Diploma Thesis

Spacer comparisons and novel antibiotic additions to PMMA cements considering their viability in endoprosthetic procedures

Submitted by

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Graz, am 29/05/2022 *Michael Abramowicz eh*

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3 Abbreviations and Glossary

ALBC	Antibiotic-loaded bone cement
BCRT	Berlin Brandenburg Centre for Regenerative Medicine
CKD	Chronic kidney disease
CNS	Coagulase negative Staphylococci
CRP	C-reactive protein
CT	Computed tomography
DAIR	Debridement, antibiotics and implant retention
DMARD's	Disease Modifying Antirheumatic Drugs
DTT	Difficult to treat
ESBL	Extended spectrum beta-lactamase
ESR	Erythrocyte sedimentation rate
i.v.	intravenous
kJ/m^2	Kilojoules per square metre
MHA	Müller-Hinton-Agar
MMA	Methyl-methacrylate
MPa	Megapascal
MRI	Magnetic resonance imaging
MRSA	Methicillin/Multi-resistant *Staphylococcus aureus*
MSIS	Musculoskeletal Infection Society
Ncm	Newton centimetre
p.o.	per os (by mouth)
PBS	Phosphate buffered saline
PJI	Periprosthetic Joint Infection
PMMA	Polymethyl methacrylate
PMN%	Polymorphonuclear neutrophil percentage
SVC	Superficial Vancomycin Coating
THA	Total Hip Arthroplasty
TKA	Total Knee Arthroplasty
TNF	Tumour Necrosis Factor
VRE	Vancomycin-resistant Enterococci
WBC	White blood cell

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6 Zusammenfassung (German)

Einleitung:

Prothetische Gelenksinfektionen sind eine schwerwiegende Komplikation der Totalendoprothetik, die oftmals in 2-zeitigen Revisionseingriffen mit antibiotika-beladenem Knochenzementen und PMMA-Spacern behandelt werden. Weil Antibiotikaresistenzen immer häufiger auftreten, werden neue antibiotika-belandene Knochenzementalternativen und verbesserte Spacer benötigt, um diese multiresistenten und polymikrobiellen Infektionen zu behandeln. Diese Dissertation untersuchte neuartige Knochenzement Kombinationen und verglich zwei kommerziell erhältliche „Spacer“, um die Behandlungsalternativen von Prothetische Gelenksinfektionen zu erweitern.

Material und Methoden:

Teil 1 analysiert die mechanischen und mikrobiologischen Eigenschaften von neuartigen Zementkombinationen. Moderate (2 g) und hohe (6 g) Dosen von Vancomycin wurden manuell zu Revisions-Zementen (Copal® G+V und G+C) hinzugefügt, und 0,5 g und 1 g Tigecycline wurden manuell zu Palacos® R- und R+G-Knochenzementen hinzugefügt. Es wurden mechanische Tests nach standardisierten Normen (ISO-5833:2002 und DIN53435:2018-09) und mikrobiologische Tests (Hemmhof-Tests) gegen die gängigsten Erreger durchgeführt.

Teil 2 verglich zwei kommerziell erhältliche „Spacer“ von Heraeus (Copal® Exchange G) und Tecres (Cemex® Spacer-K). Um die Hemmhof-Zonen der beiden Spacer zu vergleichen und um festzustellen, welche Eigenschaften bei einem Spacer wünschenswerter sind, wurden die nicht artikulierenden Oberflächen beider Produkte mit einem antibiotikafreien Knochenzement bedeckt.

Ergebnisse:

Die Knochenzement-Kombinationen erwiesen eine geringere mechanische Stabilität als die im Handel erhältlichen Zemente, erfüllten jedoch alle der Mindestanforderungen der standardisierten Normen, mit Ausnahme von Copal® G+V +6 g Vancomycin. Die mit Vancomycin beladenen Knochenzemente waren

gegen alle getesteten Pathogene wirksam und zeigten eine konstante Wirksamkeit über 42 Tage. Die mit Tigecycline beladenen Knochenzemente waren auch gegen multiresistente Pathogene wirksam und zeigten eine gute Wirksamkeit für mindestens 7 Tage. Obwohl der Tecres Spacer nach 1 Stunde größere Hemmhof-Zonen aufwies, hatte der Heraeus Spacer über die 42 Tage durchgehend größere Zonen.

Diskussion:

Tigecycline kann unbedenklich Knochenzementen zugesetzt werden und zeigt eine gute Wirksamkeit gegen multiresistente Erreger. Obwohl höhere Konzentrationen von Vancomycin die mechanische Stabilität verringern, verbessern sie ihre antimikrobielle Wirksamkeit. Copal® GC +6 g Vancomycin war die einzige Kombination mit >10% Antibiotika, die die standardisierten Normen erfüllte. Trotz seiner glatten Oberfläche scheint der Heraeus Spacer gegenüber dem Tecres für die 2-zeitige Revisionschirurgie besser geeignet zu sein, da er größere Inhibitionszonen nach 24 h zeigt und über 6 Wochen konstant seine Wirksamkeit erhält.

7 Abstract (English)

Introduction:

Prosthetic joint infections (PJI) are a severe complication of total joint arthroplasties that are often treated in 2-stage revision surgeries using antibiotic-loaded bone cements and PMMA spacers. Because antibiotic resistance is becoming more prevalent, new ALBC alternatives and improved spacers are needed to treat these multi-resistant and polymicrobial infections. This thesis investigated novel ALBC combinations and compared 2 commercially available spacers to expand PJI treatment alternatives.

Material and Methods:

Part 1 analysed the mechanical and microbiological properties of novel ALBC combinations. Moderate (2 g) and high (6 g) doses of Vancomycin were manually added to revision bone cements (Copal® G+V and G+C), and 0,5 g and 1 g of Tigecycline were manually added to Palacos® R and R+G bone cements. Mechanical tests according to standardised norms (ISO-5833:2002 and DIN53435:2018-09) and microbiological tests (inhibition zone assays) against common PJI pathogens were performed.

Part 2 compared two commercially available spacers by Heraeus (Copal® Exchange G) and Tecres (Cemex® Spacer-K). To compare the inhibition zones of the 2 spacers and determine which characteristics are more desirable in a spacer, the non-articulating surfaces of both products were covered with an antibiotic free bone cement.

Results:

The ALBC combinations showed lower mechanical stability than the plain, commercially available cements, yet all passed the minimum requirements of the standardised norms, except for Copal® G+V +6 g Vancomycin. The Vancomycin loaded bone cements were effective against all the tested pathogens and displayed a constant efficacy throughout 42 days. The Tigecycline loaded bone cements were also effective against multi-resistant pathogens and showed good efficacy for a minimum of 7 days. Even though the Tecres spacer had larger inhibition zones after

1 hour, the Heraeus spacer consistently had larger inhibition zones across the 42 days.

Discussion:

Tigecycline can be safely added to bone cements and shows good efficacy against multi-resistant pathogens. Even though higher concentrations of Vancomycin reduce mechanical stability, they improve antimicrobial efficacy. Copal® GC +6 g Vancomycin was the only combination with >10% antibiotics to fulfil the standards. Despite its smoother surface, the Heraeus spacer appears to be a better option for 2-stage revision surgery, as it exibits larger inhibition zones after 24 h and maintains consistently large inhibition zones over 6 weeks.

8 Introduction

8.1 Prosthetic Joint Infections (PJI)

8.1.1 What is PJI?

Total joint replacement surgeries or arthroplasties are life-changing procedures and the gold standard in treating, among others, end-stage osteoarthritis(1). Because they are so effective in alleviating pain and improving joint function in patients, and because over 240 million people are affected worldwide by this ailment(2), the number of total knee (TKA) and hip (THA) replacement surgeries are rising every year(3, 4). According to the German Arthroplasty Registry, over 300.000 joint replacements were performed in 2019 (5).

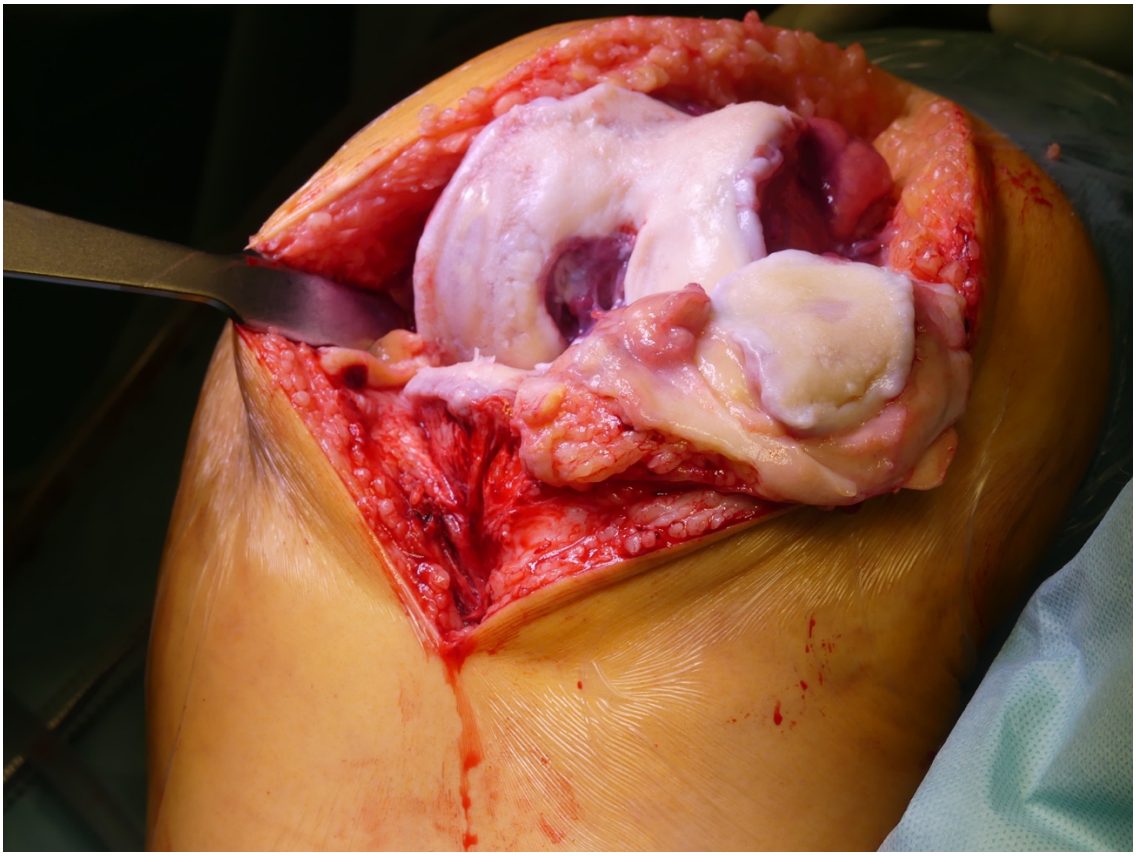

Figure 8-1: Intraoperative picture of an osteoarthritic knee undergoing total knee arthroplasty.
Picture kindly provided by Dr. Alfred Pölzl at the Department of Orthopaedic Surgery, LKH Steyr, Austria

However, as with every single medical procedure and surgery, total joint replacements carry certain complications and are not entirely risk-free. One of the most severe but rare complications of such procedures is PJI or “Periprosthetic Joint

Infection". PJI is essentially a bacterial or fungal infection of the joint prosthesis, the bone and surrounding tissue.
What makes this complication so fatal and such a burden for the patients and the entire health care system are the difficult diagnosis, the prolonged hospital stays, the lengthy anti-microbial treatments and the multiple surgeries(6).

8.1.2 Epidemiology

Even though research and papers differ on whether PJI infection rates are on the rise or the decline(7, 8), roughly 0,5-2,0% of all primary and 4% of all revision TKA's and THA's are still affected by PJI(9, 10). Additionally, PJI's are most likely to occur within the first 2 years after the procedure(11). There are also several risk factors that can increase the likelihood of PJI in patients. Here are the most relevant factors that increase the risk of a prosthetic joint infection according to Beam et al (2018) (11):

- Type of surgery (location), fixation (with or without bone cement) and type of prosthesis (e.g., metal to metal)
- Duration of surgery
- Previous surgeries and infections
- Postoperative complications
- Patient comorbidities (Diabetes mellitus, CKD, malignancy, obesity, rheumatoid arthritis, etc.)
- Smoking and the use of immunosuppressive drugs (Prednisolone, TNF-inhibitors, DMARDs etc.)
- Bacteraemia

8.1.3 What causes PJI?

As mentioned above, PJI is a fungal or bacterial infection of the joint prosthesis and the surrounding tissue. Nevertheless, bacterial infections of the joint space are far more common and will therefore be the focal point of this diploma thesis.
The first question is, how do the bacteria get into the joint space? Certain bacteria can access the joint space via 3 main mechanisms(9):

1. Intraoperative introduction during surgery
2. Continuous spread from an adjacent infection
3. Haematogenous seeding

As the most common form of infection, mechanism 1. occurs on the operating table. Here, the microorganisms enter the site of operation through either direct contact or air-borne bacteria. Infections that occur through this pathway usually develop within weeks to 1 year after surgery, depending on the virulence of the pathogen. Mechanism 2. also causes an infection within a relatively short time (weeks or months after surgery or injury). In this case, superficial surgical site infections or later trauma close to the prosthesis or operation wound can allow the pathogens to wander through a sinus tract or draining wounds into the joint space. The latter mechanism 3. usually occurs several years after surgery (late-onset infection), where bacteria from other infections within the body gain access to the bloodstream and wander towards the joint prosthesis. Although this pathway is fairly uncommon, the risk remains throughout the lifespan of the patient and their prosthesis.

Once the bacteria reach the joint space, they settle down on the surface of the prosthesis, begin propagating and form complex biofilms. Biofilms are essentially a conglomeration of bacteria (either mono- or polymicrobial) on any type of surface that are held together, nourished and protected by a self-produced extracellular matrix(12). The formation of this biofilm can be broken down into 2 phases: an immediate and reversible physical phase, and an irreversible, time-dependent cellular and molecular phase(13). Without going into further detail, these 2 phases make it clear why it is so important to quickly treat or prevent the initial formation of such biofilms. Furthermore, it is exceptionally difficult to treat or eradicate such biofilms after 3 weeks(14). As the biofilm grows over time, bacteria in the lower layers enter a slow or non-growing state that reduces the efficacy of antibiotics(15). In addition, biofilms also provide a mechanical barrier against antibiotics and immune cells. As a rule of thumb, the older and more mature the biofilm, the less effective the antibiotics and the more difficult or even impossible its treatment becomes.

Another important question is "*which bacteria cause a PJI?*" According to Tande et al. (2009) (9), *Staphylococcus aureus, Staphylococcus epidermidis* and other coagulase-negative staphylococci (CNS) are the most common cause of PJI worldwide, accounting for roughly 50-60% of all cases. Various Streptococci and Enterococci species are also relatively common and are responsible for 10% of prosthetic joint infections. Other common pathogens include Gram-negative bacilli such as *Escherichia coli* or *Pseudomonas aeruginosa*, and anaerobic bacteria such as *Propionibacterium acnes.* Furthermore, PJI's are often also caused by nosocomial pathogens such as MRSA, VRE and ESBL-bacteria. In many cases, MRSA can even be found in up 45% of all infections(16), with VRE and ESBL infections also on the rise (17, 18).

Even though most PJI's are caused by a single microorganism, 15% are caused by polymicrobial infections.

The type of pathogen also determines the clinical manifestation of PJI. Depending on the virulence and the onset of symptoms, PJI's are classified into 3 groups according to Zimmerli et al:

1. Early-onset infections (within 1-3 months after surgery),
2. Delayed-onset (3-24 months after surgery) and
3. Late-onset infections(>24 months after surgery)(15).

As such, early-onset infections are usually caused by high-virulence bacteria including the likes of *S. aureus*, Gram-negative bacilli and polymicrobial infections. Slow growing and low virulent bacteria such as CNS, anaerobic bacteria and enterococci are responsible for delayed-onset infections.

8.1.4 PJI Symptoms and Diagnosis

Despite PJI being such a severe and severe complication of arthroplasties, symptoms are usually subtle and non-specific. In addition, the diagnosis is often long and arduous due to the lack of a universally standardised definition.

In a review by Tande and Patel (2014), the authors revealed the most common clinical manifestations of PJI. These included "joint pain, joint swelling or effusion, erythema or warmth around the joint, fever, drainage, or the presence of a sinus tract."

Apart from the sinus tract, all of these symptoms aren't sufficient enough to diagnose PJI as they could also apply to a host of other orthopaedic ailments such as aseptic loosening, dislocation, adverse reactions towards the implant, gout, osteolysis and hemarthrosis, to name a few. Nevertheless, physicians can use these signs and symptoms to make a suspected diagnosis of PJI.

Once a prosthetic joint infection is assumed, physicians must take a multidisciplinary approach to confirm the ultimate diagnosis and differentiate between other possible causes for the symptoms. In an attempt to facilitate the diagnosis and define PJI, the Musculoskeletal Infection Society (MSIS) created standardised diagnostic criteria in 2011, which is the most recognised definition of PJI worldwide(19).

Table 8-1: PJI definition and diagnostic criteria according to the MSIS 2011
Table taken from "Definition of Periprosthetic Joint Infection" by Parvizi and Gehrke (2014) (19)

PJI Is Present When One of the Major Criteria Exists or Three Out of Five Minor Criteria Exist	
Major Criteria	Two positive periprosthetic cultures with phenotypically identical organisms, **OR** A sinus tract communicating with the joint, **OR**
Minor Criteria	1) Elevated serum C-reactive protein (CRP) **AND** erythrocyte sedimentation rate (ESR) 2) Elevated synovial fluid white blood cell (WBC) count **OR** ++change on leukocyte esterase test strip 3) Elevated synovial fluid polymorphonuclear neutrophil percentage (PMN%) 4) Positive histological analysis of periprosthetic tissue 5) A single positive culture

As is evident in the above table, the diagnosis of PJI is divided into major and minor criteria. At least one major or 3 minor criteria are required to diagnose PJI. However, PJI can also be present without any of the above shown criteria.

Standard X-ray and other imaging techniques (MRI, CT) are also implemented to determine whether other differential diagnoses (e.g., aseptic loosening) are relevant and is usually the first step towards the correct diagnosis. Blood serum parameters such as CRP and ESR are easy to determine and should be performed in every patient with suspected PJI. Diagnostic arthrocentesis, biopsies and intraoperative tissue sampling during revision or other procedures are more accurate, yet invasive measures in the diagnosis of PJI.

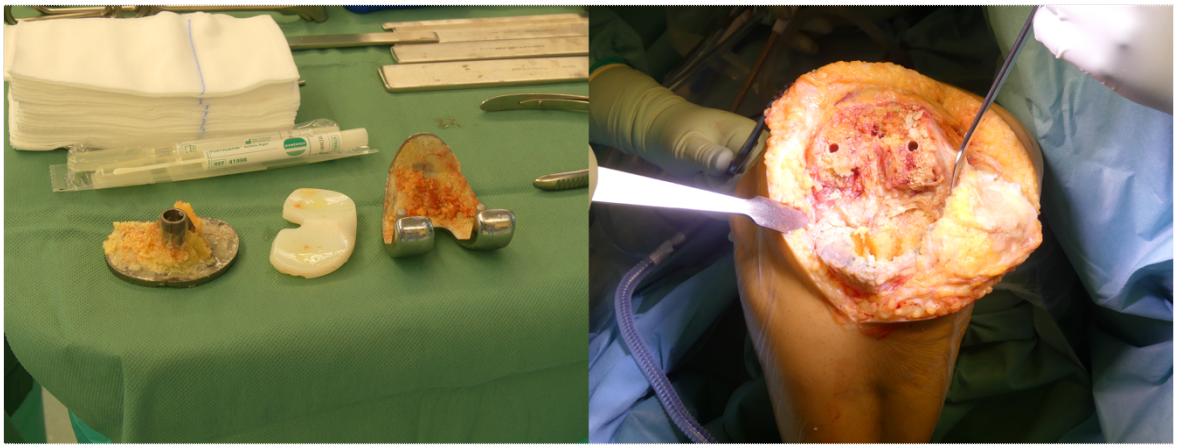

Figure 8-2: PJI of Total Knee Arthroplasty after implant removal
Left: Removed TKA; Right: Infected knee joint after prosthesis removal
Pictures kindly provided by Dr. Alfred Pölzl at the Department of Orthopaedic Surgery, LKH Steyr, Austria

8.1.5 PJI Treatment

If the diagnosis is anything to go by, then the treatment of PJI is just as difficult and challenging. If possible, patients should receive an individualised and multi-disciplinary treatment comprising of both revision surgery and antimicrobial therapy(20).

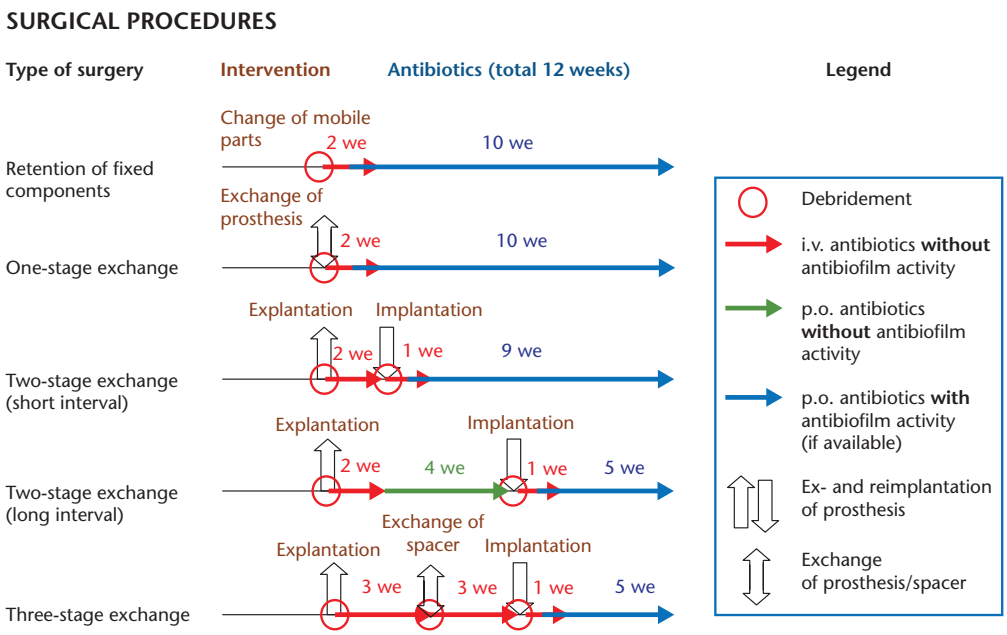

Figure 8-3: PJI Surgical Procedures
Taken from the "Pocket Guide to Diagnosis and Treatment of PJI" (2018) by the PRO-IMPLANT Foundation(20).

As per the above figure (8-1), there are several types of surgery that can be performed when treating PJI, depending on when the infection was diagnosed, the condition of the prosthesis and patient, previous surgeries and possible outcomes(21).

Debridement, antibiotics and implant retention (DAIR) is the treatment of choice in acute cases within 4 weeks after surgery while the biofilm is still immature. As the name suggests, the joint and prosthesis are cleaned, and damaged and necrotic tissue are generously removed during this procedure. Furthermore, the joint is irrigated thoroughly with sterile saline and certain mobile parts of the prosthesis are exchanged.

If PJI is diagnosed > 4 weeks after surgery, the biofilm has had a chance to mature properly, and more drastic surgical interventions become necessary. In such cases, the prostheses are exchanged in either 1 or 2 stages(22).
One-stage arthroplasty exchanges involve prosthesis removal, debridement and implantation of a new prosthesis (with the help of ALBC) within a single setting(23). Although the one-stage exchange is a more popular procedure outside of the United States, this method is recommended under the following circumstances(24):

- Immunocompetent patients
- Adequate soft tissue and bone composition
- Confirmed and antibiotic sensitive pathogen

Two-stage exchanges are considered by some to be the gold-standard in treating PJI and this type of procedure is most commonly performed in the USA. However, there is no clear evidence suggesting that a two-stage revision is superior to its one-stage counterpart(25). Two stage revisions also involve the removal of the prosthesis, extensive debridement and irrigation. Afterwards, an antibiotic impregnated PMMA spacer is inserted into the joint space for 2-6 weeks as per Figure 8-3 above. According to Vielgut et al (2015), on the other hand, the optimal duration of spacer implantation ranges between 4-11 weeks. In their study, 90% of all patients remained infection free at the final follow up, as opposed to the 70-90% success rate in other studies(26).

In a separate procedure, the spacer is then removed, and a new prosthesis is inserted(27). Below is a brief summary of two-stage revision indications:

- Patients who do not fulfil the requirements for one-stage revision and who are willing to undergo 2 surgeries
- Feasible reimplantation and functional outcome
- No previous two-stage revision

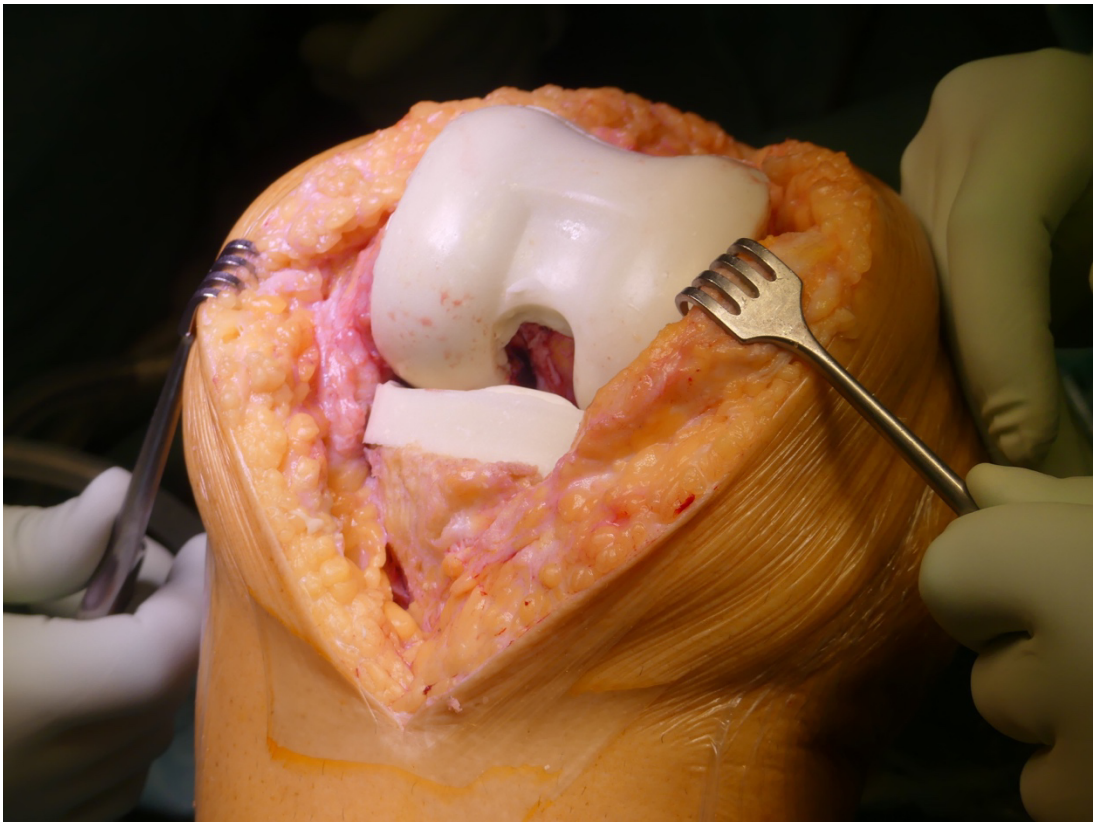

Figure 8-4: Intraoperative Knee spacer implantation
Picture kindly provided by Dr. Alfred Pölzl at the Department of Orthopaedic Surgery, LKH Steyr, Austria

In severe cases of PJI or cases in which patients don't fulfil the requirements for any of the previously mentioned procedures, permanent resection arthroplasty with arthrodesis must be performed. Amputation remains the worst case scenario and should only be performed in the presence of necrotising fasciitis, severe bone loss, inadequate tissue coverage, etc. (22).

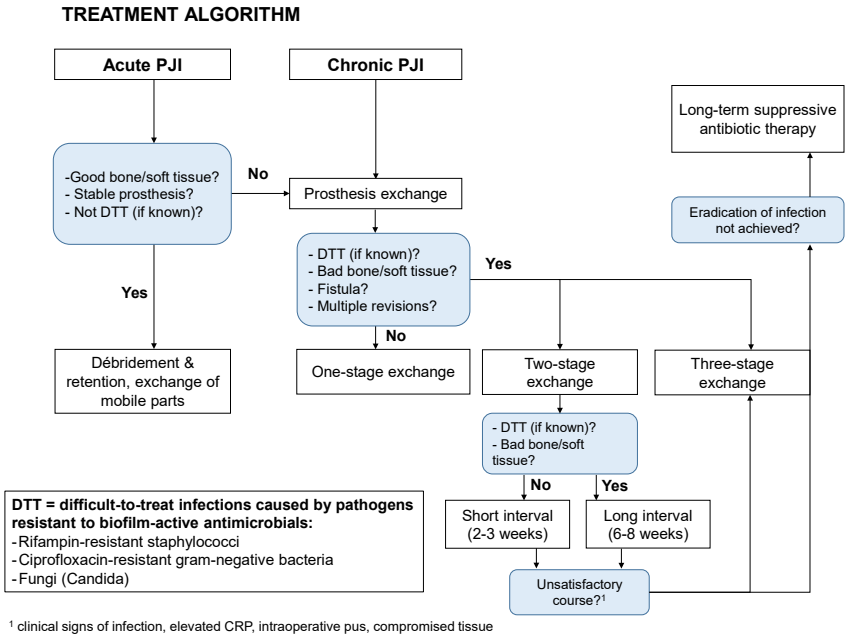

Figure 8-5: PJI Treatment Algorithm
Taken from the "Pocket Guide to Diagnosis and Treatment of PJI" (2018) by the PRO-IMPLANT Foundation(20).

Concerning the antimicrobial therapy, treatment should be tailored towards the causative pathogen and should only begin once samples for the cultures have been obtained. Empiric therapy should only be used in severe cases such as sepsis. Once the causative pathogen has been determined, antimicrobial treatment should begin as soon as possible with intravenous (i.v.) antibiotics or the oral intake of highly bioavailable antibiotics for at least 4-6 weeks and up to 12 weeks or longer depending on surgical outcome and management(9). Another method of administering antibiotics is through Antibiotic Loaded Bone Cement (ALBC) when fixating the new prosthesis or through spacer's during a 2-stage exchange. This local application of antibiotics offers a variety of benefits which shall be discussed later in this diploma thesis.

8.2 Antibiotics

Since the discovery of penicillin by Sir Alex Fleming in 1928 (28), antibiotics have become the silver bullet of medicine against bacterial and parasitic infections(29). Not only did antibiotics lower the mortality of infectious diseases, but they also improved life expectancy throughout the entire world. Antibiotics are so effective in treating bacterial infections thanks to their bactericidal or bacteriostatic effects. In

other words, they either kill off the bacteria or prevent them from growing and multiplying. There are also different classes and groups of antibiotics, each with their own method of action. For example, classic penicillin (Penicillin G, V) belong to the family of beta-lactam antibiotics and have a bactericidal effect by inhibiting the cell wall synthesis of bacteria(30).

To treat bacterial infections, antibiotics can be administered through various pathways. In orthopaedic settings, antibiotics are most commonly administered intravenously, orally or locally. For example, i.v. antibiotics such as cefazolin, vancomycin or clindamycin are given preventively during hardware implantation or targeted systemic antibiotics are given to treat PJI. A speciality of orthopaedic surgery is adding antibiotics to PMMA powders and thus creating antibiotic loaded bone cements, which allows surgeons to better treat PJI through higher local concentrations of the much-needed antibiotic(31). It is important to note, however, that not every antibiotic available on the market can be used in PMMA powders. Only antibiotics that have been extensively studied and proven for the use in bone cements should be used. Nevertheless, before treatment begins, an antibiogram should be performed to determine the efficacy of the available antibiotics against the pathogen and to prevent resistances.

As there are a plethora of antibiotics that are used regularly in medicine and orthopaedic procedures, this thesis will restrict itself to an overview of the most common types of antibiotics used in bone cements and other viable alternatives such as tigecycline.

8.2.1 Gentamicin

Gentamicin belongs to the family of glycosides, a class of antibiotic with a bactericidal effect. Aminoglycosides, and more specifically gentamicin, kill off bacteria by inhibiting bacterial protein synthesis by attaching to the 30S ribosomal unit of the bacteria. As a result, bacterial protein translation is inhibited(32) and the bacterial cell wall becomes damaged.

Thanks to this method of action, gentamicin is a broad-spectrum antibiotic that can be successfully implemented against Gram-positive (e.g. *S. auereus*), Gram-negative (mainly enterobacteria) and even atypical mycobacteria(33).

Furthermore, gentamicin is also a common component of Antibiotic Loaded Bone Cements (Palacos® R+G, Copal® G+V and Copal® G+C) and spacers (e.g., Cemex® Spacer-K, COPAL® Exchange G).

8.2.2 Clindamycin

Another effective broad-spectrum antibiotic is clindamycin. Belonging to the family of lincosamides, clindamycin prevents the growth and propagation of bacteria by binding to the 50S ribosomal subunit of bacteria and thereby inhibiting protein synthesis(34). The spectrum of clindamycin's activity includes Gram-positive bacteria such as *S. aureus*, MRSA, CNS, streptococci and anaerobes (*C. acnes*). Thanks to its excellent tissue penetration, clindamycin is most frequently used to treat bone, skin and soft tissue, head and neck, respiratory, abdominal, pelvic and polymicrobial infections.

8.2.3 Vancomycin

Another antibiotic that is commonly used in bone cement is the bactericidal vancomycin. As a glycopeptide antibiotic, vancomycin inhibits the cell wall synthesis of bacteria by forming a complex with the murein within the cell wall. This ultimately leads to a weaker bacterial cell wall and allows intracellular components to leak out, killing the bacteria. Vancomycin is only effective against Gram-positive bacteria and is used to treat infections with *S. aureus*, MRSA, streptococci and *C. difficile*(35).

	Gentamicin	Clindamycin	Vancomycin
Coagulase-positive staphylococci (CPS) (Gram +)			
***S. aureus* (MSSA)**	X	X	X
***S. aureus* (MRSA)**			X
Coagulase-negative staphylococci (CNS) (Gram +)			
***S. epidermidis* (MSSE)**	X	X	X
***S. epidermidis* (MRSE)**			X
Streptococcus spp. (Gram +)			
Strep. pneumoniae		X	X
Strep. pyogenes		X	X
Strep. agalactiae		X	X
Enterococcus spp. (Gram +)			
E. faecalis	X		X
E. faecium	X		X
***E. spp* (VRE)**	X		
Enterobacteriaceae spp. (Gram -)			
E. coli	X		
Enterobacter spp.	X		
Klebsiella spp.	X		
Proteus mirabilis	X		
Serratia spp.	X		
Other Gram -			
Pseudomonas aeroginosa	X		
Anaerobic bacteria (Gram +)			
C. difficile			X
C. perfringens			X
C. acnes		X	

Figure 8-6: Overview of Gentamicin, Clindamycin and Vancomycin efficacy spectrum
Figure taken from the Heraeus Medical Copal® Brochure
https://www.heraeus.com/media/media/hme/doc_hme/products_hme/copal_revision/COPAL_Revision_Folder_INT.pdf

8.2.4 Tigecycline

As a relatively new antibiotic, tigecycline is an excellent antibiotic for treating life threatening and complex infections with multi-resistant pathogens (MRSA, VRE, etc.). Tigecycline is the first of a new family of antibiotics, the glycylcyclins(36) and its bacteriostatic effect is based upon inhibition of bacterial protein synthesis by binding to the ribosomal 30S subunit. Furthermore, tigecycline is able to overcome many bacterial resistance mechanisms, making it an ideal broad-spectrum antibiotic(37, 38).

As of yet, there is very little research and information concerning the addition of tigecycline to ALBCs as a form of local antimicrobial therapy(39-41).

8.3 Bone cement

Polymethylmethacrylate (PMMA) cement, a.k.a. bone cement, is the most common method for fixating and anchoring implanted prostheses to the bone in orthopaedic and trauma surgery. Chemically speaking, bone cement is plexiglass that fills the space between the bone and prosthesis, and acts as an elastic zone, absorbing the forces to which the joints are exposed and distribute them evenly(42).

Commercial bone cements contain two separate and sterile components that need to be mixed together, before they can be implanted within a patient.
The first component is a PMMA powder with further additives that don't take part in the polymerisation. The additives to the powder include an initiator for polymerisation (e.g., Benzoyl peroxide) at room temperature and a contrast agent (ZrO_2, $CaCO_3$ or $BaSO_4$) for better visibility during radiological imaging. An optional component is the addition of an antibiotic powder to treat local infections (further explanation below). Nevertheless, all three of these additives influence the mechanical stability and antibiotic elution (if any is added) of the bone cement.
The second component is a liquid mainly comprised of an MMA monomer, an accelerator (to trigger polymerisation at room temperature), a stabiliser (to prevent premature polymerisation) and, depending on the manufacturer (e.g. Heraeus), chlorophyll (a green dye to improve intraoperative visibility)(42, 43).
Once both components are mixed thoroughly, free radical polymerisation occurs. This is an exothermic reaction in which the bone cement can reach temperatures of up to 86°C. While the bone cement sets, it's viscosity changes from a dough-like state into hardened PMMA polymer(42, 43).

8.3.1 Antibiotic loaded bone cement (ALBC)

As mentioned above, antibiotics are frequently added to bone cements to prevent and treat bone and joint infections. This form of drug delivery enables high local concentrations of antibiotics into areas that are difficult to access and greatly reduce the need for further revision surgeries and the likelihood of developing PJI(44). However, before an antibiotic can be added to a PMMA bone cement, it must fulfil the following criteria according to Frommelt and Kühn (2005) (45):

- No chemical interference with the PMMA
- Storage stability and possible sterilisation
- Water solubility and high elution from PMMA
- Heat resistance
- Broad-spectrum efficacy against Gram-negative and positive bacteria
- Low resistance rates
- Low allergic potential
- Low interaction with proteins

Failing to follow these principles by using an antibiotic that has not been tested for bone cement supplementation, for example, could result in failed antimicrobial treatment and be detrimental towards the mechanical stability of the bone cement. Tried and tested antibiotics that are regularly used in acrylic bone cements include Clindamycin, Gentamicin, Vancomycin, Tobramycin, etc. Furthermore, these antibiotics have a synergistic effect when combined and are therefore more effective in treating infections with multi-resistant pathogens(46). For example, bone cements for revision surgery often have more than one antibiotic with a total antibiotic concentration of over 2,5%.

ALBCs show an initial high antibiotic release with gradual elution over several weeks and even years in low doses. Once the bone cement is implanted within the patient, fluid from the body diffuses within the cement, dissolving the antibiotic and releasing it into the surrounding tissue. As a result, there are certain characteristics that can influence the elution of antibiotics within the bone cement(47):

- Additives (e.g., contrast agents) that increase the hydrophilic matrix
- Porosity (which can be influenced through fillers or mixing strategy)
- Antibiotic concentration (the higher the concentration, the higher the release)
- Superficial Vancomycin Coating

Below are 2 examples of how the elution of antibiotics into the surrounding tissue can be influenced:

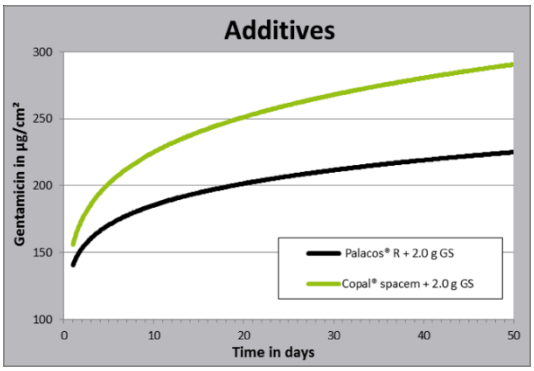

Figure 8-7: Antibiotic release comparing 2 different contrast agents
Bone cement with the additive $CaCO_3$ has a higher elution of gentamicin than the bonce cement with ZrO_2
Legend: Green = $CaCO_3$ as the additive, Black = ZrO_2 as the additive (Kühn 2014) (48)

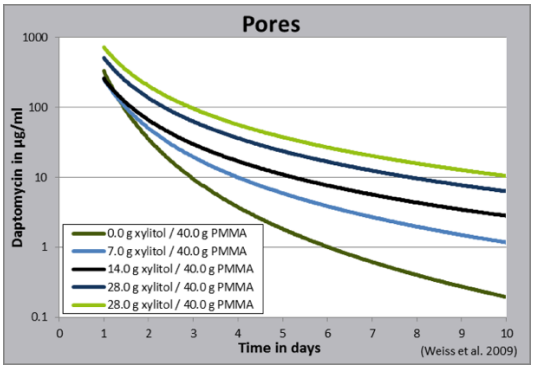

Figure 8-8: Antibiotic release comparing porosity after the addition of fillers (Weiss 2009 and Kühn 2014) (49)
The addition of xylitol increases the porosity of bone cements. The higher amount of xylitol (and therefore porosity), the higher and longer the release of daptomycin.

Therefore, antibiotic release largely depends on which type of bone cement is used as each product differs in antibiotic concentration, composition and porosity. For example, the higher the porosity of a bone cement, the better the elution of antibiotics. Another factor that influences antibiotic release is how the 2 components are mixed together. In study published in the Bone and Joint Journal, Frew et al (2017) observed that “homemade” ALBC’s, i.e. hand-mixed and manually added antibiotics, had a significantly higher antibiotic elution compared to their commercially available counterparts(50). This greater antimicrobial efficacy is largely due to the increased porosity and clumping of antibiotics within the cement that result through this manual preparation.

Nevertheless, when adding antibiotics to the PMMA powder, surgeons should not exceed an antibiotic concentration of more than 10% (e.g., >4g of antibiotics per 40g of bone cement. Concentrations >10% could result in mechanical instability of

the bone cement and implant failure(44, 46). Despite these consequences, operators still frequently opt for these high concentrations in spacers when treating severe acute or chronic infections. Although low-dose ALBC's remain local and have no systemic effect, these high-dose ALBC do reveal systemic toxicity and could even lead to acute renal failure in some patients(51, 52).

Superficial Vancomycin Coating is a novel technique that involves pressing vancomycin powder into the surface of an ALBC that is the correct position and not yet completely hardened. This method produces exceptionally high intraarticular concentrations of vancomycin without systemic side effects. SVC provides an interesting alternative if surgeons wish to enhance local doses of vancomycin without comprosing mechanical stability or exceeding the 10% antibiotic threshold according to Amerstorfer et al (2016)(53).

8.3.2 Joint Spacers

In general, antibiotic loaded joint spacers are ALBCs that can be moulded to fill the empty joint space during a 2-stage prosthesis exchange. During such 2-stage exchanges, the prosthetic joint is removed for a period of 2-6 weeks. In order to prevent re-infection of the joint space, maintain healthy tissue, and avoid tissue contraction around the joint over this period, spacers are temporarily implanted into the joint space where the previous prosthesis use to be(54).

As such, spacers follow the same characteristics that influence antibiotic elution as ALBCs. Additives, porosity and antibiotic concentration all affect the intraarticular concentration of antibiotics. Similarly to ALBCs, SVC could also potentially increase antibiotic elution from spacers and reduce reinfection. Although Amerstorfer et al. (2020) were able to achieve high intraarticular concentration of Vancomycin and observe lower reinfection rates, no statistically significant results were observed(55).

Spacers can be classified into articulating/dynamic and non-articulating/static spacers(56). Static spacers, as the name suggests, do not allow movement and fixate the joint in a certain position. For example, a static knee spacer keeps the knee either in full extension or partial flexion. Although these spacers are cheaper

and easier to insert, they can cause massive bone loss, compromise joint movement and lead to stiffness, making the re-implantation of the new prosthesis more difficult. Dynamic spacers, however, do allow for a certain amount of movement in the joint and have a similar shape to that of the prosthesis. They can make the re-implantation of the new prosthesis easier by preventing scar tissue formation, bone loss, and shortening and contracture of the surrounding tissue. Another huge advantage of dynamic spacers is the improved life quality of patients between the 2 surgeries.

Spacers can also be classified according to their manufacturing process. As such, joint spacers can be classified into:

- Self-made spacers,
- Spacer moulds and
- Commercially pre-formed spacers

Self-made spacers are moulded by the surgeons themselves out of ALBCs and are used in an “off-label” setting. Although such spacers allow for high degrees of flexibility and the addition of other components, they are less mechanically stable and may have a less consistent release of antibiotics. Furthermore, the efficacy of these spacers cannot be reproduced and marginally increase the overall duration of the procedure. Ultimately, the experience and skill of the surgeon determines the clinical outcome of such spacers.

Spacer moulds also use ALBCs that are cast into joint specific moulds that vary in size and design. Although this method doesn’t allow as much flexibility as the previous method, the surgeon can still choose the appropriate size for the patient and add other components or antibiotics. This method allows for a more homogenous spread of antibiotic throughout the spacer and results are easier to reproduce.

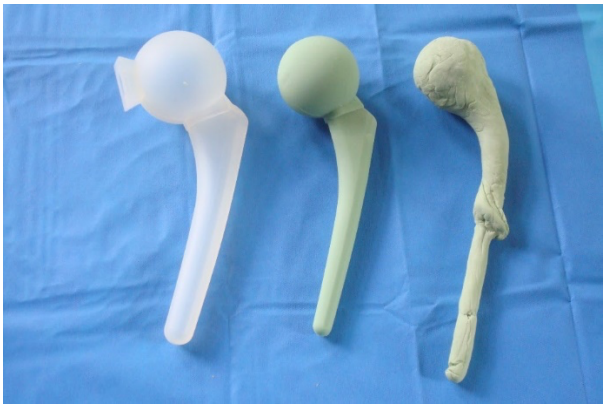

Figure 8-9: Comparison of 2 femur spacers.
Legend: Left = Hip mould for spacers; Centre = moulded hip spacer; Right = Self-made spacer

Lastly, pre-formed spacers are mixed and formed by the manufacturing company and should offer a high degree of mechanical stability and homogeneity. Although they most likely have a lower antibiotic elution and are not as individually adaptable as the previous spacers, they could offer a good reproductive value, a constant elution profile and good cement quality out of all the spacers.

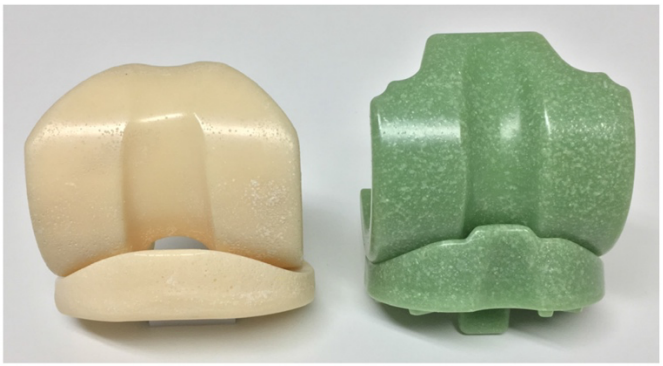

Figure 8-10: Preformed spacers(57)
Legend: Left = Tecres knee spacer; Right = Heraeus knee spacer

For this reason, we chose the pre-formed articulating spacers for our experiments, as described below.

8.4 Aims and Objectives

As mentioned above, the treatment of PJI is often complicated and difficult. Even though the use of ALBCs and Spacers are great tools to treat Prosthetic Joint Infection, the threat of bacterial antibiotic resistance looms. To improve treatment and prevent the development of such resistances and reinfection of the prosthesis, the ideal concentration of antibiotics in bone cement, as well as their ideal composition needs to be determined, and novel bone cement compatible antibiotics need to be found. For these reasons, additional Vancomycin (2 g and 6 g) was

added to the standard revision bone cement Copal® G+C and G+V. As a novel addition to bone cement, Tigecycline was added to the tried and tested Palacos® R and R+G bone cements. Furthermore, the commercially available femoral knee spacers of Heraeus and Tecres were directly compared concerning their microbiological properties. Both spacers differ in composition (surface/porosity and contrasting agent), yet it is unclear which spacer offers the most advantages.

The aims and objectives of this diploma thesis were to find such alternatives, determine whether the excessive addition of antibiotics makes sense, which type of "spacer" offers the most advantages and improve the treatment of PJI with ALBCs and spacers.

To achieve this, this study was divided into two parts:

- Part 1 compared the stability and efficacy of ALBC by adding additional Antibiotics (Vancomycin and Tigecycline) in varying doses to commercially available bone cements. To compare the stability, the new ALBC mixtures were put through internationally recognised ISO-Standard tests and DYNSTAT (DIN) tests. Overall, it was expected that the addition of more antibiotics would reduce the mechanical properties of the bone cement. In the case of excessive antibiotic addition (6g of Vancomycin), it was hypothesized that the ALBC would not comply with the ISO 5833:2002 norm. To compare the microbiological efficacy, elutions were taken at different times from ALBC mixtures and added to inhibition zone assays using the most common pathogens in PJI.
- Part 2 compared two of the leading commercially available pre-formed femoral knee spacers (by Heraeus and Tecres) according to their microbial efficacy (inhibition zone assays).

9 Material and Methods

This Diploma Thesis was conducted in 2 separate stages and locations: Stage 1 was performed in March 2019 at Heraeus Medical GmBH in Wehrheim, Germany. During this stage, all cement test bodies for the mechanical and microbiological experiments were produced, as well as all the mechanical tests on the various test bodies. The mechanical tests included the “Determination of compressive strength of polymerized cement” and “Determination of bending modulus and bending strength of polymerized cement” according to the ISO 5833:2002 Standard for bone cements(58), as well as the German Industrial Standard “DYNSTAT” (DIN 53435:2018-09) “Bending strength” and Impact strength” tests for bone cements and plastics. The second part of this thesis was carried out in July 2019 at microbiological labs at the Charité University in Berlin (PRO-IMPLANT Foundation), Germany. Here, the microbiological properties of the test bodies and spacers were examined using the “Hemmhof-Test” (inhibition zone assay) and the subsequent inhibition zone diameters of the antibiotic eluates were measured.

9.1 Materials

Below is a tabulation of the various bone cements, spacers, antibiotics, and bacteria that were used in the experiments for this diploma thesis.

Table 9-1: Bone cements and spacers

Name:	Company:	LOT:	Antibiotics:	Concentration:
COPAL® G+C	Heraeus	85294644	Gentamicin Clindamycin	1 g 1 g
COPAL® G+V	Heraeus	88234664	Gentamicin Vancomycin	0,5 g 2 g
PALACOS® R	Heraeus	91665294	None	
PALACOS® R+G	Heraeus	90344797	Gentamicin	0,5 g
MMA Monomer	Heraeus	4797	n/a	
COPAL® Exchange G Knee spacer, Size S	Heraeus	280219	Gentamicin	0,8 g
Cemex® Spacer-K Knee spacer, Size S	Tecres	AA9829	Gentamicin	0,9 g

Table 9-2: Antibiotics

Antibiotics:	LOT:	Concentration:
Vancomycin (powder)	A4240235	90,2%
Tigecylin-ratiopharm® 50 mg	ADT818, ADT820	50%

Table 9-3: Bacteria

Bacterial Strain:	LOT:
S. aureus	ATCC 29213
MRSA	ATCC 43300
E. faecalis	ATCC 29212
VRE	Van A
E. coli	ATCC 25922
ESBL	BJ HDL-1

9.2 Test Body and Spacer Production

9.2.1 Test Bodies

As per Table 9-4 below, we created 7 different groups of ALBCs (A-G) using only commercially available Heraeus Medical bone cements. In groups A-D, we added a moderate (2 g) and a high dose (6 g) of Vancomycin to Copal® G+C (Bone cement already with Gentamicin and Clindamycin) and Copal® G+V (Bone cement already with Gentamicin and Vancomycin). In groups E-F, we added 0,5 g and 1 g of Tigecycline to Palacos® R (Bone cement with no added antibiotic) and Palacos® R+G (Bone cement with added Gentamicin).

Table 9-4: Group names with their corresponding cement basis and added antibiotic

Group:	Cement type:	Added Antibiotic:	Dose of added Antibiotic:
A	Copal® G+C	Vancomycin	2 g
B	Copal® G+C	Vancomycin	6 g
C	Copal® G+V	Vancomycin	2 g
D	Copal® G+V	Vancomycin	6 g
E	Palacos® R	Tigecycline	0,5 g
F	Palacos® R+G	Tigecycline	0,5 g
G	Palacos® R+G	Tigecycline	1 g

To manufacture the test bodies with the additional Vancomycin, a Vancomycin-powder with a 90,2% antibiotic concentration was used. This meant that 2,21 g and 6,65 g of the antibiotic powder had to be added to reach 2 g and 6 g of pure Vancomycin respectively. Each Vancomycin dose was then combined with 1 sachet of Copal® G+V powder (43 g G+V-Powder containing 0,5 g Gentamicin and 2 g Vancomycin) and 1 sachet of Copal® G+C powder (42,7 g G+C-Powder containing 1 g Gentamicin and 1 g Clindamycin), and the combination was then mixed by hand in a paper cup with a spatula.

As Tigecycline is not available in powder-form for the addition to bone cement, “Tigecylin-ratiopharm® 50 mg” ampules containing a powder to produce solutions for infusions was used. Each ampule of “Tigecylin-ratiopharm® 50 mg” contained 50 mg of Tigecycline in 100 mg of powder. However, the powder inside the ampules had formed a solid, orange mass. As a result, the mass was scratched out of the ampule and ground with a mortar and a pestle to turn it into a powder again (see

Figure 9-1 below). After grinding the antibiotic into a powder, 0,5 g (10 ampules) and 1 g (20 ampules) of Tigecycline was added to 1 sachet Palacos® R and Palacos® R+G cement powder as per Table 9-1 above.

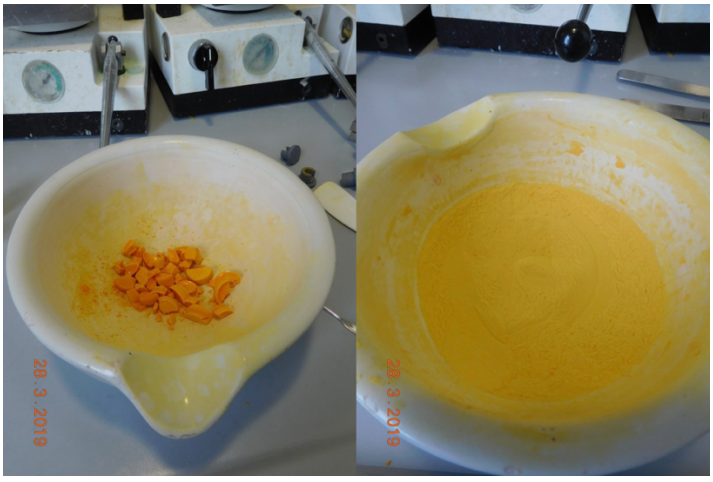

Figure 9-1: Tigecycline preparation for bone cement addition
Legend: Left = before grinding; Right = after grinding

Once the powders were thoroughly mixed with the antibiotics and were homogenous again, 20 ml of the monomer liquid included in the packaging of the respective bone cements, was added. The powder was then mixed with the liquid monomer for approximately 30 seconds and cured for 1-2 minutes. The cement was occasionally kneaded until the doughy mass wasn't sticky anymore. It was noted that during the mixing with the MMA monomer, high concentrations of additional Vancomycin (6 g) made the cement mass sandy and difficult to mix with the spatula. This problem was solved by kneading the cement by hand so that adequate polymerisation could occur. Furthermore, Tigecycline changed the typical green colour of the bone cement to an orange/brown colour (see Figures 9-3 and 9-4 below).

Afterwards, the cement was cast into moulds of stainless steel. 3 different moulds were used for the various mechanical tests according to the ISO-5833:2002 Standard(58) and DYNSTAT 53435 tests, and 1 mould for the microbiological tests. For the "Determination of compressive strength of polymerized cement" a mould that produced cement rods (height: 12 mm +/- 1, diameter: 6 mm +/- 0,1) was used. For the "Determination of bending modulus and bending strength of polymerized cement" and "DYNSTAT" test, flat moulds (depth: 3,3 mm) were used. Finally, 3 circular moulds (height: 10 mm, diameter: 25 mm) per antibiotic-cement

combination were used for the microbiological tests. A summary of the mould sizes can be seen in Table 9-5 below.

Once the cement had been cast into the moulds, the top and bottom of the moulds were covered with a plastic sheet and then with stainless steel plates. They were then squeezed together by hand and excess bone cement was removed. Next, the moulds were wedged between a clamp and a pressure of 3 bar was applied (as seen in Figure 9-2 below).

Figure 9-2: Cement mould clamps

After 30 minutes, the pressure was removed, and the test specimens were extracted from the moulds. Finally, the 2 flat moulds were cut with a “ZING” laser cutter into their final dimensions (**bending tests:** length: 75 mm, width: 10 mm; **DYNSTAT:** length: 15 mm, width 10 mm)

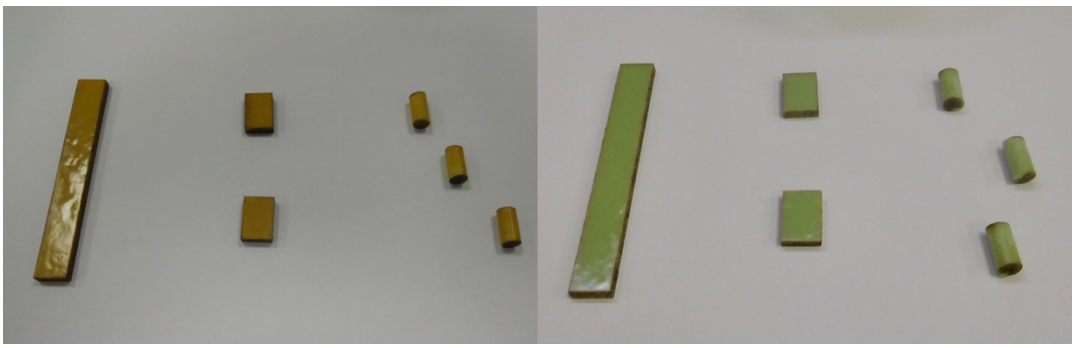

Figure 9-3: Hardened mechanical test bodies after 24h
Legend: Left picture = Tigecycline; Right picture= Vancomycin; Test bodies in both pictures from left to right = bending modulus and strength test bodies, DYNSTAT test bodies, compressive strength bodies

Figure 9-3 illustrates the forms of the mechanical test specimens. The shapes on the left were used for the bending tests, the shapes in the middle for the DYNSTAT tests, and the shapes on the right for the compression tests. All mechanical tests were performed 24 h after the test specimens were cured.

Figure 9-4: Microbiological test bodies
Legend: Left = Tigecycline; Right = Vancomycin

Figure 9-4 shows the forms of the microbiological test specimens. These were sealed in an airtight container and sent to the BCRT labs at the Charité, Berlin for later testing.

9.2.2 Spacers

To compare the efficacy and elution of the spacers, two of the leading pre-made Knee spacers on the market were used:

- Spacer A: Copal® Exchange G Knee spacer, Size S by Heraeus Medical (smooth surface; contrast agent: $CaCO_{3)}$
- Spacer B: Cemex® Spacer-K Knee spacer, Size S by Tecres (rough, porous surface; contrast agent: $BaSO_4$)

Both Knee spacers contained roughly equal concentrations of the antibiotic gentamicin.
To properly compare both spacers according to their antibiotic release and efficacy, and to simulate in-vivo conditions that occur within the joint, the concave inner, non-articulating surface of 3 femoral knee spacers of each brand were covered with an antibiotic-free bone cement (Palacos® R) (see Figure 9-5 below). Once the cement had dried and hardened, the spacers were sent to Berlin to examine their efficacy against bacteria (Charité, Berlin).

Figure 9-5: Femoral knee spacers covered with Palacos R on the non-articulating surface
Legend: Left = Spacer A; Right = Spacer B

9.3 Mechanical Properties

Commercially available bone cements and ALBCs must fulfil the 3 requirements of cured cement as specified in the ISO 5833:2002 Standards (58) as follows

- Bending modulus: Minimum 1.800 MPa
- Bending strength: Minimum 50 MPa
- Compressive strength: Minimum 70 MPa

In addition, all test specimens were tested in accordance with the German Industrial Standard DYNSTAT for impact and bending tests (DIN 53435).

Before testing, the dimensions of all the test specimens were measured (Table 9-5, length, width, diameter, and height) and visually examined (visible anomalies, structural defects). Test specimens with obvious faults or defects were excluded from the mechanical tests and discarded.

Table 9-5: Test body dimensions in mm

Test body	Height	Length	Width	Diameter
Compressive strength	12 mm	n/a	n/a	6 mm
Bending modulus and strength	3,3 mm	75 mm	10 mm	n/a
DYNSTAT	3,3 mm	15 mm	10 mm	n/a
Microbiology	10 mm	n/a	n/a	25 mm

9.3.1 Determination of compressive strength of polymerized cement

All test specimens and tests were produced and conducted following Annex E of the ISO 5833:2002 Standard. The compressive strength test is used to determine the pressure or compressive force that is needed until the cement loses its stability and breaks. For each cement combination, 10-12 cement rods were used (see above for dimensions in Table 9-5). The cement rods were then placed in the middle of a test machine capable of applying and measuring compressive force (Zwick/Roell, see Figure 9-6 below) while running on the testXpert II Zwick/Roell software. The machine applied increasing force until the test rod fractured, or until the 2% offset load or upper yield-point load were reached. Once one of these conditions was reached, the internal pressure was measured (in MPa) and the software then automatically calculated the average compressive strength of all the cement rods

and their standard deviation. To comply with the standards of the ISO 5833:2002, the ALBCs had to reach a minimum internal pressure of 70 MPa.

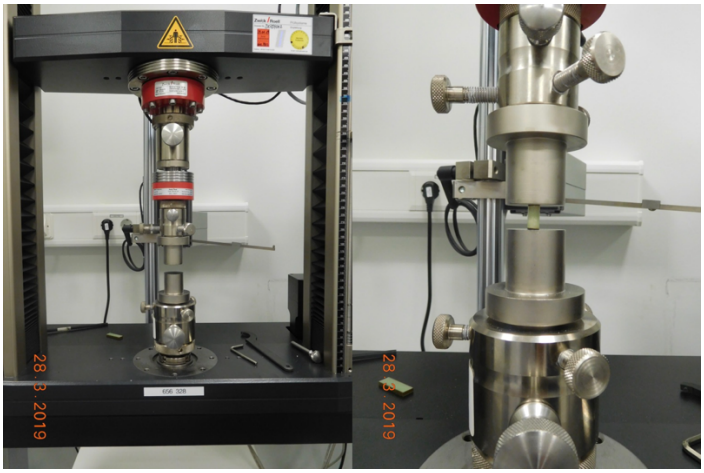

Figure 9-6: Compressive strength test rig by Zwick/Roell
Legend: Left = Test rig without test body; Right = Test rig with test body

9.3.2 Determination of bending modulus and bending strength of polymerized cement

All test specimens and tests were produced and conducted following Annex F of the ISO 5833:2002 Standard. This test was used to determine the ALBC resistance towards bending. For each cement combination, 6 rectangular cement specimens were used (see above for dimensions). After extracting the specimens from the stainless-steel moulds, they were placed in a four-point test rig (Zwick/Roell, see Figure 9-7 below) running on the testXpert II Zwick/Roell software. Extra care was taken to place the test specimens as centrally on the device as possible. The bend test machine then placed and increased the force on the test specimen while measuring its deflection until the specimens broke. The machine software then calculated the bending and strength modulus with the average and standard deviation of each cement combination in megapascals. To comply with the standards of the ISO 5833:2002, the ALBCs had to reach a minimum bending modulus of 1.800 MPa and a minimum bending strength of 50 MPa.

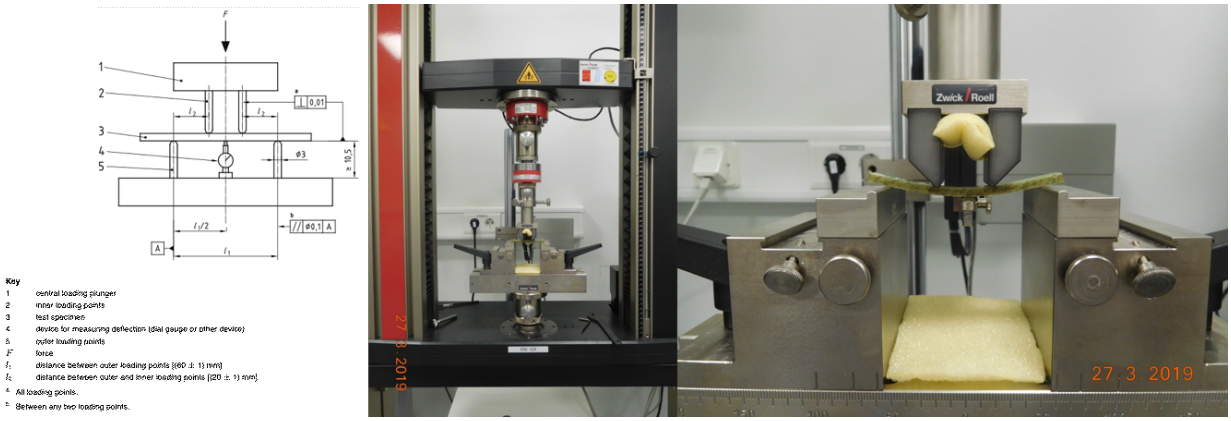

Figure 9-7: Four-point bend test rig by Zwick/Roell
Legend: Left = Basic principles of test rig; Middle =Test rig; Right = Cement body in test rig during bending measurement

9.3.3 DYNSTAT (DIN 53435)

DYNSTAT (DIN 53435) is a test to determine the mechanical stability of plastics and PMMA cements and is a good comparison of the natural forces that occur on the joint implant. This test determines 2 qualities of the bone cement:

- the bending strength and
- the impact strength.

8 test bodies (length: 15 mm, width: 10 mm, depth: 3,3 mm) were used per test.

9.3.3.1 Bending strength

The test bodies were placed in the DYNSTAT bending test apparatus (see Figure 9-8). The apparatus began to rotate at 100 °/min, applying a bending moment of 400 Ncm on the test body. Once the test specimen broke, the machine had to be stopped and the drag indicator showed the bending moment of the test body at break (Ncm).

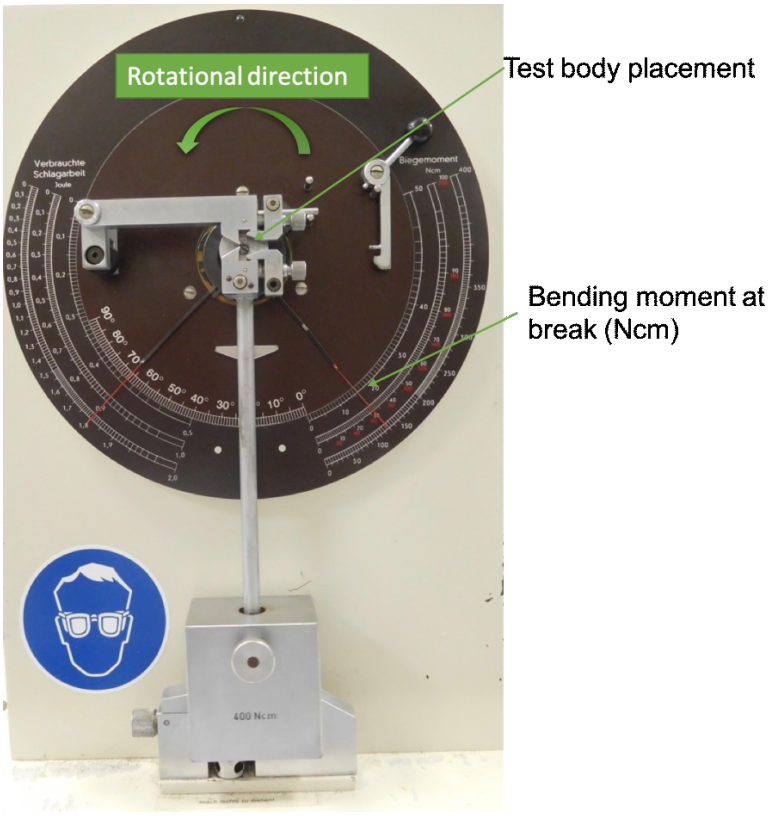

Figure 9-8: DYNSTAT bending strength test rig

To calculate the actual bending strength (MPa), the following formula was used:

$$B = 10 \cdot \frac{6 \cdot M}{b \cdot h^2}$$

B = bending strength [MPa]
M = bending moment at break [N cm]
b = measured width of specimen [mm]
h = measured thickness of specimen [mm]

Figure 9-9: DYNSTAT bending strength equation (MPa)

Finally, the average bending strength and the standard deviation were calculated.

9.3.3.2 Impact strength

The test specimens were placed in the DYNSTAT strength apparatus (see Figure 9-10) and the Pendulum was placed in its starting position. Once the pendulum was released, it collided with the test body with an impact energy of 0,5 J. The required impact energy (J) to break the test body was then displayed by the drag indicator.

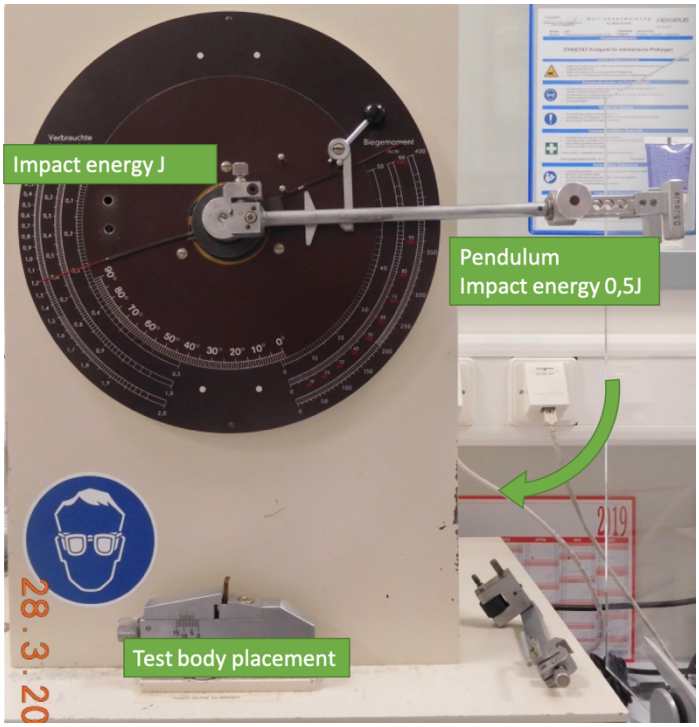

Figure 9-10: DYNSTAT impact strength test rig

To calculate the actual impact strength, the following formula was used:

$$a_n = 1000 \cdot \frac{A_n}{b \cdot h}$$

a_n = impact strength [kJ m^{-2}]
A_n = impact energy [J]
b = measured width of specimen [mm]
h = measured thickness of specimen [mm]

Figure 9-11: DYNSTAT impact strength equation (kJ/m2)

As with the bending strength, the average impact strength of the 8 test specimens and the standard deviation were calculated.

9.4 Microbiological Properties

All test specimens and spacers were sent to the BCRT, Charité, Berlin to examine the antibacterial efficacy of the test bodies and spacers via the Hemmhof-Test (inhibition zone assay). Table 9-6 (below) illustrates which test bodies and spacers were used against which bacteria.

Table 9-6: Tested bacteria and test bodies/spacers

Cement type/Spacer:	Antibiotic added:	Tested bacteria:	Group name:
Copal® G+C	2 g Vancomycin	*S. aureus* *E. faecalis* *MRSA*	A
Copal® G+C	6 g Vancomycin	*S. aureus* *E. faecalis* *MRSA*	B
Copal® G+V	2 g Vancomycin	*S. aureus* *E. faecalis* *MRSA*	C
Copal® G+V	6 g Vancomycin	*S. aureus* *E. faecalis* *MRSA*	D
Palacos® R	0,5 g Tigecycline	*MRSA* *VRE* *ESBL*	E
Palacos® R+G	0,5 g Tigecycline	*MRSA* *VRE* *ESBL*	F
Palacos® R+G	1 g Tigecycline	*MRSA* *VRE* *ESBL*	G
Copal® Exchange G	n/a	*S. aureus* *MRSA* *E. coli* *E. faecalis*	Spacer A
Cemex® K	n/a	*S. aureus* *MRSA* *E. coli* *E. faecalis*	Spacer B

For each group, 3 test specimens or spacers were tested against each bacterium. Eluates of each group were used for the tests.

9.4.1 Medium Preparation

To perform the microbiological tests, 2 different mediums had to be prepared:

- PBS as a buffer solution to extract the antibiotics as an eluate from the test specimens and spacers,
- and a Müller-Hinton-Agar (MHA) to grow the bacterial colonies and execute the inhibition zone assays.

To make 1 litre of PBS, 10 PBS tablets manufactured by VWR Chemicals (LOT: 18K1556345) containing 137 mM Sodium chloride, 2,7 mM Potassium chloride and 10 mM Phosphate buffer, were put into a glass bottle and 1.000 mL of distilled water (ddH_2O) were added (see figure 9-12). After the tablets had dissolved in the water, the lid was gently screwed onto the glass bottle so that it wasn't sealed completely and then autoclaved for 3 hours. Once the solution was autoclaved, the lids were tightened, and the bottle left at room temperature to cool down.

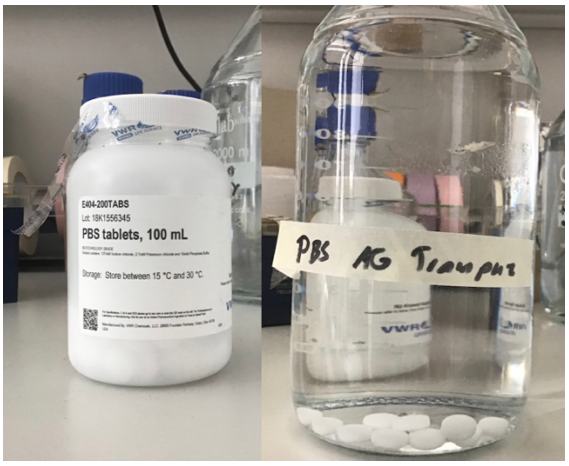

Figure 9-12: PBS-buffer preparation
Legend: Left: PBS tablets; Right = PBS tablets in 1000mL of distilled water

To make 400 ml of MHA (equivalent of 20 petri dishes), 8,4 g of MH-broth (OXIOD), 6 g of Agar (Sigma-Aldrich) and 400 mL ddH_2O were mixed in a glass bottle. Again, the lids were lightly screwed on the bottles and autoclaved for 3 hours. After tightening the lids, the liquid agar left to cool down in a 50°C water bath. Once the agar had reached 50°C, 20 mL of the agar were pipetted into petri dishes and so that the agar could solidify at room temperature (25°C).

9.4.2 Test specimen preparation and eluate extraction

To extract the antibiotics from the cement, each of the 3 test specimens (triplicates) from every group (A-G) were placed in a separate 50 ml Falcon®-Tube with 20 ml of PBS. The tubes were sealed, the lids covered with Parafilm® to prevent leakage and placed upside down so that the specimen samples were completely submerged in the PBS (Figure 9-13).

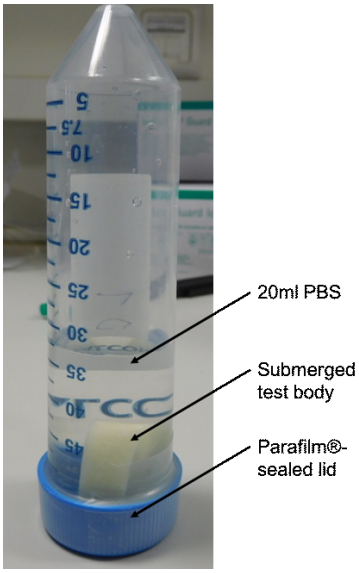

Figure 9-13: Falcon®-Tube with ALBC test body submerged in 20mL of PBS solution

The tubes were incubated at room temperature (25°C) for 1 hour, 24 hours, 7 days, 14 days, 28 days, and 42 days. After each time interval, 2 ml of the PBS (eluate) was removed and stored in a marked Eppendorf®-Tube according to group (e.g. A) and cement body number (e.g. 1). The rest of the PBS was discarded, and the Falcon®-Tubes were refilled with 20 ml of fresh PBS. The tubes were then sealed again and placed upside down until the next extraction interval.

9.4.3 Spacer preparation and eluate extraction

As the Falcon®-Tubes were too small for the spacers, they were placed in an adequately sized beaker and submerged in 120 ml of PBS. The beakers were sealed with a sheet of aluminium (Figure 9-14) and stored at room temperature (25°C). The eluate extraction was performed in the same manner as the test bodies mentioned above (Chapter 9.4.2). For each time interval (1 hour, 24 hours, 7 days, 14 days, 28 days and 42 days), 2ml of eluate was removed and the beakers were refilled with 120 ml of new PBS.

Figure 9-14: Beaker with spacer in 120mL of PBS

9.4.4 Bacteria preparation

To achieve bacterial suspensions for standardised microbial testing for all the subsequent inhibition zone assays, beads of the bacterial strains in Table 9-3 were plated and diluted on an MHA in a petri dish. These were then incubated overnight at 37°C (Figure 9-15).

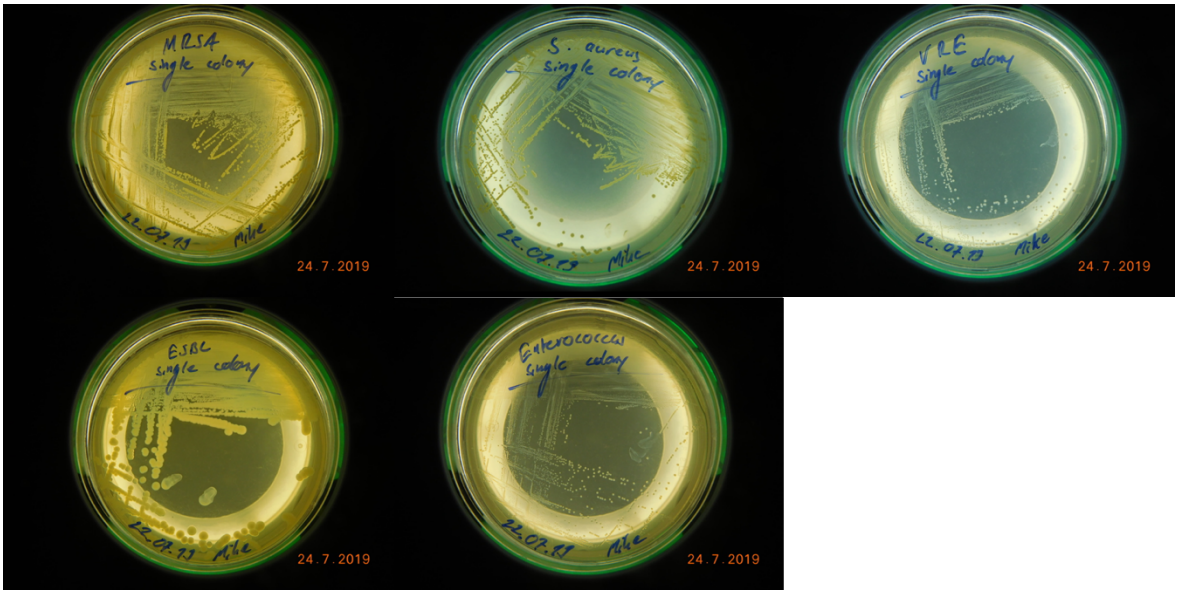

Figure 9-15: Single colony dilution smear of the various bacteria used in the experiments after overnight incubation at 37°C.

The next day, a single colony or several colonies were removed with a swab and mixed into a saline solution (0,85% NaCl) until a McFarland Standard of 0,5 (+/- 0,1) was achieved.

9.4.5 Inhibition zone assay

All the inhibition zone assays were performed on MHA. As shown in Figure 9-16, the MHAs were divided into three separate fields (one for each triplicate of eluate). For each bacterium and time interval, a fresh MHA was used. Using a cotton swab, the tested bacteria from the saline solution were then evenly divided across the petri dish. In each field, a well was punctured for the antibiotic eluate with the broad end of a Glass Pasteur's Pipette. The wells had enough distance from each other and the edges of the Petri Dish so that the inhibition zones didn't overlap.

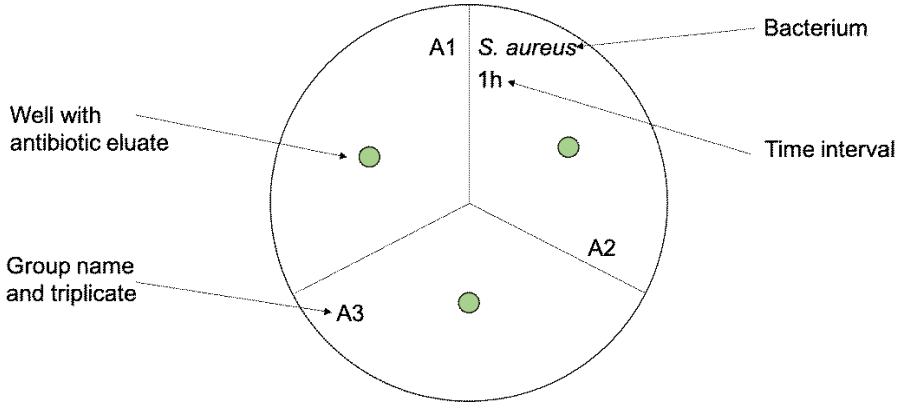

Figure 9-16: Outline of the agar plate for the Inhibition Zone Assay

60 µl of the eluates from each group per time interval were pipetted into the corresponding wells (see above). The petri dishes were incubated overnight at 37°C and the diameter of the inhibition zones measured and documented in mm the next day. The rest of the eluate was frozen and preserved.

Figure 9-17: Example of completed inhibition zone assay
The diameters of the inhibition zones were measured and documented in mm

Once all the results were documented in a table, the average diameter and standard deviation were calculated from the triplicates per group, time of elution extraction, and bacteria.

10 Results

This part of the Diploma Thesis will present the important and relevant results that were obtained. Interpretations of these results will be provided in the Discussion section.

The results will be divided into 3 categories: Visual results and handling, the mechanical results of the ALBCs, and the microbiological results of the ALBCs and spacers. Concerning the ALBCs, the results should also be viewed in 2 distinct groups:

- ALBCs with added Vancomycin (Groups A-D) and
- ALBCs with added Tigecycline (Groups E-F).

Despite all groups being displayed in the same tables and graphs in the mechanical results section, only the bone cements with the same added antibiotic can be directly compared. For example, Group A can be compared with Groups B-D, but not with Groups E-F.

10.1 Visual results and handling

As mentioned in chapters 9.2.1 and 9.2.2, the extra addition of antibiotics changed the colour and handling of the ALBCs.

As is the case with the Copal® revision cements with added Vancomycin, the addition of low doses of Vancomycin (2 g) did not change the colour nor the handling and mixing of the cement. The addition of high doses of Vancomycin (6 g), however, did make the mixing process more difficult. Once the PMMA powder and MMA Monomer were combined, the cement mass became “sandy” and required additional kneading and mixing so that an adequate polymerisation could occur.

The addition of Tigecycline to the Palacos® bone cements did not alter their handling and mixing. Nevertheless, it did change the colour from a typical green to a dark orange/brown colour (refer to Figures 9-3 and 9-4).

10.2 Mechanical results of the ALBC

Table 9-4: Groups and their corresponding cement-antibiotic combinations

Group:	Cement type:	Added Antibiotic:	Dose of added antibiotic:
A	Copal® G+C	Vancomycin	2 g
B	Copal® G+C	Vancomycin	6 g
C	Copal® G+V	Vancomycin	2 g
D	Copal® G+V	Vancomycin	6 g
E	Palacos® R	Tigecycline	0,5 g
F	Palacos® R+G	Tigecycline	0,5 g
G	Palacos® R+G	Tigecycline	1 g

Table 9-4 above (also refer to section 9.2.1), is displayed again to facilitate the reading and comprehension of the mechanical results.

All results will be compared to their corresponding standards and the mean mechanical properties of the Palacos® and Copal® commercial bone cements without additionally added antibiotics (Table 10-1 and the Figures of the individual tests below). These standards are an important factor for determining whether a certain ALBC can be used safely in a patient and whether the ALBC can cope with the loads and burdens of everyday life.

Across all the mechanical tests done, the main focus was on how higher concentrations of Vancomycin affected the mechanical properties of the PMMA cements and whether Tigecycline as an added antibiotic can fulfil the required norms.

Table 10-1: Standard mean mechanical properties of Copal® and Palacos® bone cements without additionally added antibiotics (47, 56, 57)

Test:	Copal® G+C	Copal® G+V	Palacos® R	Palacos® R+G
ISO Bending strength	63,1 MPa	63 MPa	72,3 MPa	65,79 MPa
ISO Bending modulus	2.755 MPa	2.926 MPa	2.628 MPa	2.552 MPa
ISO Compressive strength	87,3 MPa	84,9 MPa	79,6 MPa	87,46 MPa
DIN Bending strength	69,5 MPa	64 MPa	87,4 MPa	71,21 MPa
DIN Impact strength	3,1 kJ/m^2	3 kJ/m^2	7,5 kJ/m^2	3,2 kJ/m^2

10.2.1 Compressive strength

In order to comply with the ISO 5833(58) standards, the average compressive strength of an ALBC should be higher than 70 MPa. In our experiments, all ALBC combinations exceeded this requirement (see Table 10-2 and Figure 10-1).

Table 10-2: Compressive strength results of ALBC.
All compressive strength values are given as means with their corresponding standard deviation (+/-) in MPa.
Legend: ALBC = antibiotic loaded bone cement, MPa = megapascals. Min required: 70 MPa

	ALBC	Mean Compressive strength (MPa)	Standard deviation (+/-)	Minimum required value (MPa)
A	Copal® G+C + 2 g Vancomycin	83,36	2,91	**70**
B	Copal® G+C + 6 g Vancomycin	88,51	4,01	
C	Copal® G+V + 2 g Vancomycin	84,84	3,10	
D	Copal® G+V + 6 g Vancomycin	85,71	3,77	
E	Palacos® R + 0,5 g Tigecycline	84,85	2,80	
F	Palacos® R+G + 0,5 g Tigecycline	78,94	4,53	
G	Palacos® R+G + 1 g Tigecycline	78,50	2,20	

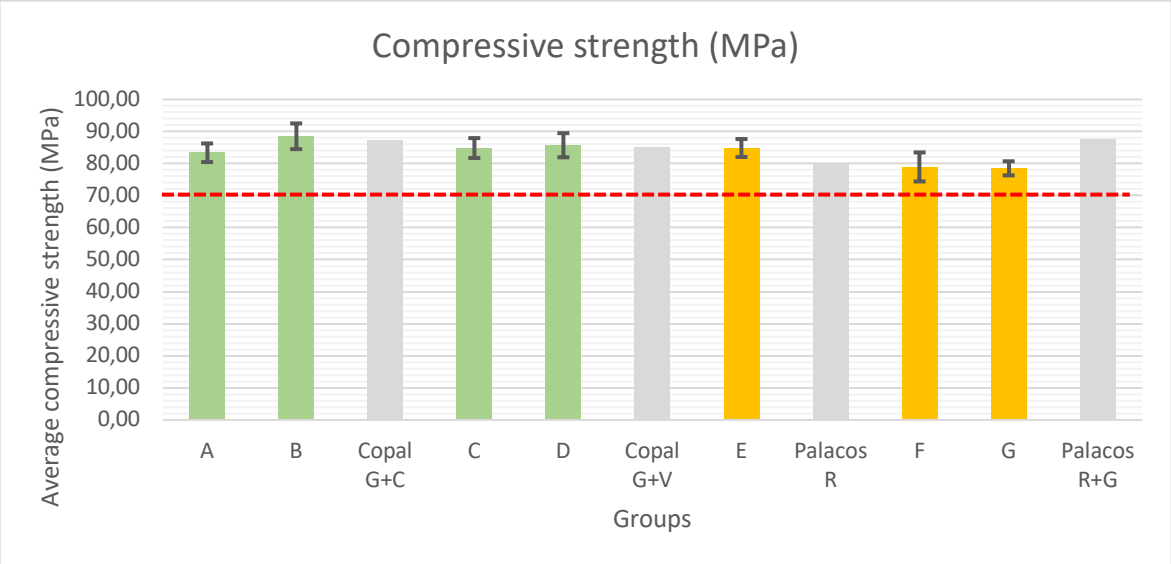

Figure 10-1: Compressive strength results of ALBC.
All listed antibiotics fulfilled the required compressive strength of 70 MPa.
Legend: green bars = ALBCs with added Vancomycin; orange bars = ALBCs with added Tigecycline; grey bars = mean compressive strength of bone cements without added antibiotics; red dotted line = minimum limit of 70 MPa; MPa = megapascals; Values are given as means with their corresponding standard deviations (+/-) in MPa.

In total, all compressive strength values between the various groups were similar, ranging between 78,50 MPa and 88,51 MPa. The standard deviation between the groups was also low, without any statistical outliers. Group B showed the highest values with an average compressive strength of 88,51 MPa, whereas Group G had the lowest with 78,50 MPa and the highest standard deviation of 4,53 MPa. Furthermore, the higher the total concentration of antibiotics was, the higher the

average compressive strength of the Copal® ALBCs with added Vancomycin. These same groups with high concentrations of Vancomycin (6 g) also produced a higher average compressive strength than their commercial counterparts (Copal® G+C and G+V) without added antibiotics. Inversely, the higher the total concentration of antibiotics in the Palacos® ALBCs with added Tigecycline, the lower the average compressive strength.

10.2.2 Bending modulus and bending strength

As with the compressive strength, the ALBC combinations had to exceed a certain minimum value according to the ISO 5833(58) Standard.

10.2.2.1 Bending modulus

To comply with the ISO 5833 standards, the test specimens had to exceed a value of 1800 MPa. As can be seen in both Table 10-3 and Figure 10-2, all ALBCs exceeded the required minimum of 1.800 MPa, thus fulfilling the ISO-Norm for bone cements.

Table 10-3: Bending modulus results of ALBC.
All bending modulus values are given as means with their corresponding standard deviation (+/-) in MPa.
Legend: ALBC = antibiotic loaded bone cement, MPa = megapascals

	ALBC	Mean Bending modulus (MPa)	Standard deviation (+/-)	Minimum Value required (MPa)
A	Copal® G+C + 2 g Vancomycin	2.707	38	**1.800**
B	Copal® G+C + 6 g Vancomycin	2.839	43	
C	Copal® G+V + 2 g Vancomycin	2.802	64	
D	Copal® G+V + 6 g Vancomycin	2.930	96	
E	Palacos® R + 0,5 g Tigecycline	2.656	34	
F	Palacos® R+G + 0,5 g Tigecycline	2.624	29	
G	Palacos® R+G + 1 g Tigecycline	2.373	46	

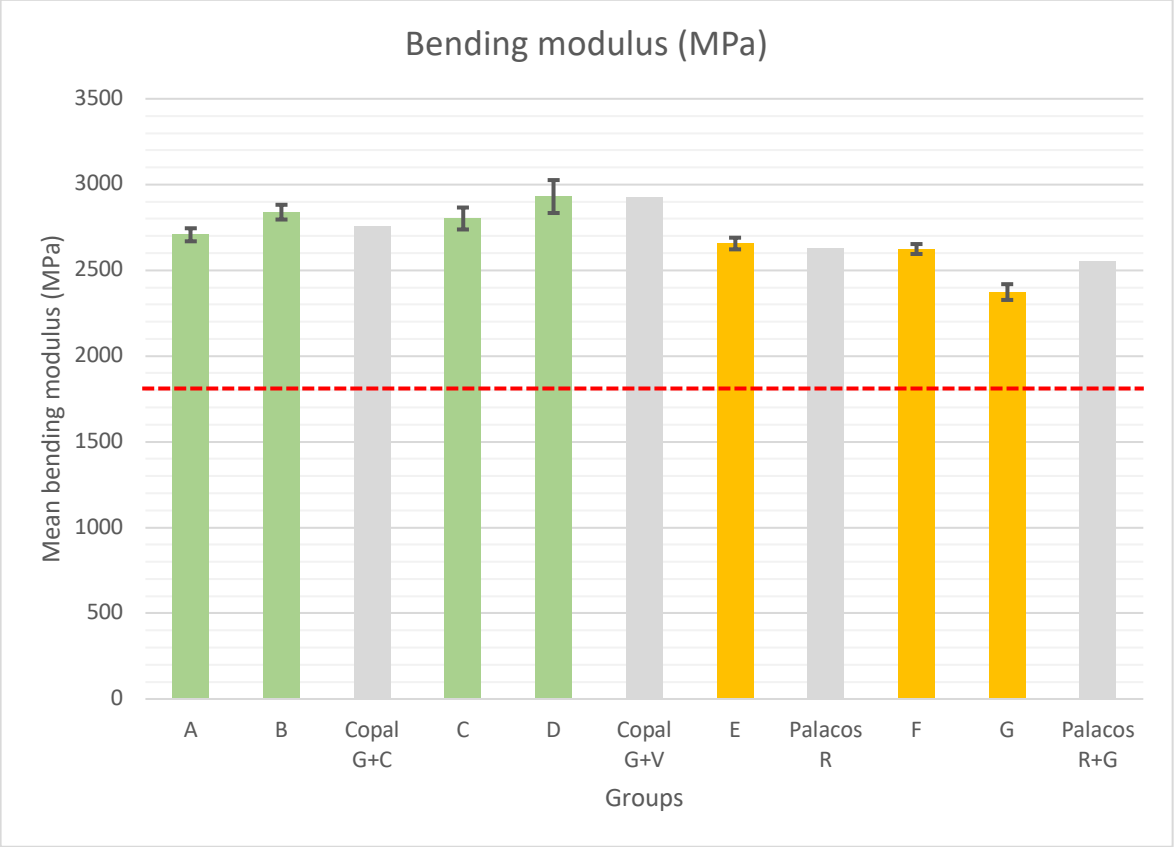

Figure 10-2: Bending modulus results of ALBC.
All listed antibiotics fulfilled the minimum required bending modulus of 1800 MPa.
Legend: green bars = ALBCs with added Vancomycin; orange bars = ALBCs with added Tigecycline; grey bars = mean bending modulus of bone cements without added antibiotics; red dotted line = minimum value of 1.800 MPa; MPa = megapascals; Values are given as means with their corresponding standard deviation (+/-) in MPa.

Again, the results of the bending modulus were all within a similar range, without any significant statistical outliers. Overall, Group D had the highest average bending modulus at 2.930 MPa and Group G the lowest with 2.373 MPa. Group D also had the largest standard deviation of 96 MPa. It should also be noted that all the Copal® ALBCs with higher overall antibiotic concentrations also had a higher bending modulus, whereas the bending modulus decreased the higher the overall antibiotic concentration in the Palacos® ALBCs. As with the compressive strength tests, the addition of antibiotics improved the mending modulus of Groups B, E and F in comparison to the bone cements without antibiotics.

10.2.2.2 Bending strength

To comply with the ISO 5833 standards, the test bodies had to exceed a value of 50 MPa. As is evident in Table 10-4 and Figure 10-3, all ALBCs exceeded the required limit, except for Group D (43,3 MPa).

Table 10-4: Bending strength results of ALBC.
All bending strength values are given as means with their corresponding standard deviation (+/-) in MPa.
Legend: ALBC = antibiotic loaded bone cement, MPa = megapascals

	ALBC	Mean Bending strength (MPa)	Standard deviation (+/-)	Minimum required value (MPa)
A	Copal® G+C + 2 g Vancomycin	57,8	3,1	**50**
B	Copal® G+C + 6 g Vancomycin	50,9	0,9	
C	Copal® G+V + 2 g Vancomycin	55,9	2,0	
D	Copal® G+V + 6 g Vancomycin	43,3	2,0	
E	Palacos® R + 0,5 g Tigecycline	67,3	2,4	
F	Palacos® R+G + 0,5 g Tigecycline	63,6	1,5	
G	Palacos® R+G + 1 g Tigecycline	52,1	2,6	

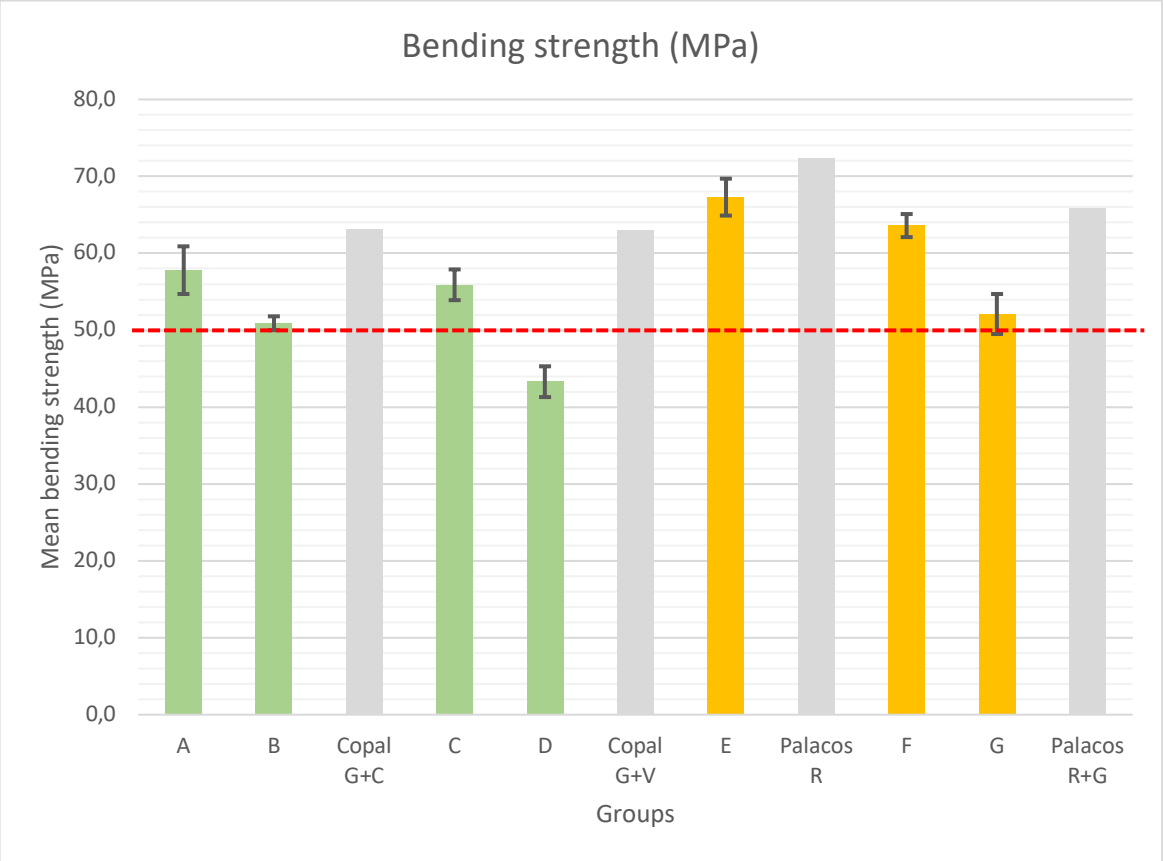

Figure 10-3: Bending strength results of ALBC.
All listed antibiotics besides Group D fulfilled the required bending strength of 50 MPa.
Legend: green bars = ALBCs with added Vancomycin; orange bars = ALBCs with added Tigecycline; grey bars = mean bending strength of bone cements without added antibiotics; red dotted line = minimum required value of 50 MPa; MPa = megapascals; Values are given as means with their corresponding standard deviation (+/-) in MPa.

As mentioned above, Group D did not fulfil the required norm. Furthermore, Groups B and G were barely within the required norm with the average bending strengths of both Groups exceeding the required minimum of 50 MPa. Within Group B, only one of the six test bodies was below the limit with a bending strength of 49,5 MPa. Group G also only showed one test body below the limit with a bending strength of 48,9 MPa. Group E had the highest bending strength at 67,3 MPa. Again, there were no significant statistical outliers, with Group A showing the highest standard deviation of 3,1 MPa. Groups B and D had the highest concentrations of added Vancomycin (6 g) and showed the lowest bending strength results. All ALBCs also showed a lower bending strength than their antibiotic-free counterparts.

10.2.3 DYNSTAT (DIN 53435)

The DYNSTAT (DIN 53435) tests do not mention any minimum requirements that PMMA bone cements and ALBC must meet. As such, there is no standardised reference to which the test bodies can be compared.

10.2.3.1 DYNSTAT Bending strength

Even though there are several differences between the DYNSTAT bending strength test (3-point-test) and the ISO 5833 bending strength test (4-point-test), both tests showed similar results and tendencies.

Table 10-5: DYNSTAT Bending strength results of ALBC.
All DYNSTAT bending strength values are given as means with their corresponding standard deviation (+/-) in MPa.
Legend: ALBC = antibiotic loaded bone cement, MPa = megapascals

	ALBC	Mean Bending strength (MPa)	Standard deviation (+/-)
A	Copal® G+C + 2 g Vancomycin	59,06	2,64
B	Copal® G+C + 6 g Vancomycin	49,80	2,50
C	Copal® G+V + 2 g Vancomycin	55,82	1,97
D	Copal® G+V + 6 g Vancomycin	47,56	2,66
E	Palacos® R + 0,5 g Tigecycline	75,32	5,94
F	Palacos® R+G + 0,5 g Tigecycline	67,03	3,13
G	Palaco®s R+G + 1 g Tigecycline	55,78	2,09

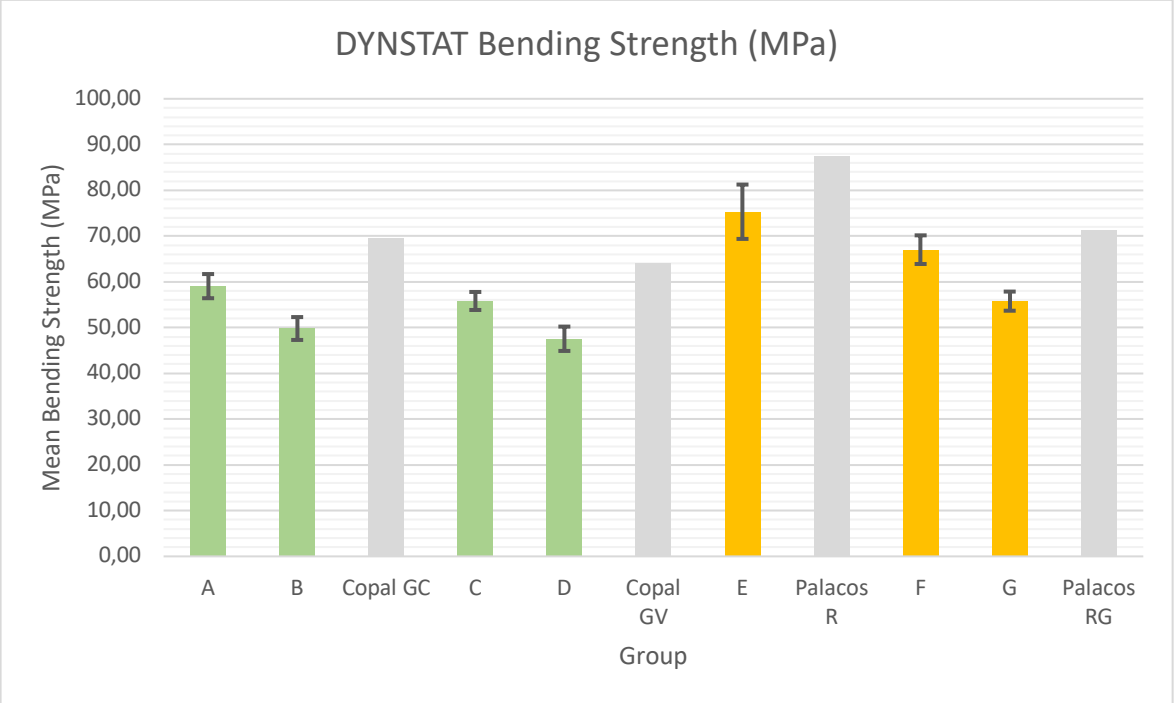

Figure 10-4: DYNSTAT Bending strength results of ALBC.
Legend: green bars = ALBCs with added Vancomycin; orange bars = ALBCs with added Tigecycline; grey bars = mean bending strength of bone cements without added antibiotics
MPa = megapascals; Values are given as means with their corresponding standard deviation (+/-) in MPa.

As with the ISO bending strength test above, Group D had the lowest values of 47,56 MPa. Group B was also just below 50 MPa, with 49,80 MPa. Group E showed the highest results with 75,32 MPa. Even though Group E had the highest standard deviation (5,94 MPa), there were no significant statistical outliers. Again, the higher the dose of total added antibiotics, the lower the overall average bending strength and in comparison, to the antibiotic-free commercial bone cements.

10.2.3.2 DYNSTAT Impact strength

As with the DYNSTAT bending strength, there is no minimum impact strength specified by these standards.

Table 10-6: DYNSTAT Impact strength results of ALBC.
All DYNSTAT impact strength values are given as means with their corresponding standard deviation (+/-) in MPa. Legend: ALBC = antibiotic loaded bone cement, kJ/m² = kilojoule per square metre

	ALBC	Impact strength (kJ/m²)	Standard deviation(+/-)
A	Copal® GC + 2 g Vancomycin	2,41	0,40
B	Copal® GC + 6 g Vancomycin	1,71	0,21
C	Copal® GV + 2 g Vancomycin	2,08	0,35
D	Copal® GV + 6 g Vancomycin	1,63	0,23
E	Palacos® R + 0,5 g Tigecycline	3,92	0,32
F	Palacos® RG + 0,5 g Tigecycline	2,72	0,43
G	Palacos® RG + 1 g Tigecycline	2,52	0,26

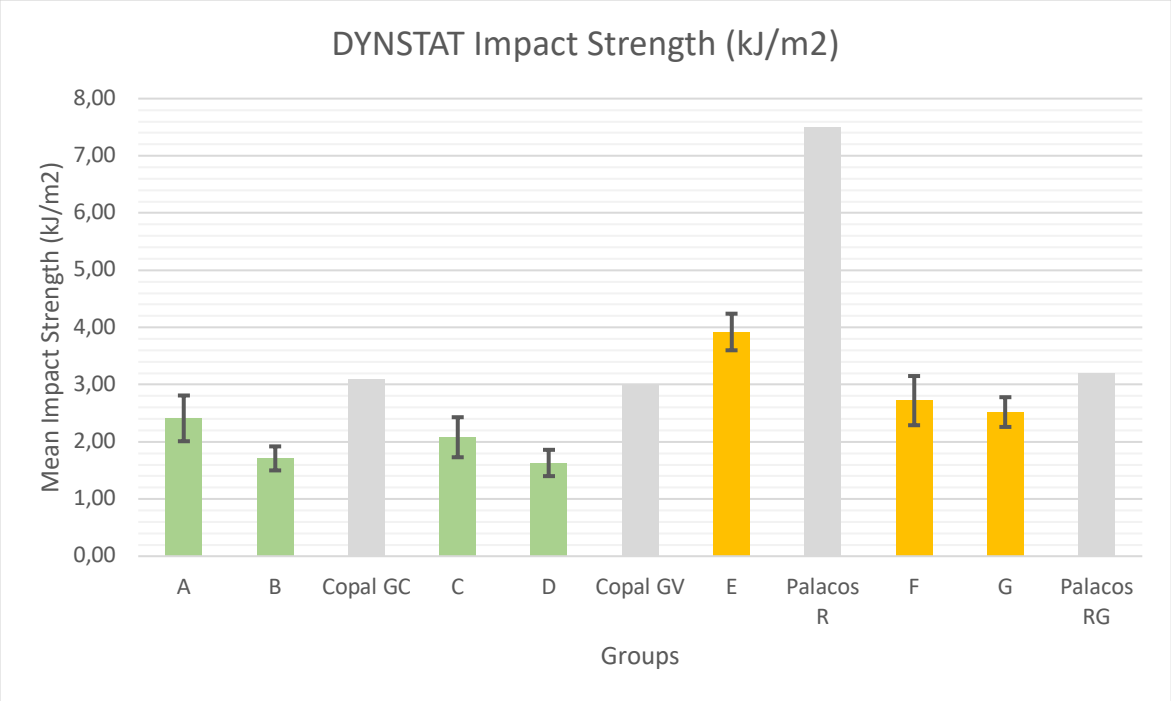

Figure 10-5: DYNSTAT Impact strength results of ALBC.
Legend: green bars = ALBCs with added Vancomycin; orange bars = ALBCs with added Tigecycline; grey bars = mean impact strength of bone cements without added antibiotics
kJ/m² = kilojoule per square metre; Values are given as means with their corresponding standard deviation (+/-) in MPa.

Nevertheless, it is clear from both Table 10-5 and Figure 10-5 that the Palacos® ALBCs with added Tigecycline have a much higher impact strength than the Copal® ALBCs with added Vancomycin. Additionally, the higher the overall antibiotic concentration, the lower the impact strength across all the tested bone cements. The addition of antibiotics also reduced the overall impact strength of all ALBCs when compared to the antibiotic free cements. Group E had the highest impact strength with 3,92 kJ/m² and Group D the lowest with 1,63 kJ/m².

10.3 Microbiology results

To determine the antimicrobial efficacy of the ALBCs and spacers, inhibition zone assays were used. Each ALBC combination was tested against a specific group of bacteria. All bone cements with added Vancomycin were tested against *S. aureus*, *E. faecalis* and MRSA. The bone cements with added Tigecycline were tested against the following multi-resistant pathogens: MRSA, VRE and ESBL. Lastly, the spacers were tested against *S. aureus*, MRSA, *E. faecalis* and *E. coli*. The efficacy of each ALBC against the individual were then compared separately.

10.3.1 Antibiotic Loaded Bone Cements (ALBC)

As with the mechanical tests, the main focus was on how the addition of the antibiotics affected the microbiological efficacy of the bone cements. More specifically: "*Do higher concentrations of Vancomycin increase efficacy and can Tigecycline even be released from bone cements?*"

10.3.1.1 Vancomycin vs *S. aureus*

All 4 Groups (A-D) with added Vancomycin were effective against *S. aureus* and showed consistently high inhibition zone diameters over the investigated time frame of 42 days (Table 10-7 and Figure 10-6).

Table 10-7: Microbiology results of commercial bone cement with added Vancomycin against S. aureus The values displayed are the mean results of the triplicates and their standard deviation in mm.

Antibiotic added	Cement type	Group	Dose	Germ	1h	24h	7d	14d	28d	42d
Vancomycin	Copal® GC	A	2 g	*S. aureus*	28,00 +/- 1,00	29,33 +/- 0,58	31,00 +/- 0,00	32,00 +/- 0,00	33,67 +/- 1,15	34,67 +/- 0,58
		B	6 g	*S. aureus*	30,00 +/- 0,00	32,00 +/- 0,00	34,67 +/- 1,15	33,67 +/- 0,58	35,00 +/- 1,00	34,00 +/- 0,00
	Copal® GV	C	2 g	*S. aureus*	19,67 +/- 0,58	20,33 +/- 0,58	19,67 +/- 0,58	18,00 +/- 0,00	18,33 +/- 0,58	17,67 +/- 0,58
		D	6 g	*S. aureus*	20,67 +/- 0,58	23,00 +/- 0,00	23,33 +/- 0,58	22,67 +/- 0,58	23,67 +/- 0,58	25,00 +/- 1,00

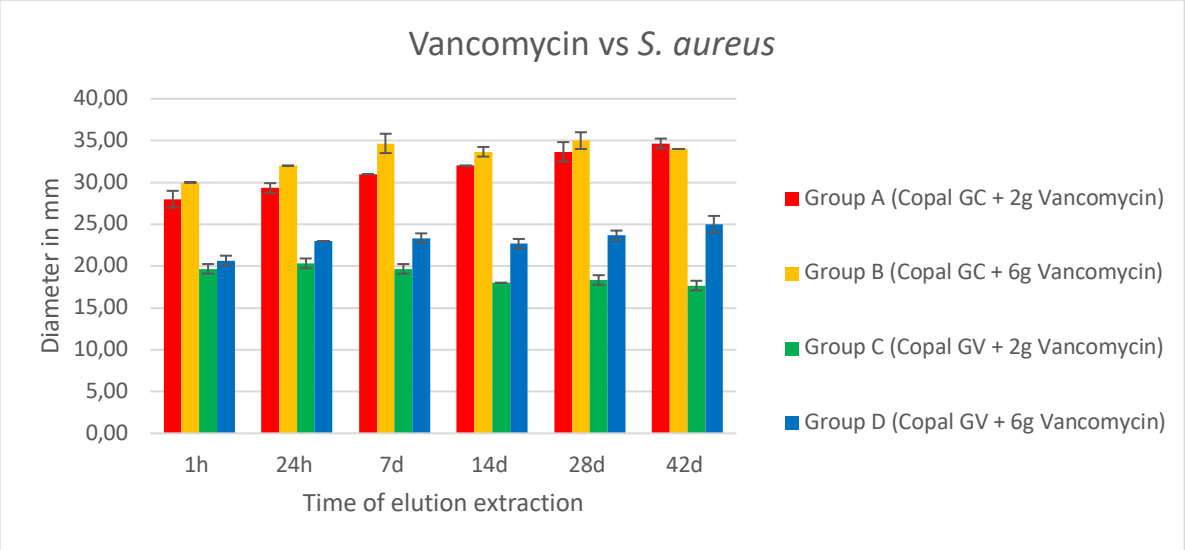

Figure 10-6: Microbiology results of commercial bone cement with added Vancomycin against S. aureus with standard deviation

As is evident in Figure 10-6, all groups besides Group C started with a smaller inhibition zone diameter that increased steadily across the 48 days. Group C was the only group whose diameter decreased after 24 hours. Group B had the highest diameters over all times of extraction, only barely dropping below Group A after 42 days. The standard deviation was also constant and small across all groups, without revealing any statistical outliers. Overall, the higher the amount of added Vancomycin (6 g vs. 2 g), the higher the antimicrobial efficacy. Also, the Groups with 3 different antibiotics (Copal® G+C + Vancomycin; Groups A and B) had a higher efficacy than those with just 2 antibiotics (Copal® G+V + Vancomycin).

10.3.1.2 Vancomycin vs E. faecalis

As in the comparison above, all Groups were effective against the pathogen *E. faecalis*. All groups were consistently effective across all times of extraction and maintained high elution profiles. The results can be seen in Table 10-8 and are visually displayed in Figure 10-7.

Table 10-8: Microbiology results of commercial bone cement with added Vancomycin against E. faecalis The values displayed are the mean results of the triplicates and their standard deviation in mm

Antibiotic added	Cement type	Group	Dose	Germ	1h	24h	7d	14d	28d	42d
Vancomycin	Copal® GC	A	2 g	*E. faecalis*	20,33 +/- 1,53	18,00 +/- 1,73	18,00 +/- 0,00	16,67 +/- 0,58	16,33 +/- 0,58	16,67 +/- 0,58
		B	6 g	*E. faecalis*	25,33 +/- 0,58	25,00 +/- 0,00	26,00 +/- 0,00	25,00 +/- 0,00	24,33 +/- 0,58	23,33 +/- 0,58
	Copal® GV	C	2 g	*E. faecalis*	21,67 +/- 0,58	21,33 +/- 0,58	21,00 +/- 1,00	18,33 +/- 0,58	16,33 +/- 0,58	16,67 +/- 0,58
		D	6 g	*E. faecalis*	24,33 +/- 0,58	25,67 +/- 0,58	26,00 +/- 0,00	26,00 +/- 0,00	25,33 +/- 0,58	24,00 +/- 1,00

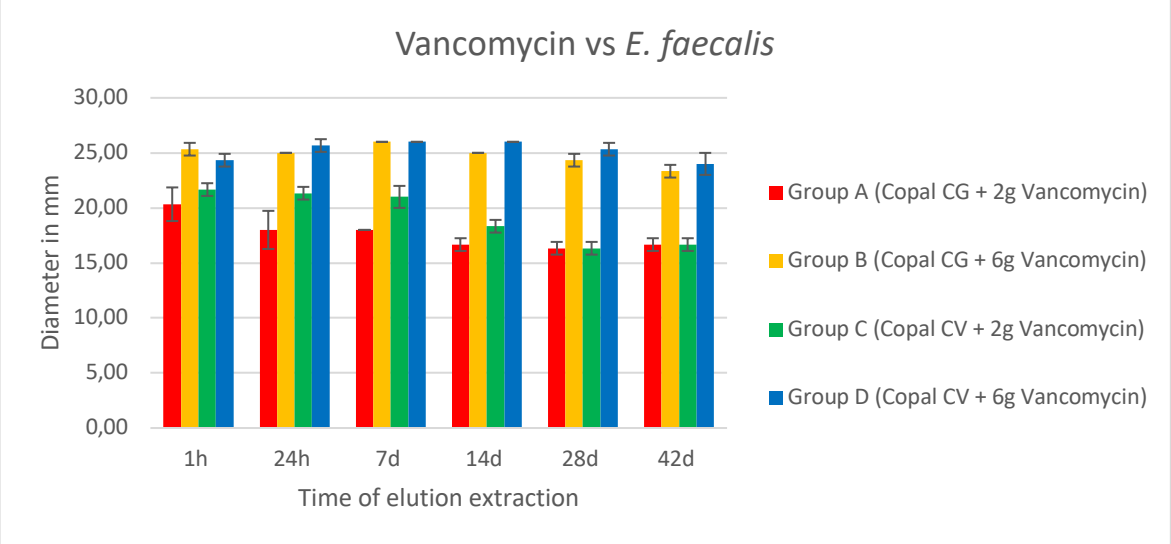

Figure 10-7: Microbiology results of commercial bone cement with added Vancomycin against E. faecalis with standard deviation

In this comparison, Groups B and D showed the highest and most consistent inhibition zone diameters at every time of elution extraction. Even though all Groups were effective against E. faecalis, it is clear that the groups (B and D) with the higher amounts of added Vancomycin (6 g) were the most effective. Groups A and C initially had larger diameters, however both decreased over time. The standard deviations also remained consistent and small across all the groups and times of elution extraction.

10.3.1.3 Vancomycin vs MRSA

In the last comparison of the ALBCs with Vancomycin, all groups were again effective against MRSA, as can be seen in Table 10-9 and Figure 10-8.

Table 10-9: Microbiology results of commercial bone cement with added Vancomycin against MRSA The values displayed are the mean results of the triplicates and their standard deviation in mm

Antibiotic added	Cement type	Group	Dose	Germ	1h	24h	7d	14d	28d	42d
Vancomycin	Copal® GC	A	2 g	MRSA	17,67 +/- 0,58	16,00 +/- 0,00	16,00 +/- 0,00	15,00 +/- 0,00	16,00 +/- 0,00	16,33 +/- 0,58
		B	6 g	MRSA	22,00 +/- 0,00	22,33 +/- 0,58	24,00 +/- 0,00	24,00 +/- 0,00	24,00 +/- 0,00	23,33 +/- 0,58
	Copal® GV	C	2 g	MRSA	20,00 +/- 0,00	19,33 +/- 0,58	19,67 +/- 0,58	18,00 +/- 0,00	18,00 +/- 0,00	17,00 +/- 0,00
		D	6 g	MRSA	21,33 +/- 0,58	22,67 +/- 0,58	24,33 +/- 0,58	23,67 +/- 0,58	24,00 +/- 0,00	24,00 +/- 0,00

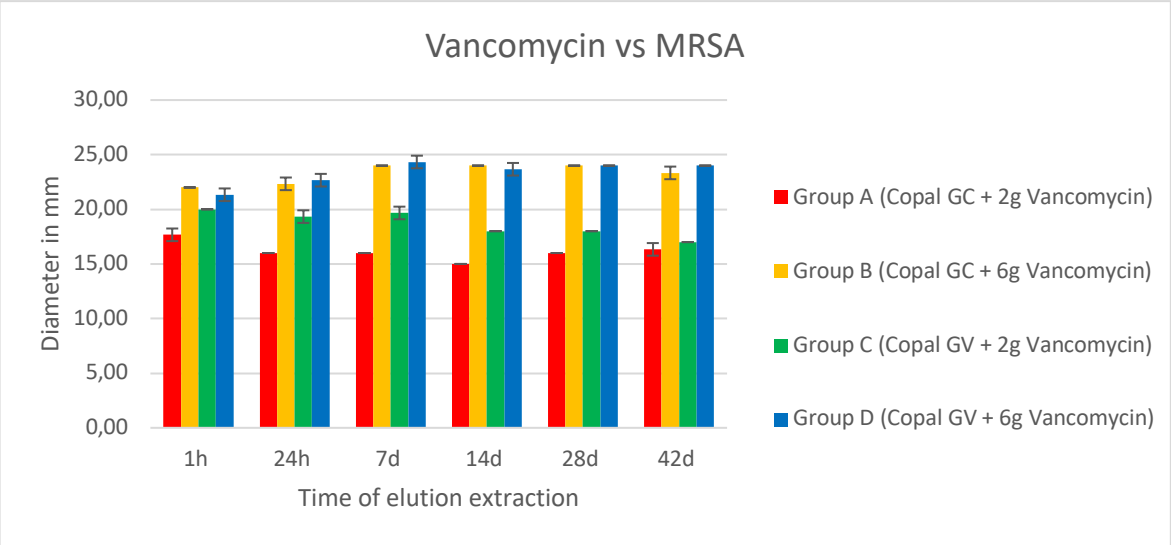

Figure 10-8: Microbiology results of commercial bone cement with added Vancomycin against MRSA with standard deviation

Groups B and D yet again had the highest elution profiles, with large initial diameters and peaking after 7 days. Both groups also continuously produced consistently large inhibition zones against MRSA for the entire duration of time after elution extraction. Groups A and C had their highest inhibition zones after 1 hour of elution extraction, but also remained constant over the 42 days. In total, Group A had the lowest inhibition zones against MRSA.

10.3.1.4 Tigecycline vs MRSA

As is evident against MRSA (Table 10-10 and Figure 10-9) and the other bacteria below, Tigecycline is indeed released from the PMMA cements and created inhibition zones against all the tested bacteria.

Table 10-10: Microbiology results of commercial bone cement with added Tigecycline against MRSA The values displayed are the mean results of the triplicates and their standard deviation in mm.

Antibiotic	Cement type	Group	Dose	Germ	1h	24h	7d	14d	28d	42d
Tigecycline	Palacos® R	E	0,5 g	MRSA	21,33 +/- 0,58	16,33 +/- 0,58	15,00 +/- 0,00	0,00 +/- 0,00	0,00 +/- 0,00	0,00 +/- 0,00
	Ralacos® RG	F	0,5 g	MRSA	20,00 +/- 0,00	16,00 +/- 0,00	13,67 +/- 0,58	0,00 +/- 0,00	0,00 +/- 0,00	0,00 +/- 0,00
		G	1 g	MRSA	23,67 +/- 0,58	18,67 +/- 0,58	20,33 +/- 0,58	6,00 +/- 5,29	0,00 +/- 0,00	0,00 +/- 0,00

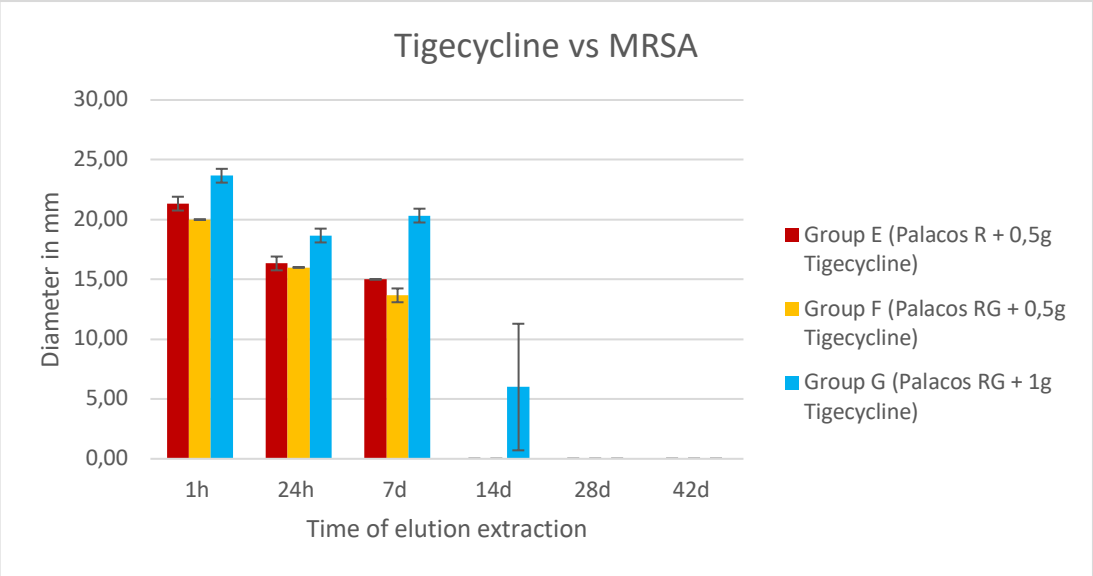

Figure 10-9: Microbiology results of commercial bone cement with added Tigecycline against MRSA with standard deviation

All groups were statistically effective against MRSA until 7 days after elution extraction. Group G created the largest inhibition zones against MRSA and was even partially effective until 14 days. However, the average inhibition zone diameter after 14 days was 6mm with a high standard deviation of 5,29 mm. Groups E and F had their highest zones after 1 hour, gradually dropped over time and showed no inhibition zones after 14 days. Group F also showed the lowest diameters against MRSA.

10.3.1.5 Tigecycline vs VRE

All groups were also effective against VRE, with high elution profiles and low standard deviations for 7 days. The results are displayed in Table 10-11 and Figure 10-10 below.

Table 10-11: Microbiology results of commercial bone cement with added Tigecycline against VRE The values displayed are the mean results of the triplicates and their standard deviation in mm

Antibiotic	Cement type	Group	Dose	Germ	1h	24h	7d	14d	28d	42d
Tigecycline	Palacos® R	E	0,5 g	VRE	21,33 +/- 1,15	15,00 +/- 1,00	15,33 +/- 0,58	0,00 +/- 0,00	0,00 +/- 0,00	0,00 +/- 0,00
	Ralacos® RG	F	0,5 g	VRE	22,00 +/- 0,00	16,33 +/- 0,58	14,67 +/- 1,15	0,00 +/- 0,00	0,00 +/- 0,00	0,00 +/- 0,00
		G	1 g	VRE	25,00 +/- 0,00	19,33 +/- 1,15	21,00 +/- 0,00	6,33 +/- 5,51	0,00 +/- 0,00	0,00 +/- 0,00

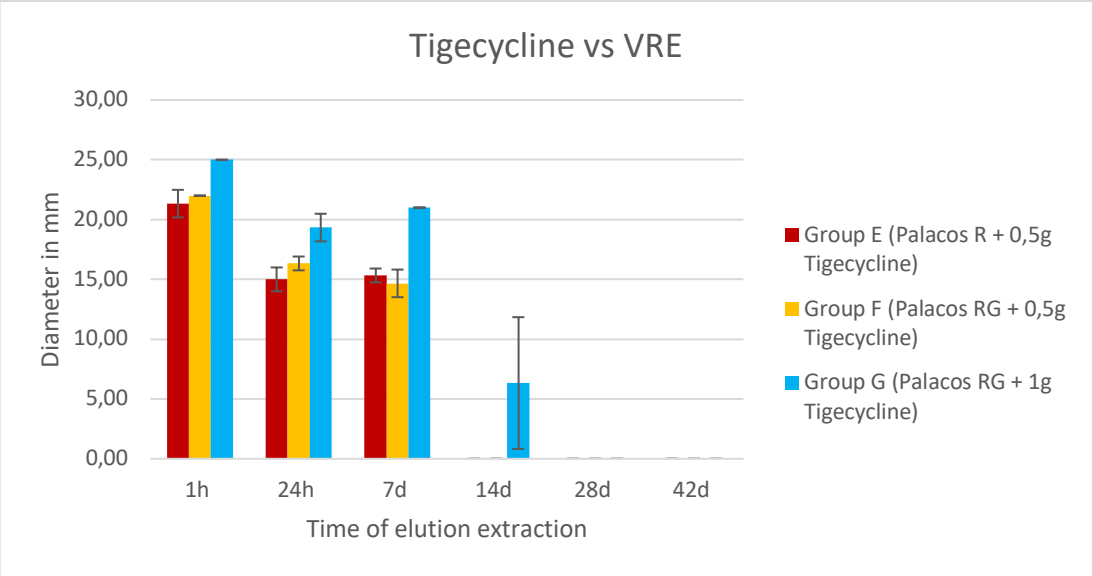

Figure 10-10: Microbiology results of commercial bone cement with added Tigecycline against VRE with standard deviation

All Groups started with their largest inhibition zone diameters after 1 hour. Group G had the largest diameters, initially starting with an average of 25 mm, dropping to 19,33 mm after 24 hours and climbing again to 21mm after 7 days. Group G also had an average diameter of 6,33 mm after 14 days, with a high standard deviation of 5,51 mm. Group F had the 2nd largest diameters and gradually dropped over time, while Group E had the smallest.

10.3.1.6 Tigecycline vs ESBL

Lastly, Groups E-G were also effective in inhibiting the growth of ESBL, as seen in Table 10-12 and Figure 10-11. In this comparison, the group with the highest concentration of Tigecycline (Group G) created inhibition zones across the entire 42 days.

Table 10-12: Microbiology results of commercial bone cement with added Tigecycline against ESBL The values displayed are the mean results of the triplicates and their standard deviation in mm

Antibiotic	Cement type	Group	Dose	Germ	1h	24h	7d	14d	28d	42d
Tigecycline	Palacos® R	E	0,5 g	ESBL	19,67 +/- 0,58	15,33 +/- 0,58	14,00 +/- 1,00	0,00 +/- 0,00	0,00 +/- 0,00	0,00 +/- 0,00
	Ralacos® RG	F	0,5 g	ESBL	20,00 +/- 0,00	15,33 +/- 0,58	15,00 +/- 0,00	0,00 +/- 0,00	0,00 +/- 0,00	0,00 +/- 0,00
		G	1 g	ESBL	22,00 +/- 0,00	18,67 +/- 1,15	19,67 +/- 0,58	9,67 +/- 2,08	13,67 +/- 1,53	14,67 +/- 1,53

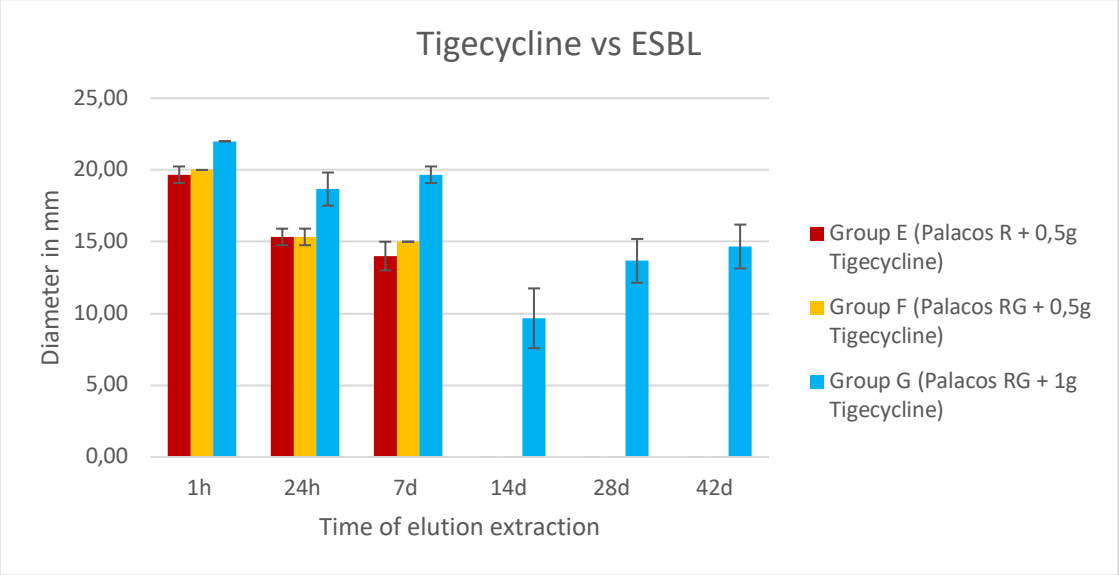

Figure 10-11: Microbiology results of commercial bone cement with added Tigecycline against ESBL with standard deviation

As with the comparisons against the other bacteria, Group G had the largest inhibition zones against ESBL. This Group started with a high average diameter after 1 hour (22 mm), dropped to 9,67 mm after 14 days and increased again after 28 and 42 days (13,67 mm and 14,67 mm respectively). The standard deviation was rather small after the first 3-time intervals, ranging between 0 mm and 1,15 mm, yet increased over the last 3-time intervals (1,53 mm to 2,08 mm). Group E and F showed similar average diameters, with initial high values after 1 hour and gradually

dropping until 7 days. Both groups showed no efficacy at 14 days of elution extraction.

10.3.2 Spacers

Spacer A (COPAL® Exchange G, Heraeus) and Spacer B (Cemex Spacer K®, Tecres) were compared according to their antimicrobial efficacy by using inhibition zone assays against predetermined bacteria. The diameter of the inhibition zones directly correlated with the antimicrobial efficacy and were measured in mm. Both spacers were tested against the following bacteria: *S. aureus*, MRSA, *E. coli* and *E. faecalis.*

The results of the inhibition zone assays on both spacers are displayed below in Table 10-13.

Table 10-13: Microbiology results of the spacers against S. aureus, MRSA, E. faecalis and E. coli. The values displayed are the mean results of the triplicates and their standard deviation in mm.

Group	Cement type	Germ	1h	24h	7d	14d	28d	42d
Spacer A	Heraeus spacer Copal® Exchange G	*S. aureus*	12,67 +/- 0,58	18,33 +/- 0,58	18,00 +/- 0,00	16,00 +/- 0,00	17,33 +/- 0,58	14,33 +/- 0,58
		MRSA	0,00	0,00	0,00	0,00	0,00	0,00
		E. faecalis	0,00	0,00	0,00	0,00	0,00	0,00
		E. coli	3,33 +/- 5,77	16,33 +/- 0,58	16,33 +/- 0,58	14,67 +/- 0,58	13,33 +/- 0,58	11,00 +/- 0,00
Spacer B	Tecres Spacer K®	*S. aureus*	17,00 +/- 1,73	15,33 +/- 1,15	12,67 +/- 0,58	12,00 +/- 1,00	13,00 +/- 0,00	11,00 +/- 0,00
		MRSA	0,00	0,00	0,00	0,00	0,00	0,00
		E. faecalis	0,00	0,00	0,00	0,00	0,00	0,00
		E. coli	13,67 +/- 1,53	11,67 +/- 0,58	10,00 +/- 1,00	8,33 +/- 0,58	0,00 +/- 0,00	0,00 +/- 0,00

As can be seen in the Table 10-13 above, both spacers showed no inhibition zones against MRSA and E. *faecalis* across all the time points of elution extraction. However, both spacers were effective against the bacteria *S. aureus* and *E. coli*. Therefore, only the results against the 2 latter bacteria were displayed in the Figures (10-12 and 10-13) below.

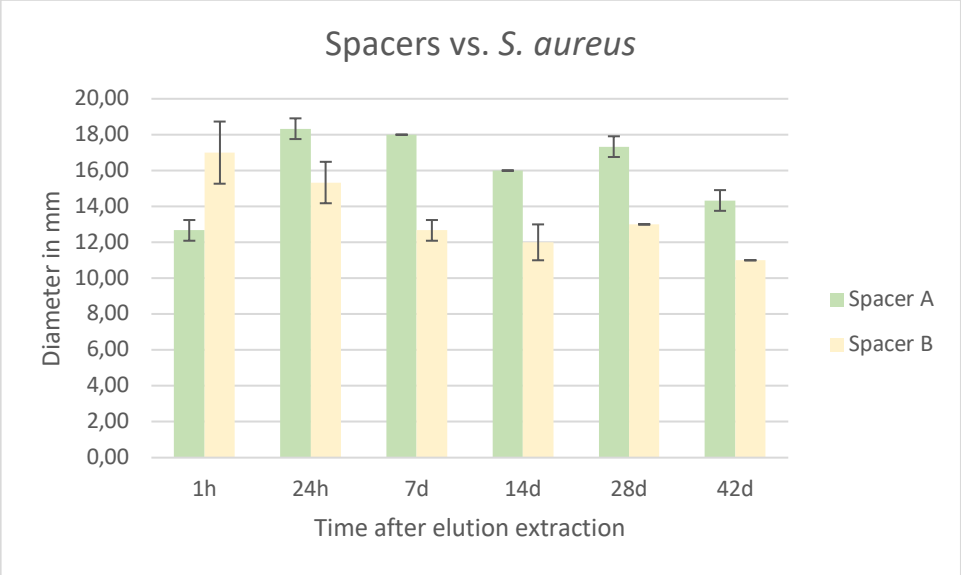

Figure 10-12: Microbiology results (diameter of inhibition zone) of Spacers A and B against S. aureus with standard deviation in mm.

The above Figure 10-12 compares the results of both spacers against *S. aureus* in a bar graph. As displayed in the figure above, Spacer B produced its highest inhibition zone diameter against *S. aureus* after 1 hour with a value of 17 mm (+/- 1,73 mm). However, the diameters of Spacer B gradually decreased over time, dropping to 11 mm (+/- 0 mm) after 42 days. Spacer A initially had a lower 1-hour inhibition zone diameter compared to Spacer B, by reaching a value of 12,67 mm (+/- 0,58 mm). Nevertheless, Spacer A overtook Spacer B after 24 hours, reaching its highest diameter. Even though the diameters of Spacer A also gradually decreased across the 42 days, it produced larger inhibition zones than Spacer B. Furthermore, neither group produced any significant statistical outliers and maintained low standard deviations.

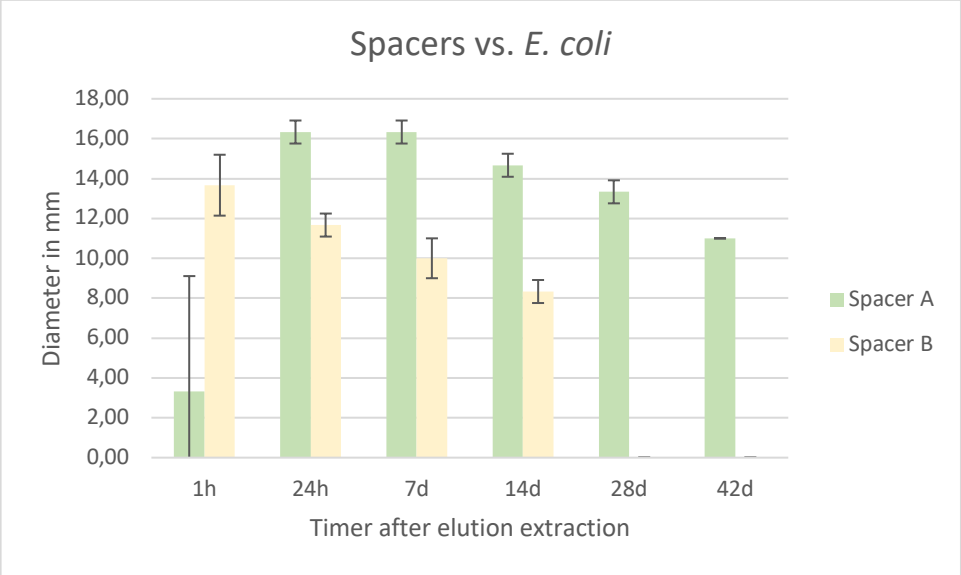

Figure 10-13: Microbiology results of Spacers A and B against E. coli with standard deviation

Figure 10-13 shows the values of both spacers against the bacteria *E. coli*. As with the results against *S. aureus*, Spacer B had the largest diameter after 1 hour. The diameters of Spacer B again decreased over time, reaching its minimum diameter after 14 days. Additionally, the Tecres spacer showed no antimicrobial efficacy from 28 days onwards.

Spacer A, on the other hand, had a low average inhibition zone diameter of 3,33 mm after 1 hour with a relatively large standard deviation of +/- 5,77 mm. The Heraeus spacer then reached its maximum average diameter after 24 hours and maintained that inhibition zone at 7 days after elution extraction. As with the previous example, Spacer A had higher diameters that gradually dropped across the 42 days.

11 Discussion

As described in the introduction of this thesis, Prosthetic Joint Infections are a feared and severe complication of total joint arthroplasties. Not only are PJI's difficult to diagnose, they also result in longer hospital stays for patients, lengthy surgeries and prolonged antibiotic treatment regimens(6). Typical yet unspecific symptoms of PJI often include joint pain, swelling and erythema. As such, the diagnosis is anything but simple and requires a plethora of measures and tools (e.g., X-ray, MRI, blood serum parameters, synovial fluid parameters, periprosthetic cultures, histological analysis of periprosthetic tissue, etc.). Even though it is very rare, a sinus tract communicating with the joint is the only pathognemonic symptom of PJI.

There are many ways to treat PJI, yet one of the most commonly used procedures is a two-stage revision surgery in which the infected prosthesis is removed during the 1st surgery. After the removal, debridement and irrigation, an antibiotic impregnated spacer with anti-biofilm activity is inserted for a period of 2-6 weeks. The spacer is then removed in a 2nd procedure and the new prosthesis is implanted. After each surgery, the patient receives an intravenous antibiotic for 1-2 weeks.

To achieve the best possible eradication of pathogens and prevent the onset of a new infection, the antimicrobial treatment must be tailored towards the causative pathogen. *S. aureus* and Coagulase negative Staphylococci (e.g., *S. epidermidis*) are the most common causes of PJI worldwide, and account for roughly 50-60% of all arthroplasty infections. And as with many other diseases across the world, antibiotic resistance is on the rise, leading towards more PJI's being caused by multi-resistant pathogens such as MRSA, VRE and ESBL.

To improve patient outcome and prevent further infection and biofilm formation after surgery, ALBC combinations and spacers need to be enhanced and upgraded.

11.1 Colour and handling

Even though mechanical stability and microbiological efficacy are undoubtedly the most important traits of any antibiotic loaded PMMA cement, the colour and handling of such bone cements also play an essential role. To improve intraoperative visibility, the implanted cement should ideally have an easily visible and contrasting colour. The preparation of the bone cements is usually done by the operating

assistant. Once preparation and mixing has begun, the operating assistant and surgeon only have a window of 10 minutes to insert and adapt the cement until it hardens.

Overall, the addition of Vancomycin to the Copal® revision cements did not change the light green colour, and so retained good intraoperative visibility. Nonetheless, the addition of high Vancomycin doses (6 g) did make the handling of the cement more difficult and lengthened the preparation time. This could potentially make the implantation and adaptation by the surgeon more difficult, leading to unsatisfactory results. As such, this thesis recommends that only experienced surgeons and operating assistants attempt the addition of high doses of Vancomycin.

On the other hand, the addition of Tigecycline to Palacos® bone cements did not influence their handling or preparation. The only noticeable change was in colour. The addition of Tigecycline powder gave the cement a dark orange/brown colour, potentially decreasing the intraoperative visibility and reducing the contrast towards the surrounding tissue, making it more difficult to adapt.

11.2 Mechanical Properties

Before the experiments in this thesis were conducted and the results documented, it was hypothesized that (A) the addition of additional antibiotics would decrease the overall mechanical stability, and that (B) the cement combinations with higher doses (6 g) of Vancomycin (Groups B and D) would not fulfil the ISO-Standard as they exceeded the maximum recommended concentration of antibiotics in bone cements (>10%)(44).

Concerning the ALBC's with added Tigecycline, there were very few studies at the time of testing and writing this thesis that examined the mechanical and microbiological properties of its addition to revision bone cements. As such, the goals were to observe and document the results of how Tigecycline effected the mechanical properties of the tried and tested Palacos® R and R+G bone cements in accordance with standardised norms.

In comparison to the ISO-Standard 5833:2002 and the DYNSTAT 53435 tests of the plain, commercially available Copal® G+C and G+V, and Palacos® R and R+G bone cements without additionally added antibiotics (Table 11-1), the tested PMMA

cements with the added antibiotics in this thesis revealed lower mechanical properties in all tests, except for the bending modulus and compressive strength tests (see chapter 10.1 Mechanical results for comparison). Furthermore, increases in antibiotic concentration within the groups did not always result in a reduction of the mechanical stability. In the compressive strength and bending modulus tests, Groups B (Copal® G+C + 6 g Vancomycin) and D (Copal® G+V + 6 g Vancomycin) had higher results than their counterparts (Groups A and C) with the lower Vancomycin addition of 2 g. Paz et al (2015) (59) found similar results in their study while comparing the compressive strength and bending modulus of Palacos® R+G with varying doses of added Vancomycin and Cefazolin. They also noted that the addition of high doses of Vancomycin barely altered the mechanical properties (bending modulus, compressive strength) of these cements. Another study by Klekamp et al (1999) (60) further confirmed that high doses of Vancomycin did not significantly reduce the compressive strength of the PMMA cements.

This could be due to an initial post-polymerization increase of the bending modulus and compressive strength by antibiotics, as described by Kühn (2014) in his book “Bone cements”(61). Water uptake (based on the higher concentration of added antibiotics) in vivo should decrease some of these mechanical parameters (Bending strength), whereas the compressive strength and bending modulus remain largely unaffected. Even though the ISO-Standard and DYNSTAT tests have no upper limit, very high parameters or an increase in certain mechanical properties (compressive strength and bending modulus) are not necessarily desirable. Bone cements and spacers require a certain flexibility and pliability to compensate the enormous forces that occur within a joint. If a bone cement or spacer is too rigid, it loses its adaptable properties and could break in vivo. Therefore, it can be confirmed that admixed antibiotics inevitably reduce the most important mechanical strength values and properties of bone cements (but not all parameters), and undermine its overall quality.

Table 10-1: Standard mean mechanical properties of Copal® and Palacos® bone cements without additionally added antibiotics (48, 61, 62)

Test:	Copal® G+C	Copal® G+V	Palacos® R	Palacos® R+G
ISO Bending strength	63,1 MPa	63 MPa	72,3 MPa	65,79 MPa
ISO Bending modulus	2.755 MPa	2.926 MPa	2.628 MPa	2.552 MPa
ISO Compressive strength	87,3 MPa	84,9 MPa	79,6 MPa	87,46 MPa
DIN Bending strength	69,5 MPa	64 MPa	87,4 MPa	71,21 MPa
DIN Impact strength	3,1 kJ/m^2	3 kJ/m^2	7,5 kJ/m^2	3,2 kJ/m^2

Overall, almost all the ALBC combinations fulfilled the required limits of the international ISO-5833:2002 Standards. As seen in Table 11-2 below and the results above (Chapter 10.1: Mechanical results), only Group D (Copal® G+V + 6 g Vancomycin) did not fulfil the required minimum limit of 50 MPa in the “Bending Strength” test and is therefore not recommended for in vivo use when manually mixed. Surprisingly, Group B, along with all other all other cement combinations passed the required limits. Therefore, they should be safe to implant in vivo and survive the everyday forces that bone cements are usually subjected to within the joint.

Table 11-2: Groups that passed the ISO-5833:2002 norms
Legend: red = failed, green = passed

Group:	Cement type:	Added Antibiotic:	Dose of added Antibiotic:
A	Copal® GC	Vancomycin	2 g
B	Copal® GC	Vancomycin	6 g
C	Copal® GV	Vancomycin	2 g
D	Copal® GV	Vancomycin	6 g
E	Palacos® R	Tigecycline	0,5 g
F	Palacos® RG	Tigecycline	0,5 g
G	Palacos® RG	Tigecycline	1 g

Even though the DYNSTAT 53435 (German industrial standard) tests do not specify any minimum values to be regarded as safe to use in vitro, the ALBC combinations performed well in comparison to other bone cements and combinations on the market (see Table 11-1 and Chapter 10: Results).

It is important to note that although the DYNSTAT tests and the ISO-standard tests both measure the bending strength in MPa of the bone cements, there are subtle differences in how these results are obtained. The DYNSTAT tests are not

considered a standard for bone cements, the test bodies are different in size and shape, and a different test rig and 3-point test are used.
Nevertheless, the DYNSTAT bending strength tests showed a similar trend and even similar values as seen in Tables 10-3 and 10-4 as well as the Figures 10-3 and 10-4.

Lastly, the addition of Tigecycline did not dramatically affect the mechanical stability of the Palacos® bone cements and fulfilled the required norms. Muratli et al (2020)(**41**) also confirmed that Tigecycline doesn't significantly decrease the mechanical stability of bone cements, despite using other bone cements and mechanical tests, as well as lower Tigecycline doses.

11.3 Microbiological Properties

ALBCs are meant to prevent and treat bone and joint infections through the local release of antibiotics into areas that are difficult to access for intravenous antibiotics. Moreover, the treatment should be tailored towards the causative pathogen. For example, Gentamicin is ideal for treating infections caused by *Pseudomonas, Enterococci, S. aureus* and CNS, among others. Clindamycin is used to treat *S. aureus*, MRSA, CNS, streptococci, and anaerobes (*C. acnes*). Vancomycin is another typical addition to ALBCs that can be used against multi-resistant pathogens and is effective against *S. aureus*, MRSA, streptococci, and *C. difficile.*
To be effective against such pathogens and prevent biofilm formation, ALBCs must release their antibiotics quickly and at a high concentration over an extended period of time. ALBCs and spacers are typically used in 2-stage revision treatment regimes. In such cases, the spacers remain in vivo for between 2 and 6 weeks, and ideally, the chosen bone cement should release high concentrations of antibiotic over the entire duration of implantation. As indicated in the Material and Methods section above, we tested various ALBC combinations and the 2 commercially available spacers against common and multi-resistant pathogens that cause PJI.

11.3.1 ALBCs result interpretation

As can be seen in the results above (Chapter 10.2.1) all the ALBC combinations were effective against their tested pathogens.

Starting with the Copal® bone cements with added Vancomycin, the results revealed no surprises. All groups had a high initial inhibition zone after 1 hour in vitro and showed consistently high inhibitions zones across the 42 days, an ideal trait for bone cements that could be implanted for up to 6 weeks. As expected, the groups with higher Vancomycin concentrations (6 g) also had larger inhibition zones than the groups with lower Vancomycin concentrations (2 g) against the 3 tested pathogens (*S. aureus, E. faecalis*, MRSA). Lee et al (2016) showed similar tendencies in their paper, revealing that ALBCs with higher antibiotic concentrations also had a higher cumulative antibiotic release(63).

When tested against *S. aureus*, the groups A and B with 3 antibiotics (Copal® G+C + Vancomycin) revealed the highest inhibition zones (Table and Figure 10-6). This is because of the added efficacy of Clindamycin and synergistic effect of all three antibiotics within this group. Similar tendencies can also be found in the book "Management of Periprosthetic Join Infection" by Kühn (2018, p. 252) (64). Nevertheless, groups C and D (Copal® G+V + Vancomycin) were only roughly 30% less effective.

Furthermore, Groups B (Copal® G+C + 6 g Vancomycin) and D (Copal® G+V + 6 g Vancomycin) were the most effective against MRSA and E. faecalis, and showed roughly similar results against both pathogens. This is due to vancomycin mainly being effective against both pathogens, whereas clindamycin is ineffective. It is also interesting to note that Group B (6 g) had a lower total concentration of vancomycin than Group D (8 g), hinting towards the fact that very high vancomycin doses only marginally increase the antimicrobial efficacy at the cost of mechanical stability. This thesis, therefore, does not recommend the use of Copal G+V + 6 g of Vancomycin, despite its good antimicrobial efficacy. Copal G+C + 6 g of Vancomycin, on the other hand, shows similar antimicrobial results, fulfills the international ISO-Norm, and has a larger antimicrobial spectrum.

The Palacos® bone cements with added Tigecycline also showed very promising results. As mentioned in the results above, all 3 groups showed consistent inhibition zones against MRSA, VRE and ESBL and were effective over a period of 7 days, showing comparable findings to a similar study by Nichol et al. (2016) (39). Moreover, the addition of 1 g of Tigecycline was the most effective against the

pathogens. Even though this group also showed inhibition zones against MRSA and VRE for a period of 14 days, the standard deviation was relatively high with a large variability between the inhibition zones. A likely explanation for these results could be a laboratory mishap during the test-specimen production with possible fluctuations or absence of antibiotic concentrations, or through a mistake during the microbiological testing.

Groups E and F also showed very similar results (inhibition zones and duration of efficacy) against all pathogens, revealing that the Gentamicin in Group F (Palacos RG® + 0,5 g Tigecycline) wasn't effective against the multi-resistant pathogens or did not work synergistically with Tigecycline.

Higher concentrations (1 g) of Tigecycline in Palacos R+G® also showed an excellent efficacy against ESBL, producing inhibition zones over the course of 42 days (Table and Figure 10-11), making it an ideal target for Tigecycline loaded bone cement during 2-stage revision surgery. Moreover, there is a dip in the inhibition zone at 14 days in this group, also with a higher standard deviation, further compounding the assumption that a mistake was made during the production of the test bodies or during microbiological testing.

It is also important to note that the Gentamicin-free Group E was effective against the tested pathogens and showed similar results to Group F (Palacos® with a Gentamicin basis). As such, Tigecycline doesn't need an additional antibiotic within the bone cement to be released from the cement matrix like other antibiotics.

Although Tigecycline is released through bone cements and the ALBC shows antimicrobial activity, further testing concerning this antibiotic in bone cements is needed. The author recommends further experimentation with the following changes:

- higher and lower doses of Tigecycline within bone cements
- the use of other commercially available bone cements for revision surgery such as Copal®
- the use of other Tigecycline preparations (e.g., Tigecycline powder)
- other methods of mixing (e.g., vacuum mixing system) to improve homogeneity throughout the revision cement
- intraoperative testing to determine visibility and contrast towards the surrounding tissue

11.3.2 Spacer result interpretations

As described and depicted in Chapter 10.2.2, both spacers were, as expected, effective against *S. aureus* and *E. coli* (gram positive and negative pathogens) and showed no efficacy against MRSA. Surprisingly, both spacers showed no inhibition zone whatsoever against *E. faecalis* despite Gentamicin also being effective against this gram-positive enterococcus. A possible explanation could be that either the Gentamicin concentration within these spacers wasn't high enough to be effective, or that the synergistic effect of another antibiotic (such as Vancomycin) was missing. Nevertheless, an adequate comparison between the efficacy of the spacers could be made. As shown in Table 10-12 and Figures 10-12 and 10-13, the Tecres Spacer K® had a significantly higher inhibition zone against *S. aureus* and *E. coli* after 1 hour. This is probably due to the rough surface of this spacer, allowing for a rapid diffusion of fluid into the bone cement and the subsequent dissolution of antibiotic molecules. However, the Spacer B inhibition zones steadily dropped after 1 hour against both pathogens. Furthermore, the efficacy of Spacer B against E. coli is only guaranteed up to 14 days, barely making it a viable option for 2-stage revision surgery and potentially posing the risk of recolonisation. These antimicrobial qualities could be caused by the modest hydrophilic properties of the spacer as well as the use of $BaSO_4$ as a contrast agent.

The Heraeus Copal® G Exchange spacer, on the other hand, had a slower initial release due to its smoother surface, yet overtook the other spacer after 24 hours. In comparison to Spacer B, Spacer A showed a 20-25% higher inhibition across the 42 days, thanks to its hydrophilic properties and $CaCO_3$ as its contrast agent. In his paper "PMMA bone cements: what is the role of antibiotics" (2016), Kühn also described the advantages of calcium carbonate cements, mentioning their lower abrasion rates, better hydrophilic matrix and higher antibiotic elution(44). A direct comparison between the spacers and which properties are most desirable in a PMMA spacer, however, could not be found in existing literature and papers.

Overall, this thesis recommends Spacer A over Spacer B for to the following reasons:

- Consistent and high inhibition zones across the 42 days, with an overall better efficacy against gram positive and negative pathogens

- Even though these characteristics were not tested in this thesis, Mueller et als determined that the smooth surface of Spacer A leads to less abrasion within the joint(57)

11.4 Limitations

It is noted that all specimen Groups and their ALBCs (not the spacers, as these were pre-formed by the manufacturing company) were mixed manually. This typically results in inhomogeneous ALBCs with varying concentrations of antibiotics throughout the bone cement, often resulting in varying inhibition zones that are difficult to reproduce. Furthermore, these inhomogeneous pockets of antibiotics within the bone cements could result in structural weak points throughout the hardened cement, potentially affecting their mechanical properties. As such, the author of this thesis recommends repeating the experiments with ALBCs that are mixed, for example, in a vacuum mixing system. This may reduce the elution of antibiotics from the bone cements, but it should be easier to reproduce the results and ALBCs such as Copal GV + 6g Vancomycin (Group D) could possibly pass the ISO 5833:2002 requirements.

This study conducted the microbiological tests in vitro instead of in vivo. Although great care was taken to simulate in vivo conditions (see chapter 9.4 Microbiological Preparation), such experiments can never replace in vivo experiments and replicate their results. For example, these in vitro experiments didn't take fluid circulation and the mechanical load and friction of the ALBCs or spacers within the joint into account. In addition, all experiments were conducted at room temperature, which is much lower than the internal human body temperature. Therefore, to reach clinically relevant conclusions, these experiments should ideally be repeated in clinical studies on patients with the in vivo implantation of the ALBCs and spacers.

The microbiological test bodies were sealed in an air-tight container after preparation, sent to Berlin and stored there for several months before the microbiological tests were conducted. Under normal, clinical circumstances, bone cements and spacers are immediately implanted within the joint after setting. It is therefore unclear if or how the transport and storage of these test bodies and spacers impacted the results of this thesis.

11.5 Conclusions

In conclusion, the main takeaways of this thesis are summarised as follows:

1. Tigecycline is a viable alternative as an antibiotic in bone cement against ESBL, MRSA and VRE. It fulfils all the required ISO 5833:2002 norms and is effective against multi-resistant pathogens for a minimum of 7 days. If a pathogen is resistant towards Vancomycin or other standard antibiotics, Tigecycline could be used in unique situations. The most effective combination was Palacos® R+G + 1 g Tigecycline against ESBL, as it maintained large inhibition zones across 42 days.
2. Extra addition of Vancomycin improves the antimicrobial efficacy of revision bone cements yet decreases their mechanical stability. The recommendation remains that the threshold of >10% antibiotic concentration should not be exceeded. The only exception can be made for Copal® GC + 6 g of Vancomycin, which complies with the ISO-Standards and shows excellent antimicrobial activity.
3. The Heraeus Copal® G Exchange spacer could perform better than the Tecres Spacer K® during 2-stage revision surgery against *S. aureus* and *E. coli*, as it exhibits larger inhibition zones after 24h and a more constant elution profile across 42 days.

12 List of references

1. Mandl LA. Determining who should be referred for total hip and knee replacements. Nature Reviews Rheumatology. 2013;9(6):351-7.
2. Nelson AE. Osteoarthritis year in review 2017: clinical. Osteoarthritis Cartilage. 2018;26(3):319-25.
3. Singh JA, Yu S, Chen L, Cleveland JD. Rates of Total Joint Replacement in the United States: Future Projections to 2020–2040 Using the National Inpatient Sample. The Journal of Rheumatology. 2019:jrheum.170990.
4. Sloan M, Premkumar A, Sheth NP. Projected Volume of Primary Total Joint Arthroplasty in the U.S., 2014 to 2030. J Bone Joint Surg Am. 2018;100(17):1455-60.
5. Grimberg A, Jansson V, Melsheimer O, Steinbrück A. German Arthroplasty Registry (EPRD) - 2019 Annual Report2020.
6. Abad CL, Haleem A. Prosthetic Joint Infections: an Update. Current Infectious Disease Reports. 2018;20(7):15.
7. Wang F-D, Wang Y-P, Chen C-F, Chen H-P. The incidence rate, trend and microbiological aetiology of prosthetic joint infection after total knee arthroplasty: A 13 years' experience from a tertiary medical center in Taiwan. Journal of Microbiology, Immunology and Infection. 2018;51(6):717-22.
8. Kurtz SM, Lau E, Watson H, Schmier JK, Parvizi J. Economic burden of periprosthetic joint infection in the United States. J Arthroplasty. 2012;27(8 Suppl):61-5.e1.
9. Tande AJ, Patel R. Prosthetic joint infection. Clin Microbiol Rev. 2014;27(2):302-45.
10. Izakovicova P, Borens O, Trampuz A. Periprosthetic joint infection: current concepts and outlook. EFORT open reviews. 2019;4(7):482-94.
11. Beam E, Osmon D. Prosthetic Joint Infection Update. Infect Dis Clin North Am. 2018;32(4):843-59.
12. López D, Vlamakis H, Kolter R. Biofilms. Cold Spring Harb Perspect Biol. 2010;2(7):a000398-a.
13. Katsikogianni M, Missirlis YF. Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. Eur Cell Mater. 2004;8:37-57.
14. del Pozo JL, Patel R. The challenge of treating biofilm-associated bacterial infections. Clin Pharmacol Ther. 2007;82(2):204-9.
15. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med. 2004;351(16):1645-54.
16. Peel TN, Cheng AC, Buising KL, Choong PFM. Microbiological Aetiology, Epidemiology, and Clinical Profile of Prosthetic Joint Infections: Are Current Antibiotic Prophylaxis Guidelines Effective? Antimicrob Agents Chemother. 2012;56(5):2386-91.
17. Si S, Durkin MJ, Mercier MM, Yarbrough ML, Liang SY. Successful Treatment of Prosthetic Joint Infection due to Vancomycin-resistant Enterococci with Tedizolid. Infect Dis Clin Pract (Baltim Md). 2017;25(2):105-7.
18. Martínez-Pastor JC, Vilchez F, Pitart C, Sierra JM, Soriano A. Antibiotic resistance in orthopaedic surgery: acute knee prosthetic joint infections due to extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae. European Journal of Clinical Microbiology & Infectious Diseases. 2010;29(8):1039-41.

19. Parvizi J, Gehrke T. Definition of Periprosthetic Joint Infection. The Journal of Arthroplasty. 2014;29(7):1331.
20. Li C, Renz N, Trampuz A. Management of Periprosthetic Joint Infection. Hip Pelvis. 2018;30(3):138-46.
21. Izakovicova P, Borens O, Trampuz A. Periprosthetic joint infection: current concepts and outlook. EFORT Open Rev. 2019;4(7):482-94.
22. Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, et al. Diagnosis and Management of Prosthetic Joint Infection: Clinical Practice Guidelines by the Infectious Diseases Society of Americaa. Clinical Infectious Diseases. 2012;56(1):e1-e25.
23. Malizos KN, Varitimidis SE. 7 - Infection in total knee arthroplasty. In: Arts JJC, Geurts J, editors. Management of Periprosthetic Joint Infections (PJIs): Woodhead Publishing; 2017. p. 133-56.
24. Thakrar RR, Horriat S, Kayani B, Haddad FS. Indications for a single-stage exchange arthroplasty for chronic prosthetic joint infection. The Bone & Joint Journal. 2019;101-B(1_Supple_A):19-24.
25. Lange J, Troelsen A, Thomsen RW, Søballe K. Chronic infections in hip arthroplasties: comparing risk of reinfection following one-stage and two-stage revision: a systematic review and meta-analysis. Clin Epidemiol. 2012;4:57-73.
26. Vielgut I, Sadoghi P, Wolf M, Holzer L, Leithner A, Schwantzer G, et al. Two-stage revision of prosthetic hip joint infections using antibiotic-loaded cement spacers: When is the best time to perform the second stage? International Orthopaedics. 2015;39(9):1731-6.
27. Mortazavi SMJ, Vegari D, Ho A, Zmistowski B, Parvizi J. Two-stage exchange arthroplasty for infected total knee arthroplasty: predictors of failure. Clin Orthop Relat Res. 2011;469(11):3049-54.
28. Fleming A. On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenzae. 1929. Bull World Health Organ. 2001;79(8):780-90.
29. Adedeji WA. THE TREASURE CALLED ANTIBIOTICS. Ann Ib Postgrad Med. 2016;14(2):56-7.
30. Yocum RR, Rasmussen JR, Strominger JL. The mechanism of action of penicillin. Penicillin acylates the active site of Bacillus stearothermophilus D-alanine carboxypeptidase. J Biol Chem. 1980;255(9):3977-86.
31. Shahpari O, Mousavian A, Elahpour N, Malahias M-A, Ebrahimzadeh MH, Moradi A. The Use of Antibiotic Impregnated Cement Spacers in the Treatment of Infected Total Joint Replacement: Challenges and Achievements. Arch Bone Jt Surg. 2020;8(1):11-20.
32. Mingeot-Leclercq MP, Glupczynski Y, Tulkens PM. Aminoglycosides: activity and resistance. Antimicrob Agents Chemother. 1999;43(4):727-37.
33. Krause KM, Serio AW, Kane TR, Connolly LE. Aminoglycosides: An Overview. Cold Spring Harb Perspect Med. 2016;6(6):a027029.
34. Smieja M. Current indications for the use of clindamycin: A critical review. Can J Infect Dis. 1998;9(1):22-8.
35. Patel S, Preuss CV, Bernice F. Vancomycin. StatPearls. Treasure Island (FL)2020.
36. Rossi F, Andreazzi D. Overview of tigecycline and its role in the era of antibiotic resistance. Braz J Infect Dis. 2006;10(3):203-16.
37. Pankey GA. Tigecycline. Journal of Antimicrobial Chemotherapy. 2005;56(3):470-80.

38. Noskin GA. Tigecycline: a new glycylcycline for treatment of serious infections. Clin Infect Dis. 2005;41 Suppl 5:S303-14.
39. Nichol T, Smith TJ, Townsend R, Stockley I, Akid R. Analysis of linezolid and tigecycline as candidates for local prophylaxis via antibiotic-loaded bone cement. Journal of Antimicrobial Chemotherapy. 2016;72(2):410-6.
40. Lastinger A, McLeod N, Dietz MJ, Guilfoose J, Sarwari AR. Clinical Experience with Tigecycline in the Treatment of Prosthetic Joint Infections. J Bone Jt Infect. 2019;4(3):126-32.
41. Muratli SK, Karatosun V, Uzun B, Günal İ. Biomechanical comparison of tigecycline loaded bone cement with vancomycin and daptomycin loaded bone cements. 2020.
42. Kuehn KD, Ege W, Gopp U. Acrylic bone cements: composition and properties. Orthop Clin North Am. 2005;36(1):17-28, v.
43. Vaishya R, Chauhan M, Vaish A. Bone cement. J Clin Orthop Trauma. 2013;4(4):157-63.
44. Kühn K-D, Lieb E, Berberich C. PMMA bone cement: what is the role of local antibiotics. Maitrise Orthopaed. 2016;243:1-15.
45. Frommelt L, Kühn K-D. Antibiotic-loaded cement. The well-cemented total hip arthroplasty: Springer; 2005. p. 86-92.
46. Schmid M, Steiner O, Fasshold L, Goessler W, Holl A-M, Kühn K-D. The stability of carbapenems before and after admixture to PMMA-cement used for replacement surgery caused by Gram-negative bacteria. Eur J Med Res. 2020;25(1):34-.
47. Kühn K-D, Renz N, Trampuz A. Lokale Antibiotikatherapie. Der Unfallchirurg. 2017;120(7):561-72.
48. Kühn KD. PMMA Cements: Springer Berlin Heidelberg; 2014.
49. Weiss BD, Weiss EC, Haggard WO, Evans RP, McLaren SG, Smeltzer MS. Optimized Elution of Daptomycin from Polymethylmethacrylate Beads. Antimicrob Agents Chemother. 2009;53(1):264-6.
50. M. FN, T. C, T. N, J. ST, I. S. Comparison of the elution properties of commercially available gentamicin and bone cement containing vancomycin with ‘home-made’ preparations. The Bone & Joint Journal. 2017;99-B(1):73-7.
51. Luu A, Syed F, Raman G, Bhalla A, Muldoon E, Hadley S, et al. Two-stage arthroplasty for prosthetic joint infection: a systematic review of acute kidney injury, systemic toxicity and infection control. J Arthroplasty. 2013;28(9):1490-8.e1.
52. Chen AF, Parvizi J. Antibiotic-loaded bone cement and periprosthetic joint infection. J Long Term Eff Med Implants. 2014;24(2-3):89-97.
53. Amerstorfer F, Fischerauer S, Sadoghi P, Schwantzer G, Kuehn KD, Leithner A, et al. Superficial Vancomycin Coating of Bone Cement in Orthopedic Revision Surgery: A Safe Technique to Enhance Local Antibiotic Concentrations. J Arthroplasty. 2017;32(5):1618-24.
54. Fehring TK, Odum S, Calton TF, Mason JB. Articulating Versus Static Spacers in Revision Total Knee Arthroplasty for Sepsis. Clinical Orthopaedics and Related Research (1976-2007). 2000;380.
55. Amerstorfer F, Schober M, Valentin T, Klim S, Leithner A, Fischerauer S, et al. Risk of reinfection after two-or multiple-stage knee revision surgery using superficial vancomycin coating and conventional spacers. Journal of Orthopaedic Research®. 2021;39(8):1700-9.

56. Mazzucchelli L, Rosso F, Marmotti A, Bonasia DE, Bruzzone M, Rossi R. The use of spacers (static and mobile) in infection knee arthroplasty. Curr Rev Musculoskelet Med. 2015;8(4):373-82.
57. Mueller U, Reinders J, Smith-Romanski S, Kretzer JP. Wear Performance of Calcium Carbonate-Containing Knee Spacers. Materials. 2017;10(7).
58. Standard I. International Standard ISO 5833, 2002, Implants for Surgery—Acrylic Resin Cements. International Standards Organization. 2002.
59. Paz E, Sanz-Ruiz P, Abenojar J, Vaquero-Martín J, Forriol F, Del Real JC. Evaluation of Elution and Mechanical Properties of High-Dose Antibiotic-Loaded Bone Cement: Comparative "In Vitro" Study of the Influence of Vancomycin and Cefazolin. J Arthroplasty. 2015;30(8):1423-9.
60. Klekamp J, Dawson JM, Haas DW, DeBoer D, Christie M. The use of vancomycin and tobramycin in acrylic bone cement: biomechanical effects and elution kinetics for use in joint arthroplasty. J Arthroplasty. 1999;14(3):339-46.
61. Kühn K-D. Bone cements: up-to-date comparison of physical and chemical properties of commercial materials: Springer Science & Business Media; 2012.
62. Caraan NA, Windhager R, Webb J, Zentgraf N, Kuehn K-D. Role of fast-setting cements in arthroplasty: A comparative analysis of characteristics. World J Orthop. 2017;8(12):881-90.
63. Lee SH, Tai CL, Chen SY, Chang CH, Chang YH, Hsieh PH. Elution and Mechanical Strength of Vancomycin-Loaded Bone Cement: In Vitro Study of the Influence of Brand Combination. PLoS One. 2016;11(11):e0166545.
64. Kühn K-D. Management of periprosthetic joint infection: Springer; 2018, p 252.