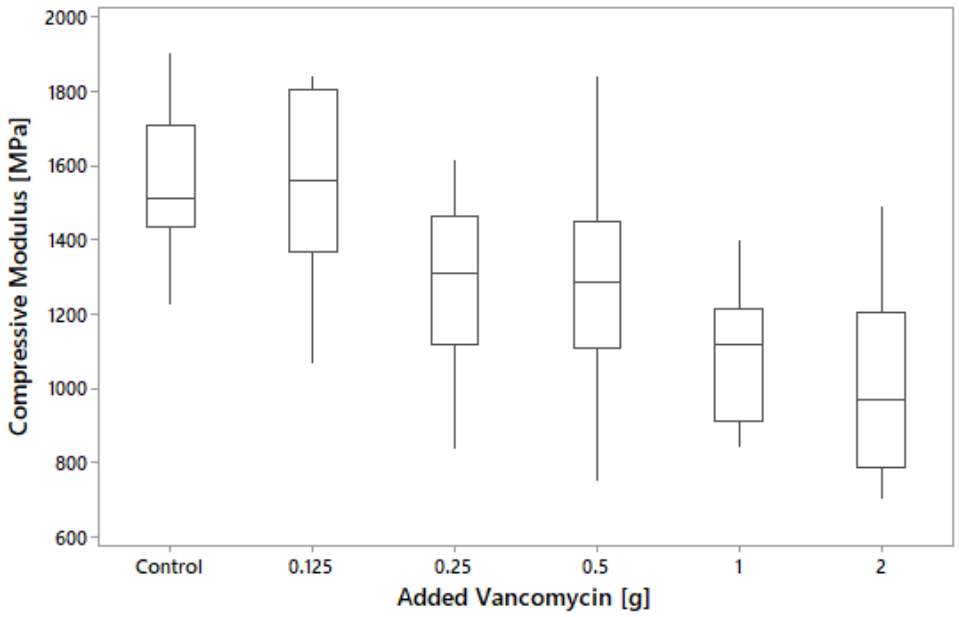


Figure III-3: Compressive modulus versus amount of vancomycin. Data showed a decreasing trend in compressive modulus with amount of vancomycin (R^2 = 0.814, S_{yc} = -279*($mass_{vanco}$) + 1470). Graph shows median, first and third quartiles, and the lowest/highest datum within 1.5 interquartile lower/higher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

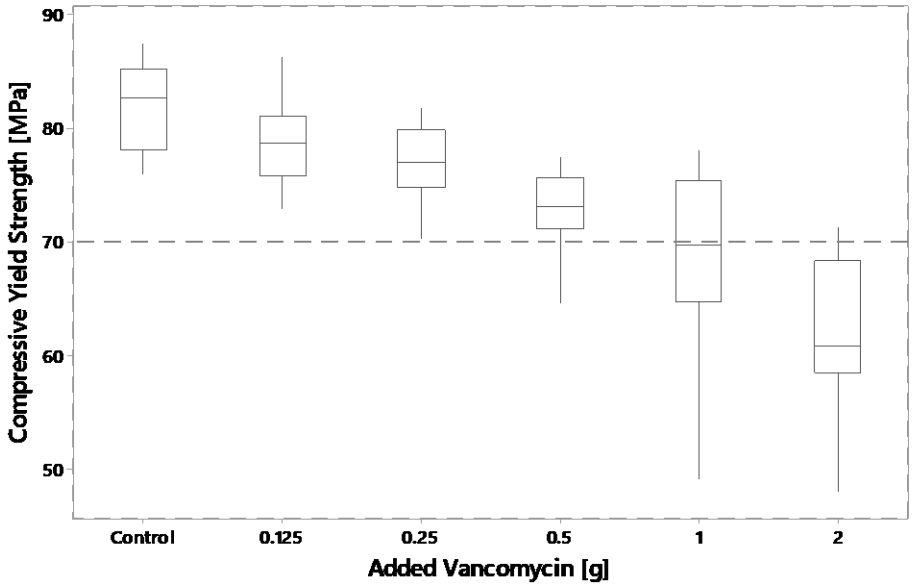


Figure III-4: Compressive yield strength versus amount of vancomycin. Dashed line at 70 MPa indicates ISO minimum requirement for compressive yield strength. Data showed a decreasing trend in compressive yield strength with amount of vancomycin ($R^2 = 0.945$, $E_c = -9.40*mass_{vanco} + 79.6$). Graph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

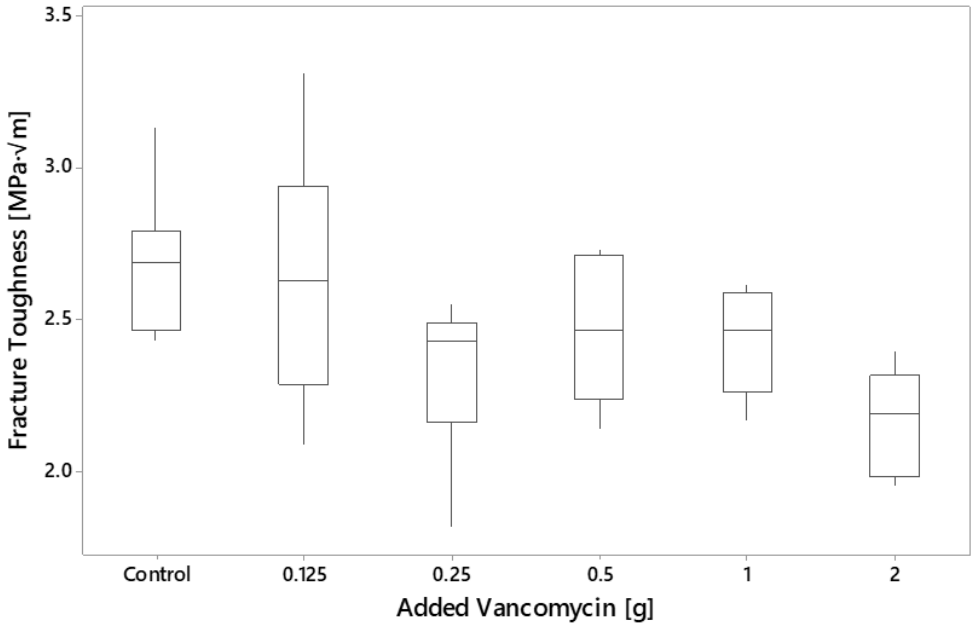


Figure III-5: Fracture toughness versus amount of vancomycin. All treatment groups were statistically not different than the control (median 2.68 ± 0.09 MPa/mm$^{1/2}$). Graph shows median, first and third quartiles, and the lowest/highest datum within 1.5 interquartile lower/higher range.

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 (Figure III-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 not statistically different from the 2.0 g samples. The 0.125 g samples released the highest percentage of total added vancomycin at 9.66%.

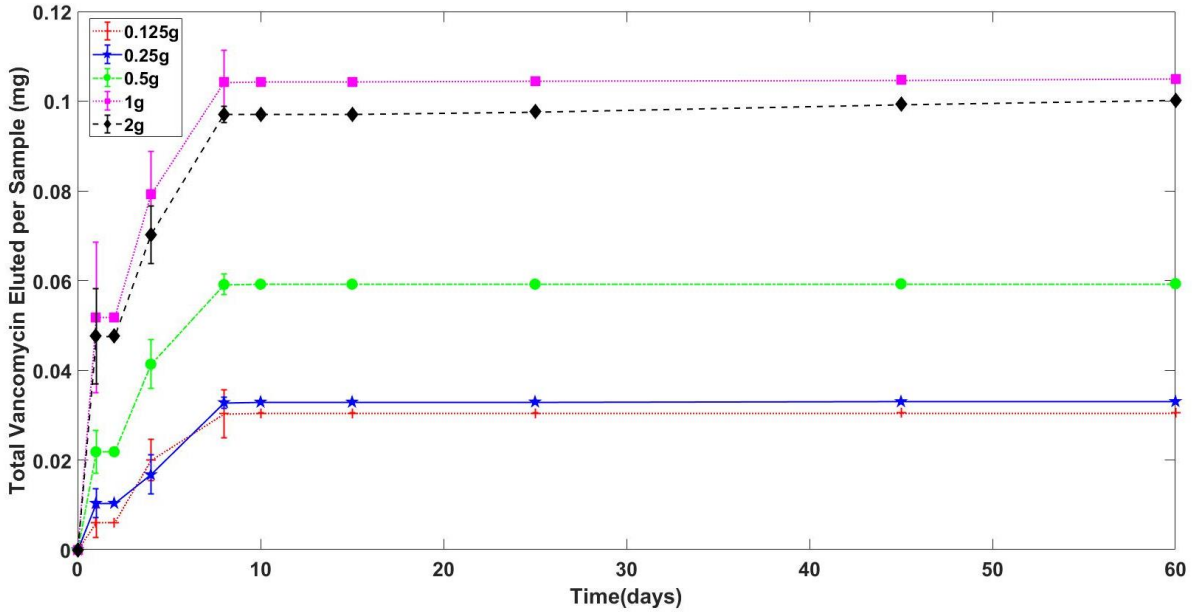


Figure III-6: Vancomycin elution (error bars show ± 1 standard deviation) from Palacos® R disks in PBS. 97% - 99% of 60-day elution occured in the first eight days.

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 (Figure III-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® R 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. (Figure III-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® R would likely not be effective at treating an active infection without additional systemic antibiotic use and implant removal.

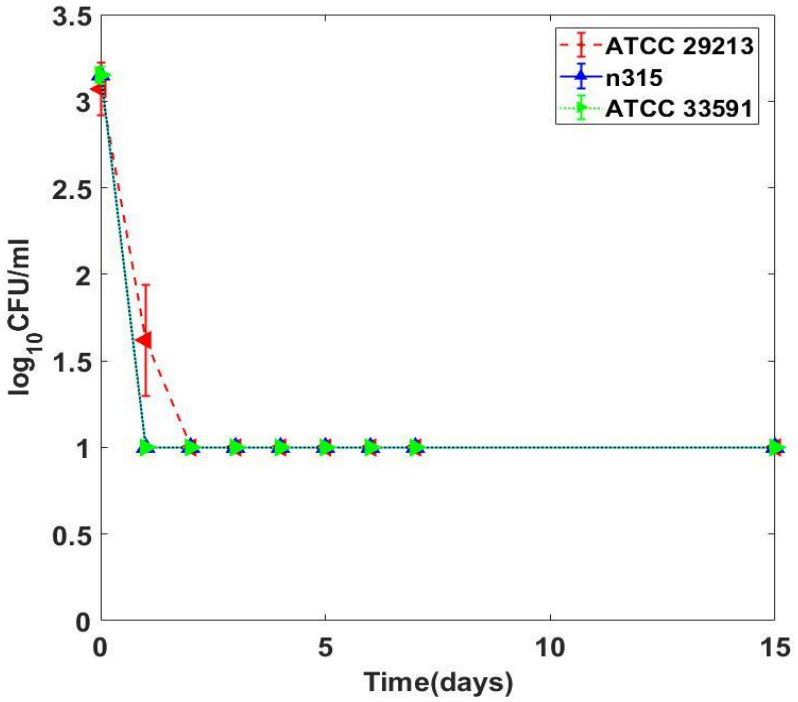


Figure III-7: Antimicrobial activity of eluted vancomycin (0.5 g) for three S. aureus strains. All bacterial colonies reduced below limit of detection by day 2 and no re-growth was observed. Error bars show ± 1 standard deviation.

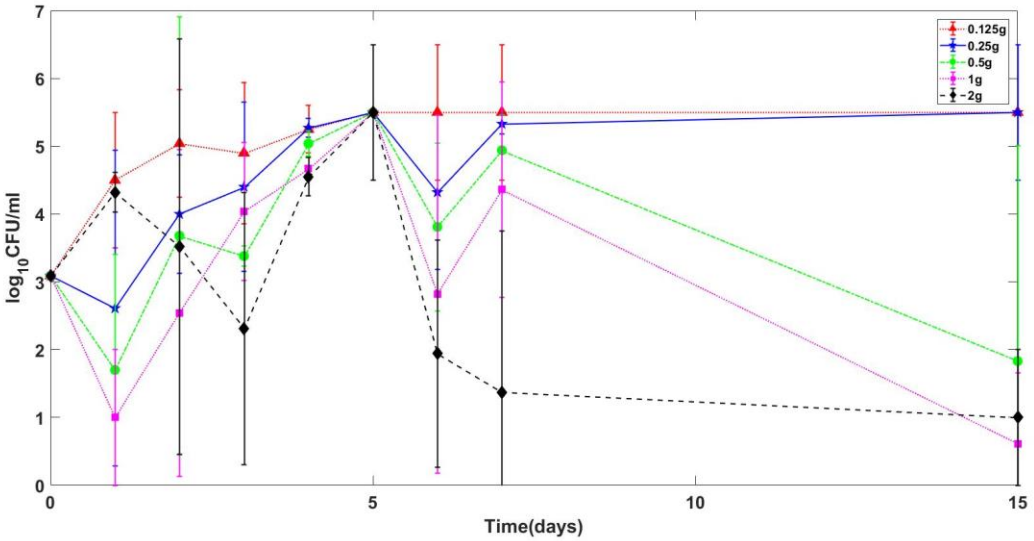


Figure III-8: Antimicrobial activity of eluted vancomycin (0.125 g – 2.0 g) for S. epidermidis 35984, a high in vitro biofilm producer. Error bars show ± 1 standard deviation.

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.

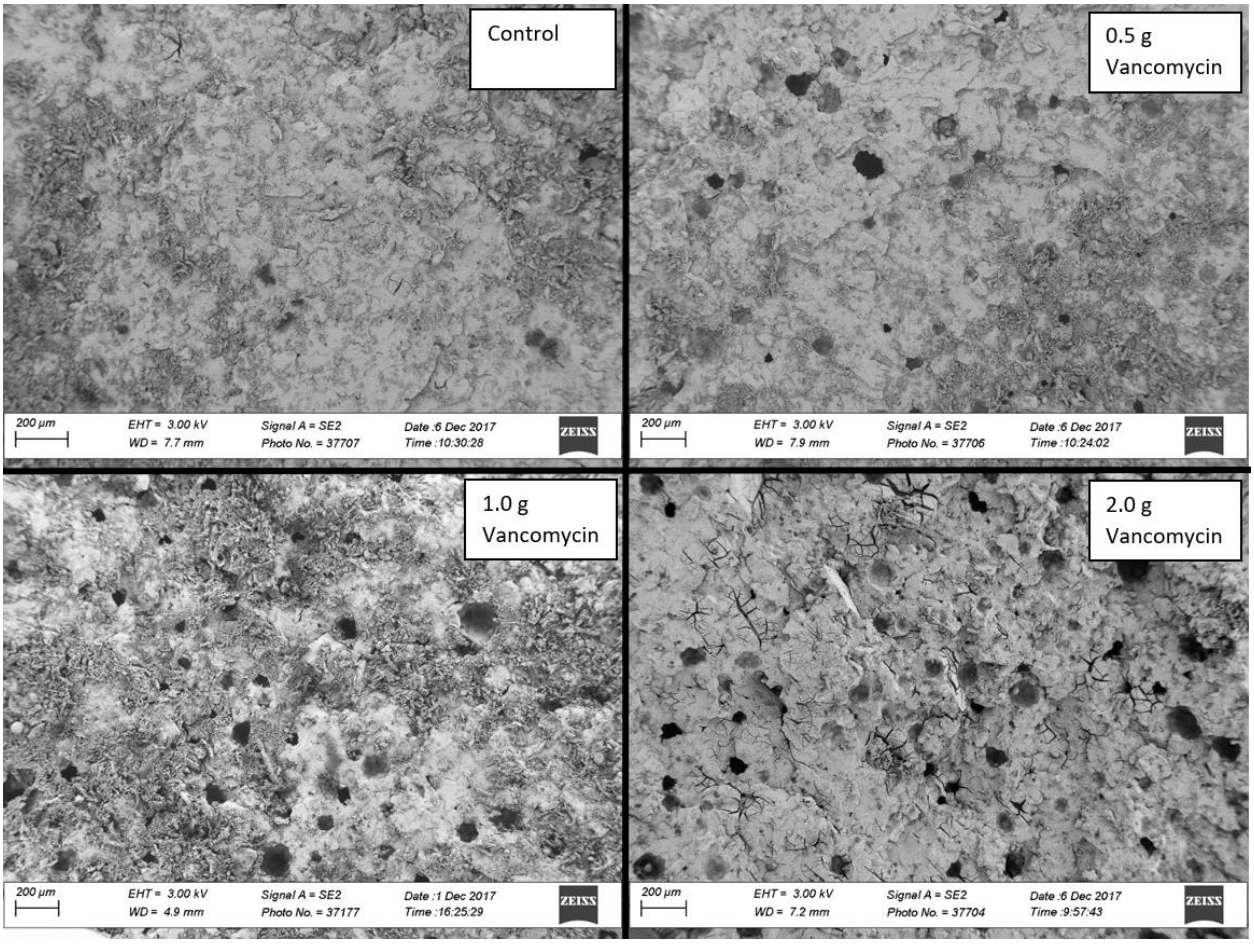


Figure III-9: SEM images of bone cement fracture surfaces from four-point bend specimens with increasing amounts of added vancomycin from control to 2.0 g. Porosity increased with increasing amount of vancomycin.

Discussion

The addition of vancomycin powder into Palacos® R 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® R 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® R [80,89] and likely due to the effect of the pores. [90] 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. [91]

All mechanical properties of the controls were similar to previously published results for 21-day wet-cured samples of Palacos® R. The greatest deviation was an approximately 10% lower compressive modulus and compressive yield strength. [41, 51] Mechanical property trends with increasing amounts of added vancomycin were similar to those found with the addition of xylitol (a poragen additive) to Palacos® R. [80] The mechanical properties of Palacos® R 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. [37, 54] A review article of bone cement studies found mechanical properties of Palacos® R were unaffected or degraded by the addition of antibiotic depending on the testing environment, wet curing process, and amount of added antibiotic. [93]

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. [56, 57] 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. [96]

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. [92] 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® R produced optimal prophylactic antibiotic activity against *S. aureus.*

Several limitations to this study are noted. Only one type of bone cement was used (Palacos® R) and other cements may produce different results. Palacos® R was chosen due to its commercial availability and popularity with surgeons in North America and Europe. [6,81] as well as at our own institution. The samples were all produced by hand. Because all vancomycin

impregnated Palacos® R 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.

Conclusions

Activity testing showed that 1.0 g of added vancomycin was optimal for drug release from wet-cured cement. However, Palacos® R 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 equal 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® R 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® R provided the maximal amount of antibiotic activity while meeting ISO mechanical property standards.

IV. MANUSCRIPT #2

Mechanical, Elution, and Antimicrobial Properties of Simplex Bone Cement Loaded with Vancomycin

Published at Journal of the Mechanical Behavior of Biomedical Materials

Abstract

Prosthetic joint infection (PJI) is one of the most devastating failures in total joint replacement (TJR). Infections are becoming difficult to treat due to the emergence of multi-drug resistant bacteria. These bacteria produce biofilm on the implant surface, rendering many antibiotics ineffective by compromising drug diffusion and penetration into the infected area. With the introduction of new antibiotics there is a need to create benchmark data from the traditional antibiotic loaded bone cements. Vancomycin, one of the commonly used antibiotics, shows activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) and *S.epidermidis*. In our study, vancomycin added to bone cement was evaluated for elution properties, antimicrobial properties, and mechanical properties of the bone cement. Vancomycin at five different loading masses (0.125, 0.25, 0.5, 1.0 and 2.0 g) was added to 40 grams of Simplex™ P cement. Addition of vancomycin affected the mechanical properties and antimicrobial activity with significant differences from controls. Flexural and compression mechanical properties were compromised with added vancomycin. The flexural strength of samples with added vancomycin of 0.5 g and greater were not greater than ISO 5833 minimum requirements. 2.0 g of vancomycin added to bone cement was able to eliminate completely the four bacterial strains tested. 2.0 g of vancomycin also showed the highest mass elution from the cement over a 60-day period. Given

the reduced flexural strength in samples with 0.5 g and greater of added vancomycin and the inability of vancomycin in amounts less than 2.0 g to eliminate bacteria, this study did not find an ideal amount of vancomycin added to Simplex™ P that meets both strength and antibacterial requirements.

Introduction

Aseptic loosening and infection after cemented total hip arthroplasty (THA) and total knee arthroplasty (TKA) are the most common and most devastating reasons for revision surgery. In 2018 the Swedish Knee Arthroplasty Register reported an increase in the proportion of TKA revisions with exchange of inlay, 27% in osteoarthritis (OA) and 24% in rheumatoid (RA), to treat early infections. [5] Furthermore, infection rates after surgical revision are 40% higher than after primary replacement [97]. Besides the considerable cost associated with prolonged hospital (on average $15,000 – $30,000 for an estimated 7 days in the U.S.), the onset of infection can cause disfigurement and psychological trauma. [65]

As a local treatment of the infection site, bone cement loaded with antibiotics has been used over the last four decades and has become a common clinical practice. Antibiotic-loaded bone cements (ALBCs) can deliver high concentrations of antibiotics at the implant site, while minimizing systemic toxicity. [92,98] However, there are several notable risk factors that lead to treatment failure. Infection with Staphylococcus species is associated with a high risk of treatment failure [99–103], likely driven by S. aureus. Methicillin-resistant S. aureus (MRSA) is also a frequent cause of infection in hospital and community settings that shows a high rate of treatment failure. MRSA is resistant to many conventional antibiotic treatments; and therefore, management of these infections is particularly difficult. [104] S. epidermidis has emerged as another pathogen implicated in PJI and causes 20% of all orthopedic device-related infections. [72]

Furthermore, the antibiotic release profile is generally characterized by an initial burst followed by a low release rate. This release profile is not ideal since biofilm formation may persist with an increased antibiotic resistance of the microorganism. [105] Because of the growing resistance of microorganisms to traditional antibiotics, a new generation of antibiotics and ALBCs are being developed. There is therefore a need to create benchmark data from traditional ALBCs against which the new formulations may be compared.

For long-term fixation of total joint replacements, the mechanical properties of ALBC are critical. Vacuum mixing [26,106] is a long established and standard method to reduce cement porosity. Vacuum mixing has been shown to increase mechanical properties with a reduction in porosity on the ALBC surface [24,107–110]. Numerous studies have investigated the reduction in mechanical properties of ALBCs. [111] However, how modifications to bone cement affect its mechanical properties and clinical performance are not well understood. For instance, Sheafi and Tanner reported a complex relationship between fatigue test specimen shape, fabrication method, and load ratio in their study with two commercial cements, Smartset GHV (contains antibiotic) and CMW1 (no antibiotic). [112] Lee and co-workers showed sample curing reduced cement mechanical properties. [92] Bishop and co-workers tested the static mechanical properties, cumulative antibiotic release, biofilm inhibition properties of cured Palacos® R bone cement with various concentrations of vancomycin. [113] Despite many studies of mechanical properties of bone cement modifications, the fracture toughness, compression and bending properties of cured Simplex™ P bone cement with added vancomycin in low doses have not been published. These data are needed as a benchmark for the next generation of ALBCs with new antibiotics.

Therefore, the purpose of this study was to quantify the static mechanical properties such as four-point bending strength, compression strength, and fracture toughness; and, antimicrobial

activity and elution tests were conducted on cured Simplex™ P bone cement loaded with low doses of vancomycin.

Materials and Method

Materials

Simplex™ P was purchased for all tests. The chemical composition of Simplex™ P is listed in Table IV-1. Simplex is commonly used in total hip and knee replacements. Clinical performance of this cement has been shown to have high rates of prosthesis survival over a 12-year period in contrast to other cements. [114]

Table IV-1. Chemical composition of the commercial bone cement (Simplex™ P). All values are given as %wt/wt, except hydroquinone which is in ppm

40 g of powder	
Poly(methymethacrylate) (PMMA)	15
Poly(methylacrylate-styrene)	73.5
Barium sulfate	10.0
Benzyl peroxide (BPO)	1.5
20 mL of liquid	
Methy methacrylate (MMA)	97.45
N,N-Dimenthyl-p-toluidine (DMPT)	2.55
Hydroquinone	80

Vancomycin powder was purchased from Sigma-Aldrich (St.Louis, MO, USA). Vancomycin, one of the commonly used antibiotics [111], was chosen for this study because the majority of the pathogens involved in orthopedic infection are gram positive and therefore treatable with vancomycin.

2.2. Mixing Method

Vacuum mixing was used to mix the bone cement. The cement powder and antibiotic were mixed by hand-shaking for 1 min in a syringe and then the liquid monomer was added into

the antibiotic cement powder. The small amount of air entrapped in the cement syringe during mixing was drawn off using a vacuum pump at -50 mbar. [80] The cement was then transferred to an aluminum mold by injection with gun pressurization. After delivery of cement to the mold, the mold cover was placed by hand and pressed against the mold.

2.3. Preparation of Antibiotic-Loaded Bone Cement

Six different experimental groups were prepared. The bone cement with no antibiotic served as a control (Simplex™ P) and five vancomycin-loaded bone cements were prepared with antibiotic masses: 0.125 g, 0.25 g, 0.5 g, 1.0 g and 2.0 g. Thus, 0.3 vol%, 0.6 vol%, 1.2 vol%, 2.4 vol%, 4.7 vol% of vancomycin powder were used for the loading in this study. Four aluminum molds were fabricated for testing samples with dimensions as described in ISO 5833 for bending and compression tests ku and ASTM-D5045 for fracture toughness tests. Rectangular prismatic samples ($75\ \text{mm} \times 10\ \text{mm} \times 3.3\ \text{mm}, \text{Width [w]} \times \text{Depth [b]} \times \text{Height [h]}$) for four-point bending tests; cylindrical samples ($6\ \text{mm} \times 12\ \text{mm}, \text{Diameter [Ø]} \times \text{Height [h]}$) for compression tests; and rectangular prismatic samples ($44\ \text{mm} \times 5\ \text{mm} \times 10\ \text{mm}, \text{Width [w]} \times \text{Depth [b]} \times \text{Heigh t[h]}$) with a crack length of 5 mm and width of 0.35 mm for fracture toughness tests were fabricated. Cracks were created using a diamond wafering blade (Buehler® IsoMet™, Lake Bluff, IL, USA), and measured using ImageJ (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, Madison, WI, USA). Cylindrical samples ($6\ \text{mm} \times 4.5\ \text{mm}, \text{Diameter [Ø]} \times \text{Height [h]}$) for drug elution and efficacy tests were also prepared.

2.4. Mechanical Testing

Quasi-static mechanical properties were measured under compression and bending. Fracture toughness was also determined. Prior to mechanical testing, the specimens were wet

cured in phosphate buffer solution (PBS) for 21days at 22°C. [113] Bending tests and compression tests were conducted in accordance with ISO 5833; whereas, fracture toughness tests were performed in accordance with ASTM-D5045. Samples sizes were seven to eight per experimental group for each test.

The mechanical properties were obtained at loading rates: 5 mm/min for bending tests; 5 mm/min for compression test; and 10 mm/min for fracture toughness. Tests were conducted with a Criterion C43.104, MTS testing machine (Eden Prairie, MN, USA) with a 1 kN load cell (LPS.103, MTS system Corp). Cylindrical bone cement samples were compressed in the axial direction until failure occurred (2% yield point) as recommended by ISO 5833. The ultimate compressive strength ($\sigma_{ys} = F_{max}/A$; F_{max} = maximum applied load in N before specimen failure, A=original cross-sectional area in mm^2) and elastic modulus in MPa (Young's modulus, E, slope at 0.2-0.4% strain) were determined from the stress-strain curves.

Four-point bend testing (Figure IV-1) was performed to measure the flexural modulus and flexural strength of the bone cement. Rectangular samples were bent until failure and the flexural modulus was determined from the stress-strain curves. The flexural strength was calculated using the force at break of the samples. The flexural modulus and strength were calculated with the following equations:

$$E_f = \left(\frac{a(3Lx - 3x^2 - a^2)}{bh^3}\right)\left(\frac{\Delta F}{\Delta D}\right) \quad [1]$$

$$S_f = \frac{3F_{max}a}{bh^2} \quad [2]$$

Where,
L = lower support distance [mm]
x = distance between the inner and outer four-point bending supports [mm]
F_{max} = applied load at failure [N]

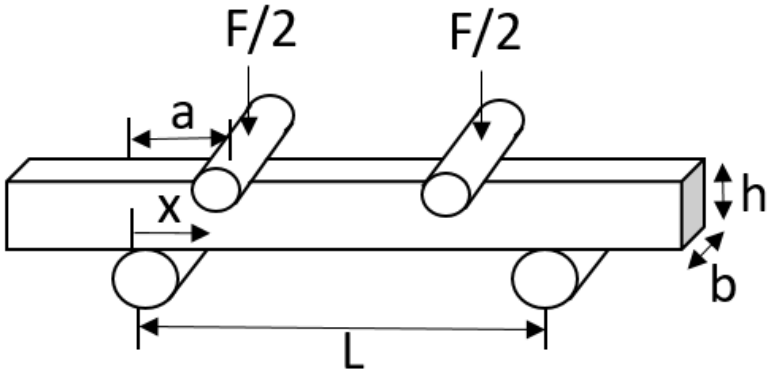


Figure IV-1. Schematic of the four-point bending test

Single edge notch bend testing (Figure IV-2) was carried out to measure the fracture toughness of the bone cement. The samples were prepared and loaded until failure. Fracture toughness in MPa√m was calculated using ASTM-D5045. [113]

$$K_{IC} = \frac{3PS}{2bh^{2/3}} f(x) \quad [3]$$

$$f(x) = 1.93\left(\frac{a_c}{h}\right)^{\frac{1}{2}} - 3.07\left(\frac{a_c}{h}\right)^{\frac{3}{2}} + 14.53\left(\frac{a_c}{h}\right)^{\frac{5}{2}} - 25.11\left(\frac{a_c}{h}\right)^{\frac{7}{2}} + 25.80\left(\frac{a_c}{h}\right)^{\frac{9}{2}} \quad [4]$$

Where,
P = maximum force [N]
S = lower span length [cm]
a_c = crack length [cm]

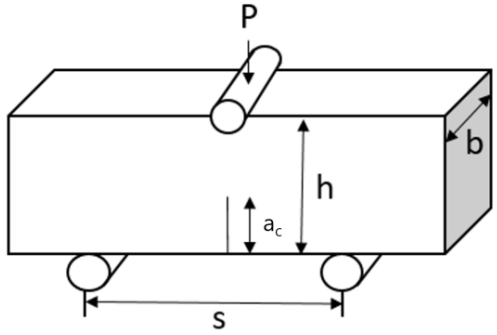


Figure IV-2. Schematic picture of single edge notch bending test

2.5. Drug Release Study

Five samples in each group were used to measure antibiotic elution. Each cylindrical sample was immersed in 5 mL of sterile PBS and stored in an incubator shaker at 37°C with constant shaking at 100 rpm. At time points, 1, 2, 4, 8, 10, 15, 25 and 45 and 60 days, cylindrical cement samples were removed from the test tubes. The eluate in each test tube was frozen at -20°C until analysis of antibiotic concentration. The cylindrical cement samples were re-immersed in test tubes containing 5 mL of fresh PBS. Vancomycin concentration in the collected eluate was quantified by high performance liquid chromatography (HPLC) with a 18 C column (10 µm analytical column and flow rate 0.5 mL/min). The isocractic mobile phase consisted of 10 mM KH_2PO_4 – Acetonitrile (composite ratio, 17/3) and absorbance was measured at 210 nm. A standard curve was constructed using known vancomycin concentrations. The release profile was characterized by fitting a two-phase exponential decay function to the elution data as recommended by Higuchi. [115,116]

$$y = A1 * e^{(-\frac{x}{t1})} + A2 * e^{\left(-\frac{x}{t2}\right)} + y_0 \quad [5]$$

Where,

A1 = Magnitude of fast phase
A2 = Magnitude of slow phase
x = Time in day
y_0 = Eluted vancomycin in mg
$\frac{1}{t1}$ = K1 = Fast decay rate in day^{-1}
$\frac{1}{t2}$ = K2 = Slow decay rate in day^{-1}

2.6. Antimicrobial Efficacy Testing

Four reference bacterial strains were evaluated for antimicrobial susceptibility testing: ATCC 33591 (Methicillin-resistant Staphylococcus aureus [MRSA]), n315 (MRSA), ATCC

29213 (*S. aureus*), ATCC 35984 (*S. epidermidis*). The minimum inhibitory concentration (MIC) of vancomycin and inoculum concentration are listed in Table IV-2. [87,88]

Table IV-2. Minimum inhibitory concentrations of vancomycin and inoculum concentration

Bacterial Strain	Characterization	MIC (mg/L)	Inoculum concentration (CFU/mL)
ATCC 33591	MRSA	2	10^3
n 315	MRSA	0.5 – 1	10^3
ATCC 29213	*S. aureus*	0.5	10^3
ATCC 35984	*S. epidermidis*	1	10^3

Three cylindrical samples were transferred into a test tube containing 3.4 mL of tryptic soy broth (TSB; Becton Dickenson, Pranklin Lakes, NJ). The broth media were inoculated with bacteria daily for 7 days and at 14 days and then two-fold serial dilutions were carried out. The diluted broth media were prepared on Mueller Hinton II agar plates (Sigma-Aldrich, St. Louis, MO, USA) for bacteria manipulation. Agar plates were incubated for 18-24 hours and bacteria colonies were then determined. The colony forming unit (CFU) quantified the ability of eluted antibiotic from the bone cement to eliminate the bacteria in the cell culture. The minimum detection of bacteria inhibitory was 10 CFU/mL. All isolates were tested in triplicate for susceptibility.

2.7. Scanning Electron Microscopy (SEM)

The external and fracture surfaces of four-point bend and fracture toughness samples, following the test, were examined using a scanning electron microscopy (SEM; Zeiss-LEO, Oberkochen, Germany). The size of pores (macro-pore and micro-pore) and the number of pores were quantified in ImageJ. A thin layer of gold was deposited on the sample surfaces for 35 s with 45 mA. Images were obtained at 200x magnification using an acceleration voltage of 3 kV.

2.8. Statistical Analysis

All the results collected from mechanical testing were statistically assessed using Minitab 18 (Minitab Inc., State College, PA). Kolmogrov-Smirnov method was used to determine normality. Kruskal-Wallis tests and post hoc Mann-Whitney U tests were conducted for non-parametric comparison between the control group and the groups with vancomycin added. Wilcoxon Signed Rank test was used to compare mechanical test results against the minimum ISO requirement. Results are presented as the mean $\pm$ standard error. A p-value of <0.05 was considered statistically significant.

Results

Mechanical Properties

The average flexural modulus for each group exceeded the ISO minimum requirement (1800 MPa) and there was no statistically significant difference between each group compared with control (mean 2459 $\pm$ 155 MPa) (Figure IV-3). The flexural strength for all treatment groups was significantly lower than the control group (mean 63.18 $\pm$ 4.77 MPa); and, the flexural strength of samples with 0.5 g and greater were statistically not greater than the ISO minimum standard (50 MPa) (Figure IV-4).

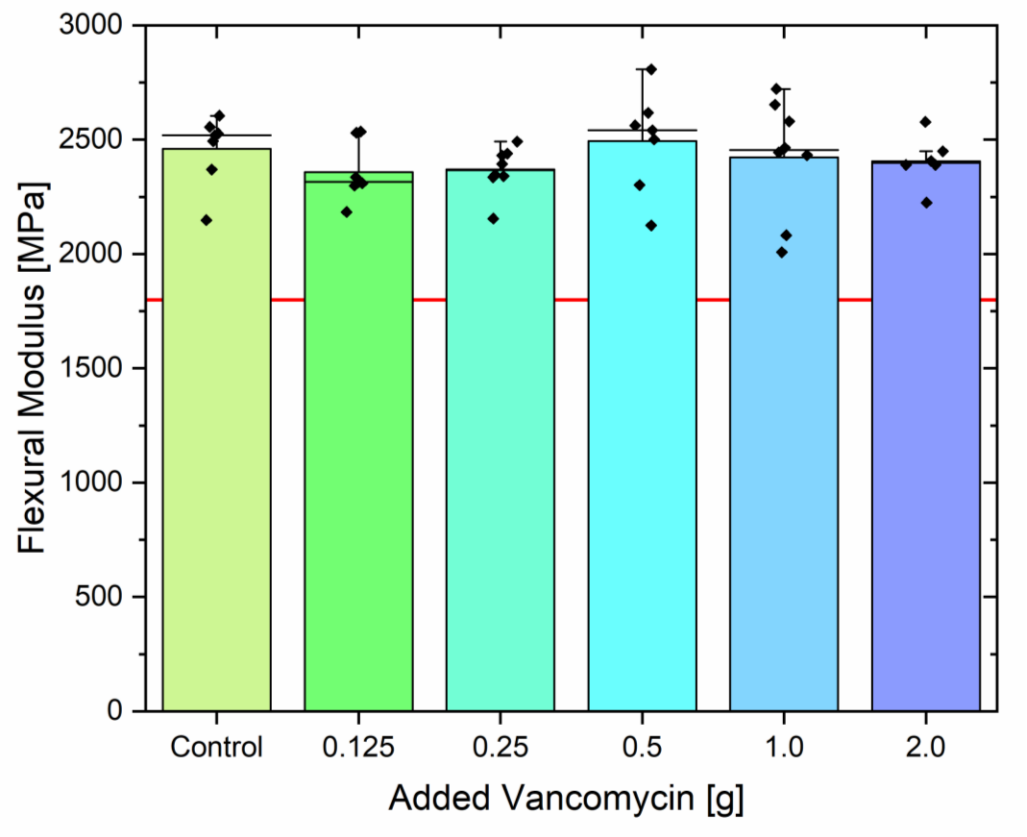


Figure IV-3. Flexural modulus of vancomycin formulated Simplex™ P bone cement after curing process. Solid red line at 1800 MPa indicates ISO 5833 standard for minimum flexural modulus. Values are shown as the mean and standard error of the mean for the specimens in each group. Treatment groups were not different than the control and all groups were above the ISO 5833 minimum and not different from the control.

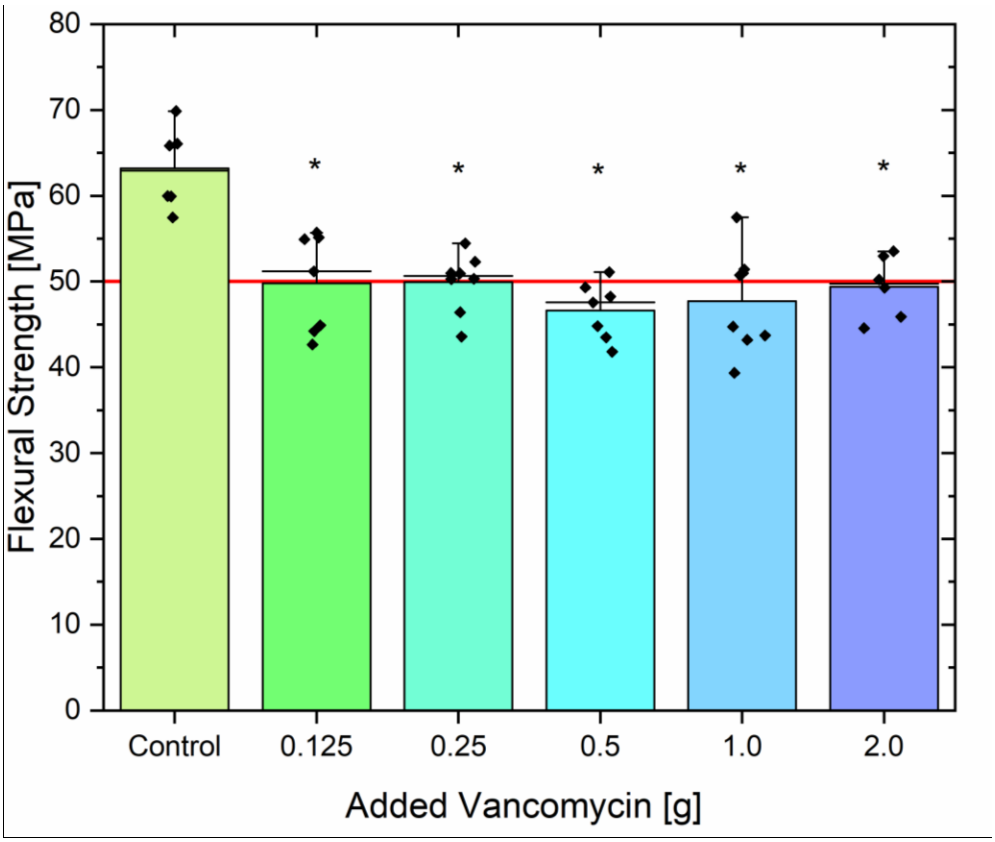


Figure IV-4: Flexural strength of vancomycin formulated Simplex™ P bone cement after curing process. Solid red line at 50 MPa indicates ISO 5833 standard for minimum flexural strength. Values are shown as the mean and standard error of the mean for the specimens in each group. The asterisk mark (for all treatment groups) represents a significant ($p<0.05$) difference from control group. Samples with 0.5 g of added vancomycin and greater were statistically not above the ISO 5833 minimum.

The compressive modulus of formulated bone cement was not significantly affected by added vancomycin as compared to the control group (mean 1694 $\pm$ 221 MPa) except for the 2 g of added antibiotic group (Figure IV-5). There is no minimum requirement specified in ISO 5833 standard for compressive modulus. The compressive yield strength was also not significantly affected by added vancomycin as compared to the control group (80.59 $\pm$ 4.48 MPa) except for the 2 g of added antibiotic group. All groups were above the minimum compressive yield strength (70 MPa) in accordance with ISO 5833 (Figure IV-6).

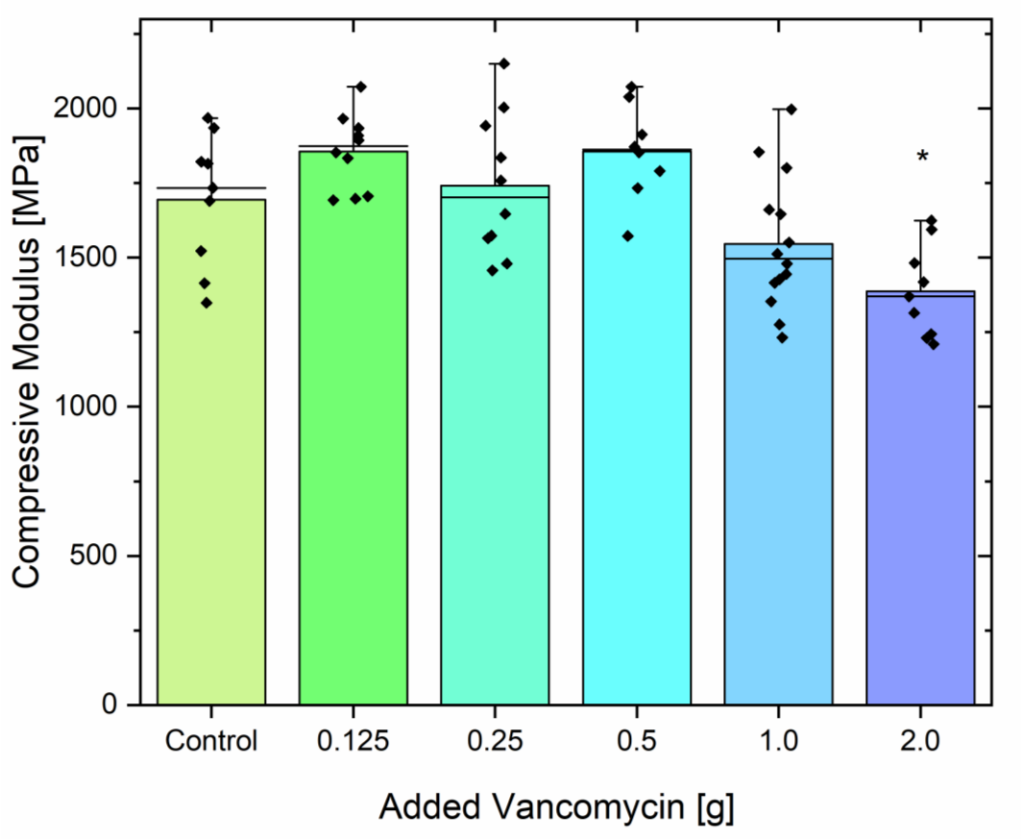


Figure IV-5: Compressive modulus of vancomycin formulated Simplex™ P bone cement after curing process. Values are shown as the mean and standard error of the mean for the specimens in each group. The asterisk mark (for 2.0 g of added vancomycin) represents a significant ($p<0.05$) difference from control group.

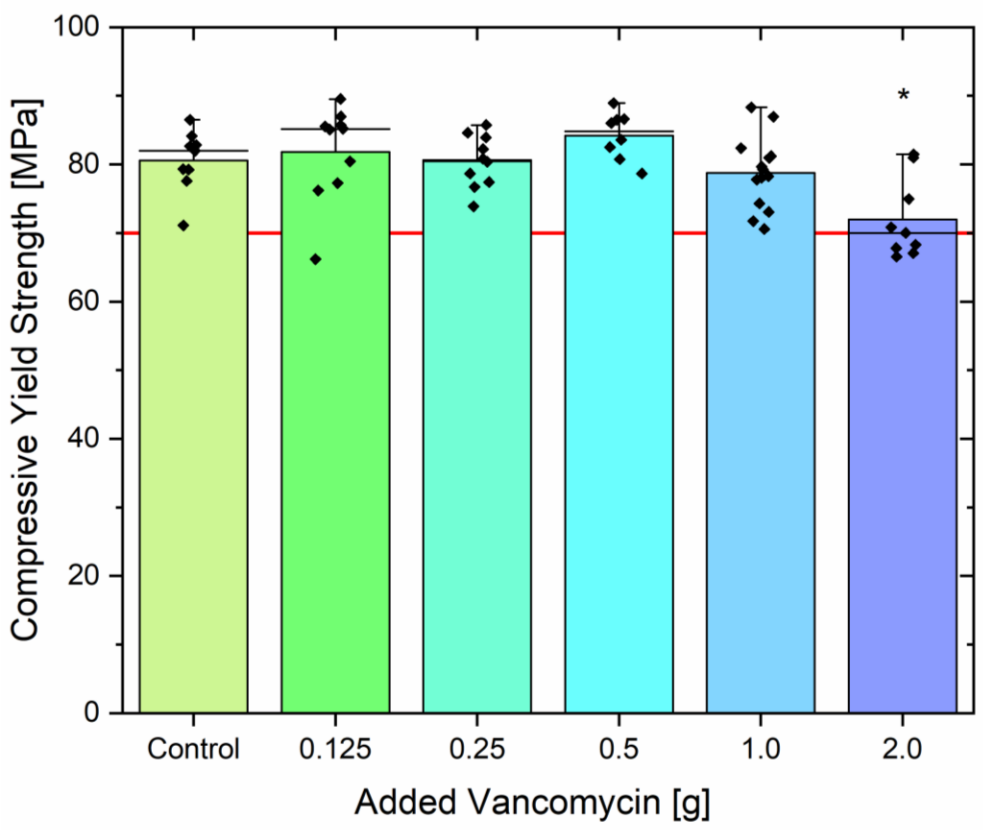


Figure IV-6: Compressive yield strength of vancomycin formulated Simplex™ P bone cement after curing process. Solid red line at 70 MPa indicates ISO 5833 standard for minimum compressive yield strength. Values are shown as the mean and standard error of the mean for the specimens in each group. The asterisk mark (for 2.0 g of added vancomycin) represents a significant ($p<0.05$) difference from control group. All groups were above the ISO 5833 minimum.

The fracture toughness, Mode I critical stress intensity factor, of Simplex™ P bone cement was statistically significantly higher ($p<0.05$) with the addition of vancomycin as compared to the control group (Mean 1.68 ± 0.16 MPa$\sqrt{m}$) (Figure IV-7).

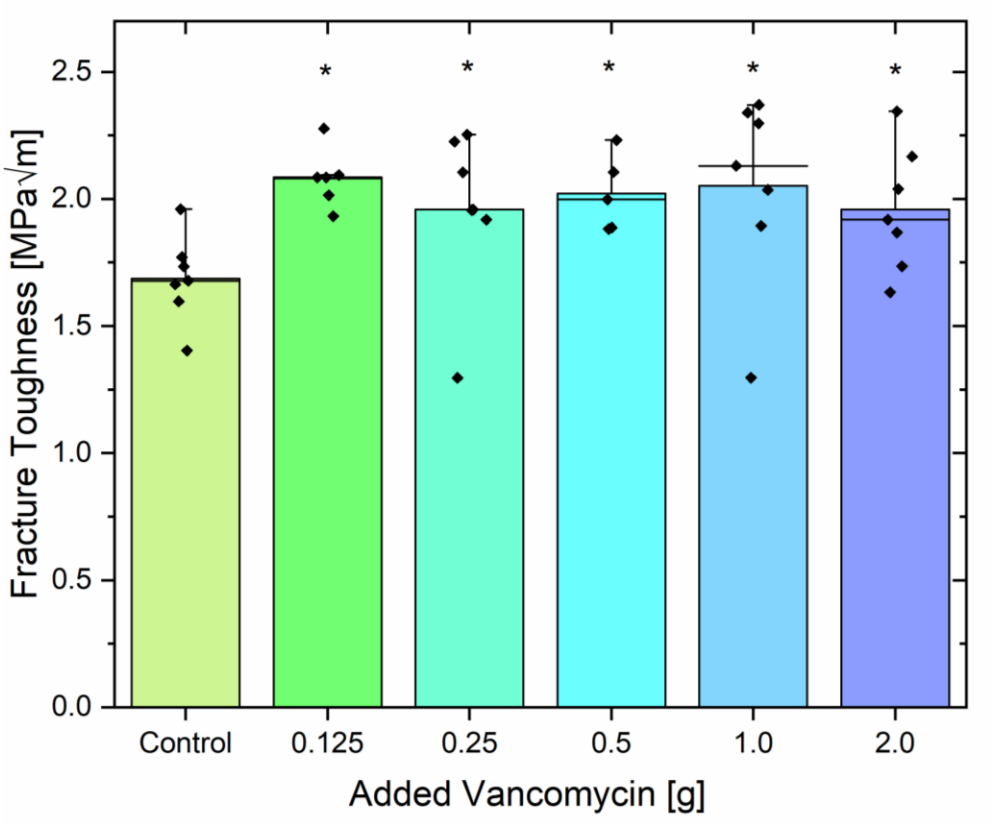


Figure IV-7: Fracture toughness of vancomycin formulated Simplex™ P bone cement after curing process. Values are shown as the mean and standard error of the mean for the specimens in each group. The asterisk mark represents a significant ($p<0.05$) difference (for all samples with added vancomycin) from control group.

Elution Properties

All groups exhibited a burst of cumulative elution of vancomycin (94% - 98% of 60-day elution) within a week and a total of 1.5% - 2.6% of vancomycin eluted over the 60-day period (Figure IV-8). For the bone cement samples containing lower amounts of antibiotic such as 0.125 g, 0.25 g and 0.5 g, the elution profile tended to level off after the initial burst. The 2 g of added antibiotic group showed the most vancomycin (0.139 ± 0.006 mg) eluted per cement disk. The groups with 1 g and 2 g of added antibiotic showed much (10x and 30x more than 0.125 g) larger amounts of vancomycin eluted from cement as compared to the 0.125 g, 0.25 g and 0.5 g of added antibiotic groups. The collected data were normalized and fit to a two-phase exponential

decay function to measure the fast and slow decay rate. All elution profiles fit the model with coefficient of determination, $R^2 > 0.99$. The parameters, A1 and A2, represent the magnitudes of the fast and slow phases, respectively. The decay rates are represented by the parameters K1 and K2, expressed by 1/t1 and 1/t2, respectively. Thus, the fast decay rate for 2 g of vancomycin (and highest elution curve) was K1 = 200 day^{-1} and the slow decay rate for 0.125 g of vancomycin was K2 = 1.01 day^{-1}.

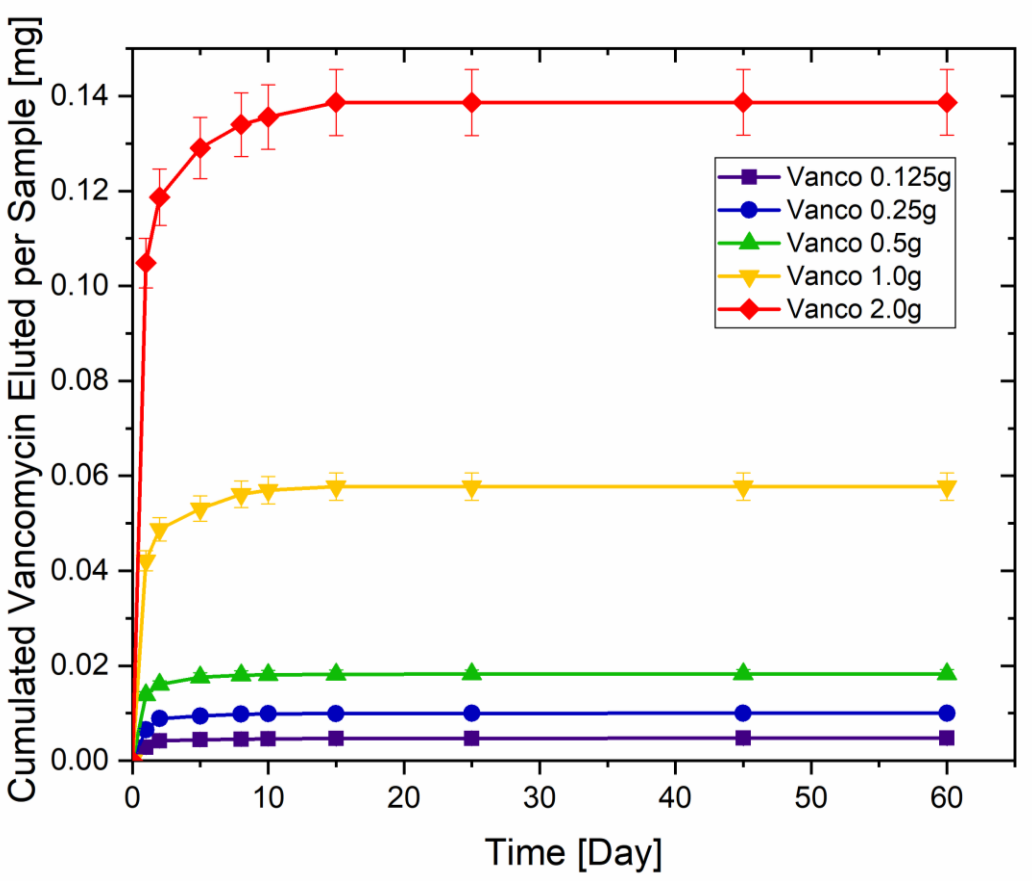


Figure IV-8: Vancomycin elution release profiles (mean ± SEM) over a 60-day period and two-phase exponential decay model.

Antimicrobial Effectiveness

0.125 g to 1.0 g of added vancomycin groups were not able to completely eliminate the bacterial inoculum below the limit of detection (10 CFU/mL). In the 2 g of added vancomycin group, the three *S. aureus* isolates tended to regrow within a week but no additional bacteria were observed after one week (Figure IV-9).

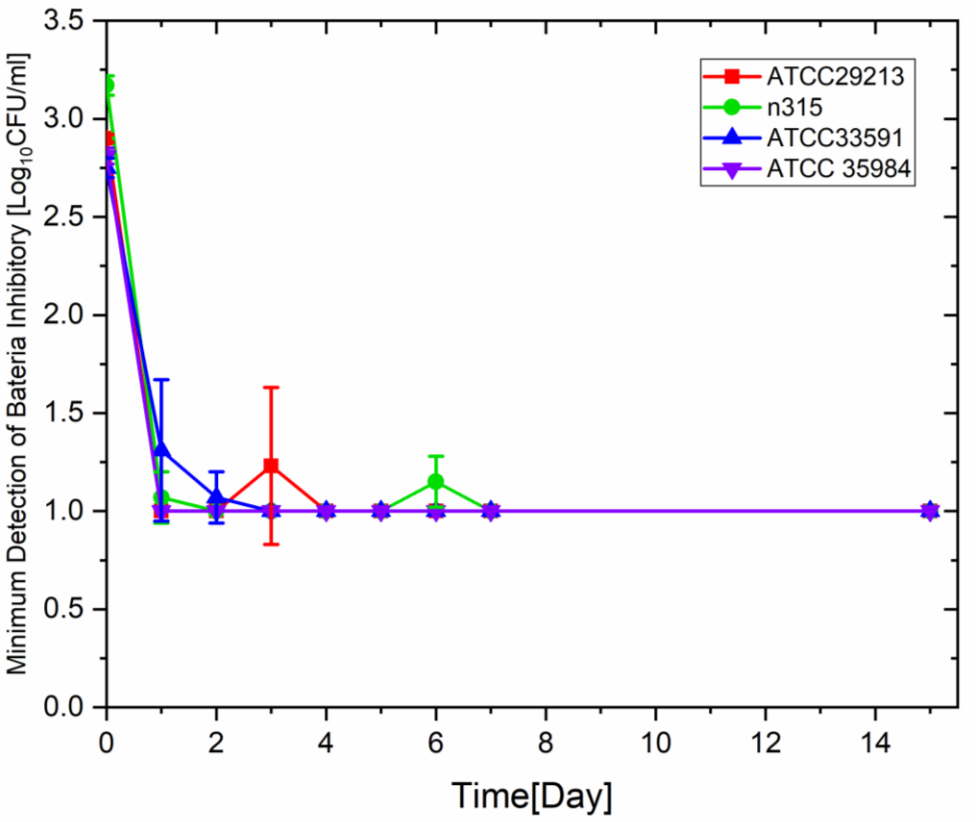


Figure IV-9: Efficacy test of eluted vancomycin (2.0 g of added vancomycin) for four strains. All bacterial colonies were completely eliminated at day 7 and no more colonies were observed afterward. Error bars show mean ± SEM.

Discussion

The results of this study showed that the mechanical properties of Simplex™ P bone cement with addition of vancomycin were changed after 21-days curing in PBS. The flexural modulus was not affected with increasing amounts of antibiotic but flexural strength was noticeably decreased with added vancomycin as compared with the control group. Samples with 0.5 g and greater of added vancomycin were statistically not above the ISO 5833 minimum. The degradation of flexural strength with added vancomycin was even more pronounced in the current study with Simplex™ P bone cement than our previous study with vancomycin added to Palacos® R.[113] In four-point bending, these material specimens are subjected to a pure bending moment without shear forces. The specimen is subjected to a linear gradient of normal stress with compression on the inner curve, tension on the outer curve and zero stress between at the neutral axis. The reduction in flexural strength with added vancomycin may be due to a decrease in the material's tensile strength with increased porosity.

The compressive modulus showed no differences between the treatment groups and control, except for the group with 2 g of added antibiotic. This group demonstrated a modulus reduction of 18%, and a strength reduction of 10%. The reduction in compressive modulus (17-37%) and yield strength (22%) was even more pronounced for vancomycin added to Palacos® R. [113] Some studies have reported that adding less than 5% of antibiotic did not significantly degrade the mechanical properties of cement. [111] Lilikakis and Sutcliffe investigated the compression strength of Palamed G and Copal cements (Heraeus Medical) adding up to 10% vol vancomycin. They found that all the samples of cement exceeded the ISO standard for minimum compressive strength. [117] However, it has been recommended that with the addition of 5% or less vancomycin, care should be taken with the mixing method, for example inhomogeneous mixing

may reduce the compressive strength. [117,118] It has also been reported that the strength of cement is reduced depending on the aging period of the cement samples. Lee and co-workers found a 5-38% reduction in ultimate compressive strength of Simplex™ P after 2-weeks curing. [92]

The fracture toughness of the control group of Simplex™ P (1.69 ± 0.16 MPa$\sqrt{m}$) was similar to previously published results. [113] The groups with added vancomycin showed a statistically significant increase of 14 – 19% in fracture toughness in comparison with the control group. This is in contrast to the flexural strength results and the author's previous results with cured Palacos® R which was not affected by added vancomycin.[113] Fracture toughness of Simplex™ P with added vancomycin has not be published; however, the results of the current study were similar to those found with the addition of graphite reinforcement [119] and addition of titanium fiber reinforcement to the bone cement. [120] It is also known that pores can increase the apparent toughness by crack tip blunting. [121,122]

The fracture surfaces were examined with SEM images (Figure IV-10). Pores on the fracture surface were identified as macro pore (diameter $\geq$ 1 mm) and were often observed in the groups with greater than 1 g of added vancomycin. Persson found from their study that pores larger than 1 mm in diameter might lead to greater scatter in the static and fatigue mechanical testing data. [118] Unlike the results from Palacos® R cement [113], the micron-sized pores (diameter $\leq$ 1 mm) were rarely found in Simplex™ P cement.

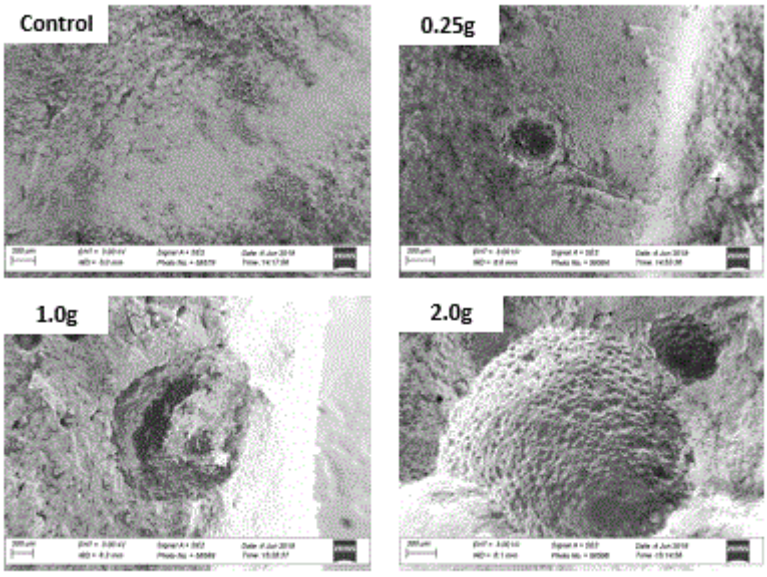


Figure IV-10: SEM images at 200× magnifications of Simplex™ P bone cement after aging in PBS showing increasing size of pores with increasing amounts of added vancomycin.

The total mass of vancomycin elution from Simplex™ P was ten times lower than the results found from Palacos® R bone cement with 1 g vancomycin. [80,113] The elution amount for 1 g of adding vancomycin to Simplex™ P was consistent with the result measured by Meeker. [61] Many studies have investigated antibiotic release dependent on type of bone cement and its manner of preparation. [111] Some authors reported that gentamicin eluted more effectively from Palacos® R than from Simplex™ P. [123,124] The poor elution of antibiotics from Simplex™ P found in these studies as well as the current study might be due to several factors, such as the physiochemical characteristics of vancomycin, size of pores and roughness of bone cement surface. [124] Van de Belt reported that a combination of surface roughness and porosity determine the release kinetics of gentamicin from bone cement. They found that surface roughness is related to the initial rates of release of antibiotics from bone cement and the porosity is related to the sustained release over a longer period of time. [125] Other studies have reported that increasing the ALBC porosity increased the antibiotic elution, so dextran and glycine were used as space

fillers to obtain more porous ALBC. [126,127] Nugent also showed that increasing the porosity of ALBC increased the elution of antimicrobials by the addition of soluble particulate poragens. [128]

Our *in vitro* elution profile demonstrated that bone cement loaded with 2 g of vancomycin provided antibacterial efficacy against MSSA, *S.aureus, S.epidermidis* within the first week. These results are consistent with previous work on antibacterial activity of vancomycin in bone cement against MSSA, MRSA, S.*epidermidis* [129] and the authors' previously published results with Palacos® R. [113]

Some limitations of the present study should be noted. First, the cement was not tested at body temperature. The test samples were wet cured and tested at lab temperature as specified in the mechanical test standards. Mechanical properties determined at body temperature will differ from the results of the current study. [121] All mechanical properties were determined by assuming linear elastic material behavior and linear elastic fracture mechanics. However, bone cement is non-linear viscoelastic material. To reduce this error, the ALBC samples were tested at relatively low loading rates. The results of this study are limited to the bone cement, antibiotic and mixing method of this study. The current study's results compliment those of other studies. Material tests as performed in the current study are important for comparing effects on the cement mechanical properties, and provide material data for finite element analyses. Cement properties, as determined from material tests such as those conducted in this study characterize the cement and not the in vivo performance of an arthroplasty anchored to the contiguous bone using the cement. Material testing, preclinical component testing and simulations provide a better understanding of implant performance, but ultimately in vivo performance can only be assessed from clinical tests.

Conclusion

The mechanical properties of Simplex™ P bone cement were significantly affected by added vancomycin. In particular, flexural strength did not meet ISO minimum requirements for 0.5 g and greater added vancomycin, and compressive modulus and compressive yield strength were compromised with 2.0 g of added vancomycin. In contrast, added vancomycin increased fracture toughness of Simplex™ P bone cement. The total mass of antibiotic elution for the 1 g and 2 g added vancomycin groups was much higher compared to other groups. However, only 2 g of added vancomycin were able to eliminate all the pathogens. Given the reduced flexural strength in samples with 0.5 g and greater of added vancomycin and the inability of vancomycin in amounts less than 2.0 g to eliminate bacteria, this study did not find an ideal amount of vancomycin added to Simplex™ P that meets both strength and antibacterial requirements.

V. MANUSCRIPT #3

Palacos and Simplex Bone Cement Loaded with Telavancin: Antibacterial and Mechanical Properties.

Submitted at Journal of Clinical Orthopedics and Related Research

Abstract

Prosthetic joint infection and aseptic loosening are the most common reasons for short-term and long-term revision of total joint replacement. Antibiotic-loaded bone cement has been used for implant fixation as a prophylactic treatment for infection. However, the rise of multidrug-resistant bacteria has reduced the efficacy of traditional antibiotics used in this way.

This study evaluated telavancin elution, stability, and antimicrobial activity when incorporated into the commercial bone cements Palacos® R and Simplex™ P. Telavancin at five loading volumes (0.3 vol%, 0.6 vol%, 1.2 vol%, 2.4 vol%, and 4.8 vol%) was added to the two cements. The release characteristics of telavancin were recorded for 60 days to estimate the elution profiles. The efficacy of eluted telavancin for eliminating four common implant pathogens was determined. Mechanical testing was also performed. Telavancin affected the elution, antimicrobial activity, and mechanical properties in a dose-dependent manner. Telavancin added to Palacos®R at 4.8 vol% was able to eliminate MSSA, MRSA, and *S. epidermidis*, while the same concentration in Simplex™ P failed to kill all tested strains. Telavancin also exhibited better elution in the former case over a 60-day period. Both cements showed reduced flexural and compression properties with added telavancin. Telavancin loaded to Palacos®R achieved better efficacy than in Simplex™ P. Under microscopic examination the two cements showed different numbers and sizes of pores, potentially affecting strength. Telavancin loaded in

Palacos®R is a promising prophylactic option. However, further consideration is required for sustainable telavancin release and increased strength.

Introduction

Periprosthetic joint infection (PJI) is one of the most challenging problems in orthopedic surgery. Infection in PJI involves the adhesion of bacteria to the implant and the proliferation of biofilm on the prosthesis[130,131]. Infections involving biofilm are difficult to treat and are capable of evading host immune response mechanisms. Staphylococci including *S. aureus* and *S. epidermidis* are the most common pathogens of device-associated infections [132,133]. Multidrug-resistant isolates of *S. aureus*, especially methicillin-resistant *Staphylococcus aureus* (MRSA), are responsible for diseases involving skin and soft tissue infection, and it is recognized as a major cause of tissue necrosis [134,135]. *S. epidermidis*, which is commonly found on skin and mucous membranes, displays high rates of antibiotic resistance and is a vigorous producer of biofilm. MRSA and *S. epidermidis* are responsible for up to of 91% PJI incidents [136].

Antibiotic-loaded bone cement (ALBC), which can effectively eliminate bacteria by delivering high levels of antibiotics to surgical sites, may prevent PJI. The antibiotic release profile is generally characterized by an initial burst followed by a lower release rate. This profile is not ideal because biofilm formation may persist, resulting in antibiotic resistance and tolerance. [105] Due to these issues, a new generation of antibiotics and ALBCs are being developed. Telavancin, a derivative of vancomycin, is a lipoglycopeptide antibiotic that was approved by the FDA in 2007 [137]. This drug kills a broad range of gram-positive bacteria, especially MRSA, and has shown effectiveness in the treatment of complicated skin and skin-structure infections and hospital-acquired pneumonia. It has a dual mechanism of action, inhibiting peptidoglycan synthesis and causing membrane depolarization.

Polymethylmethacrylate (PMMA) bone cement with telavancin has not previously been evaluated for its release characteristics, activity, or stability.

The elution of antibiotic from bone cement is a significant factor in eliminating bacteria; however, the exact mechanism is still under debate. Antibiotic elution may simply be a passive surface phenomenon, as it is significantly affected by the surface roughness [125]. Another study suggested that antibiotics eluted through voids and interconnecting cracks on the PMMA surface [138]. Furthermore, many studies have investigated antibiotic release relative to the type of bone cement and level of porosity [139]. Antibiotic elution negatively affects the mechanical properties of bone cement. A relatively low-dose ALBC is used to avoid adverse mechanical effects [140]. In previous investigations, we found that cured bone cement with vancomycin reduced mechanical properties [113,141].

For the current study we evaluated elution, mechanical properties, and antimicrobial/antibiofilm activity when telavancin was incorporated into the commercial bone cements Palacos® R and Simplex™ P.

Materials and Methods

1. Mixing Method

Two types of bone cement—Palacos® R and Simplex™ P—were prepared according to the manufacturers' instructions. These commercial products contain two main components: 40g of radiopaque polymer powder and a 20 mL ampule with liquid monomer (Table 1). Benzyl peroxide added to the powder acts as an initiator, and an accelerator, N,N-dimenthyl-p-toluidine is added to the liquid to cause polymerization at room temperature. The contrast agents barium sulfate and zirconium dioxide make the cement radiopaque. Clinical grade telavancin was provided by Theravance Biopharma (Cayman Islands) and added to the bone cements.

Table V-1. Composition of two bone cements widely used in North America. Except for hydroquinone, which is in ppm. All values are given as %wt/wt.

	Palacos® R	Simplex™ P
Manufacturer	Heraeus Medical GmbH, Wehrheim, Germany	Stryker Coporation, Kalamazoo, Michigan, USA
Powder		
Poly(methymethacrylate) (PMMA)	---	15.0
Poly(methylacrylate-methylmethacrylate)	84.0	---
Poly(methylacrylate-styrene)	---	73.5
Zirconium dioxide	15.25	---
Barium sulfate	---	10.0
Benzyl peroxide (BPO)	0.75	1.5
Liquid		
Methyl methacrylate (MMA)	97.98	97.45
N,N-Dimenthyl-p-toluidine (DMPT)	2.02	2.55
Hydroquinone	60	80

2. Mixing Method

The cement powder and antibiotic were mixed by hand shaking for 1 minute in a syringe and liquid monomer was added. The small amount of air entrapped in the cement syringe during mixing was drawn off using a vacuum pump at -50 mbar [113]. The cement was then deposited into an aluminum mold with the aid of a spatula, and the mold cover was placed by hand and pressed against the mold to aid the removal of entrapped air. The antibiotic-loaded bone cement was then left to set for 1 hour. Once removed from the mold, samples were frozen at -20°C until tested.

3. Preparation of Antibiotic – Loaded Bone Cement

Six different experimental groups were prepared for each bone cement. Cement with no antibiotic served as a control, and five antibiotic-loaded bone cements were prepared with telavancin masses of 0.125 g (0.3 vol%), 0.25 g (0.6 vol%), 0.5 g (1.2 vol%), 1.0 g (2.4 vol%), and 2.0 g (4.8 vol%). Cylindrical samples (6 mm diameter × 4.5 mm height) for drug elution and

efficacy tests were prepared. Also, rectangular prismatic samples (75 mm × 10 mm × 3.3 mm) for four point bending tests, cylindrical samples (6 mm diameter × 12 mm height) for compression tests, and rectangular prismatic samples (44 mm × 5 mm × 10 mm) with a crack length of 5 mm and width of 0.35 mm for fracture toughness tests were fabricated. Cracks were created using a diamond wafering blade (Buehler® IsoMet™, Lake Bluff, IL, USA).

4. Drug Elution

Five samples in each group were used to measure daily and cumulative antibiotic elution. Each sample was immersed in a polypropylene tube with 5 mL of sterile phosphate-buffered saline (PBS; pH 7.3) and stored in an incubator shaker at 37°C with constant shaking at 100 rpm. The study continued for 60 days, with the daily transfer of the sample into a test tube with PBS after washing with saline. At nine time points with a gradually expanding interval (1, 2, 4, 8, 10, 15, 25 and 45 and 60 days), 2 mL elution samples of PBS were collected. The eluate in each test tube was frozen at -20°C for analysis of antibiotic concentration. Antibiotic concentration in the collected eluate was quantified by high performance liquid chromatography (HPLC) with a 18 C column (10 nm analytical column with a flow rate of 0.5 mL/min). The isocractic mobile phase consisted of 10 mM KH2PO4 – Acetonitrile (composite ratio 17/3), and absorbance was measured at 210 nm. A standard curve was constructed using known telavancin concentrations.

5. Antimicrobial Efficacy

Four reference bacterial strains were selected as test organisms: ATCC 33591 (MRSA), N315 (MRSA), ATCC 29213 (MSSA), and ATCC 35984 (*S. epidermidis*). The minimum inhibitory concentrations (MIC) of telavancin according to the Clinical and Laboratory Standards Institute guidelines in Table V-2. [87,88]

Table V-2. Minimum inhibitory concentrations of both antibiotics and inoculum concentration**.**

Bacterial Strain	Characterization	MIC (mg/L)	Inoculum concentration (CFU/mL)
ATCC 33591	MRSA	2	10^3
n 315	MRSA	0.5 - 1	10^3
ATCC 29213	MSSA	0.5	10^3
ATCC 35984	*S. epidermidis*	1	10^3

[a] MIC tested at the recommended standard of 5×10^5 cfu/ml

The antimicrobial activity assay from antibiotic eluted from bone cement was performed as previously described [113]. Briefly, three samples were transferred into test tubes containing 3.4 mL of tryptic soy broth (TSB; Becton Dickenson, Franklin Lakes, NJ). The TSB were inoculated with bacteria on day 1 at a starting inoculum of 10^3 colony forming units (cfu)/mL (consistent with bacterial contamination of tissue) and evaluated daily for 7 days and again at 14 days. Serial 2-log dilutions of the culture were diluted in broth media and spot-plated on Mueller Hinton II agar plates (Sigma-Aldrich, St. Louis, MO, USA) for bacteria enumeration. Agar plates were incubated for 18-24 hours and bacterial cfu/mL determined (10 cfu/mL limit of bacterial detection).

6. Mechanical Testing

To determine mechanical performance, bending and compression tests were performed in accordance with ISO 5833 and fracture toughness tests were conducted in accordance with ASTM-D5045 [113,141]. After wet curing for 21 days in PBS at 21℃ mechanical tests were performed at the loading rates: 5 mm/min for bending tests, 5 mm/min for compression tests, and 10 mm/min for fracture toughness. Load-deflection data were measured by a Criterion C43.104

testing machine with a 1 kN LPS.103 load cell (MTS Systems Corporation, Eden Prairie, MN, USA).

Four-point bend testing was conducted to measure the flexural modulus and strength of the bone cement. Flexural modulus was determined from the stress–strain curves, and strength was calculated using the force at fracture of the sample. Cylindrical samples were compressed in the axial direction until failure occurred. Compressive modulus in MPa (slope at 0.2-0.4% strain) and yield strength (2% yield point) were determined from the stress-strain curves. Single edge notch bend testing was performed to measure fracture toughness in MPa$\sqrt{m}$ using ASTM-D5045.

7. Surface Characterization

To observe the effects of curing on the surface, samples were examined using a scanning electron microscope (SEM; Zeiss-LEO, Oberkochen, Germany). The number and size of pores were quantified in ImageJ. A thin layer of platinum was sputter coated onto the sample surfaces for 35 seconds with 45 mA. Images were obtained at 200x magnification using an acceleration voltage of 3 kV.

8. Statistical Analysis

All the results collected from experiment were statistically assessed using Minitab 18 (Minitab Inc., State College, PA). Kolmogrov-Smirnov method was used to determine normality. Kruskal-Wallis tests and post hoc Mann-Whitney U tests were conducted for non-parametric comparison between the control group and the groups with vancomycin added. Wilcoxon Signed Rank test was used to compare mechanical test results against the minimum ISO requirement. Results are presented as the mean $\pm$ standard error. A p-value of <0.05 was considered statistically significant.

Results

1. Antibiotic Elution

All tested samples showed a burst release (95%-98% of total 60-day elution) of telavancin during the first week (Figure V-1). In the low-dose group for Palacos®R (0.125g, 0.25g, and 0.5g telavancin), the specimens released antibiotics for eight days (Figure V-1A). The groups with 1g and 2g of antibiotic eluted two- and four-times higher amounts of telavancin respectively compared to the 0.25g groups. The 2g group in Palacos®R provided 15 days longer release duration versus the other groups, and the most eluted telavancin (0.0192 ± 0.001 ug). In contrast, much lower elution amounts of telavancin were observed from Simplex™P (Figure V-1B). The 2g group showed the most telavancin (0.0078 ± 0.0004 ug) eluted over 60 days, 60% less than from Palacos®R. In particular, in the Simplex™P low-dose groups, telavancin was released for a maximum of four days. The collected data were normalized and fit to a two-phase exponential decay function ($R^2 > 0.99$).

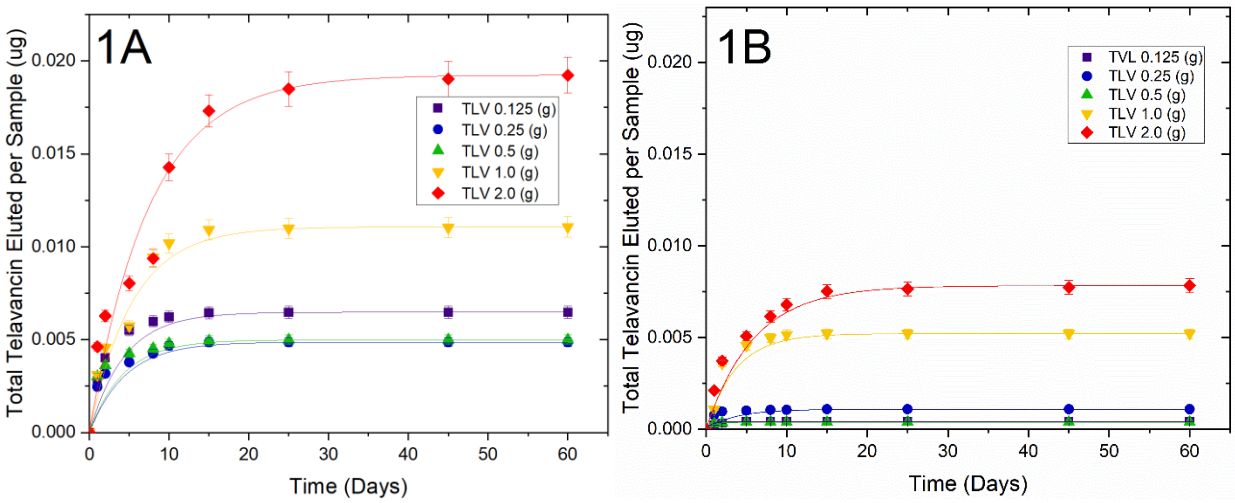


Figure V-1. Telavancin elution release profile (mean ± SEM) over a 60-day period showing a two-phase exponential decay model for Palacos® R (1A) and for Simplex™ P (1B).

2. Antimicrobial Activity

Telavancin retained its antibacterial properties after incorporation into Palacos®R. In the high-dose group (2g of telavancin), all test specimens exhibited antibacterial activity against MRSA ATCC33591, MRSA N315, MSSA, and *S. epidermidis* throughout the test period (Figure V-2). The four strains were eliminated within 14 days in this group.

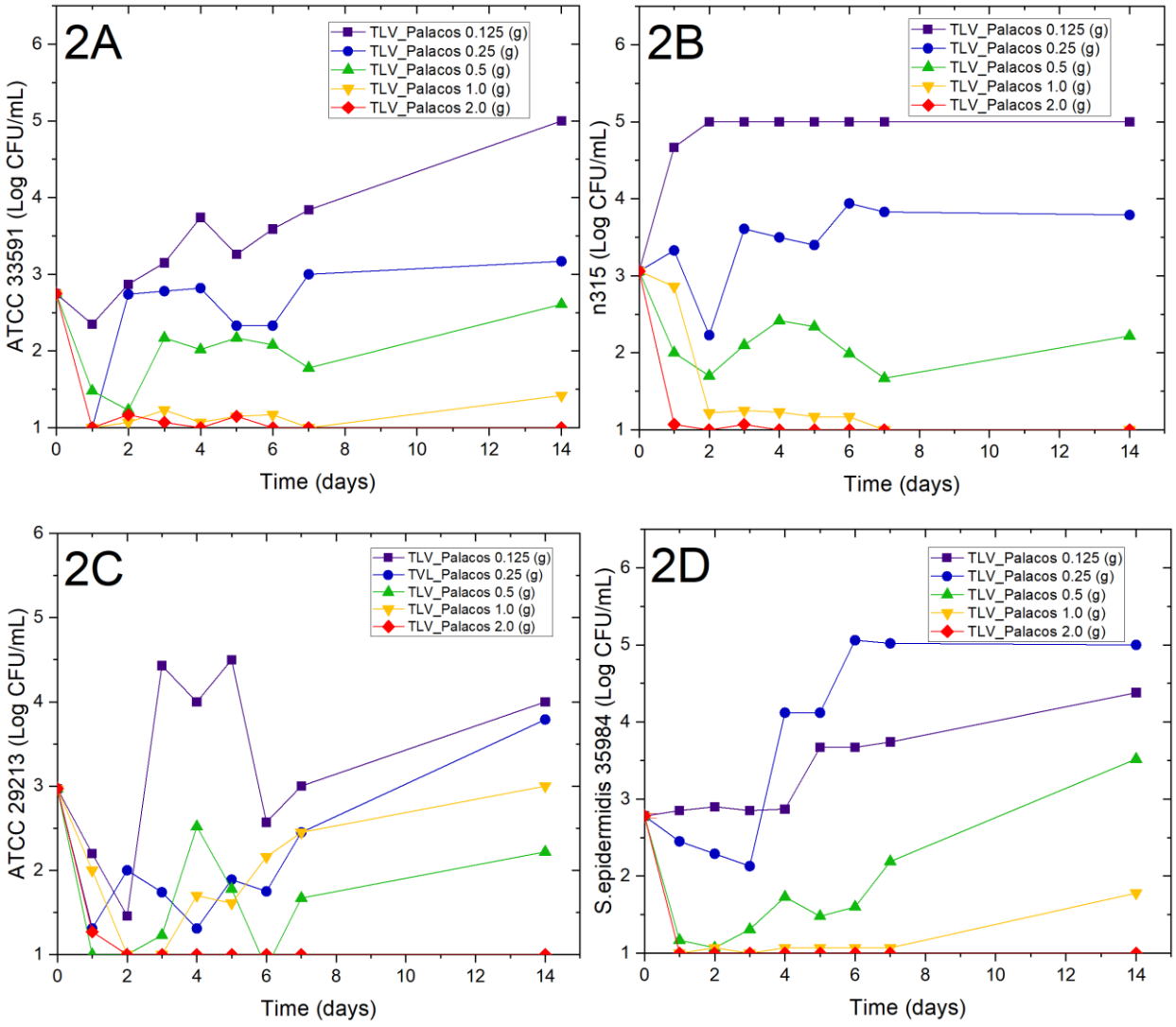


Figure V-2. Efficacy test of eluted telavancin (0.125g, 0.25g, 0.5g, 1.0g, and 2g of added telavancin) from Palacos® R for four strains of bacteria (2A – 2D). All colonies were completely eliminated, and no more bacteria were observed at day 14.

The antibacterial effect after incorporation into Simplex^{TM}P was limited to 7 days except against MRSA and N315 (Figure V-3). The specimens in the high-dose group were not able to completely eliminate MRSA ATCC33591, MSSA, and *S. epidermidis* within 7 days. These three strains regrew after 7 days regardless of the antibiotic loaded. In the low-dose group (0.125g, 0.25g, and 0.5g) in both cements, the antibacterial effect did not last longer than in the high-dose group (1g and 2g). These results align with the lower telavancin elution from Simplex^{TM}P. Overall, there was a clear dose-dependent effect on bacterial eradication with telavancin loading into both cements.

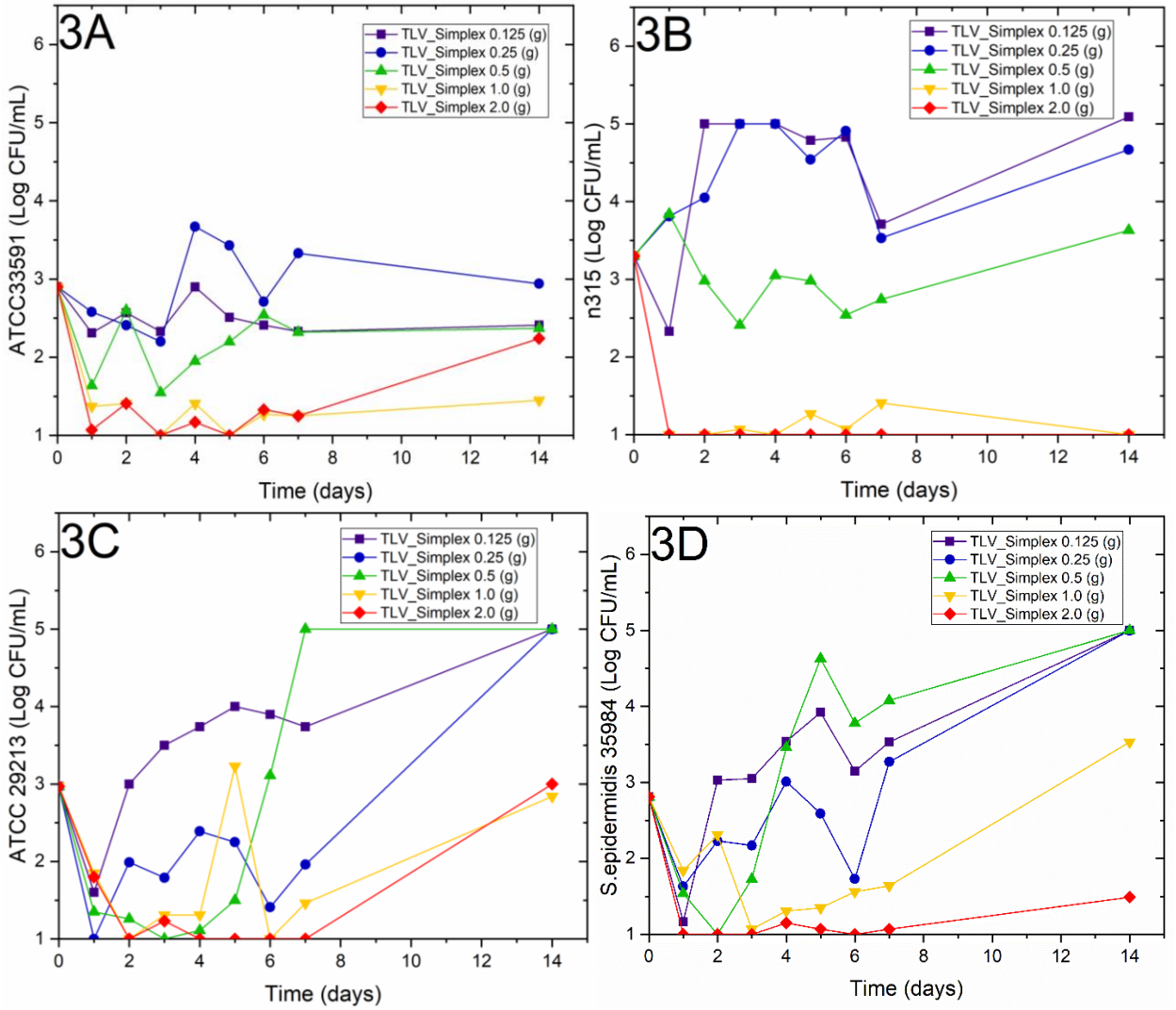


Figure V-3. Efficacy test of eluted telavancin (0.125g, 0.25g, 0.5g, 1.0g, and 2g of added telavancin) from Simplex™P for four strains of bacteria (3A-3D). No bacterial colonies were completely eliminated at day 14 except for MRSA and N315.

3. Mechanical Properties

The flexural modulus of both cements for each group exceeded the ISO minimum requirement (1800MPa). The flexural strength for groups of Palacos®R less than 2g was equal to or greater than the ISO minimum requirement (50MPa). The flexural strengths of Simplex™P groups with at least 0.25g telavancin were statistically below the ISO minimum standard and the control group's strength (63.2 ± 1.95 MPa). (Figure V-4)

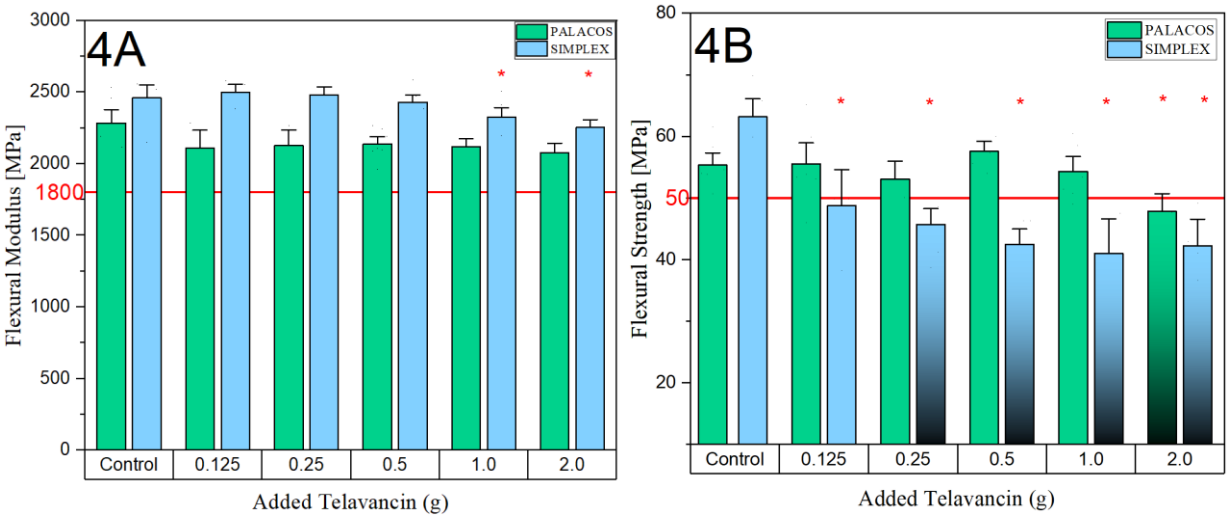


Figure V-4. Flexural modulus and flexural strength of telavancin formulated Palacos® R or Simplex™ P bone cement after the curing process. The solid red lines at 1800 MPa and 50 MPa indicate the ISO 5833 standard for minimum flexural modulus and minimum flexural strength respectively. The color gradient illustrates samples below the ISO minimum requirement. Values are shown as the mean and standard error of the mean for the specimens in each group. The asterisk mark represents a significant ($p < 0.05$) difference for all samples with added telavancin from the control group.

The compressive modulus of Palacos®R with 1g and 2g of added telavancin was significantly lower than that of the control group (1563 ± 73.2 MPa) (Figure V-5). For Simplex™P, all but the 0.5g group had a reduced compressive modulus compared with the control (1694 ± 73.9 MPa). The compressive yield strength for Palacos®R with 1g and 2g of added

telavancin were below the ISO minimum standard (70MPa). All groups of Simplex^{TM}P were above the ISO minimum standard.

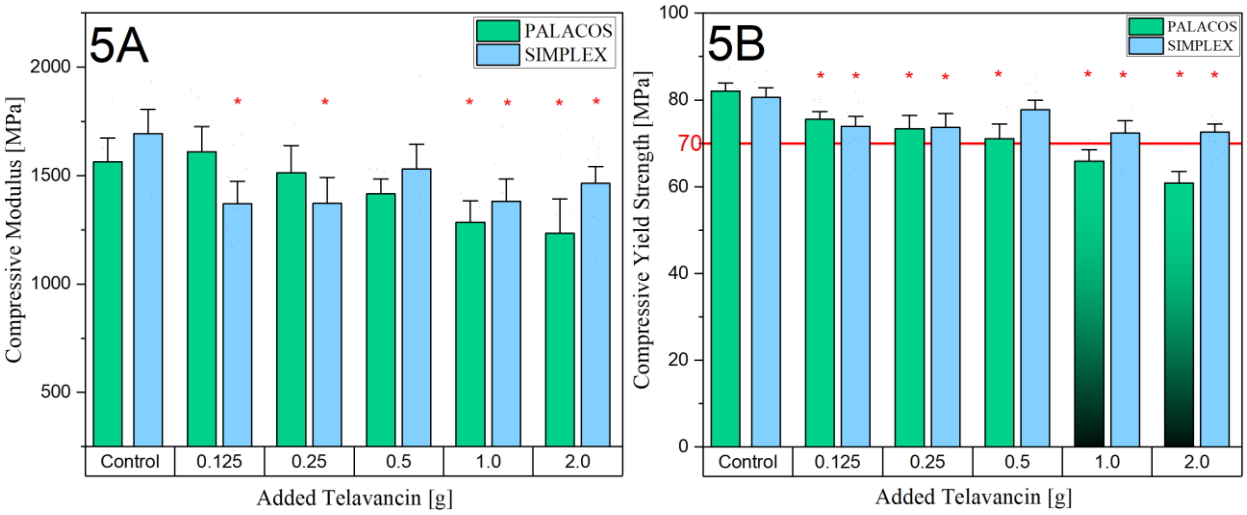


Figure V-5. Compressive modulus and compressive strength of telavancin formulated Palacos® R or Simplex^{TM} P bone cement after the curing process. The solid red line at 70 MPa indicates the ISO 5833 standard for minimum compressive yield strength. The color gradient illustrates samples below the ISO minimum requirement. Values are shown as the mean and standard error of the mean for the specimens in each group. The asterisk mark represents a significant ($p < 0.05$) difference for all samples with added telavancin from the control group.

The fracture toughness of Palacos®R was not significantly different between groups except at 2g of added telavancin. In contrast, the fracture toughness of Simplex^{TM}P was higher with 0.25g, 0.5g, and 2.0g of added telavancin as compared to the control group (1.69 ± 0.06 MPa$\sqrt{m}$) (Figure V-6).

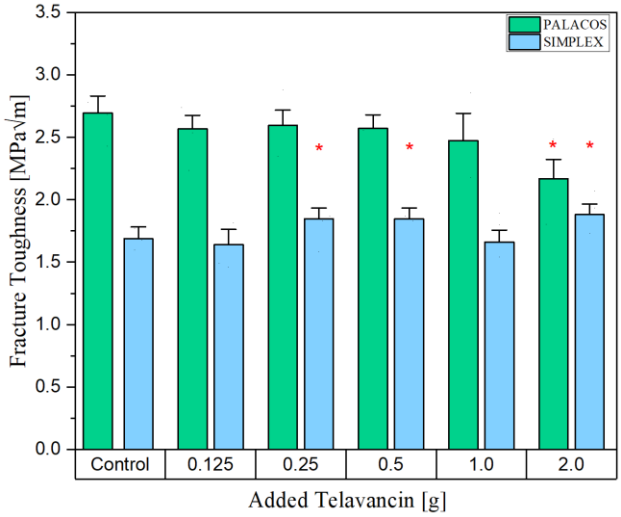


Figure V-6. Fracture toughness of telavancin formulated Palacos® R or Simplex™ P bone cement after the curing process. Values are shown as the mean and standard error of the mean for the specimens in each group. The asterisk mark represents a significant ($p < 0.05$) difference for all samples with added telavancin from the control group.

Discussion

We evaluated elution profiles and antibacterial activity of telavancin in Palacos®R and Simplex™P, which has not been previously reported. As in other cement comparison studies conducted with vancomycin, Palacos®R had the highest elution rates [92]. Kim and Bishop reported that the total vancomycin elution from Simplex™P was ten times lower than from Palacos®R [113,141]. Furthermore, Palacos®R demonstrated uniformly higher elution levels for a longer time period from total knee arthroplasty spacers *in vitro* [142].

Some limitations exist within this study. The antibiotic elution was tested under *in vitro* conditions, and the antibiotic elution *in vivo* may be different from that observed *in vitro*. Also, only one type of antibiotic was tested. New generations of antibiotics such as oritavancin, daptomycin, and linezolid have not been compared to telavancin in bone cement. Lastly, we did not measure the setting temperature of the cement and the degree of polymerization. Thus, the

degree of porosity on the cement surface or lowered antibiotic elution caused by incomplete polymerization was difficult to inspect.

The greater elution of telavancin from Palacos®R might be due to factors such as the physiochemical characteristics of the antibiotic, pore size, and bone cement surface roughness. Van de Belt reported that penetration of dissolution fluids into pores of polymer matrices depends on the wettability of the bone cement surface, making antibiotic release essentially a surface phenomenon. They also demonstrated that surface roughness is related to the initial rates of antibiotic release from bone cement and that porosity is related to a longer sustained release [125]. Other authors have stated that antibiotic elution from bone cement depends on the molecular weight of the antibiotic and the amount incorporated. Bone cement is a highly hydrophobic polymer, which limits elution. For this reason, some antibiotics are only eluted during the first hours; that is, only the antibiotic on the surface is released [143]. Furthermore, only high solubility and low molecular weight antibiotics can elute through voids and cracks [138,144,145]. For instance, when comparing the maximum elution amount for telavancin and vancomycin from Palacos®R, the maximum amount of telavancin was extremely small [113]. The reason may be that the molecular weight (1755.6 g/mol) and water solubility (0.0148 mg/mL) of telavancin are greater than those of vancomycin (1449.3 g/mol and 0.225mg/mL). In addition, telavancin is known for its ability to adhere to plastic and polymer surfaces, and therefore may have been unable to release from the cement [146].

Although the maximum amount of eluted telavancin (Palacos®R 0.0192 ± 0.001 ug and Simplex™P 0.0078 ± 0.0004 ug) was much less than for vancomycin (Palacos®R 0.1050 ± 0.000 mg and Simplex™P 0.139 ± 0.006 mg) [113,141], antibacterial activity was shown to have a similar

effect. The Palacos®R loaded with a high dose (2g) of telavancin provided a 14-day antibacterial effect against MRSA ATCC33591, MRSA N315, MSSA, and *S. epidermidis*.

The superior antibacterial potency of telavancin against staphylococci have been demonstrated by several *in vitro* and *in vivo* studies. Clous et al. compared the ability of telavancin to that of vancomycin to eliminate staphylococci from peritoneal dialysis fluid by using a static *in vitro* model to simulate the conditions of peritoneal dialysis. Telavancin exhibited better action against both methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* [147]. LaPlante and Mermel demonstrated the activities of telavancin and vancomycin against biofilm-producing staphylococci and enterococci, finding that telavancin was active against bacteria embedded in biofilm and that it inhibited biofilm formation at concentrations below the MIC, whereas vancomycin did not demonstrate the same activity [148]. In bone cements, Marsi et al. noted that high doses of antibiotic and maximal elution amount were necessary to maintain high concentrations above the breakpoint sensitivity for long-term efficacy [149]. Our results demonstrate that the amount of telavancin eluted from Simplex™P and Palacos®R was low, failing to meet the MIC concentration threshold.

The mechanical properties of Palacos®R and Simplex™P with addition of telavancin were changed after 21 days of curing in PBS. The flexural strength was decreased with added telavancin as compared with the control group. Samples with 0.25g and greater of added telavancin were below the ISO 5833 minimum. The degradation of flexural strength with added telavancin was even more pronounced with Simplex™P than with Palacos®R. However, the compressive modulus and yield strength of Simplex™P with 0.5g or greater of added telavancin was greater than that of Palacos®R. Topoleski et al. and Hoey el al. proposed that pores play a dual role in a bone cement. Pores cause stress concentration and promote crack initiation and propagation, which is

detrimental for the cement mantle. On the other hand, they divert energy to the surroundings and prevent crack propagation, which positively affects the mantle [41,122]. These findings suggest that pores in cured Simplex™P promoted cracks in bending tests; however, these effects are offset by crack inhibition in compression tests.

The fracture toughness of both cements with added telavancin offered contrasting results. The Palacos®R with 2g of added telavancin showed a statistically significant decrease of 19% in fracture toughness in comparison with the control group (2.70 ± 0.09 MPa$\sqrt{m}$), but Simplex™ P with 2g of added telavancin showed an increase of 14% in fracture toughness. These results were similar to findings in previously published studies with vancomycin [113,141]. It is known that the micron-size of pores can increase the apparent toughness through crack tip blunting [121,122]. The fracture toughness of Palacos®R was generally greater than that of Simplex™P, an outcome caused by differing pore sizes on the fracture surface. Pores on Palacos®R were identified as micro pores (diameter $<$ 1mm); in contrast, macro pores (diameter $\geq$ 1mm) were observed on Simplex™ P. Evans et al. demonstrated that larger pores are more critical because a crack emanating from a large pore is subjected to a greater volume of stress and therefore will grow more quickly [43] (Figure V-7).

Our study demonstrated that telavancin is apparently unsuitable as an antibiotic in bone cement. In Simplex™P it showed insufficient elution and an unsatisfactory antibacterial effect. Also, the mechanical strength of each cement with added telavancin was reduced in a dose dependent manner. This finding suggests telavancin cannot be considered for use in bone cement until degree of polymerization with telavancin is more clearly confirmed and the micro-structure of bone cement with pores is investigated in more detail.

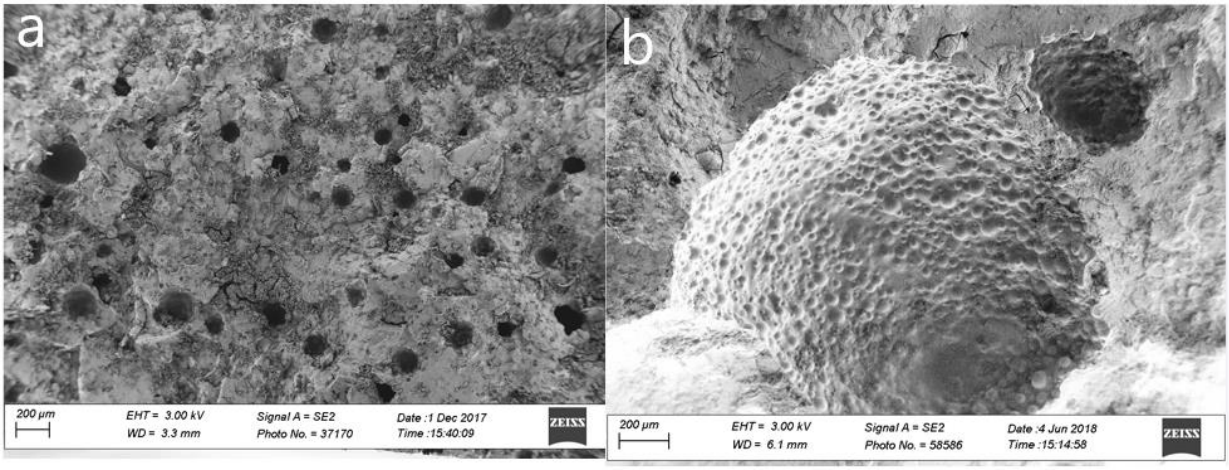


Figure V-7. 7A shows 2.0g of added telavancin loaded Palacos® R that contains more pores and relatively smaller pores, while 7B shows of 2.0g of antibiotic loaded Simplex™ P that contains fewer pores and relatively larger pores.

Conclusions

Amount of telavancin eluted from Palacos® R were greater than that of Simplex™ P. However, the amounts eluted from both cements were extremely low as compared to our previous study with vancomycin. For the efficacy tests, Palacos® R was better choice than Simplex™ P by demonstrating that the group with 2 g of added telavancin was able to completely kill four bacteria within tested period. The flexural strength of cured Palacos® R with 0.25 g and greater of added telavancin were greater than that of Simplex™ P whereas cured Simplex™ P with 0.5 g of added telavancin has greater compressive yield strength than Palacos® R. Cured Simplex™ P with added telavancin showed increased fracture toughness. Palacos® R contained more pores and relatively smaller pores while Simplex™ P contained fewer pores and relatively larger pores.

VI. MANUSCRIPT #4

Fracture Evolution in Bone Cement: XFEM Analysis of Micro-Structured Models with Pores

Submitted to Journal of Biomechanics

Abstract

Aseptic loosening is the most common reason for long-term revision of total joint replacement (TJR). Infection is the main reason for short-term revision of TJR. In our previous studies, experimental results showed that acrylic bone cement-loaded with antibiotics had a detrimental effect on cement strength such as bending strength, compressive strength, and fracture toughness. This result implied that the mechanical failure of antibiotic loaded bone cement was potentially related to porosity volume fraction. Hence, the objective of this study is to investigate the effect of porosity and its distribution on bone cement fracture toughness. The effect of pores was analyzed using the extended Finite Element Method (X-FEM) method to model crack propagation and its modulation by pore sizes and locations. Numerically obtained load-displacement response were compared to experimental results. We observe that crack propagation is affected by several porosity parameters; as expected these include pore size and pore locations (pore-pore interactions) and are related to implicit pore-crack interactions. The experimental and numerical investigations presented in the current study contribute to a better understanding of the effect of porosity on bone cement fracture toughness; key insights include the identification of a critical pore size for reduced fracture toughness, and relative insensitivity of crack propagation to stochastically distributed pore locations.

Introduction

Poly (methyl methacrylate) (PMMA) bone cement is a well-known synthetic biomaterial used for anchoring cemented arthroplasties to the contiguous bone. [150] The bone cement is used to attach an implant to a bone, and it mainly plays a role in transferring load from the prosthesis to the bone. The survival rate associated with cemented femoral stem fixation in total hip arthroplasty is over 88 % for the last 15 years. [151] Despite the high success rate of the implant, the use of this material has been controversial. One of the major drawbacks is aseptic loosening, which was the most common reason (57.5%) for mechanical failure during the period 1999-2017 regardless of whether the hip prosthesis had been revised earlier. [152–154] The cement at the interface between implant and bone is a material discontinuity; this makes the bone cement prone to fracture [155] and can lead to interface failure between the cement mantle and the implant. This failure mechanism is considered to be the primary mechanism of aseptic loosening in cemented arthroplasty. [155,156] Therefore, the fracture toughness of the cement is an essential parameter reflecting the reliability and defect tolerance of the cement.

The clinical use of bone cement has been investigated by many researchers. Numerous laboratory studies focused on antibiotic-impregnated bone cement to reduce the risk of infection that occurs after arthroplasty surgery; and, some authors have demonstrated that cement with or without antibiotics has degraded mechanical strength after conditioning in saline solution. [157,158] There is increasing concern that adding antibiotics to the bone cement weakens the cement and results in early failure of the implant.[159–161] However, it is unclear as to what factors significantly affect and decrease the cement strength. There is a general consensus in the community that porosity is highly detrimental to cement strength and has been shown to initiate failure in vivo. [42,162] Bone cement tends to contain various sizes of pores which are a result of the mixing

process and/or the elution of powders like antibiotics from the cement. Pore sizes are typically classified as either micro or macro. Micropores from 0.1 to 1.0 mm in diameter, arise from the evaporation of the monomer; and, macropores with diameters greater than 1.0 mm are caused by entrapment of air during the mixing process and insertion of the cement into the prepared bone. [25,41]

Some studies have shown that pores can act as crack initiation and propagation sites. [163,164] These findings prompted the development of mixing techniques to reduce the porosity, such as mixing the cement in a vacuum and centrifuging. These two mixing methods successfully showed a substantial reduction of the overall porosity and increased the cement strength. [165–167] Hosseinzadeh et al. (2013) noted that when the pore reached a critical size, the pores acted as sites of stress concentration which led to the weakening of the cement. Pores below this critical size acted as crack arrestors; therefore, the authors emphasized the elimination of pores greater than the critical size. The pore density and distribution, apart from overall bulk porosity, are other important factors. Hoey and Taylor (2009) demonstrated that pore clustering was detrimental to the fatigue properties of bone cement. Jeffers et al. (2005) in their simulations of cement porosity with finite element analysis (FEA) found that porosity in the cement may facilitate fatigue cracking of the cement mantle, which results in implant loosening. Despite the numerous studies which have been performed to identify the effect of porosity on bone cement, a thorough comparative study of experimental and numerical predictions to investigate the fracture toughness and crack behavior for antibiotic-loaded bone cement has not been carried out. This paper outlines such a study using fracture experiments and the extended finite element (X-FEM) numerical method [52,170–173] to investigate crack evolution in antibiotic-loaded bone cement.

This study aims to (a) investigate how the size and distribution of pores can affect the fracture toughness of antibiotic-loaded bone cement; (b) consider the effect of pores in crack initiation and propagation; (c) develop general rules governing the role of pore size on crack initiation and propagation; and, (d) compare the experimental and the simulation results for validation of simulation models.

Materials and Methods

2.1 Materials

Six different experimental groups were prepared based on different amounts of added antibiotic. The bone cement (Palacos® R) with no antibiotic served as a control group and five telavancin-loaded bone cement groups were prepared with antibiotic masses at 0.125 g, 0.25 g, 0.5 g, 1.0 g, and 2.0 g respectively. Each group had seven samples. All the specimens were fabricated under the vacuum pressure of -50 mbar. [113] An aluminum mold was created for fabricating test samples with dimensions as described in ASTM-D5045 for fracture toughness testing. Fracture toughness specimens had 44 mm width, 5 mm depth, and 10 mm height with a 5 mm pre-existing crack. Cracks were created using a diamond wafering blade (Buehler® IsoMetTM, Lake Bluff, IL, USA). Crack length was measured from images using ImageJ (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, Madison, WI, USA).

2.2 Mechanical testing

Single edge notch bend testing (Figure VI-1) was applied to the three-point bending samples with displacement rate control mode at a speed of 10 mm/min until fracture. The applied bending load was recorded synchronously with crosshead displacement and fracture toughness in $MPa\sqrt{m}$ was calculated according to ASTM-D5045. For single edge notch bending test, bending

stress (σ_b) and mode I fracture toughness (K_{Ic}) were found from Equation (1) [174,175] and Equation (2) [84,113,141], respectively.

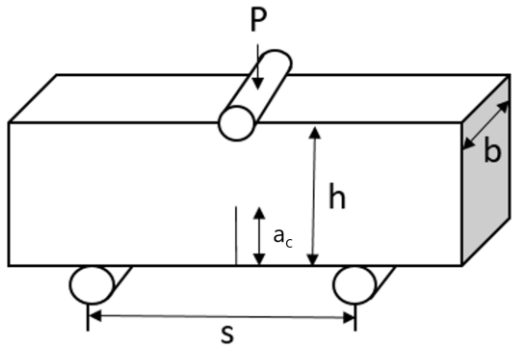


Figure VI-1. Schematic of single edge notch bending test

$$\sigma_b = \frac{3PS}{2b(h - a_c)^2} \quad (1)$$

$$K_{IC} = \frac{3PS}{2bh^{3/2}} f(x) \quad (2)$$

Where P denotes the bending load (in N), a_c is the length of the notch (in mm). b and h are, respectively, the width (in mm) and height (in mm) of the sample. S (in mm) is the span between the two lower supporting points. f is a function of a_c/h given by Equation (3) [84]:

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

In order to ensure linear elastic deformation of the specimen, the linear-elastic plane strain fracture toughness criterion given by Equation (4) [84] was used.

$$(h-a), b, a \geq 2.5\left(\frac{K_{IC}}{\sigma_{ys}}\right)^2 \quad (4)$$

2.3 Extended Finite Element Model (XFEM)

In this section, we briefly discuss XFEM and linear elastic fracture mechanics approach that is used to numerically model crack propagation in this work. XFEM is a popular advancement of the classical finite element method (FEM) that is used to model discontinuities - especially fracture of materials. In XFEM, additional degrees of freedom are added at the finite element (FE) nodes in the neighborhood of the crack path. These additional degress of freedom are used to represent the displacement discontinuity (crack opening) induced by the presence of a crack, and this is achieved without much alteration to the underlying FEM mesh. The displacement interpolation used in XFEM is given by Equation (5).

$$u(x) = \sum_{I=1}^{N} N_I(x)\left[u_I + H(x)a_I + \sum_{a}^{4} F_a(x){b_I}^a\right] \quad (5)$$

Where $u(x)$ is the displacement, $N_I(x)$ is the conventional FE shape function, $H(x)$ is the discontinuous shape function, a_I is the added set of degrees of freedom to the standard finite element model, F_a is the crack tip enrichment function, ${b_I}^a$ is the degree of freedom of the enrichment node. The four crack tip enrichment functions are given in Equation (6) [176], where r and θ are polar coordinates relative to the local coordinates of the crack tip:

$$F_a(x) = \left\{\sqrt{r}\sin\left(\frac{\theta}{2}\right), \sqrt{r}\cos\left(\frac{\theta}{2}\right), \sqrt{r}\sin(\theta)\sin\left(\frac{\theta}{2}\right), \sqrt{r}\sin(\theta)\cos\left(\frac{\theta}{2}\right)\right\} \quad (6)$$

We use the XFEM implementation available in the commercial finite element software Abaqus [Dassault Systèmes Simulia Corp., Johnston, RI, USA. Version 6.11] to generate the

simulation results in this work. We consider 2D problem geometrics and assume a plane strain material model for the bone cement. The mechanical properties (Young's modulus and yield strength) were obtained from four-point bending test and compression test experiments in our previous study. [113] Poisson's ratio was assumed to be 0.4. [177] All the relevant mechanical properties are listed in Table VI-1.

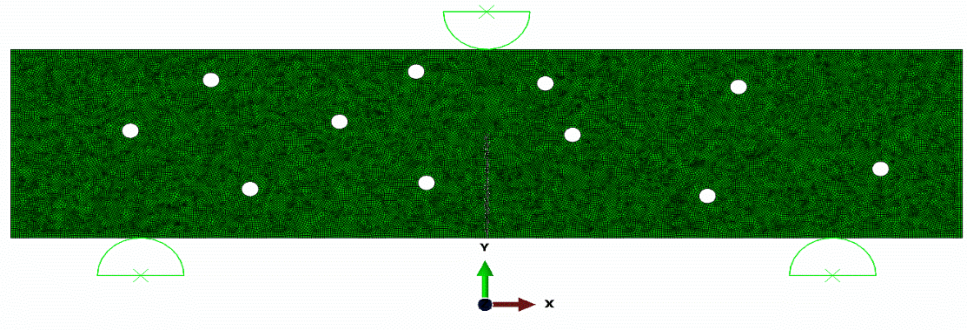


Figure VI-2. Two-dimensional mesh for XFEM of a notched bone cement sample with pores under single edge notch bending test.

Figure VI-2 shows a two-dimensional XFEM model of the bending fracture test setup of a bone cement sample with randomly distributed pores of the same size. The mesh consisted of 2,000 to 3,000 four-node bilinear plane strain quadrilateral and reduced integration elements (CPE4R). The top and bottom supports (radius 2.0 mm) were modeled as rigid surfaces. The bottom supports were fixed in all directions and the displacement of the top support was applied by controlling the displacement of the reference point. A friction coefficient of zero between steel and PMMA was assumed. The fracture zone evolution was modelled as a cohesive zone with damage evolution (Figure VI-3) as implemented in the ABAQUS software, and the maximum principal stress criterion was selected as the damage criterion for the traction separation law.

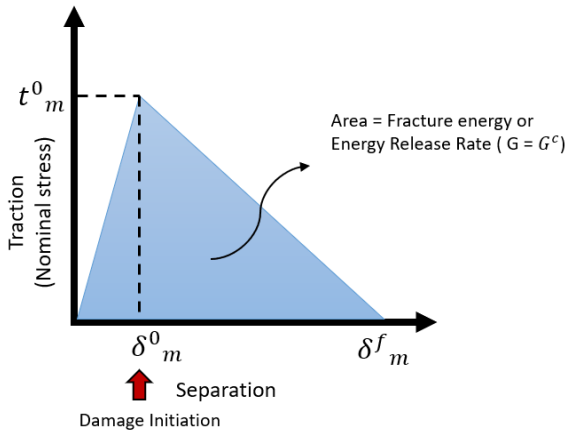


Figure VI-3. Damage evolution for traction separation law based on energy and displacement. The triangular cohesive law contains two critical parameters: the cohesive strength $t^0{}_m$ (the maximum traction the interface can endure) or the separation length $\delta^0{}_m$, and the cohesive energy which is the area of the triangle. The critical strain energy rate G_c corresponds to the area under the traction-separation curve.

Table VI-1. Bone cement mechanical properties for the XFEM models.

Young's modulus (MPa)	Poisson's ratio	Maximum Allowable Principal stress (MPa)	K_{IC} (MPa$\sqrt{m}$)	Fracture energy (J/m^2)
2080	0.4	57	2.54	3712

2.4 Pore Size and Location on XFEM Modeling

The mechanism of crack growth and toughening of bone cement is complex in part due to the porous microstructure. The pore-related parameters that effect crack growth and propagation can be divided into independent parameters such as pore shape and dependent parameters such as pore-pore and pore-crack interactions. The interactions of the dependent parameters are affected by pore size and pore location. In this study, therefore, three different conditions I, II, and III were tested:

I. Locations of pores were fixed and diameter of each pore was increased from 0.2 mm up to 1.0 mm with 0.2 mm increments. Three different fixed pore location were tested.

II. Locations of pores were randomly distributed and diameter of each pore was fixed at 0.2 mm, 0.6 mm, and 1.0 mm.

III. Locations of pores were randomly distributed and a normal distribution of each diameter (0.2 ± 0.07 mm, 0.6 ± 0.2 mm, 1.0 ± 0.18 mm) was assigned.

2.5 Micro-CT scanning for Porosity Measurement

The porosity of the antibiotic-loaded bone cement samples were examined using a micro-CT system (MicroXCT400, Xradia, Oberkochen, Germany) with an acquisition protocol that consisted of X-ray tube setting of 80 kV, 9 W and 100 μA, exposure time of 0.5 seconds, 100 frame averaging. Four specimens were mounted on a rotary stage and scanned in their entirety, being rotated -92° to 90° equiangular steps. The associated scan times were approximately an hour per scan. The stack of two-dimensional (2D) images consisted of 400 slices, 40 mm field of view, pixel size of 38 μm, and isotropic voxel size of 40 μm. Following scanning images were imported into Mimics Innovation Suite 20.0 (Materialise NV, Leuven, Belgium) to obtain three-dimensional (3D) models of the specimens. Images were segmented by an operator-selected threshold of grey scale values in order to separate the voxels representing bone cement. The 3D models were then analyzed to obtain porosity by quantifying the bulk and pore volumes in each bone cement sample. The porosity of each sample was calculated by pore volume over overall sample bulk volume. (Figure VI-4)

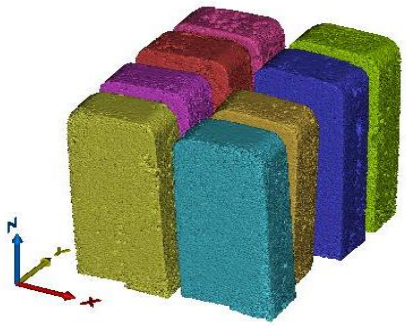


Figure VI-4. 3D modeling from micro-CT image data of fracture toughness test specimens

2.7 Scanning Electron Microscopy (SEM)

The external and fracture surfaces of fracture toughness samples following the test were examined using a scanning electron microscopy (SEM; Zeiss-LEO, Oberkochen, Germany). The size of pores (macro-pore and micro-pore) and the number of pores were quantified. A thin layer of platinum was deposited on the sample surfaces for 35 s with 45 mA. Images were obtained at 200x magnification using an acceleration voltage of 3kV.

2.8 Statistical Analysis of Mechanical Test Data

All the results collected from mechanical testing were statistically assessed using Minitab 18 (Minitab Inc., State College, PA). The Kolmogrov-Smirnov method was used to test the normality assumption. Kruskal-Wallis tests and post hoc Mann-Whitney U tests were conducted for non-parametric comparison between the control group and the groups with telavancin added. Results are presented as the mean $\pm$ standard error of mean. A p-value of <0.05 was considered statistically significant.

Results

3.1 Fracture Toughness Test Results

The fracture toughness, Mode I critical stress intensity factor, of Palacos® R bone cement was statistically significantly lower ($p<0.05$) with the addition of 2 g of telavancin as compared to the control group (Mean 2.70± 0.22 MPa√m). The fracture toughness data generated for Palacos® R acrylic bone cement for each group with different amounts of added antibiotic are presented in Figure VI-5.

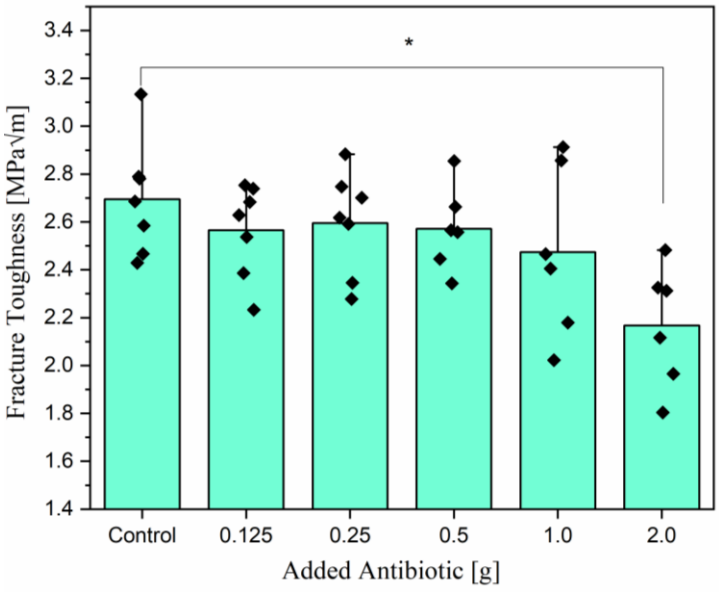


Figure VI-5: Fracture toughness of telavancin formulated Palacos® R bone cement after curing. Values are shown as the mean and standard error of the mean for the specimen in each group. The asterisk mark represents a significant ($P<0.05$) difference from control group.

The experimental load-displacement curves for the samples in the control group (Figure VI-6) overlapped more closely with each other while the groups with added telavancin showed more variance in stiffness between samples. In addition, the peak force for the 2 g of added telavancin group (137 ± 13.6 N) showed a significant decrease when compared to the control

group (160 ± 12.3 N). The load versus displacement curves for each group with different amounts of added antibiotic are plotted in Figure VI-6.

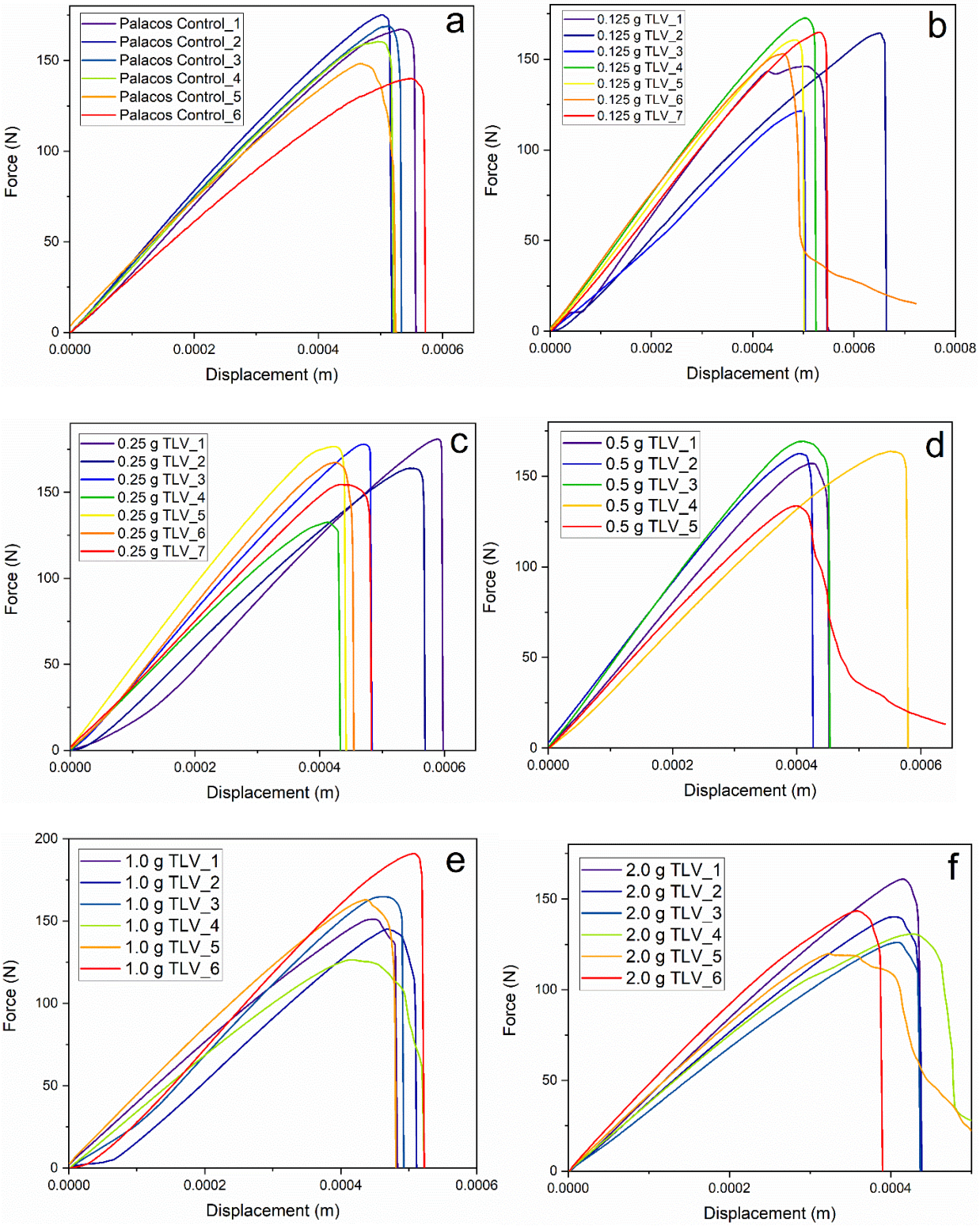


Figure VI-6: The experimental load-displacement curves for the sample groups with different amounts of added telavancin.

The SEM pictures in Figure VI-7 show that the bone cement samples have pockets of PMMA beads and matrix. The figure also presents the ‘mirror’, ‘mist’ and ‘hackle’ zones of crack propagation surface. The ‘mirror’ zone is formed during the ‘crack growth’ phase. The ‘mist’ zone is created by the slow crack propagation. However, there did not seem to be a noticeable difference between the mirror and mist zones. The ‘hackle’ zone is created by rapid crack propagation. In the hackle zone, the surface appeared much smoother and flatter when compared to the other two zones. Referring to Figure VI-7b and 7c, it can be said that cracks propagated into the bead pocket completely or they propagated through the space where pores exists (Figure VI-8b).

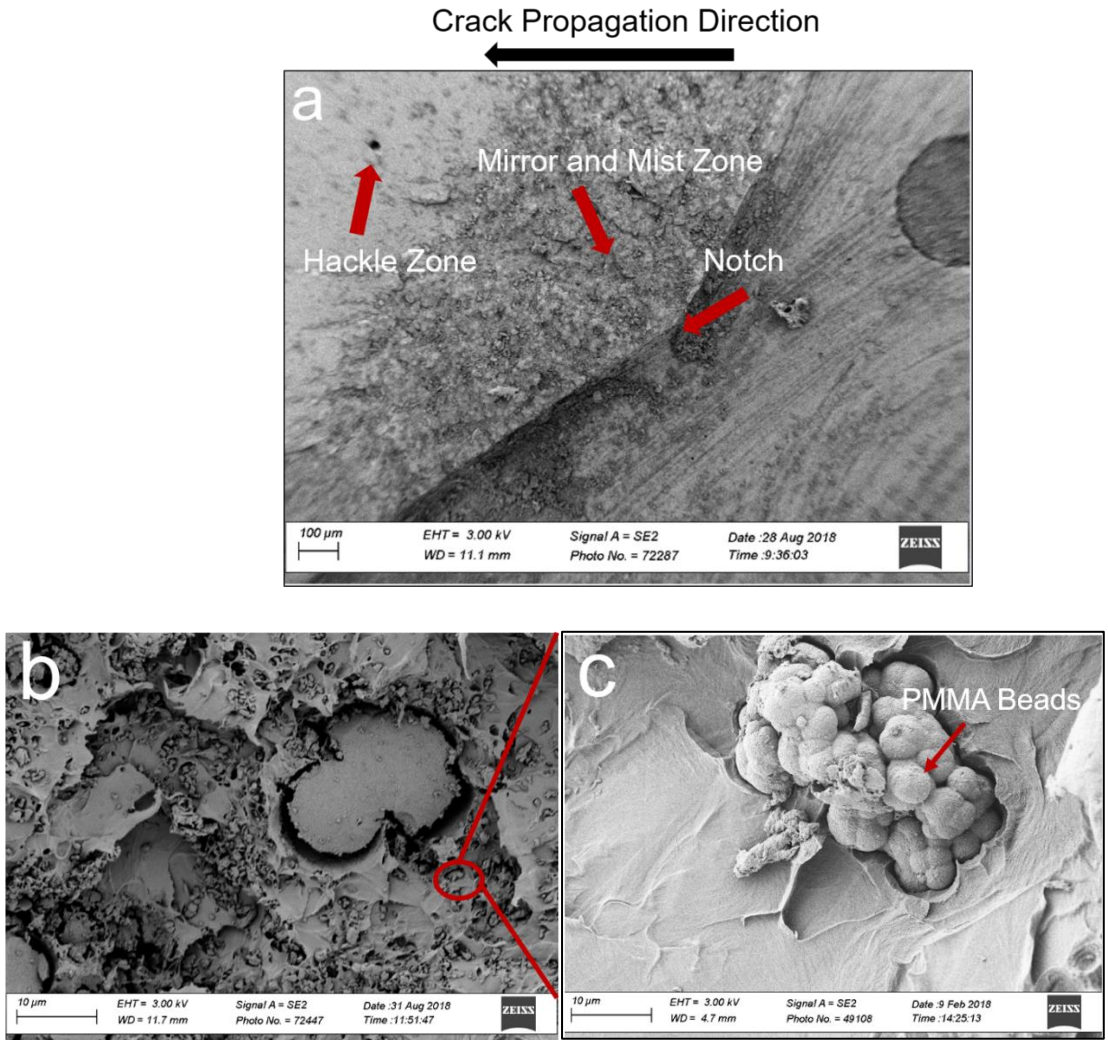


Figure VI-7: SEM pictures of fracture surface following the test at increasing magnification (a: 100x; b: 500x; c:2000x) showing the 'mirror', 'mist' and 'hackle' zones of crack propagation surface and pocket of PMMA powder beads.

3.3 Porosity Measurement Results

Larger pores (1.0 - 2.0 mm) were observed in SEM images of the fracture surface for the 2 g of antibiotic group and relatively smaller pores (200 - 500 µm) were found in the control and 0.125 g added antibiotic groups as shown in the Figure VI-8. The percent porosity as quantified from the micro-CT data (Table VI-2) showed that all of the groups were significantly greater than the control group except for 0.125 g ($p < 0.05$). The level of porosity was divided into two groups: small dose groups (0.125 g, 0.25 g and 0.5 g) and large dose groups (1.0 g and 2.0 g).

The groups with small doses of added antibiotic were not significantly different from each other and the groups with large doses of added antibiotic were not significantly different from each other ($p < 0.05$).

Table VI-2. Percent porosity of control group and five groups of different amounts of added antibiotic. Porosity was measured from micro-CT data of samples. All porosities are represented as mean ± SEM. Asterisk shows significant difference from control group ($p < 0.05$).

Bone Cement	Antibiotic Mass (n =8)					
	Control	0.125 (g)	0.25 (g)	0.5 (g)	1.0 (g)	2.0 (g)
Porosity (%)	4.49 ± 0.32	4.70 ± 0.30	5.59 ± 0.15*	5.09 ± 0.22*	5.92 ± 0.35*	7.09 ± 0.47*

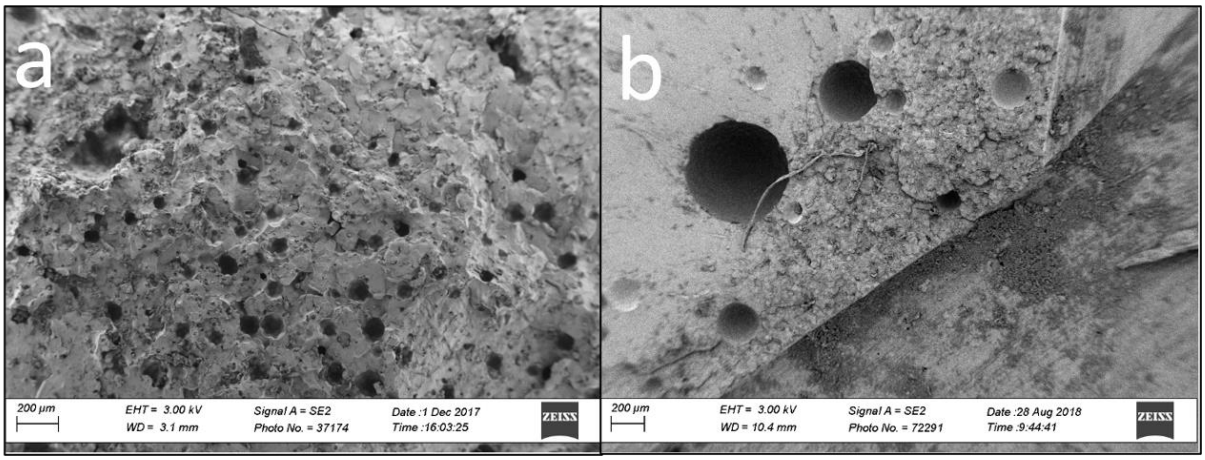


Figure VI-8: (a) shows 0.25 g of added antibiotic loaded bone cement that contains more pores and relatively smaller pores while (b) shows of 1.0 g of antibiotic-loaded bone cement that contains the fewer pores and relatively larger pores.

3.4 XFEM Simulation Results

Three different cases were simulated as described in method section 2.4. Their results were compared with that of the experimental load-displacement data. For case I, all three different models obtained similar load-displacement patterns. As shown in the Figure VI-9, the change in size of pore significantly impacted the load-displacement curves. A reduction in the peak force and stiffness were observed for the increased pore size and pore volume fraction.

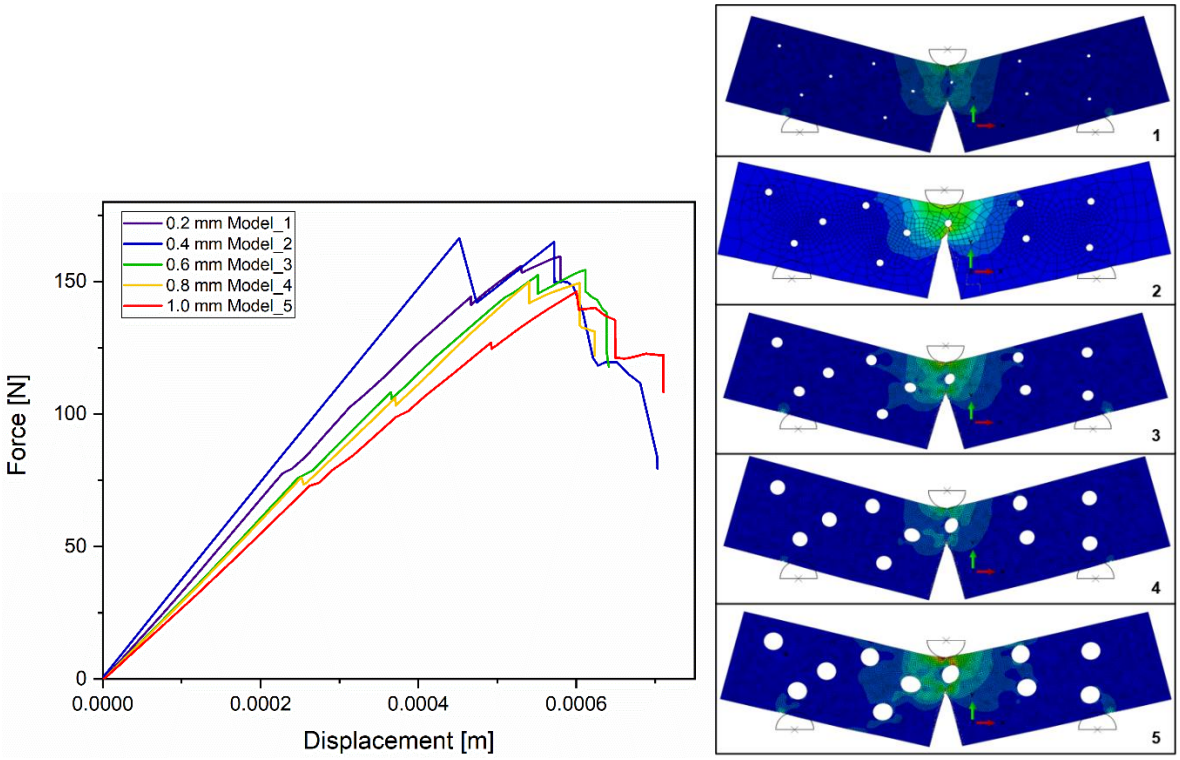


Figure VI-9: The numerical load-displacement curves for 0.2, 0.4, 0.6, 0.8 and 1.0 mm pore size with fixed locations.

The effect of pore location by testing five different models containing randomly distributed pores with fixed diameter at 0.2 mm is illustrated in Figure VI-10. The stiffness and peak force for each model was consistent regardless of pore location but the behavior following crack initiation varied. This characteristic was also found in the other models with fixed pore diameter of 0.6 mm and 1.0 mm.

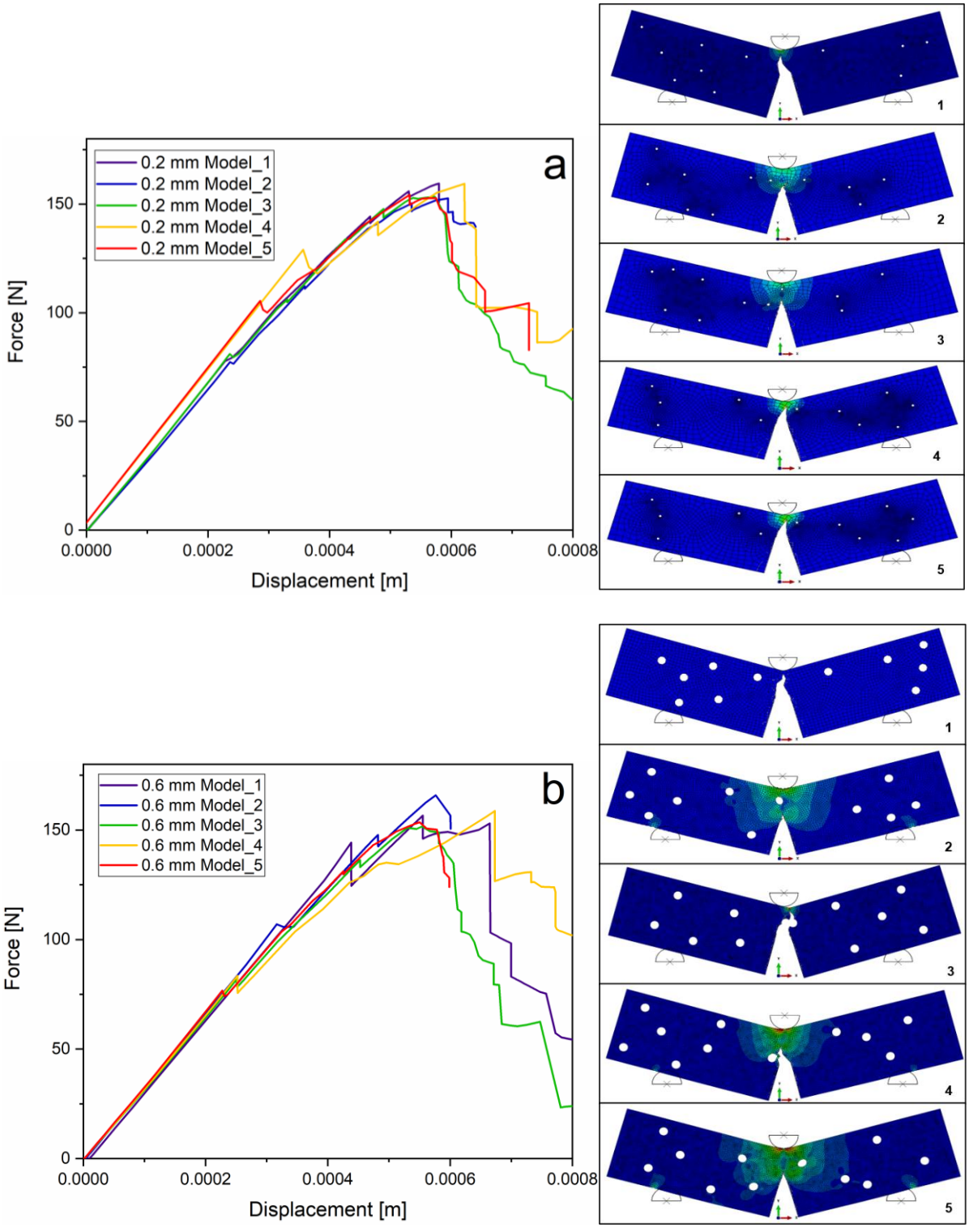
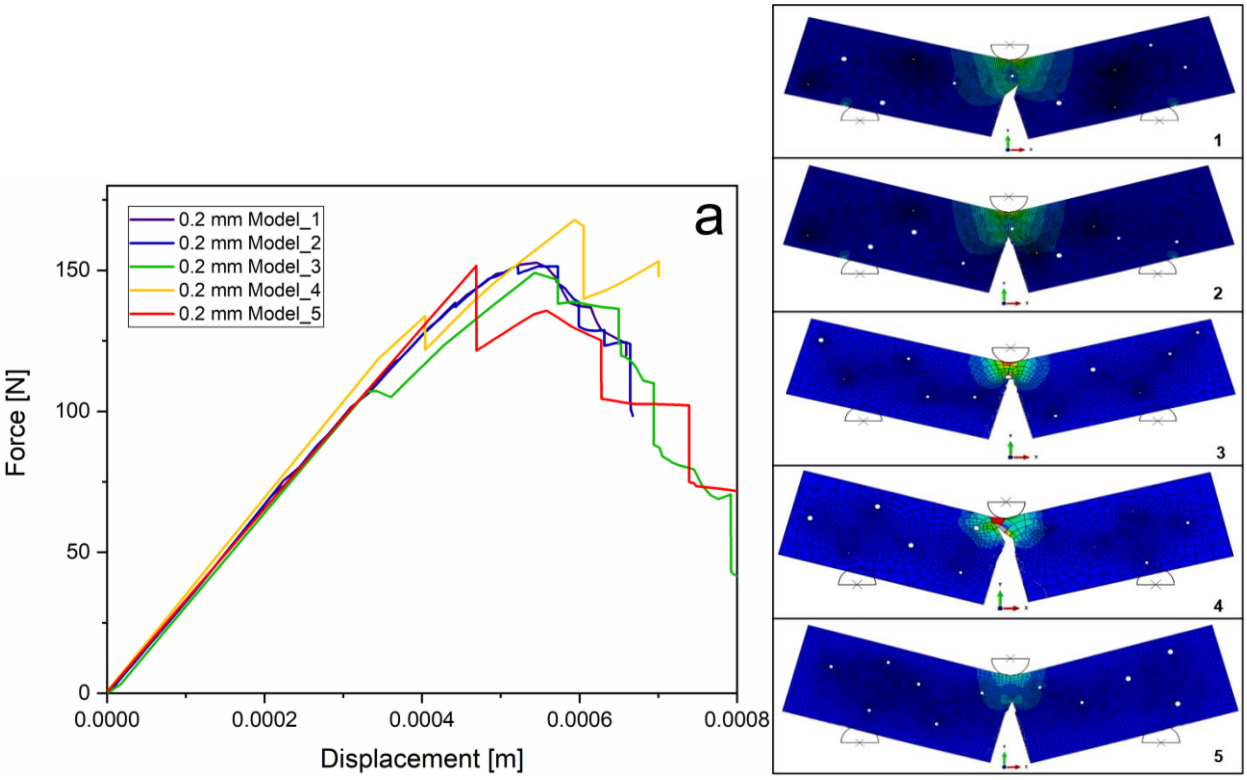


Figure VI-10*:* The numerical load-displacement curves for (a) 0.2 mm and (b) 0.6 mm pore sizes with random locations.

Figure VI-11 shows the result for case III where locations of pores were randomly distributed and a normal distribution of each diameter (0.2 ± 0.07 mm, 0.6 ± 0.2 mm, 1.0 ± 0.18 mm) were assigned. The load-displacement behavior for the 0.2 mm models closely overlapped with each other up to the peak point and then had slight differences due to variances in crack propagation patterns. On the other hand, differences in stiffness and peak force can be found in the models with larger average pore diameters. The difference between the Figure 10b and Figure 11b implies that the size of the pore affects the load-displacement behavior significantly more than randomness of the pore location.



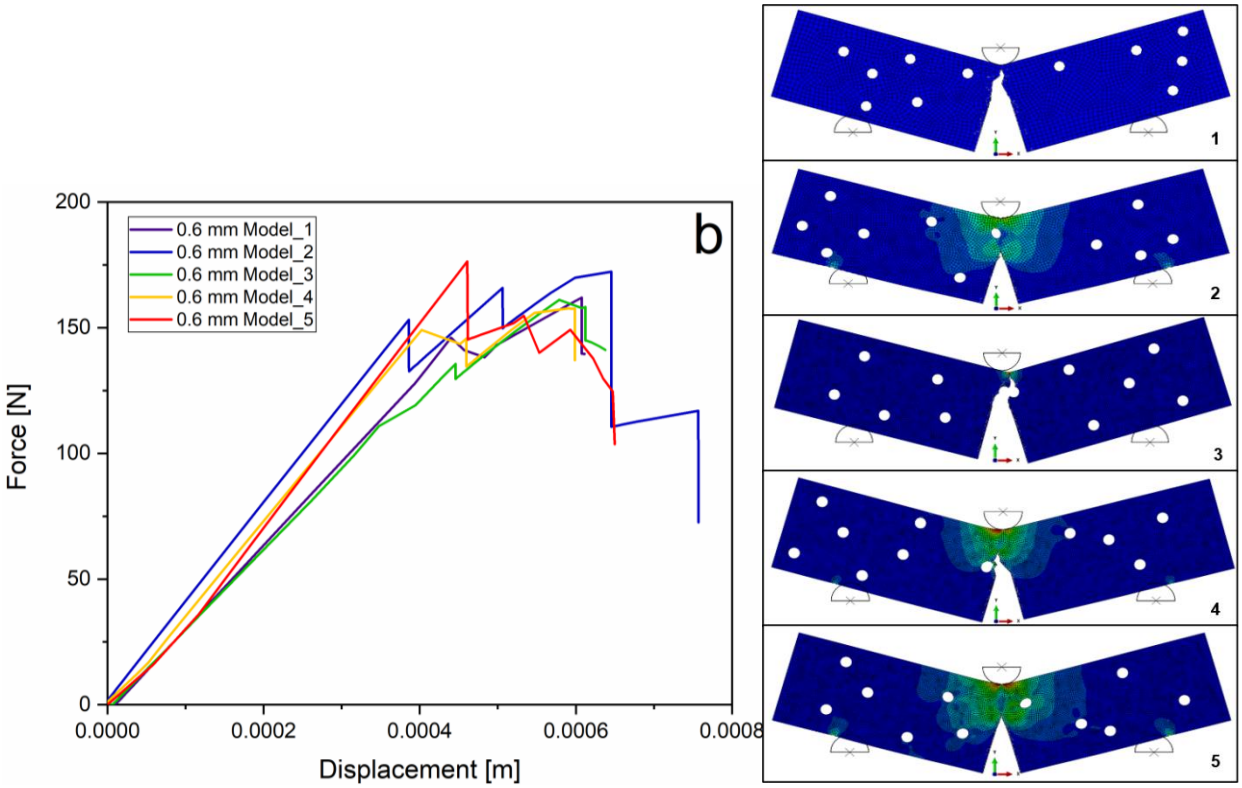


Figure VI-11: The numerical load-displacement curves for (a) 0.2 mm and (b) 0.6 mm of average pore size with random locations.

3.5 Comparison of Experimental and Simulation Results

Figure VI-12 shows experimentally measured load-displacement curves for all groups of added antibiotics together with FEA simulation results. FEA simulation results show good agreement with the experimental load-displacement response for the control and 0.125 g groups, and show variations in the stiffness for the other groups. The 0.25 g, 0.5 g, 1.0 g and 2.0 g exhibit similar peak forces as the experimental values, but with reduced stiffness. The control and 0.125 g groups show the most similar load-displacement response for the 0.6 mm of model in Case III and the 0.2 mm of model in Case II, respectively.

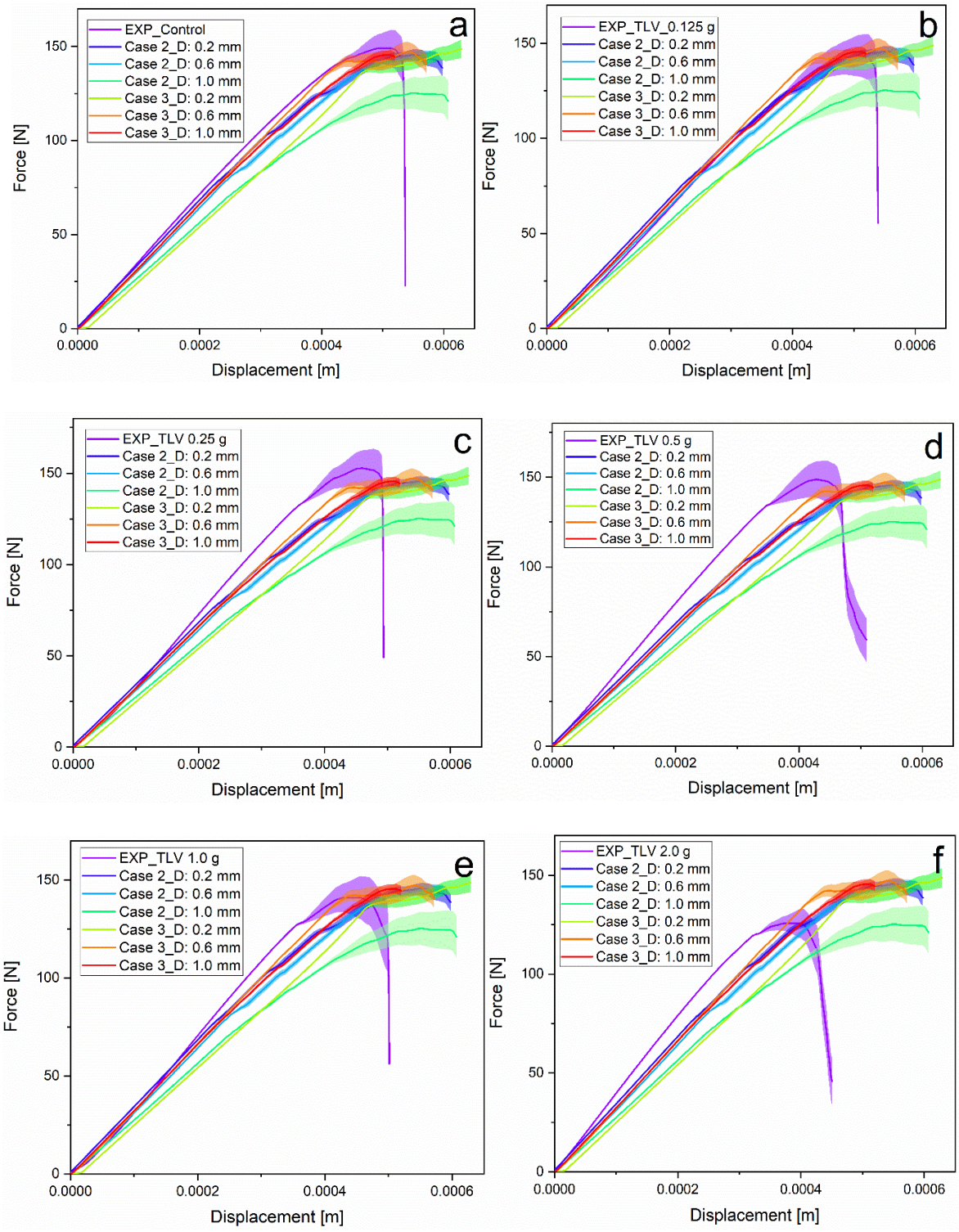


Figure VI-12: Comparison of load-displacement data from experiments and numerical simulations of fracture in bone cement for (a) control group and groups with different amounts of added antibiotic: (b) 0.125g, (c) 0.25 g, (d) 0.5 g, (e) 1.0 and (f) 2.0 g. The load-displacement curves illustrate the mean and the standard error of the mean.

Discussion

The effect of antibiotic addition on the fracture toughness of PMMA bone cement was experimentally investigated. The results obtained in the present study for control sample of Palacos® R (2.70± 0.22 MPa√m) were significantly reduced after adding antibiotic to the bone cement. The reduced fracture toughness after adding antibiotic was illustrated in other studies. Slane and co-workers investigated the effect of xylitol addition to bone cement. They found that the fracture toughness of the control sample for Palacos R+G (2.18 ± 0.07 MPa√m) was degraded up to 36 % after the addition of 10 g xylitol. [80] Bishop and co-workers studied the effects added vacomycin to the Palacos® R. They found that the fracture toughness for the 2 g of antibiotic group (2.18 ± 0.17 MPa√m) was 19 % less than the control group (2.69 ± 0.07 MPa√m). [113] Extending these studies, our observations suggest an inverse relationship between the mass of added antibiotic and fracture toughness. This finding has been supported by micro CT data. Micro CT data showed that the overall amount of porosity significantly increased by the mass of added antibiotic. This result demonstrated the relationship between the porosity and fracture toughness in different doses of added antibiotic group.

The role of porosity in the fracture toughness of bone cement is a controversial issue with some studies stating that pores are essentially defects and should be removed, while others state that pores are required for drug elution, bone ingrowth and also as a method of blunting crack propagation. The current study found that pore size was a dominant contributor to fracture toughness reduction. Although a high level of porosity was observed in the 1 g of added antibiotic group, the critical force for crack initiation remained unaffected, since pore sizes were below critical; whereas, for the 2 g group a lower critical force was required for crack initiation despite a similar level of porosity to the 1 g group. Evans et al. demonstrated that larger pores are

more critical because a crack emanating from a large pore is subjected to a greater volume of stress and therefore will grow more quickly. [43] James et al. also investigated the critical role of porosity in the fatigue life of PMMA bone cement. They found that not only the overall porosity but also individual pore size and pore distribution affect the crack initiation and fatigue behavior of bone cement. Larger pores initiate more and larger fatigue cracks than small pores, and small pores adjacent to large pores cause larger stress concentrations than single pores. [44] This size effect was also observed in the current finite element analysis. For the cases with distributed pore locations, pores under 1.0 mm, typically classified as micropores, had no effect on the cement strength; whereas, pores over 1.0 mm increased pore-pore interactions and the likelihood of failure. Thus we indentify 1.0mm as a critical size for the pores to begin impacting fracture evolution. The methodology presented here can be extended to other compositions of bone cement and a critical pore size can be estimated.

It is not clear whether porosity is the main factor of failure in the cement mantle in vivo. [42,178,179] However, it is clear that porosity is detrimental to the fracture toughness of material test specimens, with the pore sizes and pore locations in the current study. Similar results have been found by others. [42,167] Porosity has also shown beneficial effects for the clinic. Antibiotic is typically added to the bone cement for the treatment and prevention of prothetic joint infection in the surgical area. The elution of antibiotic from bone cement is increased with the surface roughness and porosity. [125] In addition, the porosity may increase fracture toughness of bone cement by dispersing the energy at the crack tip, forming a larger damage zone, and effectively blunting the crack. [122] Therefore, the compromise between the positive and negative effects is essential to find the best solution. The compromised solution as suggested by the results of the present study may be an even distribution of relatively small pores (diameter less than 1.0 mm)

for effective antibiotic elution and reduction of large pores (diameter greater than 1.0 mm) which decrease fracture toughness.

Some limitation of this study should be mentioned. The cement used in this study is a relatively high viscosity cement compared to other commercially available cements such as Simplex P™. Lower viscosity cements may induce a different crack evolution. The fracture toughness of Simplex P ™ was shown to have different results to Palacos® R as demonstrated in our previous study. [141] Another limitation is that the XFEM predictions were performed with two-dimensional models; and therefore the predictions may differ when compared to the full 3D models. However, its standard practice in the fracture mechanics community to model 2D equivalents of 3D models (especially when computationally limited), and often the 2D predictions are very close. [172,173] This seems to be the case for our model as the 2D simulation results obtained are similar to the experimental results and show an inverse relationship between the porosity and fracture toughness. Lastly, fracture toughness in vivo is rather more complex than that of a fracture toughness material test as was performed in the current study, and hence one can expect to see some deviations in the results for more complex geometries of in vivo specimens.

Conclusions

This study provides insights into modeling of porous bone cement elastic fracture using the extended finite element method and under the assumption of linear elastic fracture mechanics. The effect of pore size and pore location on crack growth such as crack initiation and propagation are presented. Our experimental result showed that the macro pores were found in groups with 1 g and 2 g of added antibiotic and reduced fracture toughness of these groups. The numerical simulations indicate the existence of a critical pore size for fracture propagation, and

that the fracture toughness of antibiotic-loaded bone cement was significantly reduced by macro pores (diameter greater than 1.0 mm) which tended to initiate cracks at a lower critical load. On the other hand, pore location with smaller pore diameters (0.2 mm, 0.6 mm and 1.0 mm) did not significantly impact fracture evolution. However, it is important to note that as the amount of the antibiotic is increased (0.25 g, 0.5 g, 1.0 g and 2.0 g groups), we observe significant variation between the experimental and numerical load displacement response, primarily in the stiffness. These findings indicate that perhaps a better representation of the pore shapes and through-thickness effects (three dimensional models) may be necessary to have a better geometric representation of the pores to more effectively model fracture evolution in the bone cement.

VII. DISCUSSION AND CONCLUSION

This section summarizes the main findings from the four manuscripts in Chapters 3, 4, 5 and 6, followed by limitations of the study and conclusions with areas for future research.

Summary of Findings

***Objective 1* – Evaluate the antibiotic release, antibacterial properties and mechanical properties of bone cements when traditional antibiotic, vancomycin, was incorporated into the bone cements Palacos® R and Simplex™ P.**

This work demonstrated that vancomycin can significantly increase antibiotic release and antibacterial properties by increasing added antibiotic to the bone cements. For the no antibiotic control up to the 2.0 g vancomycin group, at least 95 - 99% of the total antibiotic eluted over the entire 60-day period occurred in the first 8 days. The 1.0 g group in Palacos® R showed the most vancomycin (0.105 ± 0.00002 mg) while the 2.0 g group in Simplex™ P showed the most vancomycin (0.139 ± 0.006 mg) eluted over 60 days. Additionally, vancomycin significantly increased the porosity of the cements (explaining the increased antibiotic release). Unfortunately, the addition of vancomycin was found to negatively influence the mechanical properties (flexural, compressive, fracture toughness) of bone cement by increasing added vancomycin to the bone cements. Simultaneously, 2.0 g of vancomycin showed antibacterial effect in both cements. Based on these findings, it was concluded that vancomycin-loaded bone cement would be a viable option for use as a cement spacer to be used in two-stage revision procedures. It should be noted that this work was not the first to utilize vancomycin as an antibiotic-loaded bone cement. Rather, this work expands upon previously performed studies and addresses limitations and inconsistencies of other studies.

***Objective 2* – Determine the feasibility of using telavancin as an alternative to traditional antibiotics in acrylic bone cement.**

The development of antibiotic-resistant microorganisms has necessitated the development of alternative antimicrobial agents. Telavancin, a derivative of vancomycin, has emerged as an antimicrobial agent, and has shown effectiveness in the treatment of complicated skin and skin-structure infections and hospital-acquired pneumonia. The purpose of this work was to determine the feasibility of using telavancin-loaded bone cement as an alternative to traditional antibiotics within bone cement. Different loading ratios of telavancin in bone cement were used to investigate the mechanical properties, elution, and antibacterial effect. It was found that the addition of telavancin influenced the flexural, compressive, and fracture toughness properties, but the reduced mechanical properties depended on the cement type. Additionally, telavancin loaded to Palacos® R only achieved better efficacy than in Simplex™ P, but poor sustainability of release of telavancin was observed in both cements with much lower elution amounts of telavancin. This result suggested that telavancin would not be a viable antibiotic for antibiotic-loaded bone cement.

***Objective 3* – Assess the impact of porosity on the mechanical properties of bone cement using experimental and computational methods.**

This work examined the impact of porosity on the mechanical properties of bone cement. In previous investigation on mechanical properties of both bone cements, it was found that the two cements showed different numbers and sizes of pores, potentially affecting strength. Thus, this study investigated how the size and distribution of pores can affect the fracture toughness of antibiotic-loaded bone cement using X-FEM simulation. Additionally, the effects of pores in

crack initiation and propagation were also examined. It was found that the fracture toughness of antibiotic-loaded bone cement was significantly reduced by macro pores (diameter greater than 1.0 mm), which tended to initiate cracks at a lower critical load. However, pore location with the tested pore diameters did not affect stiffness or critical force. The effects of pores predicted with two-dimensional finite element analysis were in agreement with the experimental results by demonstrating reduced fracture toughness in groups with macro pores.

Discussion

Overall, the findings in this dissertation consistently demonstrate that increased volume of added antibiotics to the bone cements significantly decreased mechanical properties of bone cements, which is consistent with the results from previous studies. [78,98,121] Additionally, we found the reduced mechanical properties of bone cements result from increased porosity by adding more antibiotics to the bone cement. This detrimental effect on the bone cement was directly related to pore sizes and number of pores that exist on fracture surfaces in bone cements. Those pores play a dual role in bone cement. When the pore reached a critical size, the pores acted as site of stress concentration which led to the weakening of the cement and promoted crack initiation and propagation, which is detrimental for the cement mantle. On the other hand, pores below this critical size acted as crack arrestors; therefore, they diverted energy to the surroundings and prevented crack propagation, which positively affected the mantle. [83,122,165]

The size of pores and number of pores may reflect a combination of several factors related to the mixing method and polymerization shrinkage. First, vacuum and centrifuging mixing methods successfully showed a substantial reduction of the overall porosity and increased the cement strength. These two methods reduced macropores with diameters greater than 1.0 mm which were caused by entrapment of air during the hand mixing process and insertion of the

cement into the prepared bone. [25,41] The other most significant factor is polymerization shrinkage. Shrinkage-induced porosity occurs only if there is constraint on the cement during polymerization. When the exterior surface of the cement is allowed to move freely, no porosity development is observed. [180] Thus the question comes to mind as to what level of constraint is needed to induce porosity in the cements. This is most likely dependent on a complicated set of time-varying parameters, including the viscosity of the cement, the surface energy of the interface on which the pore nucleates, and the extent of constraint. Clinically, it has been noticed that cement tends to pull in from the proximal collar region during polymerization of a cemented femoral hip implant, implying that full constraint may not be present. Also, wet bone-bone cement interfaces may free constraint at the outer surface of the cement, limiting the quantity of bone cement porosity developed due to shrinkage in *vivo*. However, partially constrained conditions and locally varying levels of constraint still may give rise to local porosity development.

The increased number of pores was expected to act as pathways for antibiotic to leach out from the inside and in turn increase the antibiotic elution rate. However, the pores which are located deep inside the bone cement without any junction to the outside are considered to have no relation with antibiotic elution. The question then remains how deep the pores must be within the cement in order not to release relevant amounts of antibiotic. This remains hard to quantify. McLaren et al. reported that the permeability or the ease by which fluids pass through a structure is directly dependent on the porosity of that structure. They used a visualization method with phenolphthalein to evaluate the permeability.[127] In addition, Shiramizu et al. evaluated how the elution was affected by the pores on cement surface. They found that the less dense the material, the higher the porosity of the material would be, enabling more fluids to get into the surface

pores and cracks and, by this result, they achieved higher elution rates. They also noted that different viscosity of cement did not show any effect on elution rate. [181] A similar result was found in our studies in manuscript 1 and 2. Although Palacos® R and Simplex™ P were different viscosity cements, the maximum elution rate was not significantly different. Chemical compatibility to the bone cement with antibiotics were rather more critical. Telavancin in Palacos® R and Simplex™ P eluted much lower than Vancomycin in both cements.

Antibiotic loaded bone cement that has an intense initial burst of antibiotic elution and later elutes little or no antibiotics may minimize the promotion of antibacterial resistance. Antibiotic loaded bone cement with the greatest degree of early bacterial growth inhibition would likely have the greatest ability to kill or inhibit the growth of surgical wound bacterial contaminations immediately after TJR as these bacteria are still in the planktonic phase and highly susceptible to antibiotic. [182] Our study demonstrated that the activities of telavancin and vancomycin against biofilm-producing staphylococci were different. Our study findings indicated that telavancin was only active when incorporated into Palacos® R but not into Simplex™ P; whereas, vancomycin in both cements demonstrated better efficacy against same bacteria. Our results showed that the amount of telavancin eluted from Simplex™ P was extremely low, failing to meet the MIC concentration threshold. Thus, telavancin is apparently unsuitable as an antibiotic in bone cement. In Simplex™ P, it showed insufficient elution and an unsatisfactory antibacterial effect. Also, the mechanical strength of each cement with added telavancin was reduced in a dose dependent manner. This finding suggests telavancin cannot be considered for use in bone cement until degree of polymerization with telavancin is more clearly confirmed and the micro-structure of bone cement with pores is investigated in more detail.

Future Work

The investigation of porosity as it relates to curing temperature would be a valuable extension from my thesis and creates opportunities for future research. Although it is widely known that pressurization, centrifugation or vacuum mixing of bone cement have dramatically improved the porosity, mechanical properties, creep characteristics, and fatigue strength of bone cement, these techniques still do not reduce porosity at the stem-cement interface. [183] Hansen and Jensen investigated the bone cement for handling characteristics, intrusion, doughing time, setting time, and exothermic temperature under prechilling and vacuum mixing conditions. They found that some cements considerably increased exothermic temperature. [28] Such a high exothermic temperature might affect the cement porosity. Pelletier et al. also investigated pore distribution and mechanical properties of bone cement cured at different temperature. They found that pores were shown to gather near the surface of cooler molds and near the center in warmer molds for all cement brands. Small pores were more often present in cements cured at cooler temperature, with higher temperature molds producing larger pores. Therefore, a study on bone cement stored at different temperatures would be interesting for future research into the relationship between pore size and mechanical strength under these different conditions.

Another valuable follow-on study evaluates release and efficacy with nanoparticle antibiotic. Prokopovich et al. developed propyparaben nanoparticles that are hydrophilic, thus expanding the applicability of parabens to aqueous systems. They assess the possibility of employing paraben nanoparticles as antimicrobial compounds in bone cements. Nanoparticles at concentrations as low as 1% w/w in brushite bone cement were capable of preventing growth of pathogens, 5% w/w was needed for hydroxyapatite bone cement, while 7% w/w was required for PMMA bone cement. Another advantage that they found in this study was that no detrimental

effect was determined by the addition of paraben nanoparticles on bone cement compression strength and cytocompatibility. [184]

The effect of porosity and its distribution on the fatigue strength of bone cement can extend current research. David Hoey et al. analyzed the effect of pores using the Theory of Critical Distances (TCD) which was developed to explain the effect of notches and other stress concentrations on fatigue and fracture. They also investigated clusters of pores using a criterion which investigated whether or not local cracking would act to link pores together, forming a single stress concentration of a more complex shape. [41] The quantitative analysis of the effect of porosity on the fatigue strength of bone cement demonstrates the importance not only of pore size but also of pore density and distribution. Also, this approach is able to predict the cycle fatigue strength of samples containing clusters of pores.

Similar to 2D-XFEM model, a predefined straight crack front of 5 mm in 3D-XFEM model can be assumed and positioned in the mesh in the middle of the crack element ensuring similar crack propagation path. A regular crack propagation mechanism in through-thickness direction should be observed because the crack propagation direction changes and intersect the boundary of the elements after a certain crack length. When the crack front encounters the top interface of the elements, the crack starts to propagate in an irregular fashion in through-thickness direction due to which the crack size calculation becomes more complex. Gupta investigated on prediction of fatigue crack propagation in steel deck using 2D and 3D XFEM. He found that the simulated results of 3D-XFEM model showed a similar crack growth of 2D-XFEM. He also observed that the crack propagation started from the center of the thickness and propagated towards the edge of thickness for every crack length increment in the longitudinal direction. This crack mechanism held for different mesh sizes up to 1 mm and was mainly due to

the distribution of stress intensity factor along the crack front. Also, he noted that this mechanism is depended on Paris law constant, which defined the rate of crack propagation. [170] Heidari-Rarani and Sayedain studied finite element modeling strategies for 2D and 3D delamination propagation in composite double cantilever beam (DCB) specimens using VCCT, CZM and XFEM approaches. They combined the XFEM with VCCT and CXM and found that both 2D and 3D modelling with XFEM-CZM were more accurate than XFEM-VCCT. Also, the results of 2D and 3D modeling of delamination propagation were compared. They found that an oscillating behavior is observed in 3D models rather than 2D ones and concluded that this is perhaps due to stick-slip behavior during the delamination propagation. [171] Although the examples of two studies differ in materials, they raise the difference of crack pattern, crack growth, and load-displacement curve between 2D and 3D modeling. The 3D XFEM of bone cement with pores has never been studied before. Thus, 3D XFEM of bone cement will provide the more accurate modeling of the pore morphology and may predict fracture mechanisms better in comparison to 2D XFEM.

Lastly, another possible future work is antibiotic loaded bone cement with addition of soluble additives. Some soluble additives can significantly enhance the cumulative antibiotic elution from the antibiotic loaded bone cement. Slane et al. investigated the antibiotic release and biofilm inhibition properties of bone cement using water-soluble micron-sized particulate fillers. This work demonstrated that cement containing 10 g of xylitol exhibited a 353% increase in gentamicin relative to the standard cement. Additionally, xylitol significantly increased the porosity of the cement explaining the increased antibiotic release. Based on this finding, he concluded that antibiotic-loaded bone cement modified with xylitol would be a viable option for

use as a cement spacer for two-stage revision procedure. [80] This future work might demonstrate that addition of water-soluble additives can enhance the release of telavancin in bone cement.

Overall Conclusion

The results of this dissertation demonstrated that Palacos® R and Simplex™ P had different results of mechanical properties, release, and antibacterial properties with added vancomycin or telavancin. These results were related to the different volume of added antibiotics to the bone cement. The addition of increased dose of antibiotics showed increased drug release in all my studies. The efficacy testing against four different bacteria also revealed that the addition of high dose of antibiotic was effective but total amount of release of antibiotic was also related to the efficacy. Unfortunately, the addition of antibiotics was found to negatively influence the mechanical properties (flexural and compressive) due to the increased porosity. However, fracture toughness of bone cement depended on pore size. Pores less than critical size exhibited increased fracture toughness by diverting energy to the surroundings and prevented crack propagation.

These results provide more insight to the balance and proper ratio between bone cement and antibiotics. These data provide a valuable benchmark for the next generation of ALBCs with new antibiotics and cements.

VIII. REFERENCES

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IX. APPENDICES

A. Appendices for Manuscript #1

Data in Brief

Title: Data for Vancomycin elution, activity and impact on mechanical properties when incorporated into orthopedic bone cement

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Abstract

In this article, we report data on the antibiotic elution and efficacy, and mechanical properties of Palacos bone cement with different amounts of added vancomycin (0.0, 0.125, 0.25, 0.5, 1.0, 2.0 g). Mechanical testing was performed for four-point bending, compression, and fracture toughness. The release characteristics of vancomycin was recorded for up to 60 days to estimate the elution profile. The eluted vancomycin efficacy at eliminating the four most common causative orthopedic implant pathogens is also reported.

Specifications Table

Subject area	Biomechanics, Pharmacy
More specific subject area	Orthopedic, Antimicrobial agent
Type of data	Image (x-ray, microscopy, etc.), figure, tabulated
How data was acquired	SEM (Zeiss-LEO,Oberkochen, Germany), MTS (Criterion C43.104, MTS Systems, Eden Prairie, MN), High performance Liquid Chromatography (HPLC)
Data format	Analyzed data
Experimental factors	Palacos bone cement different amounts of added vancomycin: 0.0, 0.125, 0.25, 0.5, 1.0, 2.0 g
Experimental features	Mechanical testing using MTS machine measured flexural modulus flexural strength, compressive modulus, compressive yield strength, and fracture toughness, according to ISO 5833. The drug elution test was determined using high performance liquid chromatography (HPLC) with a C_{18} column. 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 inoculated with bacteria for each test condition for antimicrobial activity testing. Drug elution cements were stored in -20°C freezer and all mechanical testing cements were wet cured in a phosphate-buffer solution (PBS) for 21 days at room temperature (22°C) before testing.
Data source location	Department of Mechanical Engineering and School of Pharmacy, University of Wisconsin Madison
Data accessibility	Data is with this article.
Related research article	Bishop A.R., Kim S., Squire M.W., Rose W.E., Ploeg H., Vancomycin elution, activity and impact on mechanical properties when added to orthopedic bone cement, in press.

Value of the Data

- These data are of value in cemented joint arthroplasty using Palacos with added vancomycin as a prophylactic measure against infection.
- The mechanical test data of wet cured samples demonstrated that mechanical properties of Palacos bone cement with upto 0.5 g of vancomycin met all ISO minimum requirements.
- The release characteristic test data showed that the 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.

- The antimicrobial activity test data showed that 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)

1. Data

The data provided here are

- Mechanical test data: flexural modulus, flexural strength, compressive modulus, compressive yield strength, and fracture toughness calculated from the load-displacement curves
- Scanning electron microscope (SEM) images from the fracture surfaces of four-point bending samples
- Release characteristic test data for vancomycin added to Palacos bone cement
- Antimicrobial activity test data for eluted vancomycin efficacy at eliminating four most common causative orthopedic implant pathogens (MRSA n315, ATTC MRSA 33591, ATCC *S.aureus* 29213, and ATCC *S. epidermidis* 35984)

1.1 Mechanical Testing Data

Appendix A-1. Results from four-point bend testing. Results are reported as median ± 1 standard deviation

4 - Point Bending Test							
Cement	**Antibiotic**	**Amount of Antibiotic added [g]**	**Mixing**	**Conditioning**	**Testing Condition**	**Bending Modulus [MPa]**	**Bending Strength [MPa]**
Palacos	---	---	Vacuum	Ambient, saline, 21 days	Ambient	2192 ± 164.2 [1]	55.40 ± 3.531 [3]
Palacos	Vancomycin	0.125	Vacuum	Ambient, saline, 21 days	Ambient	2349 ± 163.9 [1]	57.72 ± 1.515 [3]
Palacos	Vancomycin	0.25	Vacuum	Ambient, saline, 21 days	Ambient	2357 ± 301.0 [1]	52.63 ± 2.221 [3]
Palacos	Vancomycin	0.50	Vacuum	Ambient, saline, 21 days	Ambient	2267± 200.4 [1]	56.71 ± 2.331 [3]
Palacos	Vancomycin	1.0	Vacuum	Ambient, saline, 21 days	Ambient	2369 ± 64.00 [1]	55.80 ± 1.541 [3]
Palacos	Vancomycin	2.0	Vacuum	Ambient, saline, 21 days	Ambient	2038 ± 164.2 [1,2]	46.80 ± 1.700 [4]

1. Significantly higher than the ISO minimum requirement of 1800 MPa
2. Significantly lower than control's bending modulus
3. Significantly higher than the ISO minimum requirement of 50 MPa
4. Significantly lower than control's bending strength

Appendix A-2. Results from compression testing. Results are reported as median ± 1 standard deviation

Compressive Test							
Cement	**Antibiotic**	**Amount of Antibiotic added [g]**	**Mixing**	**Conditioning**	**Testing Condition**	**Compressive Modulus [MPa]**	**Compressive Yield Strength [MPa]**
Palacos	---	---	Vacuum	Ambient, saline, 21 days	Ambient	1559 ± 207.4	82.71 ± 63.52 [2]
Palacos	Vancomycin	0.125	Vacuum	Ambient, saline, 21 days	Ambient	1543 ± 246.7	78.61 ± 47.91 [2,3]
Palacos	Vancomycin	0.25	Vacuum	Ambient, saline, 21 days	Ambient	1279 ± 230.1 [1]	77.01 ± 47.92 [2,3]
Palacos	Vancomycin	0.50	Vacuum	Ambient, saline, 21 days	Ambient	1282 ± 301.2 [1]	73.12 ± 33.34 [2,3]
Palacos	Vancomycin	1.0	Vacuum	Ambient, saline, 21 days	Ambient	1098 ± 182.9 [1]	69.62 ± 25.44 [3]
Palacos	Vancomycin	2.0	Vacuum	Ambient, saline, 21 days	Ambient	980.7 ± 230.4 [1]	61.72 ± 11.54 [3]

1. Significantly lower than control's compressive modulus
2. Significantly higher than the ISO minimum requirement of 70 MPa
3. Significantly lower than control's compressive yield strength

Appendix A-3. Results from Fracture toughness testing. Results are reported as median ± 1 standard deviation

Fracture Toughness Test						
Cement	**Antibiotic**	**Amount of Antibiotic added [g]**	**Mixing**	**Conditioning**	**Testing Condition**	**Fracture Toughness [MPa·m$^{1/2}$]**
Palacos	---	---	Vacuum	Ambient, saline, 21 days	Ambient	2.685 ± 0.091
Palacos	Vancomycin	0.125	Vacuum	Ambient, saline, 21 days	Ambient	2.937 ± 0.419
Palacos	Vancomycin	0.25	Vacuum	Ambient, saline, 21 days	Ambient	2.492 ± 0.267
Palacos	Vancomycin	0.50	Vacuum	Ambient, saline, 21 days	Ambient	2.467 ± 0.222
Palacos	Vancomycin	1.0	Vacuum	Ambient, saline, 21 days	Ambient	2.465 ± 0.166
Palacos	Vancomycin	2.0	Vacuum	Ambient, saline, 21 days	Ambient	2.187 ± 0.175

1.2 Drug Elution Data

Appendix A-4. Drug elution results over 60 days. Results (all units are mg) are reported as mean ± standard deviation (SD)

Days	**0.125 g**		**0.25 g**		**0.5 g**		**1.0 g**		**2.0 g**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1	0.006	0.003	0.010	0.003	0.022	0.005	0.052	0.017	0.048	0.011
2	0.006	0.000	0.010	0.000	0.022	0.000	0.052	0.000	0.048	0.000
4	0.020	0.005	0.017	0.004	0.041	0.005	0.079	0.010	0.070	0.006
8	0.030	0.005	0.033	0.001	0.059	0.002	0.104	0.007	0.097	0.002
10	0.030	0.000	0.033	0.000	0.059	0.000	0.104	0.000	0.097	0.000
15	0.030	0.000	0.033	0.000	0.059	0.000	0.104	0.000	0.097	0.000
25	0.030	0.000	0.033	0.000	0.059	0.000	0.105	0.000	0.098	0.000
45	0.030	0.000	0.033	0.000	0.059	0.000	0.105	0.000	0.099	0.000
60	0.031	0.000	0.033	0.000	0.059	0.000	0.105	0.000	0.100	0.000

1.3 Antimicrobial Activity Testing

Appendix A-5. Antimicrobial activity of eluted vancomycin (0.5 g) for three S. aureus strains. Results (all units are $log_{10}\ CFU/mL$) are reported as mean ± 1 standard deviation (SD)

Days	ATCC 29213		n315		ATCC 33591	
	Mean	SD	Mean	SD	Mean	SD
0	3.070	0.150	3.150	0.010	3.150	0.050
1	1.620	0.320	1.000	0.000	1.000	0.000
2	1.000	0.000	1.000	0.000	1.000	0.000
3	1.000	0.000	1.000	0.000	1.000	0.000
4	1.000	0.000	1.000	0.000	1.000	0.000
5	1.000	0.000	1.000	0.000	1.000	0.000
6	1.000	0.000	1.000	0.000	1.000	0.000
7	1.000	0.000	1.000	0.000	1.000	0.000
15	1.000	0.000	1.000	0.000	1.000	0.000

Appendix A-6. Antimicrobial activity of eluted vancomycin (0.125 g – 2.0 g) for S.epidermidis 35984. Results (all units are $log_{10}\ CFU/mL$) are reported as mean ± 1 standard deviation (SD)

Days	0.125 g		0.25 g		0.5 g		1.0 g		2.0 g	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	3.090	0.040	3.090	0.040	3.090	0.040	3.090	0.040	3.090	0.040
1	4.500	1.000	2.610	2.330	1.700	1.700	1.000	1.000	4.320	0.290
2	5.040	0.790	4.000	0.870	3.680	3.230	2.540	2.410	3.520	3.060
3	4.900	1.040	4.400	1.250	3.380	0.150	4.040	1.020	2.310	2.010
4	5.250	0.350	5.270	0.140	5.040	0.190	4.670	0.160	4.550	0.280
5	5.500	1.000	5.500	1.000	5.500	1.000	5.500	1.000	5.500	1.000
6	5.500	1.000	4.320	1.140	3.810	1.240	2.820	2.640	1.940	1.680
7	5.500	1.000	5.330	0.150	4.940	0.530	4.360	1.590	1.370	2.380
15	5.500	1.000	5.500	1.000	1.830	3.180	0.610	1.050	1.000	1.000

2. Experimental Design, Materials, and Methods

2.1 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 – 100 mbar. All other mixing techniques were performed as previously described [78]. 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 using a Buehler® IsoMet™ (Lake Bluff, IL, USA) low speed saw resulting in a crack width of 0.37 mm.

2.2 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 5388), with the only modification being the addition of the wet curing process. Displacement rate was 5 mm/min for four-point bending and compression test and 10 mm/min for fracture toughness tests.

2.3 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 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 [85]. 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 [86].

2.4 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 [87], 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) [88].

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 determine the inhibitory or killing properties against the bacteria in culture. The limit of bacterial detection with this method is 10 CFU/mL. All testing was performed in triplicate.

B. Appendices for Manuscript #2, 3, 4

Data in Brief

- **Article Title**: Data for Mechanical, Elution, and Antibacterial Properties of Palacos or Simplex Bone Cements Loaded with Vancomycin or Telavancin

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Abstract

In this article, we report data on "Mechanical, Elution and Antibacterial Properties of Simplex Bone Cement Loaded with Vancomycin" and also recorded data for mechanical, elution, and efficacy test of telavancin impregnated in palacos or simplex bone cement. Mechanical testing was conducted for four-point bending, compression, and fracture toughness. The elution profiles

were recorded for up to 60-day time period with high performance liquid chromatography. Efficacy tests were performed with four main pathogens that cause prosthetic joint infection.

Keywords: Bone Cement, Palacos, Simplex, Vancomycin, Telavancin, Biomaterial, Antibiotic

Specification Table

Subject	Biomedical Engineering
Specific subject area	Orthopedic, Antimicrobial agent
Type of data	Table, Figure
How data were acquired	MTS (Criterion, C43.104, MTS System, Eden Prairie, MN), High performance Liquid Chromatography (HPLC)
Data format	Raw and Analyzed
Parameters for data collection	Mechanical testing samples were cured for 21 days in phosphate buffer saline (PBS) before testing. Mechanical testing was carried out at ambient temperature.
Description of data collection	Mechanical testing using a MTS materials testing machine was conducted to measure flexural modulus, flexural strength, compressive modulus, compressive yield strength, and fracture toughness based on ISO 5833 and ASTM-D5045 testing standards. The drug elution test was determined using high performance liquid tomography (HPLC). Antimicrobial activity was evaluated by quantifying the colony forming unit.
Data source location	Department of Mechanical Engineering and School of Pharmacy, University of Wisconsin Madison, Madison, WI, USA
Data accessibility	Repository name: Scholars Portal Dataverse Data identification number: UNF:6:jnky1HWB8zHvQuu/RZcbFQ== [fileUNF] Direct URL to data: https://doi.org/10.5683/SP2/084QJP
Related research article	Kim S., Bishop A.R., Squire M.W., Rose W.E., Ploeg H., Mechanical, Elution, and Antibacterial Properties of Simplex Bone Cement Loaded withVancomycin, 10.1016/j.jmbbm.2019.103588

Value of the Data

- These data are of value in cemented joint arthroplasty using Palacos® R or Simplex™ P with added vancomycin or telavancin as a prophylactic measure against infection.

- The mechanical test data of wet cured samples demonstrated that mechanical properties of Simplex™ P and Palacos® R with 0.125 g and below of added vancomycin met all ISO minimum requirements.

- The release characteristic test data showed that the elution amount was maximized at critical first week.

- The antimicrobial activity test data showed that the eluted concentration from sample with 2.0 g of vancomycin and telavancin per Palacos® R packet sufficiently eliminated a 10^3 colony forming unit per mL. However, Simplex™ P loaded with telavancin was not able to kill all bacteria within 14 days.

Data Description

The raw and analyzed data presented here offers mechanical, elution, and efficacy test data for Simplex™ P with added vancomycin, Palacos® R or Simplex™ P with added telavancin. Appendix B-1 - Appendix B-6 show mechanical properties of each combination of two antibiotics in two cements except for Palacos® R with added vancomycin. The different elution data of three combination groups are indicated in Appendix B-7 - Appendix B-10. Appendix B-11 - Appendix B-19 show efficacy test data for Simplex™ P with added vancomycin based on the vancomycin loading from 0.125 g to 1.0 g with figures and 2 g without figure. Efficacy data for telavancin impregnated in Palacos® R are described from Appendix B-20 to Appendix B-23 based on type of strain. Efficacy data for Simplex™ with added telavancin are shown in Appendix B-24 - Appendix B-27.

Mechanical testing data for Simplex™ P loaded with vancomycin

Appendix B-1. Bending modulus and strength are provided from ISO 5833 four-point bend testing of vancomycin formulated Simplex™ P bone cement after curing process. Data are reported as mean ± standard error of the mean. Figures are provided in the original article [141]. The figure for bending modulus includes ISO 5833 standard for minimum bending modulus and the mean and standard error of the mean for the specimens in each group. Treatment groups were not different than the control and all groups were above the ISO 5833 minimum and not different from the control bending modulus. The figure for bending strength includes that the asterisk mark (for all treatment groups) represents a significant ($p<0.05$) difference from control group. Samples with 0.5 g of added vancomycin and greater were statistically not above the ISO 5833 minimum bending strength.

Four – Point Bending Test Data

Cement	Antibiotic	Amount of Antibiotic added [g]	Mixing	Conditioning	Testing Condition	Bending Modulus [MPa]	Bending Strength [MPa]
Simplex	---	---	Vacuum	Ambient, saline, 21 days	Ambient	2459 ± 144 [1]	63.2 ± 4.36 [3]
Simplex	Vancomycin	0.125	Vacuum	Ambient, saline, 21 days	Ambient	2358 ± 119 [1]	49.8± 5.31 [3,4]
Simplex	Vancomycin	0.25	Vacuum	Ambient, saline, 21 days	Ambient	2397 ± 55 [1]	49.8± 3.18 [3,4]
Simplex	Vancomycin	0.50	Vacuum	Ambient, saline, 21 days	Ambient	2493 ± 205 [1]	46.6 ± 3.09 [4]
Simplex	Vancomycin	1.0	Vacuum	Ambient, saline, 21 days	Ambient	2420 ± 255 [1]	47.2 ± 5.52 [4]
Simplex	Vancomycin	2.0	Vacuum	Ambient, saline, 21 days	Ambient	2406 ± 104 [1]	49.4 ± 3.32 [4]

1. Significantly higher than the ISO minimum requirement of 1800 MPa bending modulus.
2. Significantly lower than control's bending modulus.
3. Significantly higher than the ISO minimum requirement of 50 MPa bending strength.
4. Significantly lower than control's bending strength.

Appendix B-2. Compressive modulus and compressive yield strength are provided from ISO 5833 compression testing of vancomycin formulated Simplex™ P bone cement after curing process. Data are reported as mean ± standard error of the mean. Figures are provided in the original article [141]. The asterisk mark (for 2.0 g of added vancomycin) represents a significant ($p<0.05$) difference from control group compressive strength.

Compressive Test Data							
Cement	Antibiotic	Amount of Antibiotic added [g]	Mixing	Conditioning	Testing Condition	Compressive Modulus [MPa]	Compressive Yield Strength [MPa]
Simplex	---	---	Vacuum	Ambient, saline, 21 days	Ambient	1694 ± 209	80.6 ± 4.23 [2]
Simplex	Vancomycin	0.125	Vacuum	Ambient, saline, 21 days	Ambient	1856 ± 120	81.8 ± 6.60 [2]
Simplex	Vancomycin	0.25	Vacuum	Ambient, saline, 21 days	Ambient	1741 ± 224	80.4 ± 3.61 [2]
Simplex	Vancomycin	0.50	Vacuum	Ambient, saline, 21 days	Ambient	1856 ± 151	84.2 ± 3.21 [2]
Simplex	Vancomycin	1.0	Vacuum	Ambient, saline, 21 days	Ambient	1546 ± 214	78.8 ± 5.03 [2]
Simplex	Vancomycin	2.0	Vacuum	Ambient, saline, 21 days	Ambient	1388 ± 146 [1]	72.0 ± 5.48 [2,3]

1. Significantly lower than control's compressive modulus.
2. Significantly higher than the ISO minimum requirement of 70 MPa compressive strength.
3. Significantly lower than control's compressive yield strength.

Appendix B-3. Fracture toughness data are provided from ASTM-D5045 single edge notch bend test of vancomycin formulated Simplex™ P bone cement after curing process. Data are reported as mean ± standard error of the mean. The figure provided in the original article [141] includes the asterisk mark representing a significant ($p<0.05$) difference (for all samples with added vancomycin) from control group fracture toughness.

Fracture Toughness Test Data						
Cement	Antibiotic	Amount of Antibiotic added [g]	Mixing	Conditioning	Testing Condition	Fracture Toughness [MPa · √m]
Simplex	---	---	Vacuum	Ambient, saline, 21 days	Ambient	1.69 ± 0.16
Simplex	Vancomycin	0.125	Vacuum	Ambient, saline, 21 days	Ambient	2.08 ± 0.10 [1]
Simplex	Vancomycin	0.25	Vacuum	Ambient, saline, 21 days	Ambient	1.96 ± 0.30 [1]
Simplex	Vancomycin	0.50	Vacuum	Ambient, saline, 21 days	Ambient	2.02 ± 0.13 [1]
Simplex	Vancomycin	1.0	Vacuum	Ambient, saline, 21 days	Ambient	2.05 ± 0.35 [1]
Simplex	Vancomycin	2.0	Vacuum	Ambient, saline, 21 days	Ambient	1.96 ± 0.23 [1]

1. Significantly higher than control's fracture toughness.

Mechanical testing data for Palacos® R or Simplex™ P loaded with telavancin

Appendix B-4. Bending modulus and strength are provided from ISO 5833 four-point bend testing of telavancin formulated Palacos® R or Simplex™ P bone cement after curing process. Data are reported as mean ± standard error of the mean. The figure for bending modulus includes ISO 5833 standard for minimum bending modulus and the mean and standard error of the mean for the specimens in each group. All Palacos® R or Simplex™ P groups were above the ISO 5833 minimum. Samples with 0.25 g of added telavancin and greater were statistically not above the ISO 5833 minimum bending strength.

4 – Point Bending Test							
Cement	Antibiotic	Amount of Antibiotic added [g]	Mixing	Conditioning	Testing Condition	Bending Modulus [MPa]	Bending Strength [MPa]
Palacos	---	---	Vacuum	Ambient, saline, 21 days	Ambient	2284 ± 62.1[1]	55.33 ± 1.33[3]
Palacos	Telavancin	0.125	Vacuum	Ambient, saline, 21 days	Ambient	2111 ± 58.7[1]	55.52 ± 2.28[3]
Palacos	Telavancin	0.25	Vacuum	Ambient, saline, 21 days	Ambient	2126 ± 72.7[1]	53.07 ± 1.93[3]
Palacos	Telavancin	0.50	Vacuum	Ambient, saline, 21 days	Ambient	2136 ± 34.7[1]	57.58 ± 1.08[3]
Palacos	Telavancin	1.0	Vacuum	Ambient, saline, 21 days	Ambient	2119 ± 37.8[1]	54.30 ± 1.62[3]
Palacos	Telavancin	2.0	Vacuum	Ambient, saline, 21 days	Ambient	2075 ± 46.0[1]	47.81 ± 1.92[3,4]
Simplex	---	---	Vacuum	Ambient, saline, 21 days	Ambient	2459 ± 58.7[1]	63.18 ± 1.95[3]
Simplex	Telavancin	0.125	Vacuum	Ambient, saline, 21 days	Ambient	2497 ± 37.9[1]	48.76 ± 3.90[3,4]
Simplex	Telavancin	0.25	Vacuum	Ambient, saline, 21 days	Ambient	2481 ± 36.6[1]	45.71 ± 1.71[4]
Simplex	Telavancin	0.50	Vacuum	Ambient, saline, 21 days	Ambient	2429 ± 34.2[1]	42.46 ± 1.67[4]
Simplex	Telavancin	1.0	Vacuum	Ambient, saline, 21 days	Ambient	2325 ± 44.4[1,2]	41.00 ± 3.74[4]
Simplex	Telavancin	2.0	Vacuum	Ambient, saline, 21 days	Ambient	2252 ± 36.6[1,2]	42.25 ± 2.88[4]

1. Significantly higher than the ISO minimum requirement of 1800 MPa bending modulus.
2. Significantly lower than control's bending modulus.
3. Significantly higher than the ISO minimum requirement of 50 MPa bending strength.
4. Significantly lower than control's bending strength.

Appendix B-5. Compressive modulus and compressive yield strength are provided from ISO 5833 compression testing of vancomycin formulated Palacos® R or Simplex™ P bone cement after curing process. Data are reported as mean ± standard error of the mean. The sample with 1.0 g of added telavancin and greater did not meet ISO minimum compressive strength.

Compressive Test

Cement	Antibiotic	Amount of Antibiotic added [g]	Mixing	Conditioning	Testing Condition	Compressive Modulus [MPa]	Compressive Yield Strength [MPa]
Palacos	---	---	Vacuum	Ambient, saline, 21 days	Ambient	1563 ± 73.2	82.07 ± 1.24[2]
Palacos	Telavancin	0.125	Vacuum	Ambient, saline, 21 days	Ambient	1610 ± 77.4	75.53 ± 1.21[2,3]
Palacos	Telavancin	0.25	Vacuum	Ambient, saline, 21 days	Ambient	1514 ± 83.6	73.35 ± 2.07[2,3]
Palacos	Telavancin	0.50	Vacuum	Ambient, saline, 21 days	Ambient	1416 ± 45.5	71.09 ± 2.22[2,3]
Palacos	Telavancin	1.0	Vacuum	Ambient, saline, 21 days	Ambient	1285 ± 66.0[1]	65.89 ± 1.77[3]
Palacos	Telavancin	2.0	Vacuum	Ambient, saline, 21 days	Ambient	1234 ± 106[1]	60.88 ± 1.75[3]
Simplex	---	---	Vacuum	Ambient, saline, 21 days	Ambient	1694 ± 73.9	80.59 ± 1.49[2]
Simplex	Telavancin	0.125	Vacuum	Ambient, saline, 21 days	Ambient	1370 ± 69.7[1]	73.98 ± 1.50[2,3]
Simplex	Telavancin	0.25	Vacuum	Ambient, saline, 21 days	Ambient	1372 ± 80.2[1]	73.71 ± 2.12[2,3]
Simplex	Telavancin	0.50	Vacuum	Ambient, saline, 21 days	Ambient	1531 ± 76.4	77.77 ± 1.42[2]
Simplex	Telavancin	1.0	Vacuum	Ambient, saline, 21 days	Ambient	1383 ± 67.4[1]	72.38 ± 1.90[2,3]
Simplex	Telavancin	2.0	Vacuum	Ambient, saline, 21 days	Ambient	1464 ± 51.9[1]	72.61 ± 1.21[2,3]

1. Significantly lower than control's compressive modulus.
2. Significantly higher than the ISO minimum requirement of 70 MPa compressive strength.
3. Significantly lower than control's compressive yield strength.

Appendix B-6. Fracture toughness data are provided from ASTM-D5045 single edge notch bend test of formulated Palacos® R or Simplex™ P bone cement after curing process. Data are reported as mean ± standard error of the mean. The sample with 2 g of added telavancin to Palacos® R showed statistically significantly ($p<0.05$) lower than control group. The sample with 0.25 g, 1.0g and 2.0 g of added telavancin to Simplex™ P showed statistically significant increase in fracture toughness as compared to control group.

Fracture Toughness Test						
Cement	Antibiotic	Amount of Antibiotic added [g]	Mixing	Conditioning	Testing Condition	Fracture Toughness [MPa · √m]
Palacos	---	---	Vacuum	Ambient, saline, 21 days	Ambient	2.70 ± 0.09
Palacos	Telavancin	0.125	Vacuum	Ambient, saline, 21 days	Ambient	2.57 ± 0.07
Palacos	Telavancin	0.25	Vacuum	Ambient, saline, 21 days	Ambient	2.59 ± 0.08
Palacos	Telavancin	0.50	Vacuum	Ambient, saline, 21 days	Ambient	2.57 ± 0.07
Palacos	Telavancin	1.0	Vacuum	Ambient, saline, 21 days	Ambient	2.47 ± 0.15
Palacos	Telavancin	2.0	Vacuum	Ambient, saline, 21 days	Ambient	2.17 ± 0.10[1]
Simplex	---	---	Vacuum	Ambient, saline, 21 days	Ambient	1.69 ± 0.06
Simplex	Telavancin	0.125	Vacuum	Ambient, saline, 21 days	Ambient	1.64 ± 0.08
Simplex	Telavancin	0.25	Vacuum	Ambient, saline, 21 days	Ambient	1.85 ± 0.06[2]
Simplex	Telavancin	0.50	Vacuum	Ambient, saline, 21 days	Ambient	1.85 ± 0.06[2]
Simplex	Telavancin	1.0	Vacuum	Ambient, saline, 21 days	Ambient	1.66 ± 0.06
Simplex	Telavancin	2.0	Vacuum	Ambient, saline, 21 days	Ambient	1.88 ± 0.06[2]

1. Significantly lower than control's fracture toughness.
2. Significantly higher than control's fracture toughness.

Vancomycin elution from Simplex™ P

Appendix B-7. Vancomycin elution data from Simplex over 60 days are provided. Vancomycin elution release profiles (mean ± SEM) over a 60-day period are provided in the original article [141].

Days	0.125 g		0.25 g		0.5 g		1.0 g		2.0 g	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	0.000	0.000	0.000	0.002	0.000	0.003	0.000	0.000	0.000	0.000
1	0.003	0.001	0.006	0.004	0.014	0.001	0.042	0.003	0.105	0.028
2	0.004	0.002	0.009	0.000	0.016	0.001	0.049	0.004	0.119	0.003
4	0.004	0.000	0.009	0.000	0.018	0.000	0.053	0.002	0.129	0.003
8	0.005	0.000	0.010	0.000	0.018	0.000	0.056	0.002	0.134	0.001
10	0.005	0.000	0.010	0.000	0.018	0.000	0.057	0.001	0.136	0.000
15	0.005	0.000	0.010	0.000	0.018	0.000	0.058	0.000	0.139	0.002
25	0.005	0.000	0.010	0.000	0.018	0.000	0.058	0.000	0.139	0.000
45	0.005	0.000	0.010	0.000	0.018	0.000	0.058	0.000	0.139	0.000
60	0.005	0.000	0.010	0.000	0.018	0.000	0.058	0.000	0.139	0.000

SEM denotes standard error of the mean, and all units are $\log_{10}$ CFU/mL unless specified.

Appendix B-8. Two-phase exponential decay model and best-fit parameters for drug elution data are provided [61,116].

Equation	$y = A1 * e^{(-\frac{x}{t1})} + A2 * e^{(-\frac{x}{t2})} + y_0$, where x is time in days, and y is eluted vancomycin in mg				
Plot	Vanco 0.125	Vanco 0.25	Vanco 0.5	Vanco 1.0	Vanco 2.0
A1 (mg)	-0.00288	-0.00938	-0.0138	-0.0301	0.0727
t1 (days)	0.994	0.838	0.431	0.0251	0.00523
A2 (mg)	-0.00182	-6.62	-0.00441	-0.0266	-0.0632
t2 (days)	0.994	8.15	2.63	2.13	1.99
y_0 (mg)	0.00466	0.0101	0.0182	0.0573	0.137
R^2	0.993	0.998	0.999	0.996	0.995

Telavancin elution from Palacos® R

Appendix B-9. Telavancin elution data from Palacos bone cement over 60 days are provided. 2.0 g of added telavancin group showed the highest elution amount among groups.

Days	0.125 g		0.25 g		0.5 g		1.0 g		2.0 g	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1	0.0028	0.0001	0.0025	0.0001	0.0030	0.0002	0.0031	0.0002	0.0046	0.0002
2	0.0040	0.0002	0.0032	0.0002	0.0036	0.0002	0.0046	0.0002	0.0063	0.0003
4	0.0055	0.0003	0.0038	0.0002	0.0043	0.0002	0.0057	0.0003	0.0080	0.0004
8	0.0060	0.0003	0.0043	0.0002	0.0045	0.0002	0.0094	0.0005	0.0094	0.0005
10	0.0062	0.0003	0.0047	0.0002	0.0048	0.0002	0.0102	0.0005	0.0143	0.0007
15	0.0065	0.0003	0.0049	0.0002	0.0049	0.0002	0.0109	0.0005	0.0173	0.0009
25	0.0065	0.0003	0.0049	0.0002	0.0050	0.0002	0.0110	0.0005	0.0185	0.0009
45	0.0065	0.0003	0.0049	0.0002	0.0050	0.0002	0.0110	0.0006	0.0190	0.0010
60	0.0065	0.0003	0.0049	0.0002	0.0050	0.0002	0.0111	0.0006	0.0192	0.0010

SEM denotes standard error of the mean, and all units are $\log_{10}$ CFU/mL unless specified.

Telavancin elution from Simplex™ P

Appendix B-10. Telavancin elution data from Simplex bone cement over 60 days are provided. 2.0 g of added telavancin group showed the highest elution amount among groups.

Days	0.125 g		0.25 g		0.5 g		1.0 g		2.0 g	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1	0.0002	0.0000	0.0008	0.0000	0.0003	0.0000	0.0011	0.0001	0.0021	0.0001
2	0.0003	0.0000	0.0010	0.0000	0.0004	0.0000	0.0036	0.0002	0.0037	0.0002
4	0.0004	0.0000	0.0010	0.0001	0.0004	0.0000	0.0046	0.0002	0.0051	0.0003
8	0.0004	0.0000	0.0011	0.0001	0.0004	0.0000	0.0050	0.0002	0.0062	0.0003
10	0.0004	0.0000	0.0011	0.0001	0.0004	0.0000	0.0052	0.0003	0.0068	0.0003
15	0.0004	0.0000	0.0011	0.0001	0.0004	0.0000	0.0052	0.0003	0.0075	0.0004
25	0.0004	0.0000	0.0011	0.0001	0.0004	0.0000	0.0052	0.0003	0.0077	0.0004
45	0.0004	0.0000	0.0011	0.0001	0.0004	0.0000	0.0052	0.0003	0.0077	0.0004
60	0.0004	0.0000	0.0011	0.0001	0.0004	0.0000	0.0052	0.0003	0.0078	0.0004

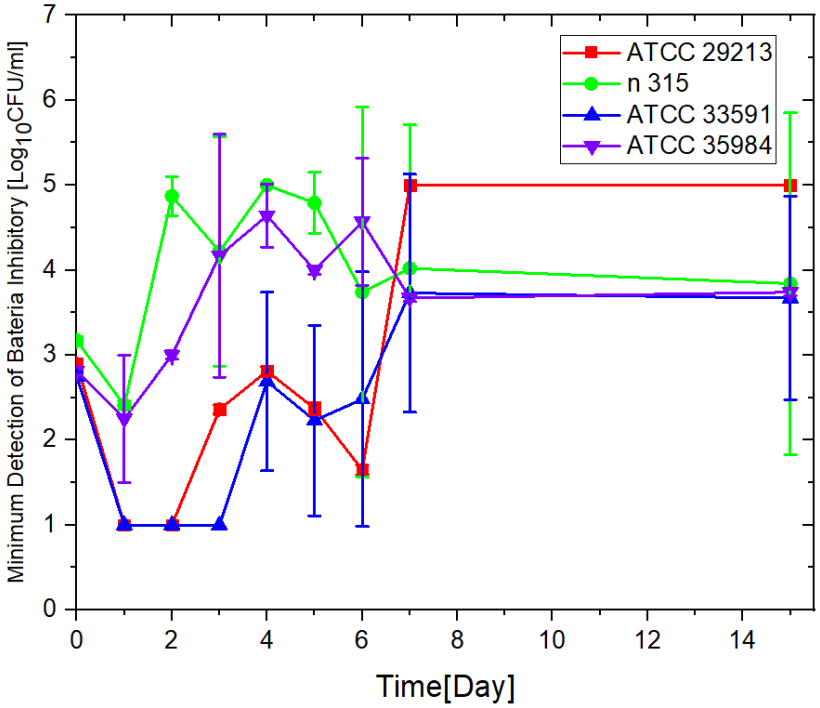
SEM denotes standard error of the mean, and all units are $\log_{10}$ CFU/mL unless specified.

Efficacy data for Simplex™ P with added vancomycin

Appendix B-11. Antimicrobial activity of eluted vancomycin (0.125 g) for four S. aureus strains. No bacterial colonies completely eliminated by day 15.

Days	ATCC 29213		n315		ATCC 33591		ATCC 35984	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	2.90	0.02	3.17	0.05	2.75	0.05	2.81	0.04
1	1.00	0.00	2.41	0.05	1.00	0.00	2.25	0.75
2	1.00	0.00	4.87	0.23	1.00	0.00	3.00	0.05
3	2.36	0.05	4.22	1.35	1.00	0.00	4.17	1.43
4	2.81	0.05	5.00	0.00	2.69	1.05	4.64	0.37
5	2.37	0.07	4.79	0.36	2.23	1.12	4.00	0.05
6	1.65	0.06	3.74	2.18	2.48	1.50	4.57	0.75
7	5.00	0.00	4.02	1.69	3.73	1.40	3.67	0.04
15	5.00	0.00	3.84	2.01	3.67	1.20	3.74	0.04

SEM denotes standard error of the mean, and all units are $\log_{10}$ CFU/mL unless specified.

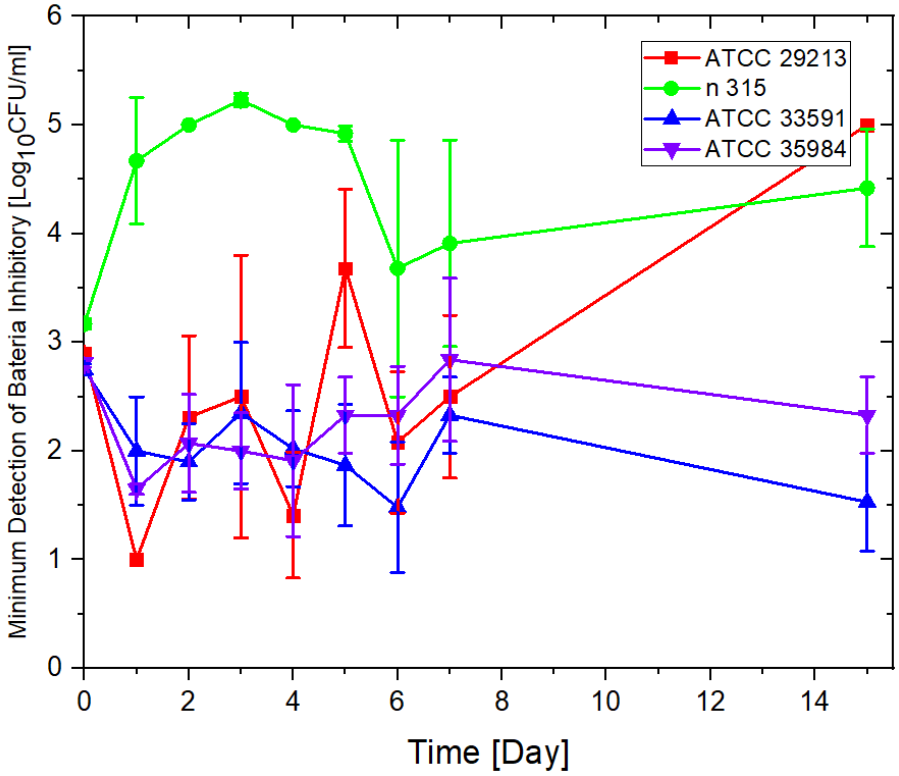


Appendix B-12: Susceptibility test of eluted vancomycin (0.125 g) for four strains. No bacterial colonies completely eliminated by day 15. Error bars show Mean ± SEM.

Appendix B-13. Antimicrobial activity of eluted vancomycin (0.25 g) for four S. aureus strains. No bacterial colonies completely eliminated at 0.25 g of vancomycin.

Days	ATCC 29213		n315		ATCC 33591		ATCC 35984	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	2.90	0.02	3.17	0.05	2.75	0.05	2.81	0.04
1	1.00	0.00	4.67	0.58	2.00	0.50	1.65	0.05
2	2.31	0.75	5.00	0.00	1.90	0.35	2.07	0.45
3	2.50	1.30	5.23	0.06	2.35	0.65	2.00	0.35
4	1.41	0.58	5.00	0.00	2.02	0.35	1.91	0.70
5	3.68	0.73	4.92	0.07	1.87	0.56	2.33	0.35
6	2.08	0.65	3.68	1.18	1.48	0.60	2.33	0.45
7	2.50	0.75	3.91	0.95	2.33	0.35	2.84	0.75
15	5.00	0.00	4.42	0.54	1.53	0.45	2.33	0.35

SEM denotes standard error of the mean, and all units are $\log_{10}$ CFU/mL unless specified.

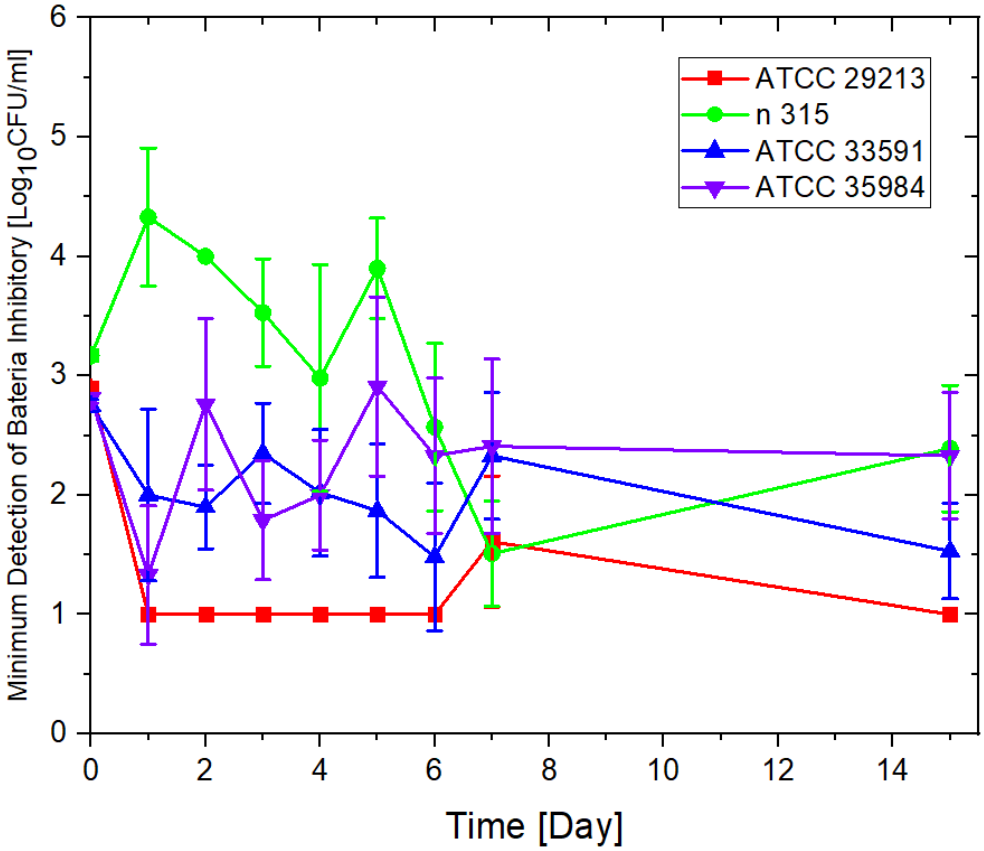


***Appendix B-14*:** Susceptibility test of eluted vancomycin (0.25 g) for four strains. No bacterial colonies completely eliminated at day 15. Error bars show Mean ± SEM.

Appendix B-15. Antimicrobial activity of eluted vancomycin (0.5 g) for four S. aureus strains Only ATCC 29213 was completely eliminated at day 15.

Days	ATCC 29213		n315		ATCC 33591		ATCC 35984	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	2.90	0.02	3.17	0.05	2.75	0.05	2.81	0.04
1	1.00	0.00	4.33	0.58	2.00	0.72	1.33	0.58
2	1.00	0.00	4.00	0.00	1.9	0.35	2.76	0.72
3	1.00	0.00	3.53	0.45	2.35	0.42	1.79	0.50
4	1.00	0.00	2.98	0.95	2.02	0.53	2.00	0.46
5	1.00	0.00	3.90	0.42	1.87	0.56	2.91	0.75
6	1.00	0.00	2.57	0.70	1.48	0.62	2.33	0.65
7	1.61	0.55	1.51	0.44	2.33	0.53	2.41	0.73
15	1.00	0.00	2.39	0.53	1.53	0.40	2.33	0.53

SEM denotes standard error of the mean, and all units are $\log_{10}$ CFU/mL unless specified.

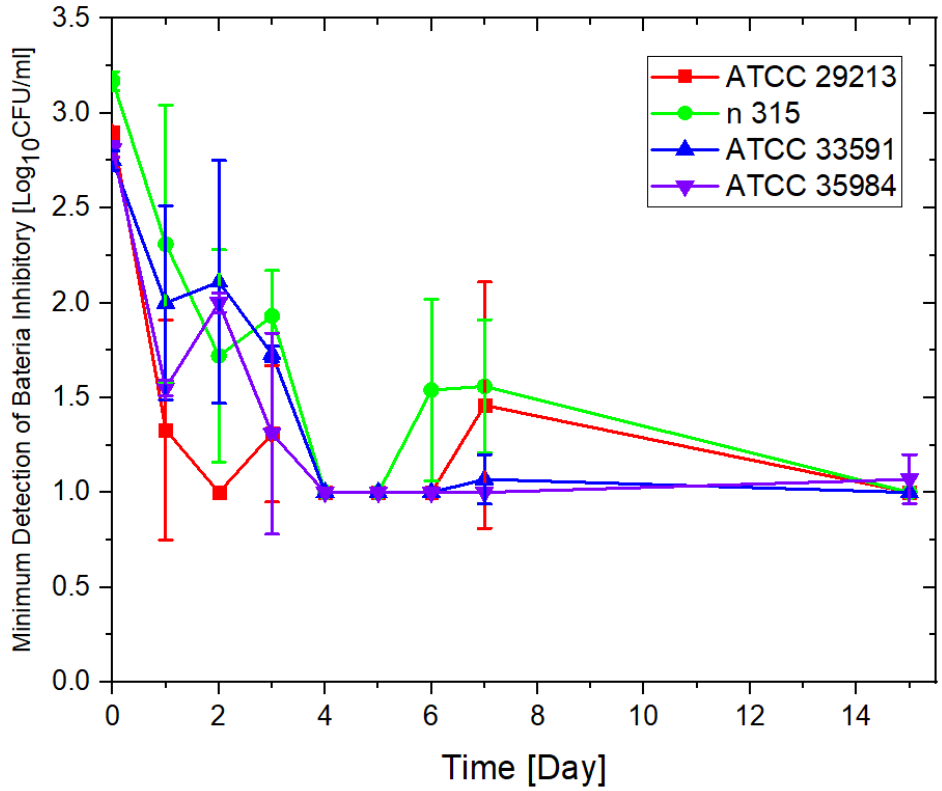


Appendix B-16: Susceptibility test of eluted vancomycin (0.5 g) for four strains. Only ATCC 29213 was completely eliminated at day 15. Error bars show Mean ± SEM.

Appendix B-17. Antimicrobial activity of eluted vancomycin (1.0 g) for four S. aureus strains. All bacterial colonies completely eliminated at day 15 and no more colonies were observed afterward.

Days	ATCC 29213		n315		ATCC 33591		ATCC 35984	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	2.9	0.02	3.17	0.05	2.75	0.05	2.81	0.04
1	1.33	0.58	2.31	0.73	2.00	0.51	1.55	0.04
2	1.00	0.00	1.72	0.56	2.11	0.64	2.00	0.05
3	1.31	0.36	1.93	0.24	1.73	0.04	1.31	0.53
4	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
5	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
6	1.00	0.00	1.54	0.48	1.00	0.00	1.00	0.00
7	1.46	0.65	1.56	0.35	1.07	0.13	1.00	0.00
15	1.00	0.00	1.00	0.00	1.00	0.00	1.07	0.13

SEM denotes standard error of the mean, and all units are $\log_{10}$ CFU/mL unless specified.



Appendix B-18: Susceptibility test of eluted vancomycin (1.0 g) for four strains. All bacterial colonies completely eliminated at day 15 and no more colonies were observed afterward. Error bars show Mean ± SEM.

Appendix B-19. Antimicrobial activity of eluted vancomycin (2.0 g) for four S. aureus strains. Data (all units are $\log_{10}$ CFU/mL) are reported as mean ± standard error of the mean (SEM). The figure for susceptibility test of eluted vancomycin (2.0 g) for four strains are provided in the original article [141]. All bacterial colonies completely eliminated at day 7 and no more colonies were observed afterward. Error bars show Mean ± SEM.

Days	ATCC 29213		n315		ATCC 33591		ATCC 35984	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	2.90	0.02	3.17	0.05	2.75	0.05	2.81	0.04
1	1.00	0.00	1.07	0.13	1.31	0.36	1.00	0.00
2	1.00	0.00	1.00	0.00	1.07	0.13	1.00	0.00
3	1.23	0.40	1.00	0.00	1.00	0.00	1.00	0.00
4	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
5	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
6	1.00	0.00	1.15	0.13	1.00	0.00	1.00	0.00
7	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
15	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00

Efficacy data for Palacos® R with added telavancin

Appendix B-20. Antimicrobial activity of eluted telavancin (0.125 g - 2.0 g) from Palacos® R for ATCC33591. Only 2 g of added telavancin completely eliminated ATCC33591.

Mass of Telavancin										
`	0.125 g		0.25 g		0.5 g		1.0 g		2.0 g	
Time (days)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	2.75	0.05	2.75	0.05	2.75	0.05	2.75	0.05	2.75	0.05
1	2.35	1.17	1.00	0.00	1.48	0.65	1.00	0.00	1.00	0
2	2.87	1.24	2.74	1.05	1.23	0.40	1.07	0.13	1.17	0.3
3	3.15	0.89	2.78	0.93	2.17	1.60	1.23	0.4	1.07	0.13
4	3.74	1.18	2.82	0.66	2.02	0.85	1.07	0.13	1.00	0.00
5	3.26	1.05	2.33	1.31	2.17	1.03	1.15	0.13	1.15	0.13
6	3.59	1.24	2.33	1.31	2.08	0.97	1.17	0.30	1.00	0.00
7	3.84	1.01	3.00	2.00	1.78	0.94	1.00	0.00	1.00	0.00
14	5.00	1.31	3.17	0.64	2.61	0.51	1.42	0.17	1.00	0.00

SEM denotes standard error of the mean, and all units are $\log_{10}$ CFU/mL unless specified.

Appendix B-21. Antimicrobial activity of eluted telavancin (0.125 g - 2.0 g) from Palacos® R for n315. Only 2 g of added telavancin completely eliminated n315.

Mass of Telavancin										
`	0.125 g		0.25 g		0.5 g		1.0 g		2.0 g	
Time (days)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	3.06	0.02	3.06	0.02	3.06	0.02	3.06	0.02	3.06	0.02
1	4.67	0.58	3.33	0.89	2.00	0.73	2.86	0.21	1.07	0.13
2	5.00	0.00	2.23	0.96	1.70	0.64	1.22	0.00	1.00	0.00
3	5.00	0.00	3.61	0.12	2.10	1.12	1.25	0.26	1.07	0.13
4	5.00	0.00	3.50	0.63	2.42	0.42	1.23	0.40	1.00	0.00
5	5.00	0.00	3.40	0.62	2.34	0.66	1.17	0.30	1.00	0.00
6	5.00	0.00	3.94	0.83	1.99	0.87	1.17	0.30	1.00	0.00
7	5.00	0.00	3.83	0.33	1.67	0.75	1.00	0.00	1.00	0.00
14	5.00	0.00	3.79	0.33	2.22	1.08	1.00	0.00	1.00	0.00

SEM denotes standard error of the mean, and all units are $\log_{10}$ CFU/mL unless specified.

Appendix B-22. Antimicrobial activity of eluted telavancin (0.125 g - 2.0 g) from Palacos® R for S.epidermidis35984. Only 2 g of added telavancin completely eliminated S.epidermidis35984.

Mass of Telavancin										
`	0.125 g		0.25 g		0.5 g		1.0 g		2.0 g	
Time (days)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	2.78	0.03	2.78	0.03	2.78	0.03	2.78	0.03	2.78	0.03
1	2.85	1.19	2.45	0.51	1.17	0.30	1.00	0.00	1.00	0.00
2	2.90	1.19	2.29	1.14	1.07	0.13	1.07	0.13	1.00	0.00
3	2.85	0.45	2.13	1.40	1.31	0.36	1.00	0.00	1.00	0.00
4	2.87	1.04	4.12	0.36	1.73	1.08	1.07	0.13	1.00	0.00
5	3.67	1.31	4.12	0.78	1.48	0.45	1.07	0.13	1.00	0.00
6	3.67	1.31	5.06	0.27	1.60	0.85	1.07	0.13	1.00	0.00
7	3.74	1.18	5.02	0.04	2.19	1.06	1.07	0.13	1.00	0.00
14	4.38	1.07	5.00	0.00	3.52	0.47	1.78	0.35	1.00	0.00

SEM denotes standard error of the mean, and all units are $\log_{10}$ CFU/mL unless specified.

Appendix B-23. Antimicrobial activity of eluted telavancin (0.125 g - 2.0 g) from Palacos® R for ATCC29213. Only 2 g of added telavancin completely eliminated ATCC29213.

Mass of Telavancin										
`	0.125 g		0.25 g		0.5 g		1.0 g		2.0 g	
Time (days)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	2.97	0.03	2.97	0.03	2.97	0.03	2.97	0.03	2.97	0.03
1	2.20	0.89	1.31	0.53	1.00	0.00	2.00	1.73	1.27	0.48
2	1.46	0.79	2.00	0.73	1.00	0.00	1.00	0.00	1.00	0.00
3	4.43	0.98	1.74	1.28	1.23	0.40	1.00	0.00	1.00	0.00
4	4.00	1.00	1.31	0.36	2.52	1.32	1.70	0.59	1.00	0.00
5	4.50	0.86	1.89	1.35	1.78	1.16	1.61	1.05	1.00	0.00
6	2.57	1.14	1.75	1.10	0.84	0.77	2.16	1.11	1.00	0.00
7	3.00	0.00	2.45	2.40	1.67	0.75	2.45	0.00	1.00	0.00
14	4.00	0.00	3.79	0.33	2.22	1.08	3.00	0.00	1.00	0.00

SEM denotes standard error of the mean, and all units are $\log_{10}$ CFU/mL unless specified.

Efficacy test for Simplex™ P with added telavancin

Appendix B-24. Antimicrobial activity of eluted telavancin (0.125 g - 2.0 g) from Simplex™ P for ATCC 33591. No group completely killed ATCC 33591 by 14-days.

Mass of Telavancin										
`	0.125 g		0.25 g		0.5 g		1.0 g		2.0 g	
Time (days)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	2.90	0.07	2.90	0.07	2.90	0.07	2.90	0.07	2.90	0.07
1	2.31	0.49	2.58	0.39	1.64	0.92	1.37	0.65	1.07	0.13
2	2.57	0.14	2.41	0.25	2.60	1.52	1.41	0.53	1.41	0.36
3	2.33	0.31	2.20	1.18	1.55	0.77	1.00	0.00	1.00	0.00
4	2.90	0.83	3.67	0.31	1.95	0.86	1.41	0.71	1.17	0.30
5	2.51	0.17	3.43	0.76	2.20	0.43	1.00	0.00	1.00	0.00
6	2.41	0.25	2.71	1.34	2.54	0.34	1.27	0.48	1.33	0.58
7	2.33	0.31	3.33	1.08	2.32	1.15	1.25	0.26	1.25	0.26
14	2.41	0.25	2.94	1.00	2.37	1.19	1.45	0.77	2.24	0.82

SEM denotes standard error of the mean, and all units are $\log_{10}$ CFU/mL unless specified.

Appendix B-25. Antimicrobial activity of eluted telavancin (0.125 g - 2.0 g) from Simplex™ P for n315. Only 2 g of added telavancin completely eliminated n315.

Mass of Telavancin										
`	0.125 g		0.25 g		0.5 g		1.0 g		2.0 g	
Time (days)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	3.30	0.02	3.30	0.02	3.30	0.02	3.30	0.02	3.30	0.02
1	2.33	0.31	3.81	1.50	3.84	1.01	1.00	0.00	1.00	0.00
2	5.00	0.00	4.05	0.93	2.98	0.38	1.00	0.00	1.00	0.00
3	5.00	0.00	5.00	0.00	2.41	0.77	1.07	0.13	1.00	0.00
4	5.00	0.00	5.00	0.00	3.05	1.07	1.00	0.00	1.00	0.00
5	4.79	0.36	4.54	0.47	2.98	0.77	1.27	0.48	1.00	0.00
6	4.83	0.30	4.91	0.27	2.54	0.34	1.07	0.13	1.00	0.00
7	3.71	0.46	3.53	0.28	2.74	0.81	1.41	0.36	1.00	0.00
14	5.09	0.16	4.67	0.58	3.63	0.85	1.00	0.00	1.00	0.00

SEM denotes standard error of the mean, and all units are $\log_{10}$ CFU/mL unless specified.

Appendix B-26. Antimicrobial activity of eluted telavancin (0.125 g - 2.0 g) from Palacos® R for S.epidermidis 35984. No group completely killed S.epidermidis 35984 by 14-days

Mass of Telavancin										
`	0.125 g		0.25 g		0.5 g		1.0 g		2.0 g	
Time (days)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	2.97	0.03	2.97	0.03	2.97	0.03	2.97	0.03	2.97	0.03
1	1.60	1.05	1.00	0.00	1.35	0.30	1.85	0.28	1.80	0.17
2	3.00	0.73	1.99	0.71	1.26	0.37	1.00	0.00	1.00	0.00
3	3.50	1.18	1.79	0.37	1.00	0.00	1.31	0.36	1.23	0.40
4	3.74	1.18	2.39	0.74	1.11	0.16	1.31	0.36	1.00	0.00
5	4.00	0.00	2.25	1.12	1.50	0.71	3.23	0.33	1.00	0.00
6	3.90	0.91	1.41	0.71	3.11	0.67	1.00	0.00	1.00	0.00
7	3.74	1.18	1.96	1.23	5.00	0.00	1.46	0.65	1.00	0.00
14	5.00	0.00	5.00	0.00	5.00	0.00	2.84	1.15	3.00	0.83

SEM denotes standard error of the mean, and all units are $\log_{10}$ CFU/mL unless specified.

Appendix B-27. Antimicrobial activity of eluted telavancin (0.125 g - 2.0 g) from Palacos® R for ATCC 29213. No group completely killed ATCC 29213 by 14-days.

Mass of Telavancin										
`	0.125 g		0.25 g		0.5 g		1.0 g		2.0 g	
Time (days)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	2.81	0.02	2.81	0.02	2.81	0.02	2.81	0.02	2.81	0.02
1	1.17	0.30	1.63	1.09	1.54	0.94	1.84	0.46	1.00	0.00
2	3.03	1.05	2.23	0.57	1.00	0.00	2.31	0.54	1.00	0.00
3	3.05	0.18	2.17	1.12	1.73	0.26	1.07	0.13	1.00	0.00
4	3.54	0.78	3.01	0.77	3.46	0.54	1.31	0.36	1.15	0.13
5	3.92	0.53	2.59	0.38	4.63	0.45	1.35	0.30	1.07	0.13
6	3.15	1.02	1.73	0.27	3.78	0.42	1.56	0.97	1.00	0.00
7	3.53	1.20	3.27	0.97	4.08	0.41	1.64	1.11	1.07	0.13
14	5.00	0.00	5.00	0.00	5.00	0.00	3.53	0.78	1.49	0.85

Experimental Design, Materials, and Methods

Mechanical Testing

Quasi-static mechanical properties were measured under compression and bending. Fracture toughness was also determined. Prior to mechanical testing, the specimen were wet cured in phosphate buffer solution (PBS) for 21days at 22°C [113]. Bending tests and compression tests were conducted in accordance with ISO 5833; whereas, fracture toughness tests were performed in accordance with ASTM-D5045 [84]. Samples sizes were seven to eight per experimental group for each test.

The mechanical properties were obtained at loading rates: 5 mm/min for bending tests; 5 mm/min for compression test; and 10 mm/min for fracture toughness. Tests were conducted with a Criterion C43.104, MTS testing machine (Eden Prairie, MN, USA) with a 1 kN load cell (LPS.103, MTS system Corp). Cylindrical bone cement samples were compressed in the axial direction until failure occurred (2% yield point) as recommended by ISO 5833. The ultimate compressive strength ($\sigma_{ys} = F_{max}/A$; F_{max} = maximum applied load in N before specimen

failure, A=original cross-sectional area in mm^2) and elastic modulus in MPa (Young's modulus, E, slope at 0.2-0.4% strain) were determined from the stress-strain curves.

Four-point bend testing was performed to measure the flexural modulus and flexural strength of the bone cement. Rectangular samples were bent until failure and the flexural modulus was determined from the stress-strain curves. The flexural strength was calculated using the force at break of the samples.

Single edge notch bend testing was carried out to measure the fracture toughness of the bone cement. The samples were prepared and loaded until failure. Fracture toughness in MPa√m was calculated using ASTM-D5045 [113].

Mixing Method

Vacuum mixing was used to mix the bone cement. The cement powder and antibiotic were mixed by hand-shaking for 1 min in a syringe and then the liquid monomer was added into the antibiotic cement powder. The small amount of air entrapped in the cement syringe during mixing was drawn off using a vacuum pump at -50 mbar [80]. The cement was then transferred to an aluminum mold by injection with gun pressurization. After delivery of cement to the mold, the mold cover was placed by hand and pressed against the mold.

Release characteristic testing

Five samples in each group were used to measure antibiotic elution. Each cylindrical sample was immersed in 5 mL of sterile PBS and stored in incubator shaker at 37°C with constant shaking at 100 rpm At specific time point (1, 2, 4, 8, 10, 15, 25 and 45 and 60 d), cylindrical cement samples were removed from test tube. The eluate in each test tube was frozen at -20°C until analysis of antibiotic concentration. The cylindrical cement samples were re-

immersed in test tubes containing 5 mL of fresh PBS. Antibiotic concentration in the collected eluates were quantified by high performance liquid chromatography (HPLC) with a 18 C column (10 μm analytical column and flow rate 0.5 mL/min) [85]. The isocractic mobile phase consisted of 10 mM KH_2PO_4 – Accetonitrile (composite ratio, 17/3) and absorbance was measured at 210 nm. Standard curves were constructed using known vancomycin concentrations.

Antimicrobial activity testing

Four reference bacterial strains were evaluated for antimicrobial susceptibility testing: ATCC 33591 (Methicillin-resistant Staphylococcus aureus [MRSA]), n315 (MRSA), ATCC 29213 (S. aureus), ATCC 35984 (S. epidermidis). Three cylindrical samples were transferred into a test tube containing 3.4 mL of tryptic soy broth (TSB; Becton Dickenson, Pranklin Lakes, NJ). The broth media were inoculated with bacteria daily for 7 days and at 14 days and then two-fold serial dilutions were carried out. The diluted broth media were prepared on Mueller Hinton II agar plates (Sigma-Aldrich, St. Louis, MO, USA) for bacteria manipulation. Agar plates were incubated for 18-24 hours and bacteria colonies were then determined. The colony forming unit (CFU) quantified the ability of eluted antibiotic from the bone cement to eliminate the bacteria in the cell culture. The minimum detection of bacteria inhibitory was 10 CFU/mL. All isolates were tested in triplicate for susceptibility.