**Diplomarbeit**

# On the Mechanical and Microbiological Aspects of PMMA with Manually Added Antibiotics in Periprosthetic Joint Infections


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Graz, 22.04.2021

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# Table of Contents

# Glossary and Abbreviations

| | |
|---|---|
| AAOS | American Academy of Orthopaedic Surgeons |
| ALBC | Antibiotic loaded bone cements |
| ASA | American Society of Anesthesiologists |
| BMI | Body mass index |
| BPO | Benzoyl peroxide |
| CI | Confidence interval |
| CRP | C-reactive protein |
| CT | Computer tomography |
| DIN | Deutsche Industrienorm |
| DM | Diabetes mellitus |
| DMPT | Di-methyl-para-toluidine |
| DTT | Difficult to treat |
| EG | Exempli gratia |
| EPS | Extracellular polymeric substances |
| ESR | Erythrocyte sedimentation rate |
| E. coli | Escherichia coli |
| HQ | Hydroquinone |
| IL-6 | Interleukin-6 |
| IL-8 | Interleukin-8 |
| ISO | International Organization for Standardization |
| LE | Leukocyte esterase |
| MIC | Minimal inhibitory concentration |
| MMA | Methyl methacrylate |
| MRI | Magnetic resonance imaging |
| MRSA | Methicillin-resistant Staphylococcus aureus |
| NGS | Next generation sequencing |
| NNIS | National nosocomial infections surveillance |
| OR | Odds ratio |
| PBP | Penicillin-binding protein |
| PBS | Phosphate buffered saline |
| PCR | Polymerase chain reaction |
| PET | Positron emission tomography |
| PJI | Periprosthetic joint infection |

| | |
|---|---|
| PMMA | Polymethyl methacrylate |
| PMN | Polymorphonuclear neutrophils |
| PoP | Plaster of Paris |
| P. aeruginosa | Pseudomonas aeruginosa |
| P. mirabilis | Proteus mirabilis |
| SMD | Standardized mean difference |
| S. aureus | Staphylococcus aureus |
| THA | Total hip arthroplasty |
| TKA | Total knee arthroplasty |
| TSS | Toxic shock syndrome |

# List of Figures

# List of Tables

# Zusammenfassung

**Einleitung:** Weltweit nimmt die Anzahl an Gelenksersatzoperationen Jahr für Jahr zu. Eine der gefürchtetsten Komplikationen dabei ist die periprothetische Gelenksinfektion. Die Therapie hierfür inkludiert meist den Wechsel der Prothese. Insbesondere bei solchen Wechseloperationen werden Knochenzemente aus Polymethylmethacrylat (PMMA) als lokale Wirkstoffträger eingesetzt. Bei Infektionen durch gram-negative Keime werden nur einige wenige Antibiotika für den lokalen Einsatz empfohlen, für deren Dosis im PMMA Zement allerdings kaum mechanische und mikrobiologische Daten vorliegend sind, die einen solchen Einsatz rechtfertigen.

**Material und Methoden:** Meropenem (Eberth) und Imipenem (Fresenius Kabi) sowie zwei Fosfomycin-Salze (Trometamol von Ebert und Natrium von Infectofos) wurden in verschiedenen Dosierungen mit unterschiedlichen PMMA-Zementen kombiniert. Mechanische Tests wurden nach ISO 5833 und DIN 53435 durchgeführt, mikrobiologisch wurde das Eluationsverhalten und die Wirksamkeit gegen verschiedene Keime, vor allem gram-negative, im Hemmhoftest untersucht.

**Ergebnis:** Meropenem eignet sich aufgrund der antibakteriellen Effektivität sowie der mechanischen Stabilität für den lokalen Einsatz im PMMA-Zement besser als Imipenem. Die mikrobiologischen Daten zeigen eine leichte Überlegenheit des Meropenems, mechanisch ist das Imipenem deutlich unterlegen. Beim Fosfomycin ist das Natriumsalz das Mittel der Wahl. In Zusammenschau der zahlreichen mikrobiologischen sowie mechanischen Ergebnisse ist sowohl in der Mechanik als auch in der Mikrobiologie das Natriumsalz dem Trometamol überlegen.

**Diskussion:** Meropenem ist für den Einsatz im PMMA Zement besser geeignet als Imipenem. Fosfomycin sollte vorzugsweise als Natriumsalz lokal im Knochenzement verwendet werden. Fosfomycin in Kombination mit dem Trometamolsalz zeiget sich sowohl in der mechanischen Stabilität als auch in der antibakteriellen Wirksamkeit unterlegen. Weitere klinische Studien sind notwendig um diese in-vitro Ergebnisse in-vivo nachvollziehen zu können.

# Abstract

**Introduction:** The number of joint replacements is increasing worldwide. Approximately 1% of all hip prosthesis get infected, which is a devastating diagnosis that includes a long treatment pathway. The therapy therefore requires in most cases changing the prosthesis. In those surgeries, bone cements consisting of polymethylmethacrylate (PMMA) utilized as local drug carrier are predominantly applicable. Especially in infections caused by gram-negative bacteria only few antibiotics are recommended, although the dosage in PMMA cement has not yet been sufficiently researched. The mechanical and microbiological properties are not yet fully understood.

**Material and Methods:** Meropenem (Eberth) and Imipenem (Fresenius Kabi) as well as two Fosfomycin salts (Trometamol from Ebert and Sodium from Infectofos) were used in different concentrations with different types of bone cement. Mechanical tests were performed according to ISO 5833 and DIN 53435. The microbiological efficacy was tested through the elution behavior. The effectiveness against predominantly gram-negative bacteria was examined in an inhibition zone assay.

**Results:** Meropenem showed better mechanical features and antimicrobial effectivity in the local usage in PMMA bone cement than Imipenem. The microbiological data indicate slightly better results, mechanically Meropenem is significantly superior to Imipenem. Of the two Fosfomycin salts, Sodium is the material of choice. In summary, not only in the mechanical testing, but also in the microbiological examination Fosfomycin-Sodium showed better properties.

**Discussion:** Meropenem is more suitable for the local usage in PMMA bone cement than Imipenem in infections caused by gram-negative bacteria. Fosfomycin-Sodium should be used locally in bone cements in periprosthetic joint infections, whereas Fosfomycin-Trometamol is inferior due to mechanical and antimicrobial properties. Furthermore, clinical studies are necessary to confirm the results of this in-vitro study in in-vivo circumstances.

# 1 Introduction

Nowadays one of the most common surgeries worldwide is joint replacement. This has led to an improved quality of life especially for elderly people with bone disease such as arthrosis. However, this development involves other problems such as the infection of the periprosthetic joint. The diagnosis of a periprosthetic joint infection (PJI) frequently means long healing processes with long during hospital stays, prolonged doses of antibiotics and revision surgery for patients. Additionally, high costs for the healthcare system are predestinated.

In Austria, for example, 210 total hip arthroplasties and 202 total knee arthroplasties per 100 000 inhabitants have been reported for 2015. From 2009 until 2015 an almost continuous increase in the number of total hip arthroplasties by 14% and 13% for total knee arthroplasties can be seen (1).

With the given circumstances, it is clear, that the number of periprosthetic joint infections will rise in total due to the higher number of arthroplasties.

The consequences of PJI can be devastating. A study conducted by Leitner et al. showed 135 patients with failed arthroplasty after PJI. After a mean follow-up of 12,8 years, patients have undergone an average of 3 revisions. The study showed that during follow-up 44% percent of total hip arthroplasties and 55% of total knee arthroplasties had to be revised after failed arthroplasty, 44% of the patients deceased during the period of follow-up and 16% could be rearranged to an infection-free implant (2).

## *1.1 Periprosthetic joint infection*

### 1.1.1 Epidemiology

With the rise of the number of joint arthroplasties, the number of postoperative complications such as periprosthetic joint infection has also increased in total. The epidemiology of periprosthetic joint infections differs from center to center and is usually under 1% for total hip replacement and under 2% for total knee arthroplasty (3).

### 1.1.2 Risk factors

There are many features which predestine individuals for a periprosthetic joint infection. Among those risk factors is clearly obesity: The odds of contracting

a periprosthetic joint infection are 2,29 (95% CI: 0,64–8,14) for patients with obesity (BMI 30–39 kg/m$^2$) and 8,96 (95% CI: 1,59–50,63) for patients with morbid obesity (BMI ≥ 40 kg/m$^2$). Additionally, patients suffering from diabetes mellitus showed an infection rate which is 6,87 times (95% CI, 2,42–19,56; p = 0.001) that of patients without DM (4). Another risk factor for PJI is malnutrition (5). A meta-analysis showed that risk factors range from male gender (OR: 1,48; 95% CI: 1,19 – 1,85), to age (SMD: −0,10; 95% CI: −0,17 – −0,03), obesity (OR: 1,54; 95% CI: 1,25 – 1,90), alcohol abuse (OR: 1,88; 95% CI: 1,32 – 2,68), ASA score >2 (OR: 2,06; 95% CI: 1,77 – 2,39), operative time (SMD: 0,49; 95% CI: 0,19 – 0,78), drain usage (OR: 0,36; 95% CI: 0,18 – 0,74), diabetes mellitus (OR: 1,58; 95% CI: 1,37 – 1,81), urinary tract infections (OR: 1,53; 95%CI: 1,09 – 2,16) and rheumatoid arthritis (OR: 1,57; 95% CI: 1,30 – 1,88). Among those significant risk factors, ASA score >2 was identified as a high risk factor. On the other side drain usage showed to be a significant protective factor (6).

A case-control study showed that the development of a surgical site infection not involving the prosthesis (OR: 35,9; 95% CI: 8,3-154,6), a National Nosocomial Infections Surveillance (NNIS) System surgical patient risk index score of 1 (OR: 1,7; 95% CI: 1,2-2,3) or 2 (OR: 3,9; 95% CI: 2,0-7,5) and the presence of a malignancy (OR: 3,1; 95% CI: 1,3-7,2) are risk factors of value too (7).

### 1.1.3 Pathogenesis

Periprosthetic joint infections are predominantly caused by microorganisms which form biofilms (8). In these biofilms, bacteria are stuck in a polymeric extracellular matrix. They organize themselves in a complex community which leads to the similarity to multicellular organisms. In these complexes different structures and functions are seen (9).

Materials used in orthopedic surgery such as titanium, stainless steel, cobalt-chromium, various polymeric materials (e.g. ceramics) and polymethylmethacrylate (PMMA) can get affected by biofilm formation (10).

| | |
|---|---|
| Central venous catheters | Pacemakers |
| Central venous catheter needleless connector | Peritoneal dialysis catheters |
| Contact lenses | Prosthetic joints |
| Endotracheal tubes | Tympanostomy tubes |
| Intrauterine devices | Urinary catheters |
| Mechanical heart valves | Voice prostheses |

**Table 1: Indwelling medical devices on which biofilms may develop** (11)

The process of biofilm formation in orthopedic implants has four stages: adhesion, proliferation, biofilm maturation and cellular detachment. In order to understand the initiation of biofilm procession the features of the substratum and the cell must be considered. Different materials such as hydrophobic or hydrophilic, rough or smooth and antibiotic-impregnated and non-antibiotic impregnated surfaces can get affected by biofilms. These properties have a significant influence on the development of a biofilm. In addition, the cell surface is of high importance. If the cell shows flagella, pili, fimbriae or glycocalyx, there is a higher probability for forming biofilms. As shown in figure 1 the first phase is the initial attachment of bacteria to the surface. After they adhesive irreversibly, bacteria start with cell division. By forming microcolonies and producing extracellular polymers, the characteristic features of biofilms are established. The extracellular polymeric substances (EPS) are primarily responsible for the structure of biofilms. In general, the structure of biofilms is heterogenous, because there are water channels which serve to nourish the bacterial cells in the biofilm layer. Bacterial cells in biofilms usually grow slower. If they detach from the biofilm layer, they can cause systemic infections (11).

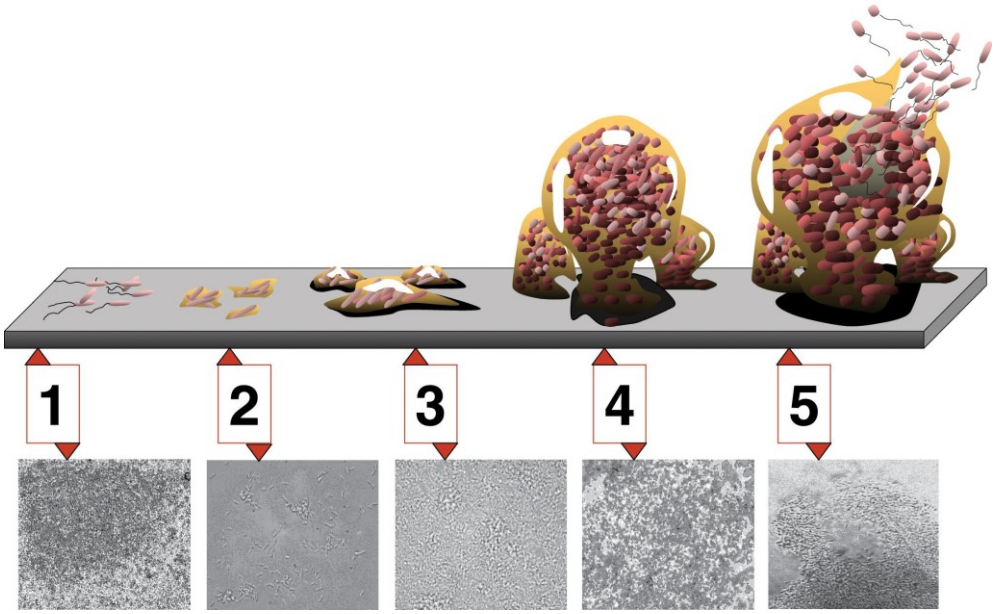



**Figure 1: 5 stages of biofilm formation**

**Each stage of development in the diagram is paired with a photomicrograph of a developing Pseudomonas aeruginosa biofilm. All photomicrographs are shown to same scale** (12)**.**

**Stage 1: initial attachment**

**Stage 2: irreversible attachment**

**Stage 3: maturation I**

**Stage 4: maturation II**

**Stage 5: Dispersion**

Infections caused by biofilms, for instance, periprosthetic joint infections are often difficult to treat. There are three main reasons: First, the antibiotic substance has to overcome the extracellular polymer matrix in order to reach the bacterial cell. Furthermore, as mentioned above the growth of bacteria in biofilms is slower, so the rate of antibiotic substance which is taken into the cell for fighting the microorganism is reduced. Third, the environment of bacterial cells can protect the bacterial cell itself (11).

### 1.1.4 Clinical manifestation

When speaking of periprosthetic joint infections we have to distinguish between early, delayed and late infections. They all show differences in their clinical manifestation. A cohort study showed that the distribution in occurrence is only slightly different: 29% of the patients showed early, 41% delayed and 30% late infections. Early infection is defined as infection within 3 months after surgery,

delayed as after 3 months until 2 years after surgery and late as infection after more than 2 years after surgery (13). While infections with virulent organisms (e.g. S. aureus and gram-negative bacilli) inoculated at implantation normally lead to acute infections, infections with less virulent organisms (e.g. coagulase-negative staphylococci) tend to reveal as chronic infections several months or even years after surgery (14).

Early-onset infections are usually acquired during implantation and they are often caused by virulent pathogens such as S. aureus or gram-negative bacilli. They can also appear due to wound dehiscence because then organisms from the superficial part of the wound can easily access to deeper structures. Most early-onset infections present with acute symptoms such as wound drainage, implant site erythema, induration or edema, joint pain, joint effusion or fever. Additionally, hematoma or superficial necrosis of the incision site can be found.

Delayed-onset infections are usually acquired during the implantation process too. Normally, less virulent organisms such as Cutibacterium species, coagulase-negative staphylococci and enterococci lead to these infections (14). In contrast to early infections, most delayed infections are indolent in the beginning and patients present with rather subtle symptoms such as implant loosening or persistent joint pain. Those infections are usually caused by less virulent bacteria such as coagulase-negative staphylococci and Propionibacterium acnes (3).

Late-onset infections are predominantly acquired by hematogenic seeding. The most frequent sources of bacteremia are infections from the skin, the respiratory tract, the dental system and the urinary tract (15). Patients usually suffer from an acute onset of symptoms of an infection in a joint which was functioning well before. In a study with 50 cases of periprosthetic joint infections, the median onset of infection was 5 years after implantation. Most of these late-onset infections are caused by S. aureus, beta-hemolytic streptococci or gram-negative bacilli (16).

### 1.1.5 Microbiology

A case-control study revealed that of all infections S. aureus caused 22%, Polymicrobial etiology 19%, Coagulase-negative staphylococci 19%, Streptococci 9%, Gram-negative bacilli 8%, Anaerobes 6%, Enterococcus species 6%, unknown 5%, Corynebacterium species 3%, Listeria monocytogenes 1%,

Mycobacterium tuberculosis 3%, Candida albicans 1%, Brucella suis 1% and Geotrichum species 1%. A negative culture was found in 12% of the cases (7). Another study showed that the microbial finding depends on the geographic site. Whereas most of the PJI in the United States were caused by S. aureus (31%), in Europe Coagulase-negative Staphylococci are responsible for most of the infections (39,3%). It also showed that the percentage of Methicillin-resistant S. aureus (MRSA) was higher at the centers in the United States than at the European centers (48,1% vs. 12,8%) (17).

### 1.1.6 Diagnosis

There are some features which should make us pay attention to a periprosthetic joint infection. Beside persistent wound drainage at the site of a joint prosthesis, acute or chronic pain at the site without a pain-free interval after surgery tend to indicate the presence of a PJI. Besides, infections or wound healing problems should make us alert too (18). The International Consensus on Orthopedic Infections proposed a concept for the diagnosis of a PJI:

| Major criteria | Diagnosis |
|---|---|
| Two positive growth of the same organism using standard culture methods | ≥ 1 Major criteria → Infected |
| Sinus tract with evidence of communication to the joint or visualization of the prosthesis | |

**Table 2: Major criteria of PJI: At least one is needed to diagnose a PJI** (19)**.**

| Minor criteria | Acute | Chronic | Score |
|---|---|---|---|
| Serum CRP (mg/L)<br>OR D-Dimer (µg/L) | 100<br>Unknown | 10<br>860 | 2 |
| Elevated Serum ESR (mm/hr) | No role | 30 | 1 |
| Elevated Synovial WBC (cells/µl)<br>OR Leukocyte Esterase<br>OR Positive Alpha-defensin<br>(signal/cutoff) | 10,000<br>++<br>1.0 | 3,000<br>++<br>1.0 | 3 |
| Elevated Synovial Polymorphonuclear Neutrophils (PMN) (%) | 90 | 70 | 2 |
| Single Positive Culture | | | 2 |
| Positive Histology | | | 3 |
| Positive Intraoperative Purulence | | | 3 |

**Table 3: Minor Criteria: Combined preoperative and postoperative score.**
**≥ 6 infected; 3-5 inconclusive (further molecular diagnostics such as Next Generation Sequencing (NGS) should be performed) ; <3 not infected** (19)**.**

There are many important tools which help diagnosing a PJI: beside plain radiography, laboratory testing for inflammatory markers (erythrocyte sedimentation rate [ESR] and C-reactive protein [CRP]), synovial fluid evaluation and tissue biopsy can be performed. In general, analysis of joint fluid or tissue is required to establish the diagnosis of PJI, identify the causative organism and guide choice of antimicrobial therapy (3).

### 1.1.6.1 Imaging studies

Imaging studies are useful for making the diagnosis PJI. If a PJI is suspected plain radiography should be performed. Other methods such as leukocyte scans, positron emission tomography (PET), computer tomography (CT) and magnetic resonance imaging (MRI) are not useful for routine diagnostic (18). Computer tomography (CT) has advantages in discovering inflammatory tissues but due to artifacts from metallic implants, it cannot be interpreted correctly in all cases. Magnetic resonance imaging has some limitations too. On the one hand, it is a technique without the need of radiation, but on the other hand not all implants are made of materials which are safe for MRI (e.g. titanium or tantalum) (3).

### 1.1.6.2 Laboratory markers

Laboratory markers such as CRP and ESR are helpful, but not diagnostic. On the one hand, they are elevated in most of the cases with PJI, which leads to the statement that normal CRP and ESR usually exclude periprosthetic joint infection (20).

On the other hand, it is necessary to be cautious because those markers can be elevated due to surgery or other chronic inflammatory diseases. CRP levels should be normal two to three weeks after surgery without complications and ESR can be abnormal up to one year after implantation (21). CRP should be performed in combination with ESR, because in one-third of chronic, low-grade infection CRP is negative (22). CRP (>10 mg/L) and ESR (>30 mm/hr) together show a high sensitivity (96%), but a low specificity (56%) for PJI (23).

### 1.1.6.3 Synovial fluid aspiration

The diagnosis of PJI is often made by isolating the pathogen from the synovial fluid before antimicrobial therapy is started. The aspiration of synovial fluid and culture are described as very exact for diagnosing a PJI (24). These methods are useful in distinguishing a periprosthetic joint infection from other circumstances such as loosening of the prosthesis without infection. However, the number of cells in a synovial fluid aspiration can show false results. The cell count can be too high due to only a short period after implantation, aseptic loosening, periprosthetic fractures or dislocation and in patients with rheumatoid arthritis.

Trampuz et al. showed that an amount of >1.7 x $10^3$/µL of leukocytes in synovial fluid has a sensitivity of 94% and a specificity of 88% for diagnosing prosthetic joint infection and a percentage of >65% neutrophils has a sensitivity of 97% and a specificity of 98%. Interestingly the bacterium S. aureus caused much higher leukocyte counts with >100 x $10^3$/µL (25).

A systematic review and meta-analysis showed that the synovial markers such as synovial fluid leukocyte count, polymorphonucleocytes % (PMN), CRP, α-defensin, leukocyte esterase (LE), IL-6, and IL-8 all demonstrate high sensitivity for the diagnosis PJI. Regarding those, α-defensin showed to be the best synovial marker (26).

Blood culture study should be performed if the patient suffers from fever or acute onset of pain (18).

### 1.1.6.4 Histopathological studies

In histopathological studies, the number of neutrophilic granulocytes in the periprosthetic tissue ranges from 1 to 10 in a high-power field at a magnification of 400. The number of cells to be found can vary extremely within different tissue sections of the same patient. Hence tissue biopsy should be performed in areas which are highly affected by infection (3).

### 1.1.6.5 Tissue biopsy

Tissue biopsy should only be performed intraoperatively if the diagnosis remains uncertain (27). Concerning gram stains, a multicenter study showed a low sensitivity of 27% in combination with a high specificity (99%) (28). Thus, the American Acadamy of Orthopaedic Surgeons (AAOS) recommended against using gram stain intraoperatively to exclude PJI (29). Cultures can be made from the aspiration of synovial fluid or from sampling periprosthetic tissue. It is assumed that cultures from periprosthetic tissue are the most reliable source for identifying the pathogen in periprosthetic joint infections. To secure the identification at least three tissue specimens should be sampled. However, cultures may be negative due to various reasons such as previous antibiotic therapy, a low amount of bacteria, a false culture medium, selective microorganisms or a long time span between sampling and the laboratory (3).

### 1.1.6.6 Sonication of removed implant

Trampuz et al. showed that the specificity and sensitivity of sonication of the removed implant are higher than for conventional periprosthetic tissue cultures. This mechanism bases on the fact that periprosthetic joint infections are caused by biofilms on the surface of the prosthesis. The approach was to obtain the samples which are cultured directly from the implant. Another advantage is that it shows good results in patients with prior antibiotic therapy. Standard culture if often negative in this special group (30).

### 1.1.6.7 New approaches

New approaches such as Multiplex PCR can be used to distinguish a PJI from prosthesis loosening without infection even if the patient has already received antibiotics. Such techniques can even detect isolated and difficult-to-culture bacteria (31).

A new method of diagnosing PJI is microcalorimetry. This technique can measure the heat microorganisms produce by growing. A study discovered that sensitivity and specificity are high for microcalorimetry of sonication fluid and so it can be used to detect a PJI early (32).

### 1.1.7 Treatment

#### 1.1.7.1 Surgical treatment

Surgical therapies include debridement with retention of the prosthesis, one-stage implant replacement and two-stage implant replacement.

#### 1.1.7.2 Debridement with retention

Earlier studies showed that debridement with retention of the prosthesis has high failure rates (33). Conversely, newer studies showed that the success rate can be higher than 80%, but special conditions are needed. In addition to the stability of the prosthesis, a pathogen with susceptibility to antimicrobial agents which are active against surface-adhering microorganisms, the absence of a sinus tract or compression of the soft tissue and duration of the infection for less than three weeks are required (34).

#### 1.1.7.3 One-stage implant replacement

This method was established 40 years ago and is characterized by requiring only one surgery with the replacement of the old prosthesis (35). The one-stage exchange is an effective surgery which not only shows a higher success rate, but also earlier mobility for patients, a shorter hospital stay and lower costs than the two-stage exchange (36). The aim of the one-stage implant replacement is to eradicate the PJI in a single surgery and thereby reduce patient morbidity by implanting a new permanent prosthesis, without having to undertake further surgeries (37). An advantage of one-stage implant replacement is that by using Antibiotic Loaded Bone Cements (ALBC) the period of systemically applicated antibiotics can be reduced to 10-24 days. If there is the suspicion that another surgery is needed, the surgeon can easily change to the process of a two-stage implant replacement. Additionally, due to the fact that only one surgery is performed, the risk of a complication is lower than for the two-stage implant replacement (38). This method is mostly used in Europe, whereas two-stage

surgery is predominantly found in the United States (18). One-stage replacement should be performed in patients with good bone conditions, no sinus tract and known bacteria with no difficult to treat (DTT) infection (34).

On the other hand there are several circumstances which tend to require using other techniques such as immune suppression of the patient, an unknown or resistant pathogen, a negative culture, a polymicrobial infection, a multimorbid patient, a major skin, soft tissue or bone defect, infection which includes the neurovascular pathways and peripheral vascular disease (39,40).

The process on which the one-stage implant replacement is based on is radical surgery with the removal of all exogenous material at the infection site. Debridement with prosthesis retention (DAIR) is usually performed using an open arthrotomy. In this case the former surgical incision is reopened. Irrigation and debridement of any necrotic or infected soft tissue follow. Furthermore, any hematoma or purulence has to be removed by the surgeon. A significant factor for the successful treatment is the thorough procedure of debridement. Usually, the stability of the prosthesis is examined during the surgery. Finally, the implant is irrigated precisely and the wound is closed by using a drain on top (41).

Eventually the implantation of a new prosthesis with ALBC follows. Next systemic antibiotic therapy for two weeks should be the standard (38).

#### 1.1.7.4 Two-stage implant replacement

Two-step implant replacement is the most commonly used method for the surgical sanitation of late and deep periprosthetic joint infections. The process involves two surgeries: The first one is necessary to remove the implant and perform debridement. The next step can be the implantation of a cement spacer. The last surgery is required to perform the reimplantation of a new prosthesis. The exact procedure differs widely. Influencing factors of two-stage implant replacement:

- Use/not use of a cement spacer
- Type of spacer used
- Type/Dosage of antibiotics in spacer
- Interval between two surgeries
- Oral or parenteral antibiotics in the interval

- Oral or parenteral antibiotics after reimplantation
- Cement or cementless revision

It is assumed that if a spacer is used the microbial eradication rate of the surgical site is good, although there may be some disadvantages when reimplantating the definitive prosthesis. Spacers can be produced by the surgeon himself during surgery or can be obtained ready to use in clinical routine. Types and doses of antibiotics vary in the literature from 0.5 g to 8 g pro 40 g polymer powder. It is recommended to use high concentrations of antibiotic in order to increase the efficacy. The decreasing mechanical properties are less essential in this case than in the one-stage implant replacement. The most common used antibiotics in spacers are Gentamicin, Tobramycin and Vancomycin. The interval between the two surgeries is of high importance. Whereas the result is poor in short periods, an interval of at least eight weeks shows promising results. The time span should be made dependent on the level of inflammatory markers (CRP, ESR). After definitive reimplantation antibiotic therapy for weeks should be performed too. Although the procedure of two-stage implant replacement can be very challenging, it offers a spectrum of wider indications for the patients and due to the recurrence rate this method is the most promising one (38).

A study conducted by Vielgut et al. investigated in a retrospective analysis the optimal period between the two surgeries. Best results were observed in patients where the two surgeries were within 12 weeks (42).

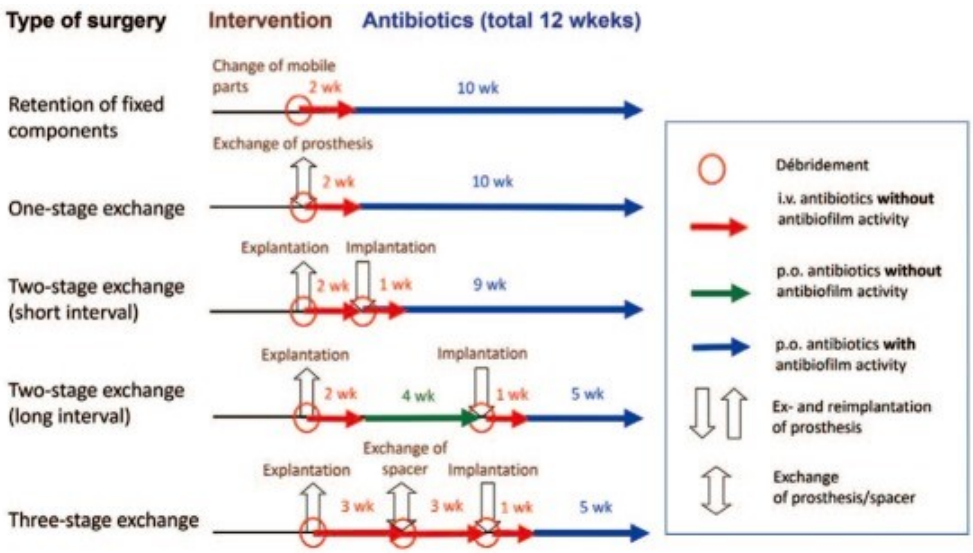



**Figure 2: Different types of surgery with time slots of exchange and antibiotic therapy** (36)

### *1.1.7.5 Antimicrobial therapy*

In general, antibiotic therapy for 12 weeks in total is recommended. There should be no microbial therapy without surgery, it should only be performed if the patient rejects surgery or the procedure of surgery itself is a high risk for the patient (36).

## *1.2 PMMA*

Polymethylmethacrylate (PMMA) is chemically seen the same as acrylic glass. Its first application was in plastic surgery to fill gaps in the skullcap in the 1940s. Because of the exceptional compatibility of PMMA with body tissue, ten years later it was first used in arthroplasty. Nowadays millions of joint replacements are performed per year and in more than half of the cases PMMA cement is used. Beside joint replacement, PMMA is also used in osteosynthesis, spongioplastic surgery and to refill bone defects.

PMMA is a two-component system. It consists of a powder, the polymer and a fluid, the monomer. Those two components are mixed intraoperatively, and surgeons can decide individually for each patient which composition or which addition they need. By mixing the two components, a homogeneous dough is produced. The viscosity of the dough in the application time is of high importance and depends on the field of application. In the process of polymerization, the dough increases its viscosity until it is a hard matrix.

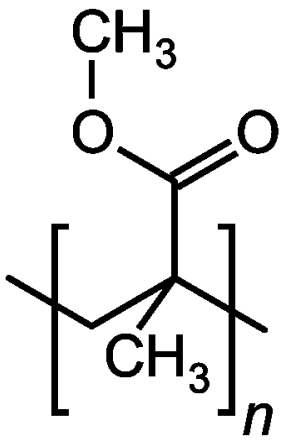



**Figure 3: Structure of Polymethylmethacrylate** (43)

### 1.2.1 Polymer

The powder (polymer) consists of one or more polymers or copolymers, which are organized in a chain. The polymer is called homopolymer if the chain consists of the same elements for example MMA. If different molecules are added, it is called copolymer. The composition of the polymer is of high importance for its features and differs from bone cement to bone cement. Other components of PMMA can be artificial coloring. Contrast medium, mostly zirconium dioxide or barium sulfate, is added to bone cements too.

To initiate the polymerization process benzoyl peroxide (BPO) is added to all PMMA bone cements. Additionally, antibiotic powder can be mixed to the PMMA powder. For this condition broad-spectrum antibiotic is mostly used. Gentamicin is often of use in this case because it is sensitive to approximately 75% of the bacteria which arise in periprosthetic joint infections. There are other mixtures ready to use and in addition the surgeon can add antibiotic powder to the cement powder individually. As most of the bone cements tend to have the same color as bones (beige to grey), it is useful for the surgeon to simplify the implantation process by visualization (44).

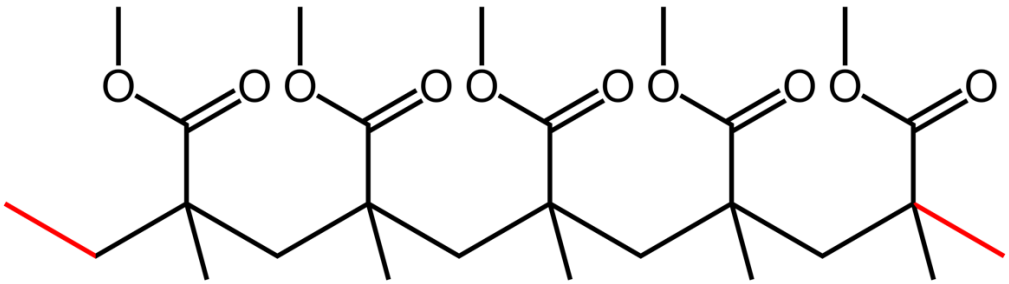


**Figure 4: Homopolymer of PMMA** (45)

### 1.2.2 Monomer

In contrast to the polymer, the monomer fluid consists of one main substance which is methylmethacrylate (MMA). MMA is chemically an ester of the methacrylic acid and polymerization is found due to the C=C double bond. MMA is often stabilized with Hydrochinon (HQ). Besides, there is the co-initiator di-methyl-para-toluidine (DMPT). There can be artificial coloring in the fluid component too in order to enhance the coloring of the bone cement (44).

**Figure 5: Methylmethacrylate (MMA)** (46)

### 1.2.3 Polymerization process

The ratio between polymer and monomer is mostly 2:1. A higher amount of polymer reduces the shrinkage of MMA and decreases the development of heat. The order of the components for mixing the cement dough differs from bone cement to bone cement (44).

**1)** The polymerization process starts with the splitting off BPO from the polymer in a redox process through the DMPT of the monomer. The results are benzoyl radicals. Then carbon dioxide is separated from the radicals (47).

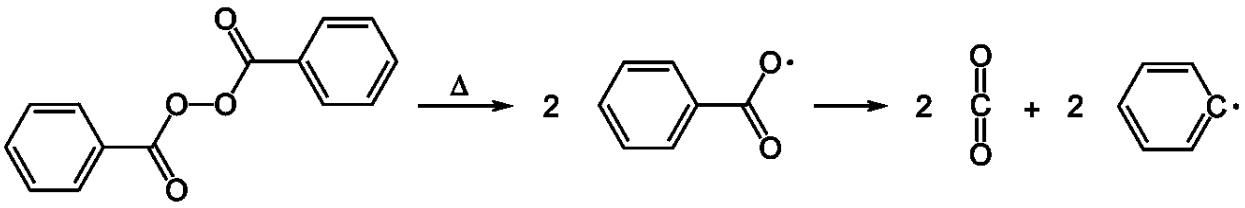



**Figure 6: Step 1 of polymerization** (48)

**2)** As a second step the radicals bind on the C=C double bond of MMA and a new, bigger radical is formed (47).

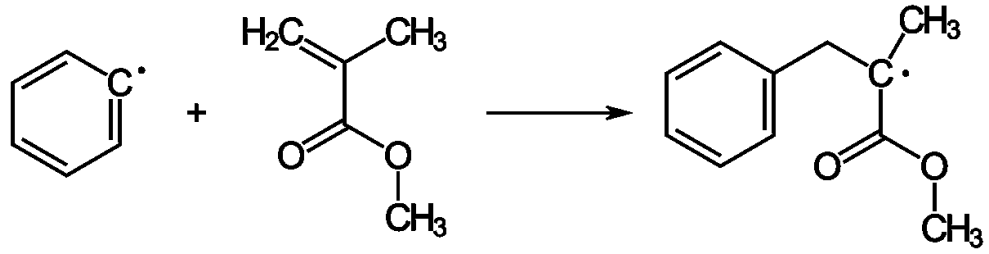



**Figure 7: Step 2 of polymerization** (49)

**3)** The growth of the chain uses the same step as the forming of the first bond. The radical binds MMA (47).

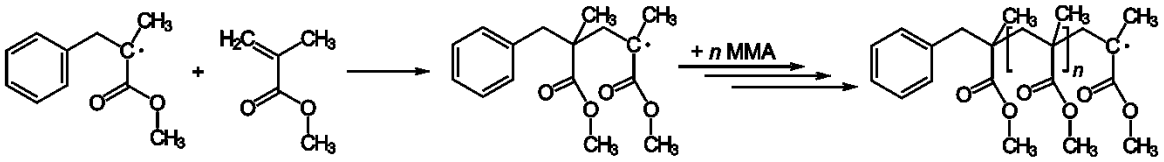



**Figure 8: Step 3 of polymerization** (50)

**4)** The termination of the chain can occur through different mechanisms. Two growing chains can for example hit each other and fuse (47).

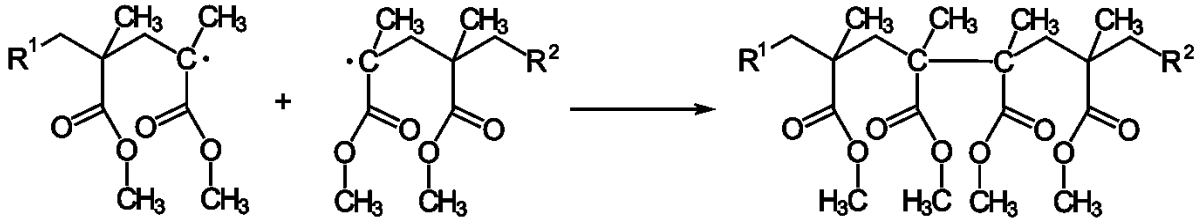


**Figure 9: Step 4 of polymerization** (51)

With the increasing viscosity in polymerization, also the temperature of the cement rises. In-vitro the heat peaks reach 80-90°C, but in the human body only 42-46°C, which is below the temperature limit which is critically for human protein. The heat in vivo depends on various factors such as the thickness of the bone cement, metallic implants and blood flow. Heated up metallic implants inhibit the conductive effect and the temperature increases significantly. The blood flow reduces the produced heat.

Besides, the high temperature can be used therapeutically for killing tumor cells in the area of the PMMA cement.

## *1.3 Antibiotic loaded bone cements (ALBC)*

The history of antibiotic-loaded bone cements (ALBC) is based on the experiments of Buchholz and Engelbrecht who first mixed polymethylmethacrylate (PMMA) bone cement with antibiotic powder at the end of the 1960s. They achieved a massive reduction of periprosthetic joint infections (52). Even though most of the data exist for local antibiotic usage in PMMA cement, other materials are used intraoperatively too. The advantages of using PMMA bone cements is the delayed release, long-existing clinical know-how and the high amount of clinical studies. PMMA cements are medical devices which serve as fixing of

artificial joints in the human body. The aim to fix the prosthesis is the same for the usage of antibiotic-loaded bone cements, but additionally they aim to prevent bacterial growth in the operation site. ALBC has two functions: the prevention of bacterial growth on the surface of the bone cement and secondarily the support of bacterial reduction in the bone and in the soft tissue. An advantage of using ALBC is that antibiotics which normally can cause devastating side effects can be used locally in a high concentration and will not reach the toxic drug level in serum (53). Generally, the maximum of added antibiotics in bone cements is 4 g which relates to the 10%-rule: only a limit of 10% of the 40 g polymer should be added (54).

### 1.3.1 Antibiotic considerations

To begin with, not all antibiotics available are suitable for the usage in bone cements, because there are some criteria that need to be considered. The elected antibiotic substance should be stable at high temperatures, because polymerization process of PMMA can cause temperature peaks to 80-90 °C (44). Studies show that the application of liquid antibiotics increases the amount of antibiotic release, but then the mechanical properties do not match the standards anymore (55). In general, antibiotics added in bone cements should be powders. Nonetheless, the mechanical properties should be tested for each antibiotic. The antibiotic used should have a broad antimicrobial efficacy spectrum, because it should be sensitive to bacteria which are common causes of periprosthetic joint infections (56). The elution of the antibiotic depends on various factors. Therefore antibiotics should be well-soluble in water in order to increase the release of them(57). The chosen antibiotic should be effective at low dosage since the lesser the added substance, the better are the mechanical properties. However, it has to be considered, that 1 g of for example Gentamicin is not equipotent to 1 g of amoxicillin, which means that different antibiotics require various dosages to obtain the same drug level in the bone cement (56). The added antibiotic should have no or low risk of allergy. Furthermore, they should not influence the mechanical properties significantly and they should show low serum protein binding (58).

### 1.3.2 Elution process

The mechanism of the release of the microbial active substance was first investigated in Palacos® R bone cement containing Gentamicin by Buchholz et al in 1970 (52).

The elution of antibiotics from bone cements shows a biphasic process. First, there is a high rapid phase of releasing and then a period of slower but constant elution follows. ALBC has been used in orthopedic surgery for centuries but the exact mechanism is not understood fully yet. Some studies demonstrate that antibiotics are released by diffusion through solid PMMA cement (59). Others assume that the antimicrobial substance is released through small cracks and gaps in the PMMA cement (60). Then again others suggest that the elution of antibiotics is only a mechanism of the surface and there is no diffusion process through the cement (61). A study combines those thesis and assumes that first the release is a procedure of the surface and after a time span the antibiotic which is eluted depends on the porosity of the cement (62).

### 1.3.3 Influencing factors of elution process

| Cement-related factors | Antibiotic-related factors | Test-related factors |
|---|---|---|
| Brand | Type/category | Fluid refreshment |
| Shape | Dose | Load bearing |
| Powder/liquid ratio | Combination | Temperature |
| Porosity | Volume | |
| Roughness | State (powder/liquid) | |
| Viscosity | | |

**Table 4: Contributing factors of elution process** (57)

There are many influencing factors which contribute to the elution process of antibiotic-loaded bone cements. First, the type of bone cement is one of the most important determinants and it is generally assumed that Palacos shows the best results.

Secondly, the mixing technique is an important influencing factor. Mixing manually is regarded as being advantageous for the better release of antibiotics,

because more air is trapped in the cement in this process and as a result the porosity is increased. The possibilities to enhance the elution are limited with the usage of biantibiotic combinations or fillers being the most common ones. Liquid antibiotics would increase the release, but unfortunately, have negative effects on the mechanical properties. The combination of aminoglycosides with glycopeptides shows promising synergistic effects, although it depends on the dosage of each antibiotic of those groups. Fillers such as dextran, glycine or xylitol seem to have positive effects on the release, but the definitive filler is not found yet and also the right amount is challenging. Fillers and the application of ultrasound show promising results in animal studies, but further studies should be performed prior to clinical usage (57).

A study conducted by Labmayr et al. assessed a technique where custom-made cement was covered with vancomycin powder (superficial vancomycin coating (SVC)). This in-vitro study showed that the antimicrobial effect in the first 24 hours of the ALBC is amplified, while mechanical stability remains unchanged (63). A retrospective cohort study conducted by Amerstorfer et al. showed that the prevalence of having a reinfection was not significantly different between the study group using SVC spacers and conventional spacers (64). A longitudinal case series compared the Vancomycin drug level in blood samples and locally from the drain after revision surgery using SVC and conventional spacers. The results of the study conducted by Amerstorfer et al. showed, that SVC of bone cement does not lead to systemic antibiotic side effects, but is highly effective to augment local antimicrobial concentrations at the site of infection (65).

## *1.4 Antibiotics*

The history of antibiotics is based on the discoveries of Alexander Fleming, who noticed the antibiotic effect of Penicillin isolated from the fungus Penicillium notatum. Although there are many highly effective substances nowadays, the development of resistances challenges scientists all over the world.

### 1.4.1 Carbapenems

The Carbapenems Meropenem and Imipenem are part of the beta-lactam antibiotics, which are characterized by the beta-lactam ring in their structure. To this group of antibiotics belong besides penicillin derivatives, cephalosporins, monobactams and Carbapenems. The beta-lactam ring is the antibiotic active

center. All beta-lactam antibiotics work by inhibiting the biosynthesis of the bacterial cell wall. More specifically they inhibit the synthesis of the peptidoglycan layer in the bacterial cell wall which is necessary for its cell wall integrity. Most of the beta-lactams inhibit penicillin-binding proteins (PBP) which are useful in the final transpeptidation step in the synthesis of peptidoglycans. Beta-lactam antibiotics are bacteriocidal. The most common resistance mechanism of bacteria is the synthesis of beta-lactamases. These are enzymes which destroy the beta-lactam ring by hydrolysis and inactivate the antibiotics. The effectiveness of these enzymes against a special antibiotic differs from bacterium to bacterium. There are ones who preferably destroy Penicillins (Staphylococci) and ones who preferably split Cephalosporins (Pseudomonas).

Carbapenems differ from other beta-lactams in the carbon atom (“carba-“) instead of Sulphur and a double-bound in the five-membered ring (“-penem”). Carbapenems are stable to beta-lactamases and have a broad antibacterial spectrum. They were first isolated from Streptomyces cattleya leading to the discovery of the original substance Thienamycin. They are highly effective against nearly all gram-positive bacteria, gram-negative bacteria and anaerobia. Meropenem is more effective against gram-negative bacteria than Imipenem and Imipenem slightly more effective against gram-positives. Imipenem is only available in combination with Cilastatin, which is an enzymatic inhibitor. The enzyme dehydropeptidase I which is found in the tubular cells of the kidney splits the Imipenem. Cilastatin is necessary because otherwise Imipenem would be inactivated before therapeutic retention period in plasma could be achieved. Because of improved stability, Meropenem and the other available Carbapenems do not depend on Cilastatin as enzyme inhibitor.

All of these antibiotics are only for parenteral use, as they are not sufficiently resorbed in the gastrointestinal tract. Imipenem is only available in a fix 1:1 combination together with Cilastatin (66).

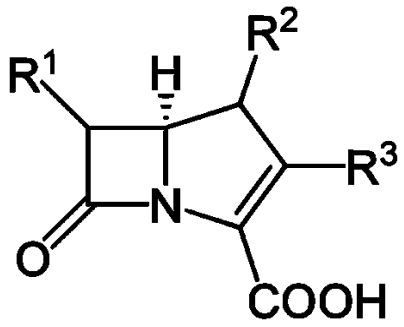



**Figure 10: Backbone structure of Carbapenems** (67)

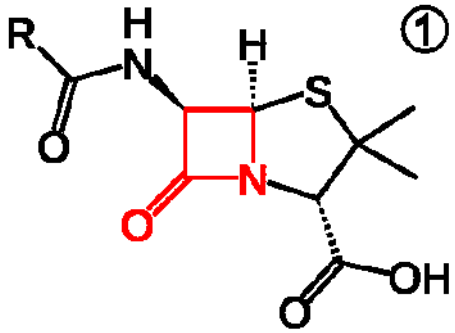



**Figure 11: Differences to Penicillin backbone: Carbapenems have a carbon atom instead of Sulphur and a double bound in the ring structure** (68)

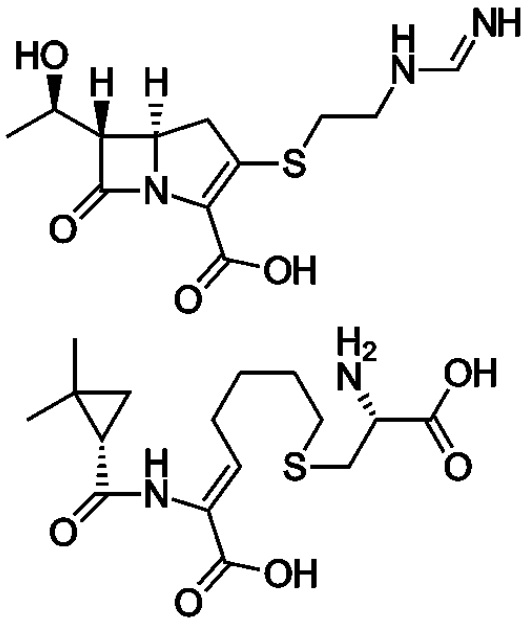



**Figure 12: Structure of Imipenem (above) and Cilastatin (below)** (69)

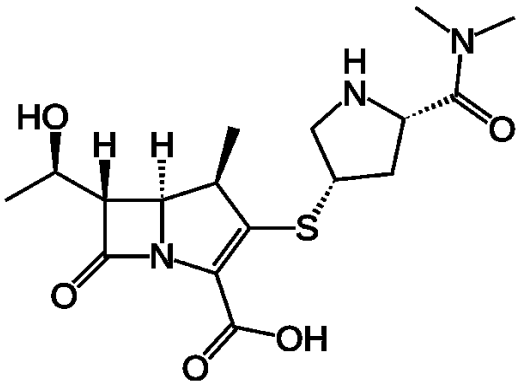



**Figure 13: Structure of Meropenem** (70)

### 1.4.2 Fosfomycin

Fosfomycin was discovered centuries ago by isolating phosphonic acid through cultures of Streptomyces spp in 1969 (71). Until now it was not often used, but due to the increasing resistance development rate, there is a new upturn. Fosfomycin was first isolated from Streptomyces sorts but now it is produced synthetically. The substance is stable and is well-soluble in water. Fosfomycin is only effective in an area, where there is enough glucose-6-phosphate, which is necessary to activate the transport system in the bacterium. Fosfomycin then blocks the pyruvyltransferase which is needed to build peptidoglycan. Besides S. aureus haemolyticus, coagulase-negative staphylococci and E.coli are sensitive to Fosfomycin (66). Fosfomycin is available in different drug formulations. There are two oral formulations, Fosfomycin-Trometamol and Fosfomycin calcium. There is also one intravenous formulation: Fosfomycin disodium (72). Fosfomycin-Trometamol (Monuril®) is used in the oral therapy of urinary tract infection of women. In this indication it is available in a granulated form (66).

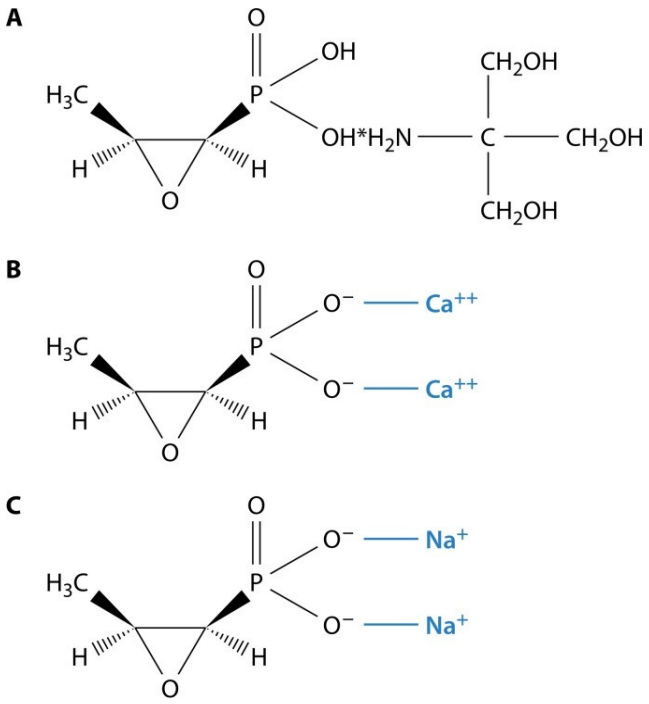



**Figure 14: Molecular structure of Fosfomycin in three different forms:**
**A=Fosfomycin-Trometamol**
**B=Fosfomycin-Calcium**
**C= Fosfomycin-disodium** (72)

## *1.5 Currently available data*

### 1.5.1 Meropenem

In half of the studies, Meropenem was tested in combination with another antibiotic, mostly Vancomycin, but also Tobramycin and Daptomycin (73–76).

A study conducted by Galvez-Lopez et al. showed that the elution kinetics of Meropenem in the concentration of 10% and 20% (1 g pro 10 g polymer and 2 g pro 10 g polymer) was a triphasic pattern. The peak was lower and the decrease faster than in other antibiotics, but it remained above the MIC (minimal inhibition concentration) of the tested bacterium E. coli. They also tested for compressive strength using an IBERTEST® electromechanical twin-screw machine and found that the mechanical properties were similar to bare acrylic bone cement (77).

Another in vitro study by Samuel et al. showed that Meropenem was eluted in measurable concentrations from 3-27 days. As the sample with the highest concentration of Meropenem (10% of 10 g polymer) showed the longest period of elution, the elution kinetics was assumed to be depending on the dosage of added antibiotics (78).

Baleani et al. performed a study which revealed that a combination of 1 g Meropenem and 1 g Vancomycin added to acrylic bone cements the showed best results among the tested combinations. Whereas all ALBCs met the ISO for compressive strength, the combination of 1 g Meropenem and 1 g Vancomycin did not reach the requirement for bending strength (73).

Another study performed by Andollina et al. showed in a chromatographic assay that the percentage of elution in a sample containing 0,5 g Vancomycin and 0,5 g Meropenem was higher than that of a sample with 1 g Vancomycin and 1 g Meropenem after 5 weeks (74).

A study which exclusively investigated the mechanical properties of ALBC (1,25% Meropenem and 1,25% Vancomycin) was conducted by Persson et al. This combination is promising in its combination of mechanical and microbiological properties with unchanged compressive and fatigue strength and slightly reduced bending strength and bending modulus, but all above the ISO 5833 limit (75).

Another study by Charlton-Ouw et al. was with the intention of investigating the efficacy of ALBC for the treatment of infections in abdominal aortic vascular grafts. The methods, however, are exactly the same. They showed that the addition of Daptomycin is useful in the therapy of gram-positive bacteria, whereas the addition of tobramycin showed reduced efficacy of Meropenem. It was found that the combination of 10% Meropenem and 2,5% Daptomycin was most suitable in the treatment of typical bacterial species (76).

### 1.5.2 Imipenem

Cerretani et al. discovered that the total amount of Vancomycin released is dependent on the type of cement and highest in CMW 1. With the addition of Imipenem-Cilastatin, the total amount of elution increased and the duration of elution was also higher in the combination. Palacos® R and Simplex show better results in the total amount of Vancomycin released when the combination is used (79).

A study by Chang et al. showed that ALBC containing Imipenem had a significantly lower cumulative antibiotic release efficacy and the release duration was worse than for other ALBCs. The compressive strength of Imipenem was worse than other ALBCs hence it is suggested that Imipenem is not usable in PMMA cements (80).

Bowyer et al. conducted a study comparing the release of different antibiotics in plaster of Paris (PoP) and PMMA. The release of Imipenem was much lower than that of other antibiotics with only 10,3 microgram/pellet/hour compared to, for instance, Ciprofloxacin with 200 microgram/pellet/hour. It has to be noticed, that the amount of added Imipenem with 3,7 mg/pellet was inferior to that of other substances. The reason for this remains unclear (81).

### 1.5.3 Fosfomycin

A study by Eick et al. demonstrated that Fosfomycin had excellent activity against MSSA, S. epidermidis and E. coli. They stated the company from which they obtained the Fosfomycin (Sigma-Aldrich Chemie GmbH) but it remains unclear which formulation exactly was used (Fosfomycin-Trometamol, sodium, calcium or pure) (82).

The aim of another study conducted by Roth et al. was to find out whether the addition of dextran fluid increased the elution of antibiotics in ALBC. They showed that Fosfomycin in Palacos® R+G is the most sensitive among the tested antibiotics for MRSA and MSSA as measured by inhibition zones (83).

On the contrary, a Study by Yuenyongviwat et al. compared the efficacy of Vancomycin and Fosfomycin ALBC and found out that the Fosfomycin ALBC are inferior to those with Vancomycin. The bone cement with Fosfomycin only showed inhibition zones for three days whereas in Vancomycin loaded bone cement they could be observed for up to four weeks (84).

All the three studies named the company of the obtained Fosfomycin, but not the exact formulation.

## *1.6 Experimental approach*

The aim of this study was to produce solid data for the usage of Meropenem, Imipenem, Fosfomycin-Trometamol and Fosfomycin-Sodium in PMMA cements for treating PJIs. Due to the fact that those antibiotics are often used in bone cements without reliable clinical data, the goal is to create a guideline for the usage of the right amount of antibiotics, the dosage form and the bacterial indication.

We hypothesized that Meropenem shows promising results for microbiological activity and mechanical stability since studies have been conducted on this issue, whereas Imipenem has not been investigated enough.

Furthermore, Imipenem is composed half of non-antimicrobial active substance, which eventually leads in less antimicrobial activity and/or reduced mechanical stability.

The aim of this study was to distinguish between the different Fosfomycin salts and create clear guidelines for the usage of the appropriate formulation too.

# 2 Material and Methods

## *2.1 Overview*

For the experiments four different antibiotics (Meropenem, Imipenem, Fosfomycin-Trometamol and Fosfomycin-Sodium) were combined with four different PMMA-cements (Palacos® R, Palacos® R+G, Copal® G+V and Copal® G+C). The aim was to evaluate the mechanical characteristics by using tests according to ISO 5833 and DIN 53435. Furthermore, cement bodies were produced to verify the microbiological effectiveness of the ALBCs.

### 2.1.1 Bone cements

All bone cements used in this study were produced by Heraeus Medical GmbH Wehrheim, Germany.

| Product | Company | Charge |
|---|---|---|
| Palacos® R 40 g | Heraeus | 8972, 9066, 9067 |
| Palacos® R+G 40 g | Heraeus | 8952, 8895, 9030, 9032, 9033, 9034, 9036, 9037, 9039, |
| Copal® G+V 40 g | Heraeus | 8536 |
| Copal® G+C 40 g | Heraeus | 9049 |
| Monomer liquid 20 ml | Heraeus | 4733, 4741, 4774, 4772, 9733, |

**Table 5: Bone cements with charges**

Palacos® R is an acrylic bone cement containing no antibiotic substance. 0,5 g of Gentamicin is added in Palacos® R+G bone cement. The antibiotic substance is already dispersed in the polymer powder beforehand in the production process. In Copal® G+V 0,5 g Gentamicin and 2,0 g Vancomycin were added, whereas in Copal® G+C 1,0 g Gentamicin and 1,0 g Clindamycin were admixtured.


**Figure 15: Bone cements: Palacos® R, Palacos® R+G, Copal® G+C, Copal® G+V from Heraeus Medical GmbH Wehrheim, Germany** (85)

## 2.1.2 Antibiotics

### 2.1.2.1 Meropenem

There is 1 g of Meropenem in a vial. The other ingredient is sodium carbonate. It is a white to pale yellow powder. 1 g of Meropenem means in the following tests the content of one vial.

### 2.1.2.2 Imipenem

A vial of Imipenem contains 500 mg Imipenem (equivalent to 530 mg Imipenem-Monohydrate) and 500 mg Cilastatin (equivalent to Cilastatin-sodium). Additionally, there is sodium hydrogen carbonate. To obtain 1 g of Imipenem two vials (each containing 500 mg) are needed.

### 2.1.2.3 Fosfomycin-Trometamol

In a sachet of Fosfomycin-Trometamol there are in total 8 g. 5,631 g of Fosfomycin-Trometamol are equivalent to 3 g of pure Fosfomycin. Additionally, the white to light yellow granulate contains sucrose, saccharin sodium, orange aroma and calcium hydroxide. To obtain the right amount of pure Fosfomycin, the amount to weight out has to be calculated. In the following tests 1 g Fosfomycin means pure Fosfomycin.

| | |
|---|---|
| 1 g Fosfomycin | 2,67 g substance |
| 2 g Fosfomycin | 5,33 g substance |
| 3 g Fosfomycin | 8 g substance |

**Table 6: Conversion table Fosfomycin-Trometamol into pure Fosfomycin**

#### 2.1.2.4 Fosfomycin-Sodium

A vial contains 5 g effective Fosfomycin. The 5 g are found in 6,73 g powder in total which is equivalent to 6,6 g Fosfomycin-Sodium which is effectively 5 g of pure Fosfomycin. The rest includes succinic acid. Fosfomycin-Sodium is a white to cream powder. The right amount of powder to add has to be calculated in order to receive the right amount of pure Fosfomycin.

| | |
|---|---|
| 1 g Fosfomycin | 1,35 g substance |
| 2 g Fosfomycin | 2,7 g substance |
| 5 g Fosfomycin | 6,73 g substance |

**Table 7: Conversion table Fosfomycin-Sodium into pure Fosfomycin**

| Product | Company | Pharmaceutical form | Charge |
|---|---|---|---|
| Meropenem | Eberth | Powder in a vial | 70B0417 |
| Imipenem | Fresenius Kabi | Powder in a vial | IDEA1167 |
| Fosfomycin-Trometamol | Eberth | Granulate in a sachet | 752063 |
| Fosfomycin-Sodium | Infectofos | Powder in a vial | F101658.1 |

**Table 8: Antibiotics with company and charge**

### 2.1.3 Agar and buffers

For the inhibition zone assay Petri dishes with Müller Hinton Agar was used.

| Product | Company | CAT# | LOT |
|---|---|---|---|
| Müller Hinton Agar | Oxoid | CM0405 | 1900545 |
| PBS tablets | Amresco | E404-200TABS | 1034C412 |
| Falcon tubes | | | |
| Petri dishes | | | |
| Eppendorf tubes | | | |

**Table 9: Material used for microbiological testing**

## *2.2 Tested strains*

The used strains of bacteria for the microbiological testing are listed in table 10.

| Bacteria | Strains |
|---|---|
| P. aeruginosa | ATCC 27853 |
| P. mirabilis | ATCC 12453 |
| E. coli | BjHDE-1 |
| MRSA | ATCC 43300 |

**Table 10: Tested strains**

### 2.2.1 Pseudomonas aeruginosa

Pseudomonas aeruginosa is a gram-negative bacilliform shaped bacterium of 1-3 µm length and has a monotrichous polar flagellum. It is a facultative anaerobe and an opportunistic bacterium, which in nature predominantly grows in humid areas. A common feature of Pseudomonas is their intrinsic antibiotic resistance and multiresistant strains. Pseudomonas aeruginosa is resistant to most beta-lactam antibiotics, macrolides and aminoglycosides. This leads to the fact, that the sensitivity to antibiotics must be tested in advance. Furthermore, it is worldwide one of the most common causes of nosocomial pneumonia, wound infections and urinary tract infections especially in immunocompromised individuals (86).

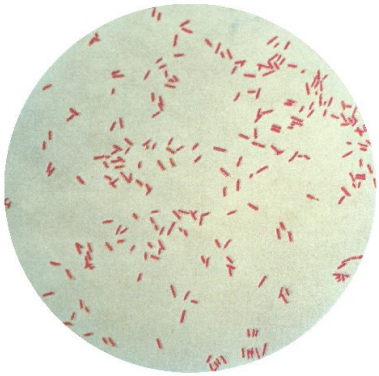


**Figure 16: Pseudomonas aeruginosa gram stain** (87)

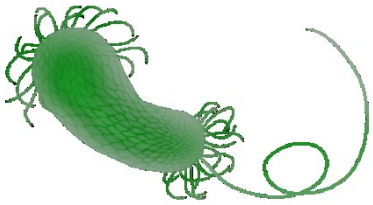


**Figure 17: Pseudomonas aeruginosa** (88)

### 2.2.2 Proteus mirabilis

Proteus mirabilis belongs to the Enterobacteriae such as E. coli and is a gram-negative bacillus. The special features of Proteus are the numerous peritrichous flagella which are responsible for the high mobility. Interestingly, Proetus produces the enzyme urease, which can split urea. Through this process ammonia is produced which augments the urine pH-value in order to secure a better growth environment. Proteus as putrefying bacteria is to be found in wastewater, carcasses and some food such as in overripe cheese. Proteus is often seen in the gastrointestinal tract of healthy people. Extraintestinal we see opportunistic infections such as urinary tract or systemic infections (89).

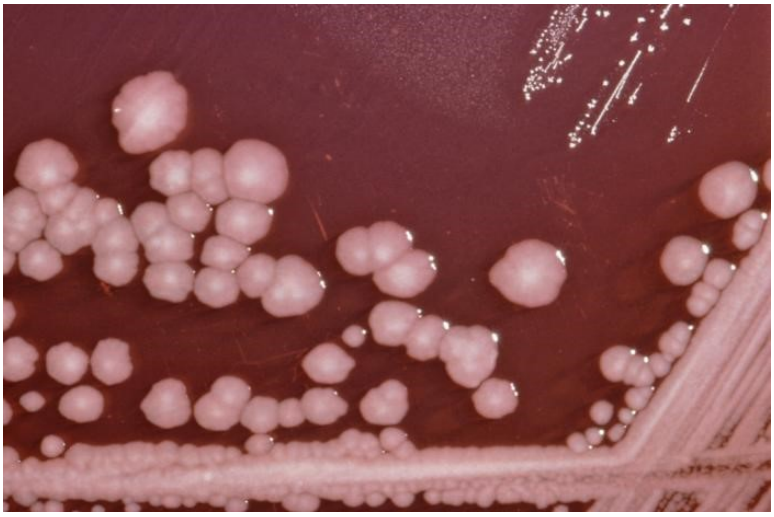


**Figure 18: Proteus mirabilis colonies on an agar plate** (90)

### 2.2.3 E. coli

Escherichia coli is a gram-negative bacillus and facultative anaerobic. Apathogenic and facultative pathogenic strains belong to the human gastrointestinal tract. The optimal growth temperature is 37°C and the generation time is approximately 20 minutes. E. coli can be causative for sepsis, urinary tract infection, meningitis and wound infections when it is taken away in the body. One of the most important resistance mechanisms is the synthesis of β-lactamases which can split penicillins and cephalosporins easily, but many β-lactamases of E. coli can be made ineffective by β-lactamase-inhibitors. E. coli is in most cases sensible to cephalosporins from the 2$^{nd}$ and 3$^{rd}$ generation, Carbapenems, gyrase inhibitors and cotrimoxazole. However, numerous strains are resistant to ampicillin and in a lower percentage to piperacillin (89).

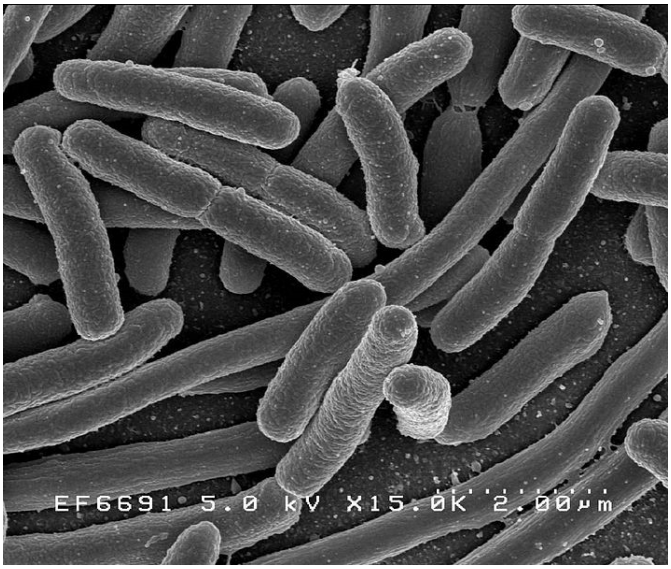



**Figure 19 Electron microscope picture of E.coli** (91)

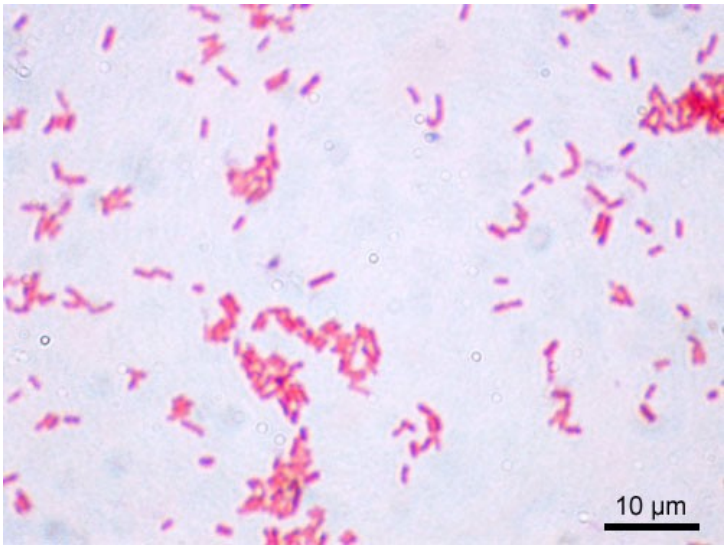



**Figure 20: Escherichia coli gram stain** (92)

### 2.2.4 Methicillin-resistant Staphylococcus aureus (MRSA)

Staphylococcus aureus is a gram-positive, round-shaped bacterium. MRSA differs from Staphylococcus aureus through another resistance mechanism. They have an additional penicillin-binding protein (PBP2a) to which beta-lactam antibiotics can only bind weakly. In Germany approximately 30% of all Staphylococcus aureus isolates are MRSA. MRSA is resistant to Penicillins, Carbapenems and Cephalosporins and can also be resistant to other antibiotics. Staphylococcus aureus can cause not only local infections (superficial purulent or deep), but also sepsis and toxin-caused syndromes (Toxic shock syndrome = TSS) (93).

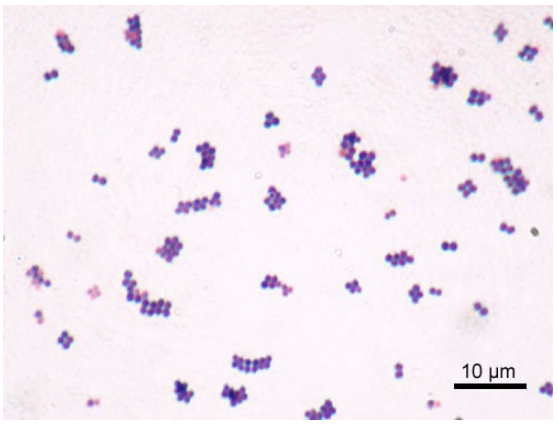



**Figure 21: Staphylococcus aureus gram stain** (94)

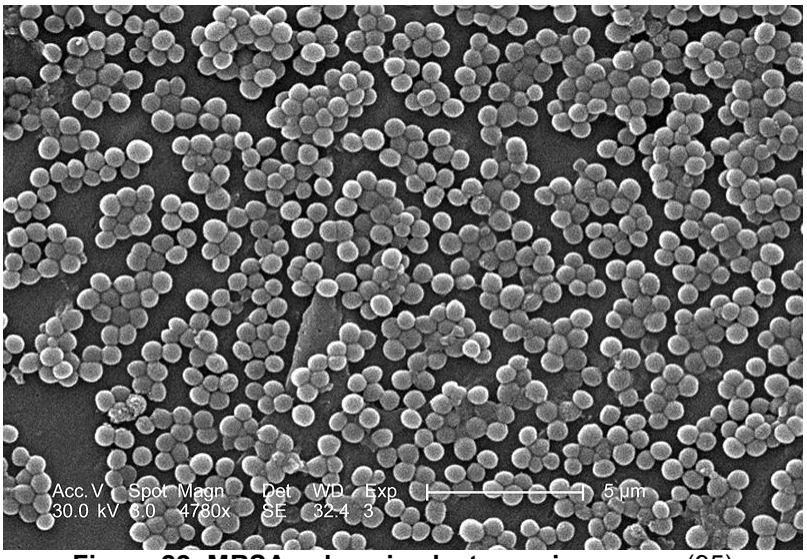


**Figure 22: MRSA colony in electron microscope** (95)

## *2.3 Preparation of cement bodies*

Cement bodies were produced to evaluate the mechanical and microbiological properties at Heraeus Medical GmbH in Wehrheim (Germany). PMMA cement which is also available in clinical routine was used. 40 g of polymer powder (one bag) was mixed with 20 ml of monomer (one vial) plus the additionally added antibiotics.

First, the right amount of antibiotic powder was weighted out with a precision scale (FRANK PTI GmbH, Germany®). Three of the antibiotics used were powders, but the Fosfomycin-Trometamol was only available in granulated form. The antibiotics which are powders were homogenized using fractional admixture. In order to prevent irregularities in the cement, it was decided to work on the granules in a mortar. After this process, the Fosfomycin-Trometamol was easy to homogenize too.

The next step was to mix the polymer powder and the antibiotic powder in a single-use plastic cup with a small spoon. In order to get comparable results, the whole process was standardized in using a timer. As the following step, the polymer powder containing antibiotics was added to the monomer liquid. In this step, the order is of great importance. Now the mixture was stirred for 30 seconds. Then it was examined using gloves if the cement dough was still sticky and after 20 seconds it was ready to use. Subsequently, it was hand-kneaded for 60 seconds.

Next, the doughy cement was put in the mold and then in a hydraulic press at 3 bars for 30 minutes using a timer again.

Then a hammer was used to remove the cement bodies from the molds. Afterwards, they were put in a laser shaper in order to put them in a standardized shape. Four different shapes were generated in this way: cylindric cement bodies for the microbiological examination, two types of rectangular bodies and another type of cylindric body for the mechanical tests. The cement bodies for microbiological testing did not have to be cut in the shaper and so we put them in plastic bags and welded them. The bodies for mechanical examination were incubated at 23°C for 24 hours.


**Figure 23: Molds containing cement in the hydraulic presses at 3 bars for 30 minutes**


**Figure 24: Experimental setup for producing cement bodies: from left to right: monomer liquid, polymer powder, spatula, spoon, ceramic bowl and two plastic bowls**

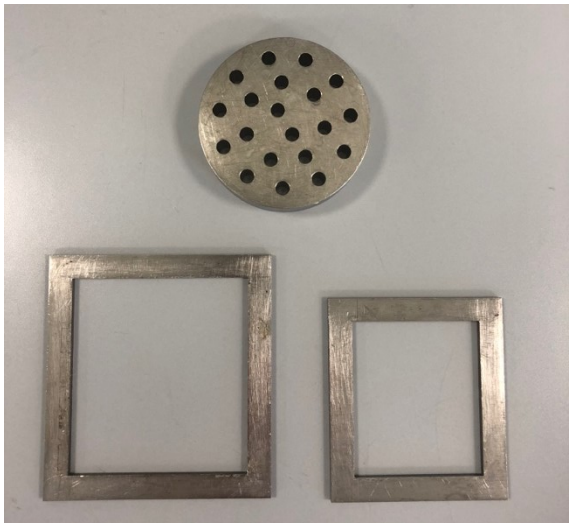


**Figure 25: Molds for preparing cement bodies for mechanical testing**

## *2.4 Mechanical tests*

| Antibiotic | Cement | Bending strength, bending modulus and 4PB | Compressive strength |
|---|---|---|---|
| Meropenem 2 g | Palacos® R+G | + | |
| Imipenem 4 g | Palacos® R+G | + | |
| Fosfomycin-Trometamol 2 g | Palacos® R+G | + | + |
| Fosfomycin-Trometamol 3 g | Palacos® R+G | + | |
| Fosfomycin-Sodium 2 g | Palacos® R+G | + | + |
| Fosfomycin-Sodium 5 g | Palacos® R+G | + | + |

**Table 11: Experimental plan mechanical testing (4PB = 4-point-bending strength)**

Mechanical tests were performed after the cement bodies were stored 24 hours in a room, in which the temperature was fixed at constant 23°C. All mechanical tests were performed in the same room in regular air at the same temperature. Compressive strength, bending modulus and bending strength were conducted applying a Zwick Roell® material testing tool and performed according to ISO 5833 standard, whereas impact strength and bending strength were performed using DIN 53435.

### 2.4.1 Impact strength (DIN 53435)

The test for impact strength is necessary to evaluate the properties of the cement if suddenly a force in the form of a strike occurs. The following test was performed using a Dynstat configuration and according to DIN 53435.

As the first step, the broadness and the thickness of the rectangular cement body were measured using a caliper and documented. The device called for a width of 10 mm, a length of 15 mm and a height of 3,3 mm.

A 0,5 J weight was used and the swinging element was fixed in the mount at 90° height of fall. Then the cement body was placed in the intended notch facing the pendulum. The needle which is used to read off the scale was set at 0,3 Joule because that is the result of a swing without a cement body.

Now to release the mechanism a lever is pressed and the element pumps against the body, which releases the needle and the result could be read off the scale. For this test, it was necessary that the cement body breaks in order to get reliable results. Eight replicas of the same ALBC combination were measured. The actual impact strength had to be calculated using the impact in Joule, the broadness and the thickness of the cement body with the following equation:

$$a_n = 1000 * \frac{A_N}{B * T}$$

$a_n$... Dynstat impact strength in kJ/m$^2$

$A_N$...Impact in Joule

$B$... Breadth in mm

$T$... Thickness in mm

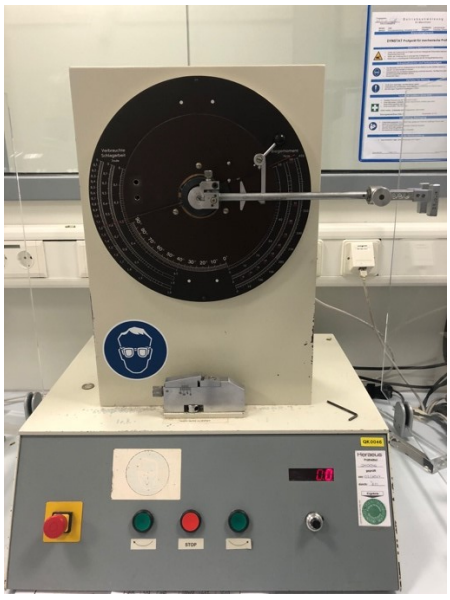


**Figure 26: Starting position of Dynstat impact strength test**

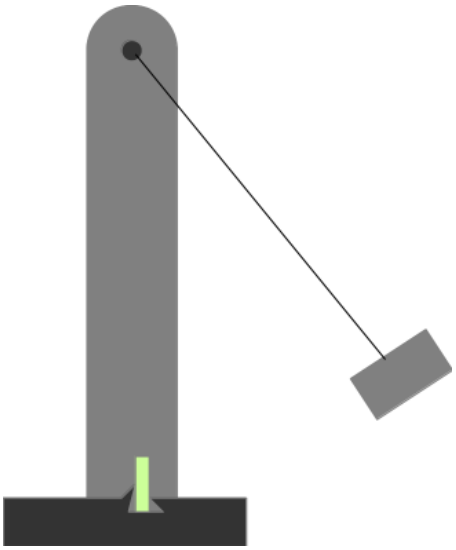


**Figure 27: Modell of the starting position of Dynstat impact strength test**

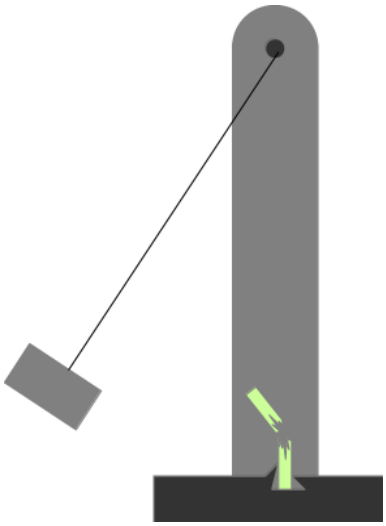


**Figure 28: Modell of the finishing position of Dynstat impact strength test**

### 2.4.2 Bending strength (DIN 53435)

The bending strength test was performed using a Dynstat configuration according to DIN 53435 and rectangular cement bodies (10 x 15 x 3,3 mm).

The bodies were placed in the suspension device. There were two screws, in order to fix the cement bodies not too loose and not too tight to get comparable results. Then they were bent at a rate of 150°/min. The bending moment [Ncm] was read off a scale using a drag pointer and the bending strength in MPa was calculated afterwards. This procedure was repeated seven times for each combination of ALBC.

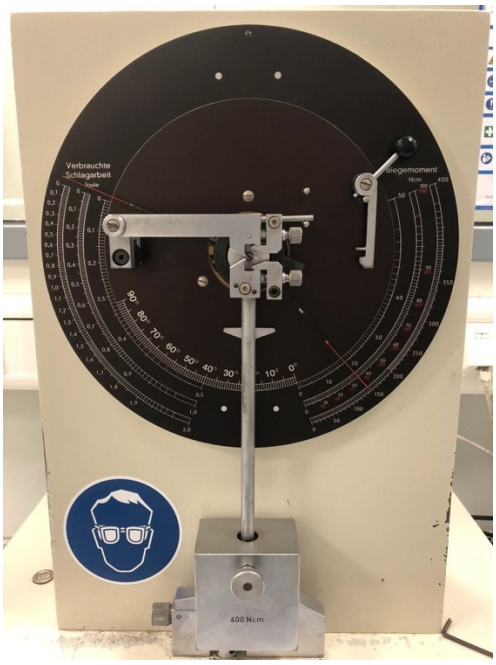



**Figure 29: Starting position of Dynstat bending strength test**

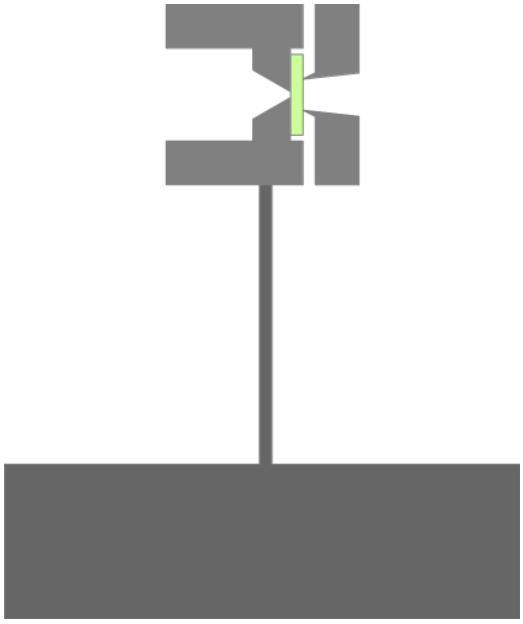


**Figure 30: Modell of the starting position of Dynstat bending strength test**

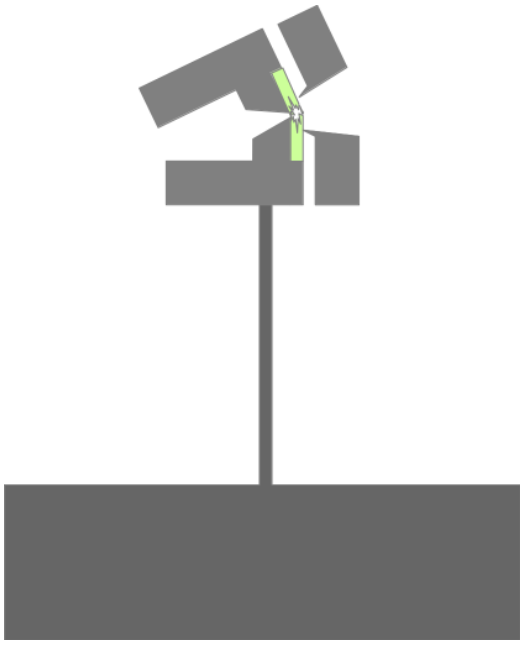


**Figure 31: Modell of the finishing position of Dynstat bending strength test**

### 2.4.3 Compressive strength (ISO 5833)

To get information concerning the compressive stability of the ALBC, this test according to ISO 5833 standards was performed. For this test, a Zwick Roell® materials testing machine was used.

Prior to conducting the test, the machine hat to be configurated in advance. After measuring its height and diameter with a digital caliper (height: 12 mm, diameter: 6 mm), the bone cement cylinder was placed upright in the middle of the intended plane.

When starting the test, a cylindrically shaped component lowers towards the fixed one where the cement body is placed at a rate of 20 mm/min. The machine constantly applies a load on the cement cylinder. The maximum compressive strength is measured at the point when a sudden reduction in force is registered. The maximum force is necessary to calculate the compressive strength.

The machine sometimes does not stop automatically at this point and lowers completely towards the lower limit. In this case, it had to be stopped and the breaking point had to be determined manually. Compressive strength is measured in Megapascal (force per area). The minimum compressive strength according to ISO 5833 should be at least 70 MPa.

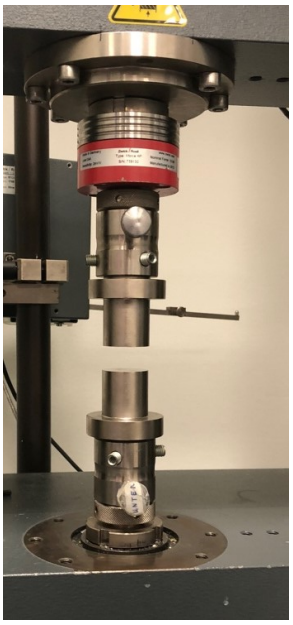


**Figure 32: Starting position of compressive strength test**

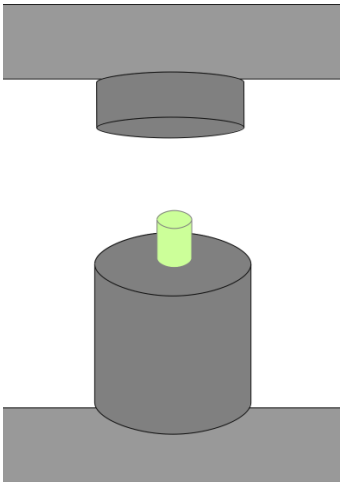


**Figure 33: Modell of the starting position of compressive strength test**

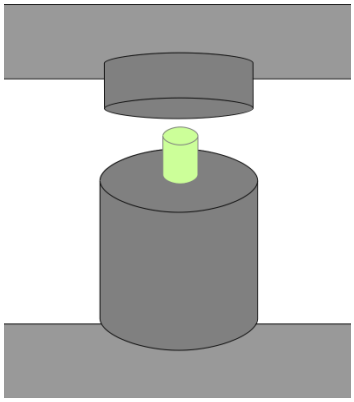


**Figure 34: Modell of the process of compressive strength**

### 2.4.4 4-point-bending strength and bending modulus (ISO 5833)

The bending modulus and the bending strength are indicators for the amount of stress that can be tolerated by the ALBC. This test is performed with the Zwick Roell® materials testing machine according to ISO 5833 too. Before starting the test, it has to be specially set up, the right starting position has to be evaluated and the distance measurer has to be placed in the middle. The rectangular cement bodies need to be placed centrally in order to get comparable results. They also have to be measured with a caliper in advance (width: 10 mm, height: 3.3 mm, length: 75 mm).

An automatic feed with 5 mm/min is used. Two pins constantly apply load to the ALBC, which is placed on two supports. The maximum load and the amount of bending quantified with the distance measurer are noted. Six replicates of each combination of the cement bodies were measured using this test. Documentation and measurement were performed with a computer running the testXpert®II software. The bending strength according to ISO 5833 should meet 50 MPa and the bending modulus 1800 MPa.

**Figure 35: Starting position of four-point bending test**

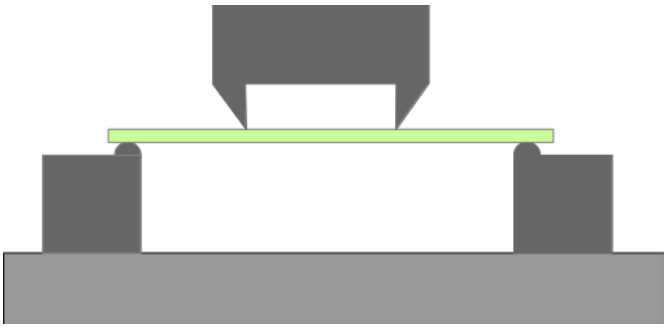


**Figure 36: Modell of starting position of four-point bending test**

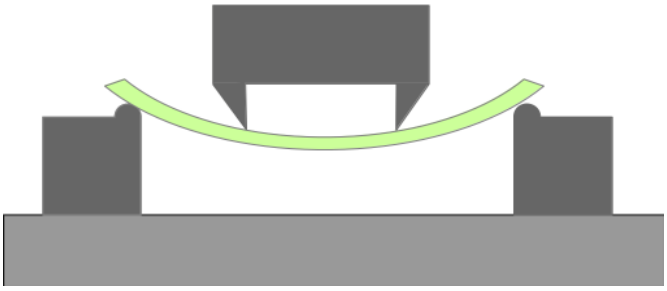


**Figure 37: Modell of finishing position of four-point bending test**

## *2.5 Microbiology tests*

The tested combinations of antibiotics in varying concentrations and different cement types are listed in table 12.

| Antibiotic | Cement |
|---|---|
| Meropenem 1 g | Palacos® R |
| Meropenem 2 g | Palacos® R+G |
| Meropenem 1 g | Copal® G+C |
| Imipenem 2 g | Palacos® R |
| Imipenem 4 g | Palacos® R+G |
| Imipenem 2 g | Copal® G+C |
| Fosfomycin-Trometamol 1 g | Palacos® R |
| Fosfomycin-Trometamol 3 g | Palacos® R |
| Fosfomycin-Trometamol 1 g | Palacos® R+G |
| Fosfomycin-Trometamol 2 g | Palacos® R+G |
| Fosfomycin-Trometamol 2 g | Palacos® R+G Pro |
| Fosfomycin-Trometamol 3 g | Palacos® R+G |
| Fosfomycin-Trometamol 2 g | Copal® G+V |
| Fosfomycin-Sodium 1 g | Palacos® R |
| Fosfomycin-Sodium 5 g | Palacos® R |
| Fosfomycin-Sodium 1 g | Palacos® R+G |
| Fosfomycin-Sodium 2 g | Palacos® R+G |
| Fosfomycin-Sodium 5 g | Palacos® R+G |
| Fosfomycin-Sodium 2 g | Copal® G+V |

**Table 12: Tested combinations of antibiotics and cements**

Each antibiotic was tested against three different strains of bacteria. The exact list is shown in table 13.

| Antibiotic | Strain 1 | Strain 2 | Strain 3 |
|---|---|---|---|
| Meropenem | Escherichia coli | Proteus mirabilis | Pseudomonas aeruginosa |
| Imipenem | | | |
| Fosfomycin-Trometamol | MRSA | | |
| Fosfomycin-Sodium | | | |

**Table 13: Strains the antibiotics were tested against**

To evaluate the release of the antibiotics from the bone cement, inhibition zone assays were performed. The following experiments were conducted in an S2-laboratory. Firstly, phosphate-buffered saline (PBS) was produced and autoclaved. Three replicates of each composition had to be tested. Antibiotic loaded bone cement cylinders with a diameter of 25 mm and a height of 9 mm were used for performing the microbiological assessment


**Figure 38: Example of cement bodies used for microbiological testing**

### 2.5.1 Preparation of the tubes

The PBS buffer was prepared using phosphate-buffered saline tablets of Amresco® in advance. First, 20 ml of PBS was put in a Falcon tube using an

electric pipetting aid. Then the cement bodies were placed in the tubes using a sterile tweezer. Afterwards, the tubes were closed with screw closure and additionally with a Parafilm® in order to avoid liquid flowing out when they were turned over in the next step. The tubes were stored upside down and the cement bodies should be fully covered with fluid. The tubes were then incubated for one hour at room temperature. Then the tubes were changed for the first time.

### 2.5.2 Changing of the tubes

First, 1 ml of the eluate was put in an Eppendorf. The cement bodies were taken out of the Falcon-tube using a sterile tweezer and then they were put on a sterile swab. The rest of the fluid in the falcon-tube was not needed anymore. The cement bodies now should be dabbed with the swab in order to lose the redundant fluid on the surface of the body. Then the cement body was put in a new Falcon tube with 20 ml of PBS. To secure the tightness of the falcon tubes, the cover was wrapped in Parafilm®. Now the tubes were turned over. Since in this step the cement body often got stuck in the middle of the tube, it was important to verify that it was fully covered with fluid. Finally, the tubes were incubated at room temperature for a predefined time span (24 h, 48 h, 7 d, 14 d, 28 d, 42 d).

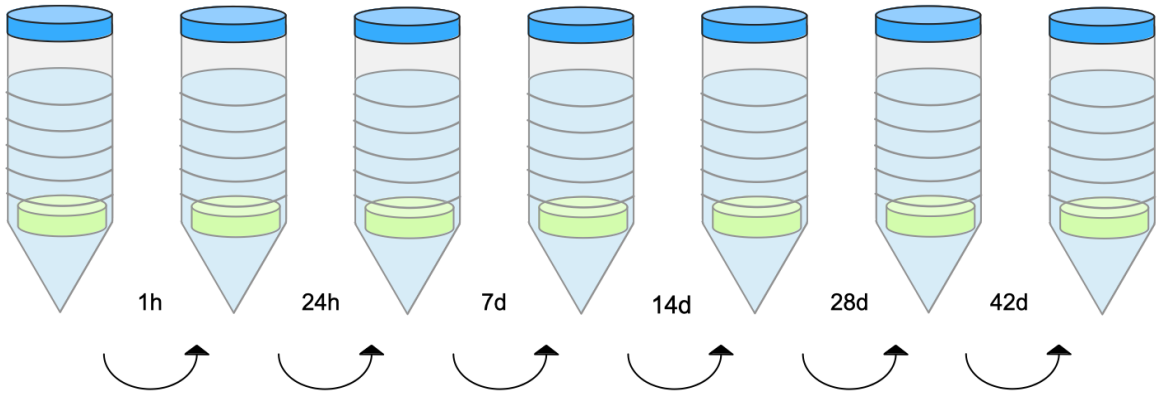



**Figure 39: Time points of tube changing**

### 2.5.3 Inhibition zone assay

The inhibition zone assay was performed on sterile Agar plates using Müller-Hinton agar without antibiotics. For this purpose, Müller-Hinton agar was prepared and autoclaved. For each plate, 20 ml of agar using an electric pipetting aid was poured paying attention to the prevention of producing air pockets in the agar. Afterwards, the dishes were let dry. In order to compare the results, the bacteria had to be prepared so that McFarland was 0.5. This corresponds to a

concentration of 1,5 x $10^8$ colony-forming units per milliliter [CFU/ml] of bacteria. Subsequently, the prepared suspension was smeared using a sterile cotton bud for four times to get a dense microorganic layer. After that, the plates were dried with half-open lid for a few minutes. Next, a hole with a diameter of 6 mm was pricked in the center of the plate using a glass-Pasteur pipette.

Then 60 µl of the eluate sampled from the Falcon tubes with the cement bodies in the step before was put in the hole. After that, the plates were incubated at 37°C for 24 hours under aerobic conditions. After the time span, the inhibition zones could be measured and the data was put in an Excel sheet. For this reason, a ruler was used to read off the diameter of the zone where no bacterial growth could be evaluated. The diameter was rounded to the nearest millimeter and the central hole was included in the measurement. To improve the precision of the results, they were measured in three varying angles and the mean value was calculated afterwards.

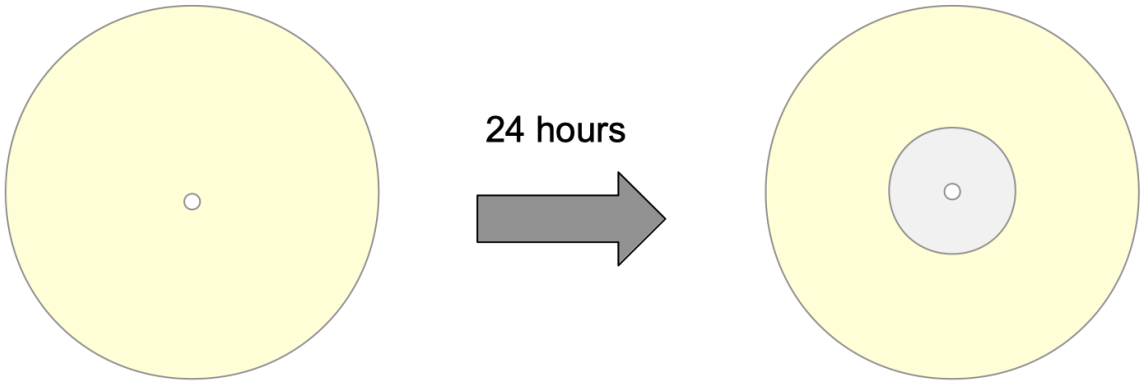



**Figure 40: Agar plate with inhibition zone after 24 hours**

## *2.6 Statistical Analysis*

The statistical analysis was conducted with the Microsoft software Excel. To evaluate the differences in mechanical properties the Student's t-test was performed. By using this method, it could be found out if there was a statistical significance in the reduction of the mechanical characteristics. The test was conducted using a two-sided distribution and checked for unpaired distribution. To identify the microbiological effectiveness, descriptive statistical analysis was performed since there were only three replicas of each combination of ALBC.

# 3 Results

## *3.1 Visual evaluation*

Inhomogeneities produced in the mixing process can have a significant impact on the mechanical stability of the hardened ALBC. On the contrary, it has been discussed, that small cracks and entrapped air facilitate the elution process. Fosfomycin-Trometamol is available in granulated form and all the other antimicrobial substances are powders. In order to improve homogenization, it was decided to ground Fosfomycin-Trometamol in a mortar. Afterwards, it was easy to homogenize by using fractured admixing.

Notably, the antibiotics containing Fosfomycin formed small aggregates with PMMA. After adding the monomer liquid to the antibiotic-loaded polymer powder inhomogeneities were minimally visible.

**Figure 41: from left to right: Meropenem, Fosfomycin-Trometamol, Fosfomycin-Sodium. Meropenem is homogenous, whereas ALBS with Fosfomycin formed small aggregates.**

Before using the hardened cement bodies for mechanical or microbiological testing, they were visually investigated whether they had inhomogeneities, cracks, entrapped air or other visible damage. Those were excluded for the following experiments.

## *3.2 Mechanical assessment*

### 3.2.1 Impact strength

The test for impact strength was performed according to DIN 53435 and revealed the values in table 14. Since there is no exact limit that should be reached given by the DIN 53435 guideline, it was decided that the ALBC should

not undercut the limit of 80% of the reference Palacos® R+G without the added antibiotic powder.

| Type of combination | Mean value (kJ/m2) | Percentual decrease % | p-value |
|---|---|---|---|
| Palacos R+G reference | 3 | | |
| Palacos R+G Meropenem 2g | 2,56 | -15% | 0,026 |
| Palacos R+G Imipenem 4g | 1,9 | -37% | 5,11*10-6 |
| Palacos R+G Fosfo-Trom 2g | 2,33 | -22% | 0,00049 |
| Palacos R+G Fosfo-Trom 3g | 2,14 | -29% | 5,51*10-5 |
| Palacos R+G Fosfo-Sodium 2g | 3,07 | 2% | 0,72 |
| Palacos R+G Fosfo-Sodium 5g | 2,39 | -20% | 0,00088 |

**Table 14: Results of impact strength test**

The mean value for Palacos® R+G bone cement without manually added antibiotics showed 3 kJ/m2 in the impact strength test. All of the other combinations except for Fosfomycin-Sodium 2 g showed a significant decrease in the impact strength. This combination yielded a 2% higher mean impact strength than the reference.

Meropenem 2 g showed the lowest reduction which is by 15%. Fosfomycin-Sodium 5 g with 20%, Fosfomycin-Trometamol 2 g with 22% and Fosfomycin-Trometamol 3 g with 29% follow. Imipenem shows the highest reduction in impact strength shows with 37%.

Given the limit of 80% of the reference Palacos® R+G without added antibiotics, only the combination with Meropenem 2 g, Fosfomycin-Sodium 2 g and Fosfomycin-Sodium 5 g reached the expectation limit. With a decrease of 22%, Fosfomycin-Trometamol 2 g is slightly below the limit.

Table 15 shows the direct difference between ALBC containing Meropenem and Imipenem in contrast to the reference Palacos® R+G without manually added antibiotic powder. Whereas Meropenem shows the lowest reduction (15%) in impact strength test, Imipenem shows the highest one with 37%. Both decreases are statistically relevant with a p-value <0,05.

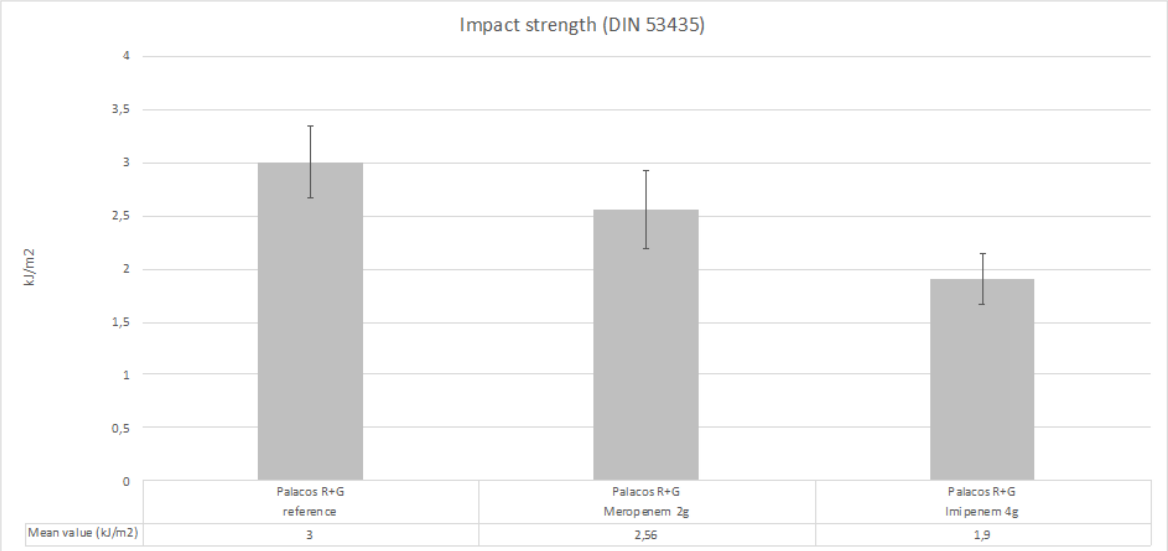



**Table 15: Comparison of ALBC with Meropenem and Imipenem in impact strength test with the reference Palacos® R+G**

In table 16 the differences between the two Fosfomycin salts are depicted. Fosfomycin-Sodium 2 g even shows an increase of 2% in comparison with the reference bone cement. The results for Fosfomycin-Sodium 5 g and Fosfomycin-Trometamol 2 g are nearly equal with 20% for Sodium and 22% for Trometamol. With an average of 2,14 kJ/m2 Fosfomycin-Trometamol 3 g showed the lowest impact strength stability.

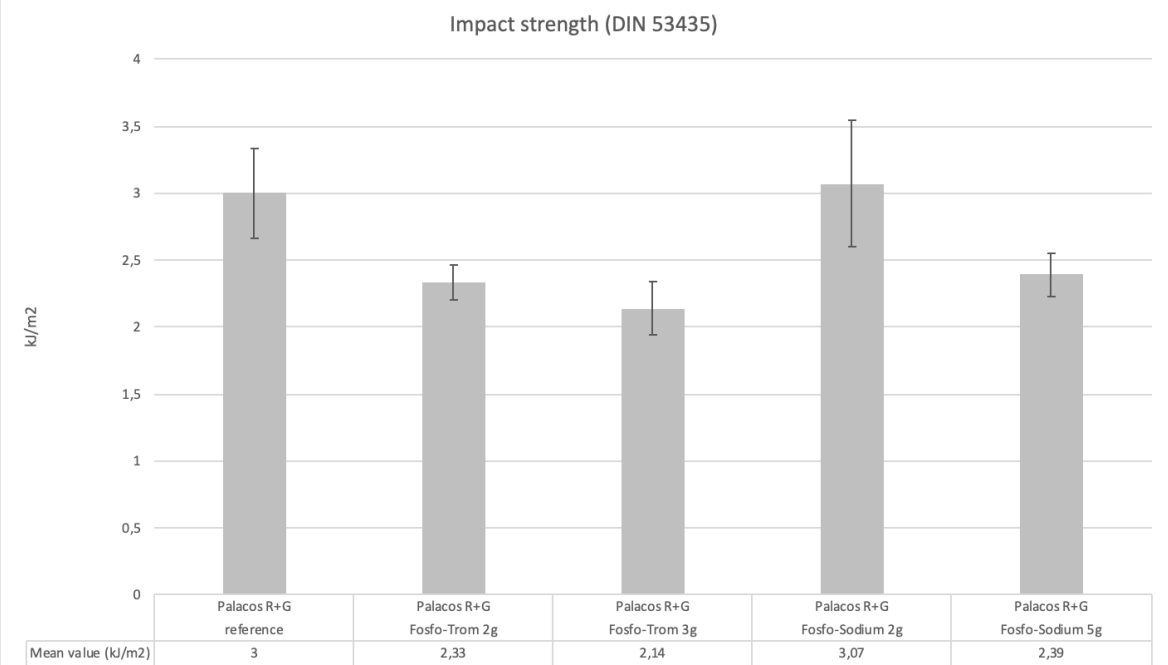
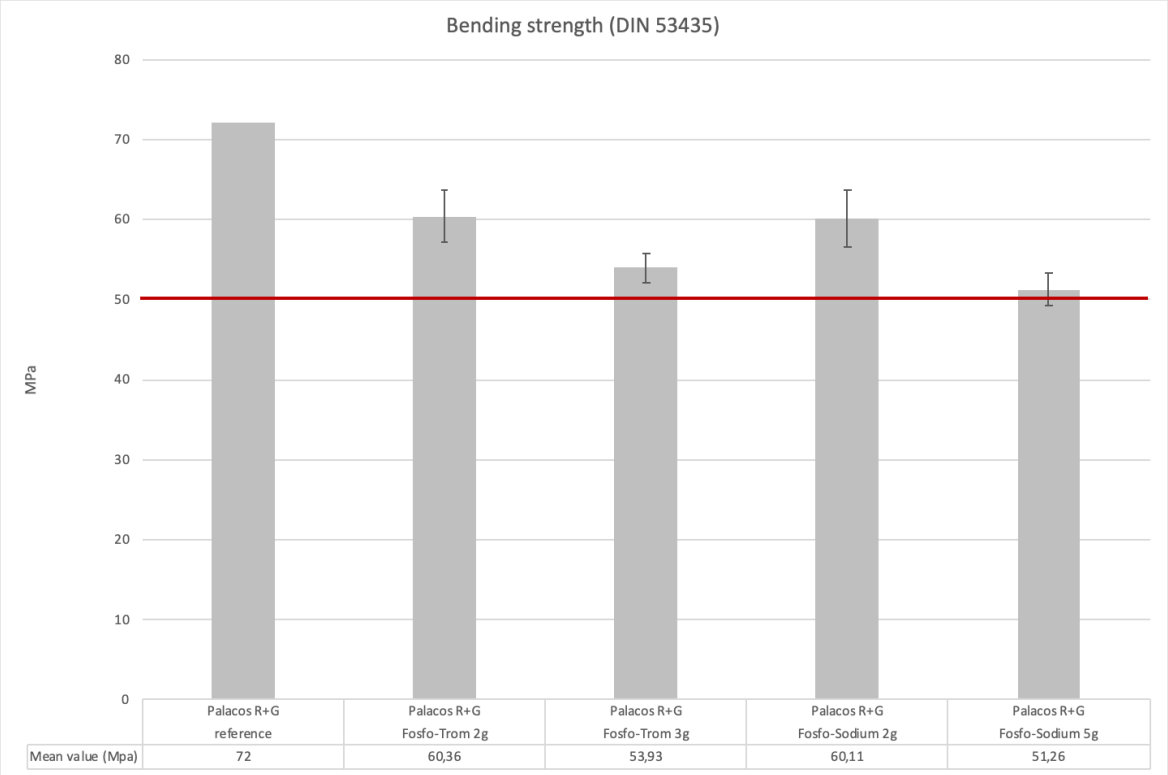



**Table 16: Comparison of ALBC with Fosfomycin-Sodium and Fosfomycin-Trometamol in impact strength test with the reference Palacos® R+G**

### 3.2.2 Bending strength

The Bending strength test was conducted according to DIN 53435. The limit of 50 MPa should be reached in order to secure the mechanical features of acrylic bone cements. The mean value of Palacos® R+G without manually added antibiotic powder is 72 MPa.




| | Palacos R+G reference | Palacos R+G Fosfo-Trom 2g | Palacos R+G Fosfo-Trom 3g | Palacos R+G Fosfo-Sodium 2g | Palacos R+G Fosfo-Sodium 5g |
|---|---|---|---|---|---|
| Mean value (Mpa) | 72 | 60,36 | 53,93 | 60,11 | 51,26 |

**Table 17: Results of Fosfomycin salts in bending strength test**

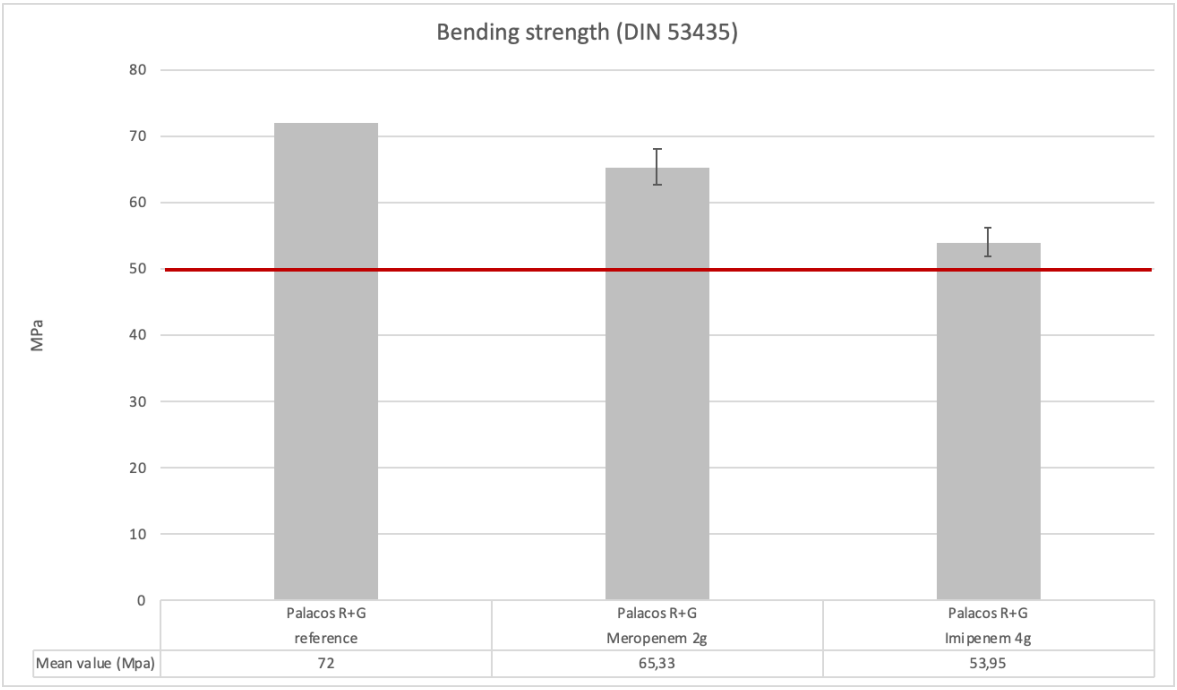



**Table 18: Results of Meropenem and Imipenem in bending strength test**

### 3.2.3 Compressive strength

This test was performed according to ISO 5833 and the standards to be met should be at least 70 MPa.

| Replica | Palacos R+G reference | Fosfo-Trom 2g | Fosfo-Sodium 2g | Fosfo-Sodium 5g |
|---|---|---|---|---|
| **1** | 89,72 | 79,37 | 77,52 | 71,72 |
| **2** | 86,01 | 80,89 | 74,89 | 71,11 |
| **3** | 84,98 | 81,53 | 72,87 | 72,33 |
| **4** | 81,2 | 81,16 | 72,22 | 71,45 |
| **5** | 86,1 | 79,91 | 76,64 | 70,72 |
| **6** | | 78,34 | 74,48 | 70,57 |
| **7** | | 79,64 | 74,18 | 72,21 |
| **8** | | 79,32 | 75,4 | 69,94 |
| **Mean value** | 85,60 | 80,02 | 74,78 | 71,26 |
| **Standard deviation** | 3,05 | 1,08 | 1,77 | 0,83 |
| **Percentage decrease** | | 7% | 13% | 17% |
| **P-value** | | 0,013 | 0,00044 | 0,00031 |

**Table 19: Results of compressive strength test**

The mean value of the reference Palacos® R+G is 85,6 MPa. All the other combinations show a statistically significant reduction in compressive strength. Fosfomycin-Trometamol 2 g shows the lowest decrease by 7%, Fosfomycin-Sodium 2 g by 13% and Fosfomycin-Sodium 5 g by 17%. The direct comparison between the same amount of Fosfomycin-Trometamol and Fosfomycin-Sodium shows, that Fosfomycin-Trometamol has more compressive strength (decrease of 7%) than Fosfomycin-Sodium (decrease of 13%). However, the mean values of all

of the tested combinations lay above the mark of 70 MPa and so they can be recommended for clinical use.

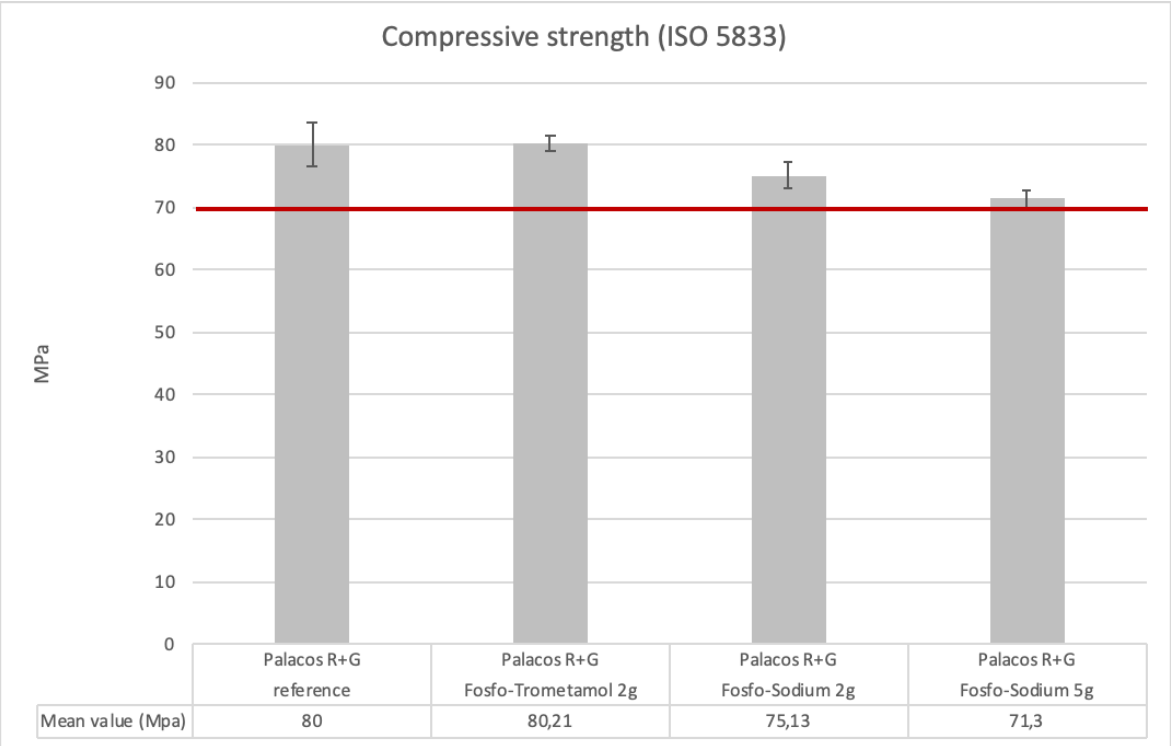



**Table 20: Comparison of ALBC with Fosfomycin-Trometamol 2 g, Fosfomycin-Sodium 2 g, Fosfomycin-Sodium 5 g and Palacos® R+G. The red line marks the test limit of 70 MPa.**

### 3.2.4 4-point bending strength

| Replica | Palacos R+G reference | Meropenem 2g | Imipenem 4g | Fosfo-Trom 2g | Fosfo-Trom 3g | Fosfo-Sodium 2g | Fosfo-Sodium 5g |
|---|---|---|---|---|---|---|---|
| 1 | 67,8 | 60,3 | 53,3 | 58,2 | 52,4 | 58,7 | 46,8 |
| 2 | 69,1 | 55,6 | 52,8 | 57,1 | 50,5 | 55,5 | 48,4 |
| 3 | 69,1 | 64 | 50,6 | 56,3 | 49,5 | 58 | 47 |
| 4 | 66,4 | 64,1 | 52,9 | 56 | 50 | 59 | 48,2 |
| 5 | 67,5 | | 51,7 | 57,3 | 50,9 | 58,6 | 48,9 |
| 6 | | | 50,8 | 55,7 | 51,6 | 56,7 | 47,3 |
| Mean value | 67,98 | 61 | 52,02 | 56,77 | 50,82 | 57,75 | 47,77 |
| Standard deviation | 1,15 | 4,01 | 1,15 | 0,94 | 1,06 | 1,37 | 0,85 |
| Percentage decrease | | 10% | 23% | 16% | 25% | 15% | 30% |
| P-value | | 0,036 | 4,72E-09 | 1,61E-07 | 3,23E-09 | 2,89E-07 | 3,70E-09 |

**Table 21: Results of 4-point bending strength test**

This test was performed according to ISO 5833 and to fulfill the criteria 50 MPa are needed.

The mean value of Palacos® R+G bone cement without additionally added antibiotics is 67,98 MPa. The addition of Meropenem 2 g reduced the bending strength by 10% to an average of 61 MPa, which is already statistically significant. The same amount of antimicrobial active substance of Imipenem showed a reduction of 23% (52,02 MPa). Fosfomycin-Trometamol 2 g and Fosfomycin-Sodium 2 g showed approximately the same mean value in bending strength (16%

vs. 15%). Fosfomycin-Trometamol 3 g yielded a reduction of 25% and Fosfomycin-Sodium 5 g of 30%, meaning that Fosfomycin-Sodium 5 g does not reach the limit of 50 MPa and cannot be recommended for unhesitating clinical usage.

Although both Meropenem 2 g and Imipenem 4 g show a statistically significant reduction in bending strength, both exceed the limit of 50 MPa (table 22).

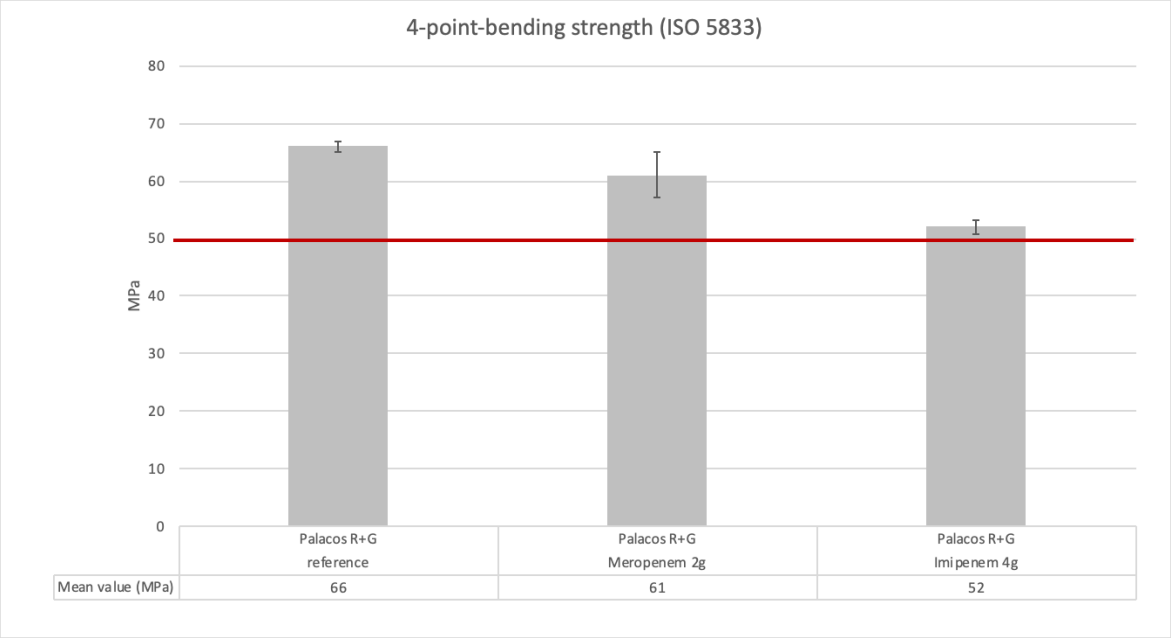



**Table 22: Comparison of 4-point-bending strength of Meropenem 2 g and Imipenem 4 g to the reference Palacos® R+G**

Fosfomycin-Trometamol 2 g and Fosfomycin-Sodium 2 g show nearly equal results and can be recommended, whereas Fosfomycin-Trometamol 3 g barely reaches the limit of 50 MPa and Fosfomycin-Sodium 5 g cannot be recommended since the limit is not reached anymore with an average bending strength of 47,8 MPa (table 23).

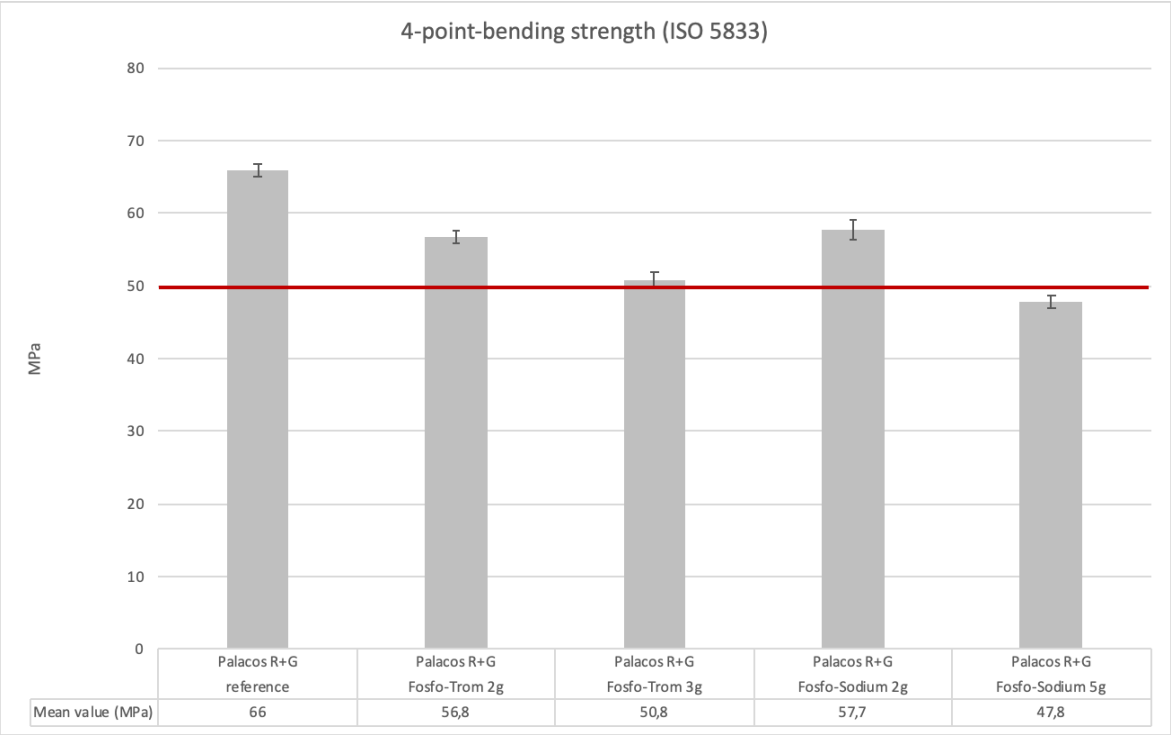



**Table 23: Comparison of Fosfomycin-Trometamol and Fosfomycin-Sodium in 4-point-bending strength with the reference Palacos® R+G**

### 3.2.5 Bending modulus

According to ISO 5833, PMMA bone cements should meet a limit for bending modulus of 1800 MPa.

Interestingly, the experiment showed that bone cement with 2 g Meropenem and with 4 g Imipenem augments the bending modulus by 3% vs. 6%. On the contrary, the Fosfomycin salts showed lower bending modulus. Fosfomycin-Trometamol 2 g yielded a slight decrease of 1%, which is not statistically significant. Fosfomycin-Trometamol 3 g with a reduction of 4%, Fosfomycin-Sodium 2 g with 8% and Fosfomycin-Sodium 5 g with 16% follow, which is already statistically significant. In the bending modulus test the Fosfomycin-Trometamol mixtures showed better results than the Fosfomycin-Sodium ones.

| Replica | Palacos R+G reference | Meropenem 2g | Imipenem 4g | Fosfo-Trom 2g | Fosfo-Trom 3g | Fosfo-Sodium 2g | Fosfo-Sodium 5g |
|---|---|---|---|---|---|---|---|
| **1** | 2770 | 2911 | 3040 | 2806 | 2740 | 2633 | 2362 |
| **2** | 2829 | 2850 | 2964 | 2793 | 2681 | 2508 | 2356 |
| **3** | 2775 | 2915 | 2933 | 2809 | 2715 | 2563 | 2367 |
| **4** | 2834 | 2870 | 3015 | 2682 | 2705 | 2651 | 2301 |
| **5** | 2780 | | 2993 | 2829 | 2654 | 2633 | 2373 |
| **6** | | | 2908 | 2760 | 2573 | 2500 | 2371 |
| **Mean value** | 2797,6 | 2886,5 | 2975,5 | 2779,83 | 2678 | 2581,33 | 2355 |
| **Standard deviation** | 31,20 | 31,71 | 50,04 | 53,09 | 59,25 | 67,11 | 27,17 |
| **Percentage decrease** | | 3% | 6% | -1% | -4% | -8% | -16% |
| **P-value** | | 0,005 | 7,073E-05 | 0,510 | 0,003 | 0,0002 | 6,507E-09 |

**Table 24: Results of bending modulus test**

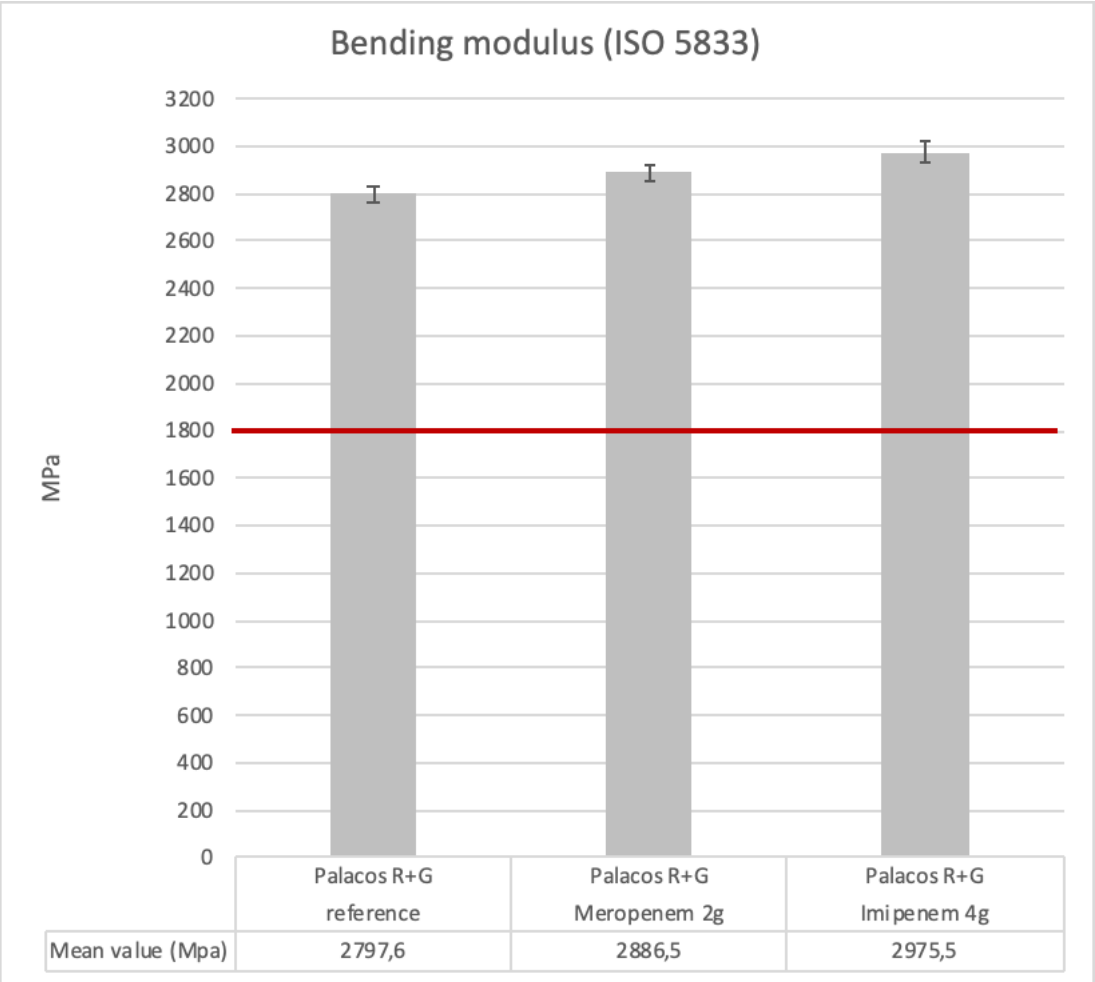



**Table 25: Results of Meropenem and Imipenem in bending modulus test**

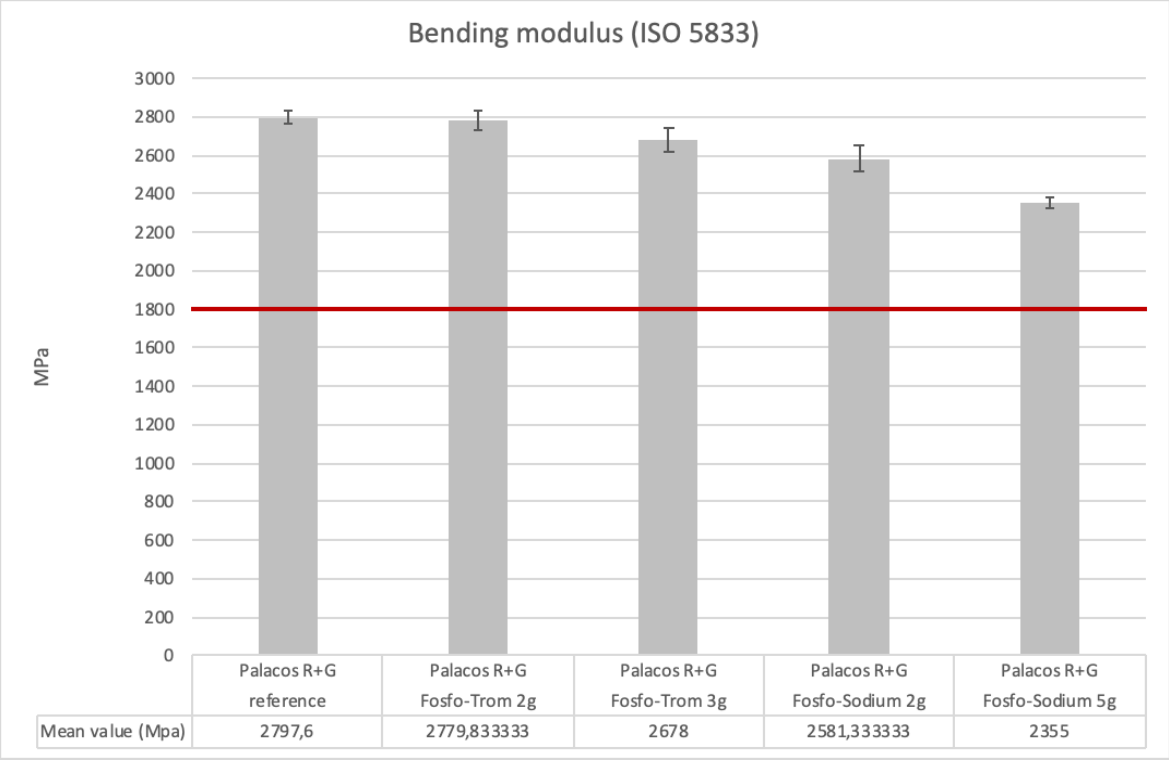



**Table 26: Results of Fosfomycin salts in bending modulus test**

## *3.3 Summary of mechanical test results*

### 3.3.1 Meropenem and Imipenem

In direct comparison, Meropenem shows better mechanical features than Imipenem in 4-point bending strength test, impact strength test and bending strength test according to ISO 5833 and DIN 53435. Nevertheless, Imipenem shows a higher bending modulus than Meropenem, given the fact, that both lay above the results of the reference Palacos® R+G. Meropenem 2 g reaches every limit given by ISO 5833 and DIN 53435 in every test, so the clinical usage is mechanically safe. For Imipenem 4 g, the impact strength lies under the 80% limit with a decrease of 37%.

### 3.3.2 Fosfomycin-Trometamol and Fosfomycin-Sodium

Fosfomycin-Trometamol 2 g shows the best results among the Fosfomycin salts in compressive strength and bending modulus, whereas Fosfomycin-Sodium 2 g has better mechanical features in impact strength. The results in bending strength according to DIN 53435 and 4-point-bending strength are nearly equal. Fosfomycin-Sodium 2 g and Fosfomycin-Sodium 5 g reach the limit of 80% of the reference data in impact strength test, whereas Fosfomycin-Trometamol 2 g and 3 g do not reach the limit with a decrease of 22% and 29%. Mechanical features of all Fosfomycin salts reach the given limit in compressive strength, bending strength according to DIN 53435 and bending modulus. In 4-point-bending test 5 g of Fosfomycin-Sodium does not meet the requirement.

## *3.4 Microbiological assessment*

The microbiological properties of the tested ALBC were investigated in an inhibition zone assay. Each combination of antibiotic added PMMA was tested three times. The diameter was measured with a ruler and recorded. Then the mean value was calculated and listed in a table. To improve the graphical illustration the values are accentuated with colors. Red shades stand for small inhibition zones. Orange and yellow follow whereas green ones stand for large inhibition zones.

### 3.4.1 Meropenem

Three different combinations of Meropenem were tested: Palacos® R with 1 g Meropenem, Palacos® R+G with 2 g Meropenem and Copal® G+C with 1 g Meropenem. Meropenem ALBC shows an inhibition zone against all tested strains over the whole testing period of 42 days.

| E.coli | 1h | 24h | 7d | 14d | 28d | 42d |
|---|---|---|---|---|---|---|
| Palacos R + Meropenem 1g | 24 | 26 | 24 | 18 | 16 | 17 |
| Palacos R+G + Meropenem 2g | 26 | 28 | 28 | 20 | 20 | 20 |
| Copal G+C + Meropenem 1g | 25 | 26 | 25 | 21 | 20 | 21 |

**Table 27: Inhibition zone in mm after the listed time spans. (h=hour, d= day)**

For E. coli (table 27) the inhibition zones are approximately the same for all of the combinations for each measuring point, but the combination of Palacos® R+G with Meropenem 2 g shows a slightly larger inhibition zone. At the 24 h and 7 d time point the maximum was registered with a mean diameter of 28 mm. After 42 d the inhibition zone against E. coli does not fall below 17 mm diameter. Overall, the inhibition zones for E. coli show excellent results until the time span of 7 days and good results until 42 days.

| Proteus mirabilis | 1h | 24h | 7d | 14d | 28d | 42d |
|---|---|---|---|---|---|---|
| Palacos R + Meropenem 1g | 24 | 26 | 24 | 19 | 18 | 17 |
| Palacos R+G + Meropenem 2g | 25 | 28 | 27 | 17 | 20 | 21 |
| Copal G+C + Meropenem 1g | 25 | 27 | 26 | 22 | 21 | 14 |

**Table 28: Inhibition zones in mm after the specific time spans. (h=hour, d=day)**

Next, the antimicrobial activity against Proteus mirabilis was tested. Similar results as for E. coli can be seen. Over the first seven days the elution and activity against the tested strain is excellent with a maximum of 28 mm in diameter for Meropenem 2 g with Palacos® R+G after 24 hours. In general, the different combinations of antibiotic and PMMA cement show nearly equal inhibition zones. The activity against Proteus mirabilis lasts until the last measuring point of 42 days and does not undercut 14 mm in diameter. Palacos® R+G with Meropenem 2 g even shows an inhibition zone of 21 mm after 42 days.

| Pseudomonas aeruginosa | 1h | 24h | 7d | 14d | 28d | 42d |
|---|---|---|---|---|---|---|
| Palacos R + Meropenem 1g | 24 | 24 | 19 | 6 | 6 | 5 |
| Palacos R+G + Meropenem 2g | 27 | 31 | 26 | 16 | 18 | 10 |
| Copal G+C + Meropenem 1g | 25 | 24 | 23 | 19 | 20 | 14 |

**Table 29: Inhibition zones in mm after specific time spans. (h=hour, d=day)**

For Pseudomonas aeruginosa, a difference to the inhibition zones of E. coli and Proteus mirabilis can be seen. The combination of 1 g Meropenem with Palacos® R shows inhibition zones from 19 mm – 24 mm, but after 14 days they are reduced to only 6 and 5 mm in diameter. The second combination shows excellent results though with a maximum diameter of 31 mm. After 14 d a cut-off can be seen and the inhibition zones are much smaller with maximum of only 18 mm after 28 days. Combination C with Copal® G+C shows continuous inhibition and does not fall under 14 mm after 42 days.

In general, Meropenem ALBC shows excellent results against the tested gram-negative bacteria.

### 3.4.2 Imipenem

Imipenem was also tested in three different combinations: Palacos® R with 2 g Imipenem, Palacos® R+G with 4 g Imipenem and Copal® G+C with 2 g Imipenem. As Imipenem is dependent on Cilastatin, which shows no antimicrobial activity itself, more substance was added to get the same amount of active antibacterial substance. In general, the inhibition zones for Imipenem range from 0 – 20 mm in diameter.

| E.coli | 1h | 24h | 7d | 14d | 28d | 42d |
|---|---|---|---|---|---|---|
| Palacos R + Imipenem 2g | 0 | 0 | 0 | 6 | 4 | 0 |
| Palacos R+G + Imipenem 4g | 17 | 18 | 18 | 16 | 17 | 14 |
| Copal G+C + Imipenem 2g | 18 | 19 | 18 | 18 | 18 | 17 |

**Table 30: Inhibition zones in mm after a specific time span. (h=hour, d=day)**

For E. coli the activity of Palacos® R with 2 g Imipenem can only be seen in the measuring point of 14 days and 28 days and is very small with only 4 mm or rather 6 mm in diameter. However, the inhibition zones for Palacos® R+G with 4 g Imipenem and Copal® G+C with 2 g Imipenem show constant elution and antimicrobial activity for the full time span of measurement. The zones range from 14 – 19 mm.

| Proteus mirabilis | 1h | 24h | 7d | 14d | 28d | 42d |
|---|---|---|---|---|---|---|
| Palacos R + Imipenem 2g | 0 | 0 | 0 | 7 | 6 | 0 |
| Palacos R+G + Imipenem 4g | 18 | 17 | 20 | 17 | 17 | 17 |
| Copal G+C + Imipenem 2g | 19 | 20 | 20 | 16 | 18 | 18 |

**Table 31: Inhibition zones in mm after a specific time span. (h=hour, d=day)**

For Proteus mirabilis, similar results can be seen. Whereas the combination of Palacos® R + Imipenem 2 g only shows measurable inhibition zones after 14 days and 28 days, steady zones were found for Palacos® R+G with Imipenem 4 g and Copal® G+C with Imipenem 2 g. The maximum inhibition zones were measured in the combination of Palacos® R+G with Imipenem 4 g after 7 days with 20 mm and in Copal® G+C with Imipenem 2 g in 24 hours and 7 days with 20 mm too.

| Pseudomonas aeruginosa | 1h | 24h | 7d | 14d | 28d | 42d |
|---|---|---|---|---|---|---|
| Palacos R + Imipenem 2g | 0 | 0 | 0 | 2 | 3 | 0 |
| Palacos R+G + Imipenem 4g | 13 | 14 | 16 | 13 | 15 | 12 |
| Copal G+C + Imipenem 2g | 13 | 15 | 18 | 16 | 17 | 13 |

**Table 32: Inhibition zones in mm after a specific time span. (h=hour, d=day)**

For Pseudomonas aeruginosa, the inhibition zones do not reach the same amount of diameter as for E. coli or Proteus mirabilis. The maximum of 18 mm in diameter can be seen in combination Copal® G+C and Imipenem 2 g after 7 days of elution. The combination Palacos® R with Imipenem 2 g shows barely measurable zones in the two time spans already mentioned.

### 3.4.3 Fosfomycin-Trometamol

Fosfomycin-Trometamol was tested in six different combinations and one extra combination mixed with the Pro-system.

| MRSA | 1h | 24h | 7d | 14d | 28d | 42d |
|---|---|---|---|---|---|---|
| Palacos R + Fosfomycin-Trom 1g | 11 | 13 | 18 | 0 | 0 | 7 |
| Palacos R + Fosfomycin-Trom 3g | 24 | 19 | 28 | 11 | 8 | 0 |
| Palacos R+G + Fosfomycin-Trom 1g | 12 | 19 | 19 | 0 | 0 | 0 |
| Palacos R+G + Fosfomycin-Trom 2g | 18 | 15 | 19 | 6 | 5 | 0 |
| Palacos R+G + Fosfomycin-Trom 2g (Pro) | 16 | 19 | 27 | 4 | 6 | 0 |
| Palacos R+G + Fosfomycin-Trom 3g | 16 | 21 | 26 | 12 | 14 | 0 |
| Copal G+V + Fosfomycin-Trom 2g | 19 | 20 | 29 | 19 | 20 | 18 |

**Table 33: Inhibition zones in mm after a specific time span. (h=hour, d=day)**

For MRSA Copal® G+V with 2 g Fosfomycin-Trometamol shows best results in the inhibition zone assay with a maximum of 29 mm in diameter after 7

days. This combination is the only one which shows good results even after 14 days with inhibition zones of 19 mm after 14 days, 20 mm after 28 days and 18 mm after 42 days. Palacos® R and Palacos® R+G each combined with Fosfomycin-Trometamol 1 g show similar results. Nevertheless, the inhibition zones were small with a maximum of 18 and 19 mm and no inhibition zone after 14 days, except for Palacos® R with 1 g of Fosfomycin-Trometamol which showed an inhibition zone of 7 mm after 42 days. The combination of Palacos® R and R+G with Fosfomycin-Trometamol 3 g showed good results. A cut-off with much smaller inhibition zones after 14 days can be seen too. The combination of Palacos® R+G with Fosfomycin-Trometamol 2 g mixed manually and mixed with the Pro-system showed equal results, but there is an outlier after 7 days with an inhibition zone which is 8 mm bigger in the sample produced with the pro-system than the manually mixed one.

| Proteus mirabilis | 1h | 24h | 7d | 14d | 28d | 42d |
|---|---|---|---|---|---|---|
| Palacos R + Fosfomycin-Trom 1g | 21 | 24 | 19 | 21 | 18 | 12 |
| Palacos R + Fosfomycin-Trom 3g | 26 | 31 | 30 | 27 | 25 | 19 |
| Palacos R+G + Fosfomycin-Trom 1g | 24 | 27 | 28 | 21 | 22 | 16 |
| Palacos R+G + Fosfomycin-Trom 2g | 24 | 30 | 32 | 26 | 27 | 21 |
| Palacos R+G + Fosfomycin-Trom 2g (Pro) | 25 | 30 | 33 | 26 | 27 | 22 |
| Palacos R+G + Fosfomycin-Trom 3g | 27 | 30 | 33 | 27 | 29 | 23 |
| Copal G+V + Fosfomycin-Trom 2g | 26 | 31 | 33 | 27 | 27 | 21 |

**Table 34: Inhibition zones in mm after a specific time span. (h=hour, d=day)**

In general, all combinations tested had inhibition zones even after 42 days against Proteus mirabilis. The smallest inhibition zone (12 mm) was shown by Palacos® R+G with the minimum amount of Fosfomycin-Trometamol which is 1 g after 42 days. All combinations with more than 1 g Fosfomycin-Trometamol added showed nearly equal results. Nevertheless, the combination Palacos® R + Fosfomycin-Trometamol 1 g and Palacos® R+G + Fosfomycin-Trometamol 1 g showed good results too, but they are inferior to the ones with a higher admixture of antibiotic substance. The largest inhibition zone (33 mm) was shown by Palacos® R+G with 2 g of Fosfomycin-Trometamol mixed with the pro-system together with Palacos® R+G with Fosfomycin-Trometamol 3 g and Copal® G+C with Fosfomycin-Trometamol 2 g after a time span of 7 days.

| Pseudomonas aeruginosa | 1h | 24h | 7d | 14d | 28d | 42d |
|---|---|---|---|---|---|---|
| Palacos R + Fosfomycin-Trom 1g | 22 | 18 | 20 | 11 | 10 | 0 |
| Palacos R + Fosfomycin-Trom 3g | 21 | 28 | 32 | 23 | 27 | 19 |
| Palacos R+G + Fosfomycin-Trom 1g | 16 | 19 | 21 | 12 | 14 | 8 |
| Palacos R+G + Fosfomycin-Trom 2g | 24 | 26 | 27 | 21 | 20 | 13 |
| Palacos R+G + Fosfomycin-Trom 2g (Pro) | 25 | 25 | 29 | 22 | 25 | 14 |
| Palacos R+G + Fosfomycin-Trom 3g | 25 | 31 | 35 | 24 | 28 | 14 |
| Copal G+V + Fosfomycin-Trom 2g | 23 | 27 | 32 | 22 | 25 | 14 |

**Table 35: Inhibition zones in mm after a specific time span. (h=hour, d=day)**

Inhibition zones against Pseudomonas aeruginosa showed similar results as against Proteus mirabilis. All combinations showed zones at each point of measurement, except Palacos® R + Fosfomycin-Trometamol 1 g after the longest time span of 42 days. The best combination in this line was Palacos® R+G with Fosfomycin-Trometamol 3 g. The combinations to which 1 g of antibiotic powder was added are inferior to those with more manually added antimicrobial substance. The combination mixed with the pro-system shows equal results in comparison with the manually mixed one.

### 3.4.4 Fosfomycin-Sodium

Six different combinations of PMMA bone cement (with and without loaded antibiotics) and manually added antimicrobial substances (Fosfomycin-Sodium) were tested against MRSA, Proteus mirabilis and Pseudomonas aeruginosa.

| MRSA | 1h | 24h | 7d | 14d | 28d | 42d |
|---|---|---|---|---|---|---|
| Palacos R + Fosfomycin-Sodium 1g | 13 | 21 | 18 | 2 | 5 | 0 |
| Palacos R + Fosfomycin-Sodium 5g | 28 | 30 | 36 | 23 | 27 | 21 |
| Palacos R+G + Fosofomycin-Sodium 1g | 9 | 13 | 20 | 4 | 8 | 0 |
| Palacos R+G + Fosofomycin-Sodium 2g | 27 | 21 | 26 | 13 | 15 | 3 |
| Palacos R+G + Fosofomycin-Sodium 5g | 30 | 28 | 35 | 28 | 26 | 18 |
| Copal G+V + Fosfomycin-Sodium 2g | 27 | 21 | 27 | 19 | 19 | 18 |

**Table 36: Inhibition zones in mm after a specific time span. (h=hour, d=day)**

Large inhibition zones were shown by combinations of Palacos® R with Fosfomycin-Sodium 5 g and Palacos® R+G with the same amount of antibiotic powder (Fosfomycin-Sodium). Those combinations showed the largest inhibition zone with a diameter of 36 and 35 mm after 7 days. Those two combinations had a sufficient antimicrobial activity even after 42 days. The combinations with 1 g Fosfomycin-Sodium showed good activity until 7 days, but after 14 days there were only small inhibition zones left. 2 g of Fosfomycin-Sodium showed better

activity against MRSA combined with Copal® G+C than together with Palacos® R+G PMMA bone cement.

| Proteus mirabilis | 1h | 24h | 7d | 14d | 28d | 42d |
|---|---|---|---|---|---|---|
| Palacos R + Fosfomycin-Sodium 1g | 22 | 25 | 29 | 23 | 23 | 19 |
| Palacos R + Fosfomycin-Sodium 5g | 34 | 34 | 37 | 33 | 35 | 31 |
| Palacos R+G + Fosofomycin-Sodium 1g | 25 | 25 | 35 | 25 | 27 | 21 |
| Palacos R+G + Fosofomycin-Sodium 2g | 27 | 29 | 33 | 27 | 29 | 25 |
| Palacos R+G + Fosofomycin-Sodium 5g | 35 | 33 | 37 | 34 | 34 | 31 |
| Copal G+V + Fosfomycin-Sodium 2g | 32 | 29 | 33 | 31 | 29 | 24 |

**Table 37: Inhibition zones in mm after a specific time span. (h=hour, d=day)**

The activity measured in inhibition zones of Fosfomycin-Sodium against Proteus mirabilis showed excellent results, with a minimum diameter of 19 mm after 42 days in the combination of Palacos® R + Fosfomycin-Sodium 1 g. The maximum inhibition zones (37 mm) were shown by combinations with the highest amount (5 g) of manually added antimicrobial substance. Each combination with the same amount of added antibiotic powder showed equal inhibition zones than the other one.

| Pseudomonas aeruginosa | 1h | 24h | 7d | 14d | 28d | 42d |
|---|---|---|---|---|---|---|
| Palacos R + Fosfomycin-Sodium 1g | 18 | 23 | 25 | 19 | 24 | 16 |
| Palacos R + Fosfomycin-Sodium 5g | 32 | 32 | 36 | 33 | 35 | 29 |
| Palacos R+G + Fosofomycin-Sodium 1g | 18 | 22 | 26 | 18 | 25 | 14 |
| Palacos R+G + Fosofomycin-Sodium 2g | 25 | 30 | 28 | 26 | 30 | 21 |
| Palacos R+G + Fosofomycin-Sodium 5g | 32 | 33 | 37 | 33 | 34 | 29 |
| Copal G+V + Fosfomycin-Sodium 2g | 27 | 30 | 30 | 26 | 28 | 19 |

**Table 38: Inhibition zones in mm after a specific time span. (h=hour, d=day)**

In general, in testing Fosfomycin-Sodium against Pseudomonas aeruginosa, no inhibition zones with a diameter below 14 mm were detected. The best antimicrobial activity was shown by both combinations with 5 g of additionally added antibiotic substance with maximum inhibition zones of 36 mm and 37 mm after 7 days. 5 g added to Palacos® R and Palacos® R+G showed nearly equal results. The combinations with 2 g Fosfomycin-Sodium are superior to those with only 1 g of antibiotic substance.

# 4 Discussion

The treatment of periprosthetic joint infection is often challenging and includes surgical debridement, one or two-stage revision surgery and adequate antimicrobial therapy. If there is a two-stage revision surgery including the implantation of a spacer, the spacer itself can be used as a local antibiotic carrier. Using antibiotic powder in acrylic bone cement increases the drug level locally in the tissue because of the releasement from the bone cement itself.

Only a few antibiotics have been tested so far for the local usage in PMMA cement. Hence the study was designed to investigate two Carbapenems and two Fosfomycin salts and whether they are suitable for local antibiotic therapy in ALBC for the treatment of PJI. The PMMA bone cements Palacos®R, Palacos®R+G and Copal®G+C were admixtured with different dosages of Meropenem, Imipenem, Fosfomycin-Trometamol and Fosfomycin-Sodium. The aim of this study was to find the compromise between an ALBC with effective antimicrobial efficacy while maintaining its mechanical features.

To investigate the mechanical features of the different antibiotic-loaded bone cements, standardized mechanical tests according to DIN 53435 and ISO 5833 specifications were performed. Therefore, DIN impact strength, ISO bending strength, ISO bending modulus and ISO compressive strength served as indicators of preserved mechanical properties.

The elution of antibiotic substance was tested for each combination of ALBC in inhibition zone essays to evaluate the antimicrobial efficacy and predict the clinical effectiveness of different bacteria. Three gram-negative bacteria (Proetus mirabilis, Pseudomonas aeruginosa and E. coli) and one gram-positive (MRSA) were tested since those are often potential triggers of periprosthetic joint infections.

## *4.1 Mechanical aspects*

As expected in advance, the addition of antibiotic powder to PMMA cement reduces the mechanical features in most of the combinations tested in our experiments. The combination of Fosfomycin-Sodium 2 g in Palacos® R+G yielded a 2% higher mean impact strength than the reference without manually added antibiotic substance. Interestingly, the experiment showed that bone cement with 2 g Meropenem and with 4 g Imipenem each admixtured to Palacos®

R+G augments the bending modulus by 3% vs. 6%. Fosfomycin-Sodium 5 g cannot be recommended in clinical usage since the minimum limit in the bending strength test is not reached anymore with an average bending strength of 47,8 MPa.

In a study conducted by Gálvez-López et al., ALBC was produced by admixing antibiotics (Meropenem) at a weight/weight ratio of 10 and 20% (1 g of antibiotic per 10 g of PMMA and 2 g of antibiotic per 10 g of PMMA) to the PMMA. In general, they found similar mechanical properties for ALBC in comparison to pure acrylic bone cement in compression tests (IBERTEST® electromechanical twin-screw machine). The mean compression values were greater than 70 MPa for every sample (77). For this study, compressive strength tests were performed only with the Fosfomycin salts and yielded comparable results by topping the limit of 70 MPa for every tested combination.

A study conducted by Baleani et al. tested the effectiveness and mechanical property of ALBC containing Meropenem exclusively in combination with Vancomycin. The experiments showed that the admixture of antibiotics did not significantly reduce the compressive strength and all tested combinations of ALBC reached the minimum requirement of 70 MPa given by ISO 5833. The combination of the bone cement with 0.5 g of Vancomycin and 0.5 g of Meropenem did not reach the limit of 50 MPa for bending strength, hence the combination of 1 g of Vancomycin and 1 g of Meropenem was not even tested (73). The combination of 2 g Meropenem in Palacos® R+G reached the limit of 50 MPa with 61 MPa, but a direct comparison is not possible, since a higher dosage of Meropenem with no additional Vancomycin was tested.

A combination of an ALBC containing 1.25% Vancomycin and 1.25% Meropenem showed unchanged compressive strength and fatigue strength properties in a study by Persson et al. Bending strength (-14%) and the bending modulus (-9%) were only slightly reduced but still reached the limits set by ISO 5833 standard (75). There is no direct comparison possible since ALBC containing Vancomycin was not tested in this study in mechanical testing.

A study conducted by Chang et al. reported significant adverse effects on the mechanical aspects in compressive strength of ALBC containing 1 g of Imipenem. ALBC containing 1 g of Imipenem did not reach the 70 MPa limit with 67 MPa before elution and 55 MPa after (80). While for this study combinations

with Imipenem were not tested for their compressive strength, a higher dosage of Imipenem (4 g) showed mechanically safe results with 52 MPa according to ISO 5833 in 4-point bending strength test.

When compared directly, Meropenem shows better mechanical features than Imipenem in the 4-point bending strength test, impact strength test and bending strength test according to ISO 5833 and DIN 53435. ALBC containing Meropenem 2 g reaches every limit given by ISO 5833 and DIN 53435 in every test, so the clinical usage is mechanically safe. For Imipenem 4 g, the impact strength lies under the 80% limit with a decrease of 37%.

Mechanical features of all Fosfomycin salts reach the given limit in compressive strength and bending strength according to DIN 53435 and bending modulus. In the 4-point-bending test 5 g of Fosfomycin-Sodium does not meet the requirement anymore and hence cannot be recommended for safe clinical usage.

In our studies, we showed that ALBC containing up to 5% of Meropenem and ALBC containing up to 10% Imipenem did reach all the mechanical limits set by ISO 5833 and DIN 53435. For ALBC containing Fosfomycin, a combination of PMMA cement admixtured with up to 7,5% Fosfomycin-Trometamol is mechanically safe, whereas an ALBC containing 12,5% Fosfomycin-Sodium did not reach the limit in the 4-point-bending test and hence cannot be recommended for safe clinical usage. Fosfomycin-Sodium up to 5% showed sufficient mechanical stability.

There are no studies that investigate the mechanical aspects of Fosfomycin loaded bone cement. Therefore, no comparison of the results is feasible.

## *4.2 Microbiological activity*

### 4.2.1 E. coli

A study conducted by Sumant et al. described the biological effectiveness of Meropenem loaded ALBC against E. coli for a duration of three weeks (78). In addition, in our study antimicrobial activity for a total time span of six weeks could be observed.

In a study by Baleani et al. both, a combination of ALBC containing 0.5 g of Vancomycin and 0.5 g of Meropenem and a formulation with 1 g of Vancomycin and 1 g of Meropenem was highly effective against E. coli, whereas Vancomycin alone did not combat the growth of bacteria (73).

A study conducted by Andollina et al. tested ALBC containing the same combinations of Vancomycin and Meropenem as in the study by Baleani et al. mentioned above against E. coli. There was no growth of bacteria detectable for the whole time period of six days (74).

In a study by Chang et al. high-performance liquid chromatography was used in order to detect the concentrations of eluted antibiotics after the specific periods. The study showed that the antibiotic activity of ALBC containing 1 g of Imipenem only lasted for two days against E. coli. The study by Chang et al. revealed that the elusion efficacy of Imipenem from PMMA cement is inferior to ALBC containing other antimicrobial substances. In this study, other ALBCs containing other antibiotics had a preferable efficacy over a longer time span (80). In contrast, an acceptable antimicrobial efficacy of ALBC containing Imipenem could be detected in our experiments. In our experiment, PMMA cement containing 2 g of Imipenem did only produce inhibition zones with limited diameters. Conversely, ALBC prepared with Copal® G+C cement, which is admixed previously with Gentamicin and Clindamycin, containing the same amount of Imipenem does create constant inhibition zones with an average of 17 mm for the full time span of 42 days. In direct comparison with those two combinations, synergistic effects of improved elusion of Imipenem combined with Gentamycin and Clindamycin are to be assumed.

In testing Fosfomycin against E. coli a study conducted by Eick et al. reported that PMMA bone cement containing gentamicin in addition to Fosfomycin showed better antimicrobial activity of the eluate and lasted longer against strains being sensitive to both antibiotics when compared to Fosfomycin alone. In the study mentioned above, Fosfomycin showed excellent effectiveness against the tested E. coli strains (82). Although the study used inhibition zone assays too, a direct comparison between the results of our experiments is not possible, since Fosfomycin was not tested against E.coli.

In our experiments direct comparison of ALBC containing Meropenem and Imipenem is feasible. Comparing the diameter of the inhibition zones, Meropenem clearly shows better antimicrobial activity against E. coli than Imipenem loaded ALBC for all combinations tested. Best results showed the combination of Palacos® R+G with 2 g of Meropenem with a maximum inhibition zone of 26 mm after a time span of 24 hours. Inhibition zones with a diameter of 20 mm can be

detected even after six weeks of testing. Conversely, Imipenem loaded ALBC with the same amount of antibiotic active substance (4 g of Imipenem in Palacos® R+G) did create inhibition zones with a similar diameter to those with Meropenem, but much more substance is added which does not inhibit bacterial growth directly but does reduce the mechanical stability of the produced PMMA bone cement. The mechanical properties of all tested combinations containing Meropenem and Imipenem do meet the standards according to ISO 5833 and DIN 54345, but in direct comparison, Meropenem does affect the mechanical stability more than ALBC containing Imipenem.

To conclude, if a periprosthetic joint infection occurs, ALBC containing Meropenem is superior to those containing Imipenem, because more substance is needed to achieve the same amount of antibacterial growth effect in ALBC containing Imipenem. Meropenem should be used locally in the bone cement, whereas Imipenem can be used systematically to combat PJIs.

### 4.2.2 Proteus mirabilis

To our knowledge, at the moment, there has not been a study which investigating the effectivity of ALBC containing Fosfomycin against Proteus mirabilis until now.

In our study, both Fosfomycin salts and both Carbapenems were tested against Proteus mirabilis. Best results in the inhibition zone assay were shown by Palacos® R+G and Palacos® R with Fosfomycin-Sodium 5 g. Despite the excellent antimicrobial effectivity, the combination of Palacos® R+G and Fosfomycin-Sodium 5 g does not meet the mechanical requirements for bending strength and should therefore not be utilized in clinical usage. Slightly better results than Fosfomycin-Trometamol 2 g added to Palacos® R+G were shown by Fosfomycin-Sodium 2 g. In addition, less powder has to be added to PMMA cement in Fosfomycin-Sodium than in Fosfomycin-Trometamol to obtain the same amount of active antimicrobial substance. The Fosfomycin salts show better results in the inhibition zone assays than both Carbapenems. However, Meropenem shows larger inhibition zones than Imipenem. The most effective dosage of Meropenem with 2 g active antibiotic substance added to Palacos® R+G bone cement does reach every limit in the mechanical testing, whereas the best combination of Imipenem (Imipenem 4 g with Palacos® R+G) does not reach

the limit in impact strength. Hence this combination should not be used in clinical routine. If there is an infection caused by Proteus mirabilis, the best combination in an overall view of the antimicrobial activity, the mechanical features and the antibiotic properties for all of the tested combinations in this study is the usage of Palacos® R+G with Fosfomycin-Sodium 2 g.

### 4.2.3 Pseudomonas aeruginosa

In a study by Sumant et al., the effectiveness of Meropenem against Pseudomonas aeruginosa was tested. Meropenem elutes from acrylic bone cement for a period of 3-27 days depending on the concentration of antibiotic. Liquid chromatography was used in this study to measure the antibiotic drug concentration in the elution liquid (78).

A combination of ALBC with 0.5 g of Vancomycin and 0.5 g of Meropenem and an ALBC with 1 g of Vancomycin and 1 g of Meropenem were tested against Pseudomonas aeruginosa in a study conducted by Baleani et al. While the first combination did not inhibit the growth of bacteria at all three measurement time spans, the following one did stop the increase after 24 hours (73). In our experiments, all tested combinations of Meropenem were effective against Pseudomonas aeruginosa for up to 42 days. Best results could be seen from the combination of 2 g Meropenem in Palacos® R+G acrylic bone cement.

A study conducted by Andollina et al. still observed bacterial growth of Pseudomonas aeruginosa with ALBC containing 0,5 g of Vancomycin and 0,5 g of Meropenem. In doubling the dosage of both antibiotic substances, there was no bacterial growth seen at the first time span (74).

In a study by Chang et al., the ALBC containing 1 g Imipenem did not inhibit the growth of Pseudomonas aeruginosa bacteria (80). Conversely, in our experiments inhibition zones which are constant in diameter can be seen for up to 42 days. It has to be noted, however, that a concentration of Imipenem four times higher than in the experiments conducted by Chang et al. was used.

There are no studies which test the effectivity of ALBC containing Fosfomycin at the moment, so there is no point of comparison.

In our experiments, Meropenem, Imipenem and both Fosfomycin salts were tested against Pseudomonas aeruginosa using inhibition zone essays. In a direct comparison between the two Carbapenems, Meropenem 2 g added to Palacos®

R+G bone cement showed the best antimicrobial effectivity with an inhibition zone of 31 mm after 24 hours. Antimicrobial activity lasted for up to 42 days in this combination. ALBC containing 1 g of Meropenem did also show good antibiotic activity, but there was a drop in diameter of the inhibition zones for Meropenem 1 g in Palacos® R after 14 days with diameters decreasing to 6 mm. In comparison, ALBC containing Imipenem did only reach a maximum diameter of 18 mm in inhibition zone essays and the antimicrobial effectivity is inferior to Meropenem. Interestingly, whereas a combination of Palacos® R and Imipenem 2 g did not show any antimicrobial effect in the first time spans, the combination of the same amount of antibiotic substance to Copal® G+C bone cement showed acceptable effectiveness with diameters of up to 18 mm in the first time spans. The reason for this difference can be synergistic effects since in Copal® G+C Gentamicin and Clindamycin premixed to the PMMA cement can be found. In combating a PJI caused by Pseudomonas aeruginosa, of the two Carbapenems, Meropenem is the antibiotic substance of choice.

All combinations with Fosfomycin-Sodium inhibit bacterial growth for up to 42 days. Since the combinations with 5 g of Fosfomycin-Sodium show unsafe mechanical properties, Fosfomycin-Sodium 2 g in Palacos® R+G or Copal® G+V show the best results in the inhibition zone essays. Those two combinations are preferable to Fosfomycin-Trometamol since the antimicrobial activity for Fosfomycin-Trometamol is inferior to Fosfomycin-Sodium and also less additional substance, which does not inhibit antimicrobial growth, but does reduce the mechanical stability is included in Fosfomycin-Trometamol.

### 4.2.4 MRSA

A study conducted by Sumant et al. demonstrated resistance of Staphylococcus aureus ATCC 43300 (MRSA) to the eluate of two samples of ALBC containing different concentrations of Meropenem (5 and 10%) after one week and hence further testing was stopped (78).

In a study conducted by Charlton-Ouw et al. ALBC containing 2 g Meropenem alone was highly effective against all tested bacteria, with an average inhibition zone of 17 mm against MRSA ATCC 43300. Interestingly, the study showed that the admixture of daptomycin to Meropenem did not significantly alter

the average inhibition zones compared with Meropenem alone in MRSA cultures (76).

A study conducted by Chang et al. did not prove the effectivity of Imipenem loaded ALBC against MRSA (ATCC 43300) (80).

In our studies, neither Meropenem nor Imipenem were tested against MRSA since further studies must be done to cover this scientific issue.

A study conducted by Eick et al. found that all of the cement tested containing Fosfomycin inhibited the growth of MRSA bacteria within a time span up to two days. The ALBC in the study contained a mixture of Gentamicin in addition to Fosfomycin. The results in this study showed that the antimicrobial effectiveness of the eluate was higher and lasted for a longer time span against MRSA for the combination than compared to Fosfomycin alone. In testing Fosfomycin against MRSA, antimicrobial activity depending on the bacterial concentration was revealed. A low bacterial count was killed by ALBC containing Fosfomycin (82).

In testing Refobacin®-Palacos®R bone cement loaded with Fosfomycin against MRSA a study by Roth et al. consistently large zones of inhibition could be detected against both MSSA and MRSA could also be detected. The exact diameters of inhibition zones are not visible. In this study Refobacin PalacosR40® (0.8 g gentamicin sulfate and 0.5 g gentamicin) was admixtured with 2 g of Fosfomycin (83).

A study conducted by Yuenyongviwat et al. compared the effectiveness of ALBC containing Vancomycin and Fosfomycin directly against MRSA. Results showed that ALBC containing Fosfomycin showed large inhibition zones on the first day of testing with an average diameter of 10,3 mm on the first day. However, after the first day, the effectivity dropped rapidly and only a maximum inhibition zone of 1,22 mm could be detected (84).

A study by Lu et al. showed that Vancomycin has a bigger molecular size than Fosfomycin and the solubility in water is higher for Fosfomycin than Vancomycin. In conclusion, those factors are relevant for the elution of Fosfomycin from PMMA bone cement (96).

In our experiments, the aim was to compare the two Fosfomycin salts with each other. Interestingly, a difference in antimicrobial activity measured by inhibition zones in ALBC containing Fosfomycin and Gentamycin and ALBC with

Fosfomycin alone was not found. The largest inhibition zones could be detected in testing Palacos® R+G with Fosfomycin-Sodium 5 g and Palacos® R with Fosfomycin-Sodium against MRSA with a maximum diameter of 36 mm after a time span of seven days. Nevertheless, Fosfomycin-Trometamol showed good antimicrobial effectiveness with inhibition zones of 28 mm for Palacos® R with Fosfomycin-Trometamol 3 g and 29 mm in Copal® G+V with Fosfomycin-Trometamol 2 g. Most of the Fosfomycin-Trometamol formulations showed antimicrobial activity for up to 28 days, the formulation with Copal® G+V and Fosfomycin-Trometamol 2 g even up to 42 days. As the study by Yuenyongviwat et al. revealed, we could also detect that there was a rapid drop in effectiveness against MRSA. That study described a decrease in inhibition zones after only one day, whereas we found a drop in antibiotic activity after a time span of 14 days (84).

To conclude, the best antimicrobial effective and mechanically safe combinations are Fosfomycin-Trometamol 2 g and Fosfomycin-Sodium 2 g in Copal® G+V bone cement. Since Fosfomycin in Copal® G+V cement shows prolonged antimicrobial effectivity, synergistic effects with Gentamicin and Vancomycin are assumed.

The combinations of Fosfomycin-Sodium 5 g showed antimicrobial activity in producing inhibition zones for the full test time span of 42 days. A possible reason for the difference in comparison with Fosfomycin-Trometamol is that there is more antibiotic substance included in the combination of Fosfomycin-Sodium 5 g than in the maximum concentration of Fosfomycin-Trometamol 3 g used in our experiments.

Interestingly, in our experiments we compared ALBC with Palacos® R+G containing Fosfomycin-Trometamol 2 g that was admixtured manually to a sample of the same ALBC that was admixtured using the Pro-System. Inhibition zones showed equal results for all time spans, except for the time span after seven days. The formulation which was mixed manually showed an inhibition zone with a diameter of 19 mm, whereas the combination that was mixed with the Pro-System did produce an inhibition zone which was 8 mm larger. The reason for this circumstance remains unclear.

### 4.2.5 Limitations

Our experiments investigated mechanical stability and antimicrobial activity against different common bacteria in PJIs using in vitro conditions only. When performing the experiments, realistic conditions such as manufacturing the PMMA bone cement in the operating room were attempted to be recreated. Nevertheless, in vivo conditions may change the results of our experiments.

Since mean values were used in our studies, eventually standard deviation describes a broad distribution of results.

In this study only PMMA bone cement provided by Heraeus GmBH Wehrheim, Germany was used. The results may differ if antibiotic substances are added to PMMA bone cements from different manufacturers. Furthermore, antibiotic substance provided by other companies may show other properties and concentrations of active antibiotic substance. In addition, the amount of non-antibiotic active substance may differ from the antibiotics used which may in particular influence the mechanical stability of the produced ALBC.

The antibiotic substances provided contain different amounts of antibiotic active substance and non-antimicrobial ingredients. To compare the results to other studies, the exact definition of the amount of whether used active antibiotic substance or total antibiotic powder is of existential need. We depicted the exact amount used in our experiments in section 2.1.2 Antibiotics.

In this study antimicrobial activity was measured using the diameter of inhibition zone essays as an indirect indicator of antibiotic activity. The measurement of the direct amount of eluded antibiotic substance was not part of our study but can be measured in addition using high-performance liquid chromatography.

In this experiment, the mechanical stability was assessed with cement bodies which do not relate to in vivo situations since the impact of strength over a long time period is not simulated. Eventually, the mechanical testing is to be evaluated again at a later timepoint.

A study conducted by Chang et al. did evaluate the mechanical properties twice: before and after a two-week immersion. That study showed a decrease in compressive strength after the time period (80).

In addition, the size and shape of cement bodies do influence the amount of eluted antibiotic substance. The produced cement bodies differ in size and shape from the cement spacers which are usually produced for in vivo use.

## *4.3 Secondary conclusions*

### 4.3.1 Synergistic effects

In general, for some combinations of antibiotic substances, synergistic release effects are prescribed. That effect describes a situation where the effectivity of the antibiotic is lower alone than in combination with other substances. Hence some antibiotics can influence one another's elution rate and therefore the antimicrobial activity.

A study by Minelli et al. produced PMMA cement bodies containing gentamicin alone, Vancomycin alone and both drugs together. The combination of the two antibiotics showed synergistic antibiotic effectiveness against Escherichia coli and Enterococcus faecalis. Interestingly, Gentamicin alone and in combination with Vancomycin showed approximately the same elution rates, whereas Vancomycin release from PMMA cylinders in the combination was lower than for Vancomycin alone (97).

Of interest is the combination of Meropenem 1 g in Palacos® R bone cement in comparison to Copal® G+C cement in testing against Pseudomonas aeruginosa. The combination with Copal® G+C does show better antimicrobial activity for the time spans starting from 14 days. Imipenem 2 g combined with Copal® G+C show larger inhibition zones than combined with Palacos® R+G for all tested bacteria. Of interest is the combination of Fosfomycin-Trometamol 2 g with Copal® G+V, because prolonged antimicrobial effectiveness could be detected.

# 5 Conclusion

Worldwide the usage of antibiotic-loaded bone cements in revision surgery is increasing. Surgeons augment bone cement with the specific antibiotic in an individual dosage themselves. Therefore, the surgeon has to be certain that the combination of the PMMA bone cement and the antibiotic powder is reliable. The aim is to provide tailored antimicrobial therapy locally admixtured in PMMA bone cement by the surgeon himself or herself.

We have been able to demonstrate that if a PJI caused by E. coli occurs the best combination of cement and antibiotic with remained mechanical stability is Palacos® R+G with 2 g of Meropenem. If the infection is caused by P. mirabilis, Palacos® R+G with Fosfomycin-Sodium 2 g is the combination of choice. P. aeruginosa related PJIs should be treated by using Fosfomycin-Sodium 2 g in Palacos® R+G or Copal® G+V. For MRSA infections Fosfomycin-Trometamol 2 g and Fosfomycin-Sodium 2 g in Copal® G+V show best results.

To conclude, clinical study data is of need to prove the efficacy in combatting periprosthetic joint infection but the results of this study are a promising approach that the tested antibiotic substances can be used in PMMA bone cements to treat periprosthetic joint infections.

# 6 Bibliography


1. Leitner L, Türk S, Heidinger M, Stöckl B, Posch F, Maurer-Ertl W, et al. Trends and Economic Impact of Hip and Knee Arthroplasty in Central Europe: Findings from the Austrian National Database. Sci Rep [Internet]. 2018 Mar 16 [cited 2019 Apr 23];8(1):4707. Available from: http://www.nature.com/articles/s41598-018-23266-w
2. Leitner L, Posch F, Amerstorfer F, Sadoghi P, Leithner A, Glehr M. The Dark Side of Arthroplasty: Competing Risk Analysis of Failed Hip and Knee Arthroplasty With Periprosthetic Joint Infection. J Arthroplasty [Internet]. 2020 Sep 1 [cited 2021 Apr 9];35(9):2601-2606.e1. Available from: https://pubmed-1ncbi-1nlm-1nih-1gov-10013b51e0492.han.medunigraz.at/32451282/
3. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-Joint Infections. N Engl J Med [Internet]. 2004 Oct 14 [cited 2019 May 28];351(16):1645–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15483283
4. Dowsey MM, Choong PFM. Obese diabetic patients are at substantial risk for deep infection after primary TKA. Clin Orthop Relat Res [Internet]. 2009 Jun 8 [cited 2019 Apr 23];467(6):1577–81. Available from: http://link.springer.com/10.1007/s11999-008-0551-6
5. Jaberi FM, Parvizi J, Haytmanek CT, Joshi A, Purtill J. Procrastination of wound drainage and malnutrition affect the outcome of joint arthroplasty. Clin Orthop Relat Res [Internet]. 2008 Jun 11 [cited 2019 Apr 23];466(6):1368–71. Available from: http://link.springer.com/10.1007/s11999-008-0214-7
6. Kong L, Cao J, Zhang Y, Ding W, Shen Y. Risk factors for periprosthetic joint infection following primary total hip or knee arthroplasty: a meta-analysis. Int Wound J [Internet]. 2017 Jun [cited 2019 Apr 23];14(3):529–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27397553
7. Berbari EF, Hanssen AD, Duffy MC, Steckelberg JM, Ilstrup DM, Harmsen WS, et al. Risk Factors for Prosthetic Joint Infection: Case-Control Study. Clin Infect Dis [Internet]. 1998 Nov [cited 2019 May 28];27(5):1247–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9827278
8. Gristina AG. Biomaterial-centered infection: microbial adhesion versus

tissue integration. Science [Internet]. 1987 Sep 25 [cited 2019 May 30];237(4822):1588–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3629258

9. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science [Internet]. 1999 May 21 [cited 2019 May 30];284(5418):1318–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10334980
10. Rochford ETJ, Richards RG, Moriarty TF. Influence of material on the development of device-associated infections. Clin Microbiol Infect [Internet]. 2012 Dec [cited 2019 Jun 5];18(12):1162–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1198743X14608016
11. Donlan RM. Biofilm Formation: A Clinically Relevant Microbiological Process. Clin Infect Dis [Internet]. 2001 Oct 15 [cited 2020 Jul 21];33(8):1387–92. Available from: https://pubmed.ncbi.nlm.nih.gov/11565080/
12. Monroe D. Looking for chinks in the armor of bacterial biofilms [Internet]. Vol. 5, PLoS Biology. PLoS Biol; 2007 [cited 2020 Jul 20]. p. 2458–61. Available from: https://pubmed.ncbi.nlm.nih.gov/18001153/
13. Giulieri SG, Graber P, Ochsner PE, Zimmerli W. Management of Infection Associated with Total Hip Arthroplasty according to a Treatment Algorithm. Infection [Internet]. 2004 Aug [cited 2019 Apr 23];32(4):222–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15293078
14. Del Pozo JL, Patel R. Infection Associated with Prosthetic Joints. N Engl J Med [Internet]. 2009 Aug 20 [cited 2019 Apr 23];361(8):787–94. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMcp0905029
15. Maderazo EG, Judson S, Pasternak H. Late infections of total joint prostheses. A review and recommendations for prevention. Clin Orthop Relat Res [Internet]. 1988 Apr [cited 2019 May 28];(229):131–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3280197
16. Rodríguez D, Pigrau C, Euba G, Cobo J, García-Lechuz J, Palomino J, et al. Acute haematogenous prosthetic joint infection: prospective evaluation of medical and surgical management. Clin Microbiol Infect [Internet]. 2010 Dec [cited 2019 May 28];16(12):1789–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21077986

17. Aggarwal V, Bakhshi H, Ecker N, Parvizi J, Gehrke T, Kendoff D. Organism Profile in Periprosthetic Joint Infection: Pathogens Differ at Two Arthroplasty Infection Referral Centers in Europe and in the United States. J Knee Surg [Internet]. 2014 Jan 10 [cited 2019 May 28];27(05):399–406. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24414388
18. Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, et al. Executive Summary: Diagnosis and Management of Prosthetic Joint Infection: Clinical Practice Guidelines by the Infectious Diseases Society of Americaa. Clin Infect Dis [Internet]. 2013 Jan 1 [cited 2019 May 28];56(1):1–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23230301
19. Shohat N, Bauer T, Buttaro M, Budhiparama N, Cashman J, Della Valle CJ, et al. Hip and Knee Section, What is the Definition of a Periprosthetic Joint Infection (PJI) of the Knee and the Hip? Can the Same Criteria be Used for Both Joints?: Proceedings of International Consensus on Orthopedic Infections. J Arthroplasty [Internet]. 2019 Feb [cited 2019 May 28];34(2S):S325–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0883540318308283
20. Spangehl MJ, Masri BA, O'Connell JX, Duncan CP. Prospective analysis of preoperative and intraoperative investigations for the diagnosis of infection at the sites of two hundred and two revision total hip arthroplasties. J Bone Joint Surg Am [Internet]. 1999 May [cited 2019 May 28];81(5):672–83. Available from: https://insights.ovid.com/crossref?an=00004623-199905000-00008
21. Honsawek S, Deepaisarnsakul B, Tanavalee A, Sakdinakiattikoon M, Ngarmukos S, Preativatanyou K, et al. Relationship of serum IL-6, C-reactive protein, erythrocyte sedimentation rate, and knee skin temperature after total knee arthroplasty: a prospective study. Int Orthop [Internet]. 2011 Jan 21 [cited 2019 May 28];35(1):31–5. Available from: http://link.springer.com/10.1007/s00264-010-0973-0
22. Pérez-Prieto D, Portillo ME, Puig-Verdié L, Alier A, Martínez S, Sorlí L, et al. C-reactive protein may misdiagnose prosthetic joint infections, particularly chronic and low-grade infections. Int Orthop [Internet]. 2017 Jul 21 [cited 2019 May 30];41(7):1315–9. Available from: http://link.springer.com/10.1007/s00264-017-3430-5

23. Tande AJ, Gomez-Urena EO, Berbari EF, Osmon DR. Management of Prosthetic Joint Infection. Infect Dis Clin North Am [Internet]. 2017 Jun [cited 2019 May 30];31(2):237–52. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0891552017300090
24. Ahmad SS, Becker R, Chen AF, Kohl S. EKA survey: diagnosis of prosthetic knee joint infection. Knee Surgery, Sport Traumatol Arthrosc [Internet]. 2016 Oct 16 [cited 2019 Jun 4];24(10):3050–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27637855
25. Trampuz A, Hanssen AD, Osmon DR, Mandrekar J, Steckelberg JM, Patel R. Synovial fluid leukocyte count and differential for the diagnosis of prosthetic knee infection. Am J Med [Internet]. 2004 Oct 15 [cited 2019 Jun 4];117(8):556–62. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0002934304004401
26. Lee YS, Koo K-H, Kim HJ, Tian S, Kim T-Y, Maltenfort MG, et al. Synovial Fluid Biomarkers for the Diagnosis of Periprosthetic Joint Infection. J Bone Jt Surg [Internet]. 2017 Dec 20 [cited 2019 May 28];99(24):2077–84. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29257013
27. Springer BD. The Diagnosis of Periprosthetic Joint Infection. J Arthroplasty [Internet]. 2015 Jun [cited 2019 Jun 4];30(6):908–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25913561
28. Morgan PM, Sharkey P, Ghanem E, Parvizi J, Clohisy JC, Burnett RSJ, et al. The Value of Intraoperative Gram Stain in Revision Total Knee Arthroplasty. J Bone Jt Surgery-American Vol [Internet]. 2009 Sep [cited 2019 Jun 4];91(9):2124–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19723988
29. Della Valle C, Parvizi J, Bauer TW, DiCesare PE, Evans RP, Segreti J, et al. American Academy of Orthopaedic Surgeons Clinical Practice Guideline on. J Bone Jt Surgery-American Vol [Internet]. 2011 Jul [cited 2019 Jun 4];93(14):1355–7. Available from: https://insights.ovid.com/crossref?an=00004623-201107200-00011
30. Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, Osmon DR, et al. Sonication of Removed Hip and Knee Prostheses for Diagnosis of Infection. N Engl J Med [Internet]. 2007 Aug 16 [cited 2019 May 30];357(7):654–63. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/17699815

31. Portillo ME, Salvadó M, Sorli L, Alier A, Martínez S, Trampuz A, et al. Multiplex PCR of sonication fluid accurately differentiates between prosthetic joint infection and aseptic failure. J Infect [Internet]. 2012 Dec [cited 2019 Jun 3];65(6):541–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0163445312002502
32. Borens O, Yusuf E, Steinrücken J, Trampuz A. Accurate and early diagnosis of orthopedic device-related infection by microbial heat production and sonication. J Orthop Res [Internet]. 2013 Nov [cited 2019 Jun 3];31(11):1700–3. Available from: http://doi.wiley.com/10.1002/jor.22419
33. Deirmengian C, Greenbaum J, Lotke PA, Booth RE, Lonner JH. Limited success with open debridement and retention of components in the treatment of acute Staphylococcus aureus infections after total knee arthroplasty. J Arthroplasty [Internet]. 2003 Oct [cited 2019 May 28];18(7 Suppl 1):22–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14560406
34. Trampuz A, Zimmerli W. Prosthetic joint infections: update in diagnosis and treatment. Swiss Med Wkly [Internet]. 2005 Apr 30 [cited 2019 May 28];135(17–18):243–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15965826
35. Buchholz HW, Elson RA, Engelbrecht E, Lodenkämper H, Röttger J, Siegel A. Management of deep infection of total hip replacement. J Bone Joint Surg Br [Internet]. 1981 [cited 2019 May 30];63-B(3):342–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7021561
36. Li C, Renz N, Trampuz A. Management of Periprosthetic Joint Infection. Hip Pelvis [Internet]. 2018 [cited 2019 May 28];30(3):138. Available from: https://synapse.koreamed.org/DOIx.php?id=10.5371/hp.2018.30.3.138
37. George DA, Konan S, Haddad FS. Single-Stage Hip and Knee Exchange for Periprosthetic Joint Infection. J Arthroplasty [Internet]. 2015 Dec [cited 2019 May 30];30(12):2264–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26096072
38. Jahoda D. Clinical Strategy for the Treatment of Deep Infection of Hip Arthroplasty. In: Kienapfel H, Kühn K-D, editors. The Infected Implant. Heidelberg; 2009. p. 27–42.

39. URE KJ, AMSTUTZ HC, NASSER S, SCHMALZRIED TP. Direct-Exchange Arthroplasty for the Treatment of Infection after Total Hip Replacement. An Average Ten-Year Follow-up*. J Bone Jt Surg [Internet]. 1998 Jul [cited 2019 May 30];80(7):961–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9698000
40. Zahar A, Gehrke TA. One-Stage Revision for Infected Total Hip Arthroplasty. Orthop Clin North Am [Internet]. 2016 Jan [cited 2019 May 30];47(1):11–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26614916
41. Tande AJ, Patel R. Prosthetic joint infection. Clin Microbiol Rev [Internet]. 2014 [cited 2021 Apr 8];27(2):302–45. Available from: https://pubmed-1ncbi-1nlm-1nih-1gov-10013b51e0449.han.medunigraz.at/24696437/
42. Vielgut I, Schwantzer G, Leithner A, Sadoghi P, Berzins U, Glehr M. Successful two-stage exchange arthroplasty for periprosthetic infection following total knee arthroplasty: The impact of timing on eradication of infection. Int J Med Sci [Internet]. 2021 [cited 2021 Apr 9];18(4):1000–6. Available from: https://pubmed-1ncbi-1nlm-1nih-1gov-10013b51e0492.han.medunigraz.at/33456357/
43. Roland.chem. Polymethylmethacrylat [Internet]. [cited 2020 Jul 21]. Available from: https://commons.wikimedia.org/wiki/File:Polymethylmethacrylat.svg
44. Kühn K-D, Höntzsch D. Augmentation mit PMMA-Zement. Unfallchirurg [Internet]. 2015 Sep 28 [cited 2019 May 30];118(9):737–48. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26315391
45. Pngbot. PMMA-chain [Internet]. [cited 2020 Jul 21]. Available from: https://commons.wikimedia.org/wiki/File:PMMA-chain.png
46. Emeldir. Methyl methacrylate 200 [Internet]. [cited 2020 Jul 21]. Available from: https://commons.wikimedia.org/wiki/File:Methyl_methacrylate_200.svg
47. Helmich U. Plexiglas [Internet]. 2020 [cited 2020 Jul 21]. Available from: http://www.u-helmich.de/che/Q2/kunststoffe/verbindungen/plexiglas.html
48. Roland.chem. Dibenzoylperoxid-Zerfall [Internet]. 2016 [cited 2020 Jul 21]. Available from: https://commons.wikimedia.org/wiki/File:Dibenzoylperoxid-Zerfall.svg
49. Roland.chem. PMMA-Start [Internet]. 2016 [cited 2020 Jul 21]. Available from: https://commons.wikimedia.org/wiki/File:PMMA-Start.svg

50. Roland.chem. PMMA-Wachstum [Internet]. 2016 [cited 2020 Jul 21]. Available from: https://commons.wikimedia.org/wiki/File:PMMA-Wachstum.svg
51. Roland.chem. PMMA-Abbruch [Internet]. 2016 [cited 2020 Jul 21]. Available from: https://commons.wikimedia.org/wiki/File:PMMA-Abbruch.svg
52. Buchholz HW, Engelbrecht H. [Depot effects of various antibiotics mixed with Palacos resins]. Chirurg [Internet]. 1970 Nov [cited 2019 May 30];41(11):511–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/5487941
53. Kühn K-D, Renz N, Trampuz A. Lokale Antibiotikatherapie. Unfallchirurg [Internet]. 2017 Jul 22 [cited 2019 Jun 5];120(7):561–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28643095
54. Hanssen AD. Prophylactic use of antibiotic bone cement: an emerging standard--in opposition. J Arthroplasty [Internet]. 2004 Jun [cited 2019 Jun 5];19(4 Suppl 1):73–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15190554
55. Penner MJ, Masri BA, Duncan CP. Elution characteristics of vancomycin and tobramycin combined in acrylic bone-cement. J Arthroplasty [Internet]. 1996 Dec [cited 2019 Jun 5];11(8):939–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8986572
56. Martínez-Moreno J, Merino V, Nácher A, Rodrigo JL, Climente M, Merino-Sanjuán M. Antibiotic-loaded Bone Cement as Prophylaxis in Total Joint Replacement. Orthop Surg [Internet]. 2017 Nov [cited 2019 Jun 5];9(4):331–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29178309
57. Anagnostakos K, Kelm J. Enhancement of antibiotic elution from acrylic bone cement. J Biomed Mater Res Part B Appl Biomater [Internet]. 2009 Jan 14 [cited 2019 Jun 5];90B(1):467–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19145626
58. Anagnostakos K, Fürst O, Kelm J. Antibiotic-impregnated PMMA hip spacers: Current status. Acta Orthop [Internet]. 2006 Aug 8 [cited 2019 Jun 5];77(4):628–37. Available from: https://www.tandfonline.com/doi/full/10.1080/17453670610012719
59. Bayston R, Milner RD. The sustained release of antimicrobial drugs from bone cement. An appraisal of laboratory investigations and their

significance. J Bone Joint Surg Br [Internet]. 1982 [cited 2019 Jun 5];64(4):460–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7096423

60. Baker AS, Greenham LW. Release of gentamicin from acrylic bone cement. Elution and diffusion studies. J Bone Joint Surg Am [Internet]. 1988 Dec [cited 2019 Jun 5];70(10):1551–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3198680
61. Kuechle DK, Landon GC, Musher DM, Noble PC. Elution of vancomycin, daptomycin, and amikacin from acrylic bone cement. Clin Orthop Relat Res [Internet]. 1991 Mar [cited 2019 Jun 5];(264):302–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1705191
62. van de Belt H, Neut D, Uges DR, Schenk W, van Horn JR, van der Mei HC, et al. Surface roughness, porosity and wettability of gentamicin-loaded bone cements and their antibiotic release. Biomaterials [Internet]. 2000 Oct [cited 2019 Jun 5];21(19):1981–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10941919
63. Labmayr V, Lerchbaumer MH, Kuehn K-D, Kittinger C, Amerstorfer F, Leithner A, et al. Comparison of elution characteristics and mechanical properties of acrylic bone cements with and without superficial vancomycin coating (SVC) in the late phase of polymerization. Orthop Traumatol Surg Res [Internet]. 2021 Mar [cited 2021 Apr 9];102908. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1877056821001274
64. 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. J Orthop Res [Internet]. 2020 [cited 2021 Apr 9]; Available from: https://pubmed-1ncbi-1nlm-1nih-1gov-10013b51e0492.han.medunigraz.at/33118642/
65. 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 [Internet]. 2017 May 1 [cited 2021 Apr 9];32(5):1618–24. Available from: https://pubmed-1ncbi-1nlm-1nih-1gov-10013b51e0492.han.medunigraz.at/28111125/
66. Aktories K, Förstermann U, Hofmann F, K S. Pharmakologie und

Toxikologie. 11. München: Urban & Fischer; 2013. 753–775 p.

67. Yikrazuul. Carbapenems structure [Internet]. 2009 [cited 2020 Jul 21]. Available from: https://commons.wikimedia.org/wiki/File:Carbapenems_structure.svg
68. Fvasconcellos. Beta-lactam antibiotics example 1 [Internet]. 2007 [cited 2020 Jul 21]. Available from: https://commons.wikimedia.org/wiki/File:Beta-lactam_antibiotics_example_1.svg
69. Vaccinationist. Imipenem + cilastatin [Internet]. 2016 [cited 2020 Jul 21]. Available from: https://commons.wikimedia.org/wiki/File:Imipenem_%2B_cilastatin.svg
70. Fvasconcellos. Meropenem [Internet]. 2006 [cited 2020 Jul 21]. Available from: https://commons.wikimedia.org/wiki/File:Meropenem.svg
71. Hendlin D, Stapley EO, Jackson M, Wallick H, Miller AK, Wolf FJ, et al. Phosphonomycin, a new antibiotic produced by strains of streptomyces. Science (80- ) [Internet]. 1969 [cited 2021 Apr 8];166(3901):122–3. Available from: https://pubmed-1ncbi-1nlm-1nih-1gov-10013b51e0449.han.medunigraz.at/5809587/
72. Falagas ME, Vouloumanou EK, Samonis G, Vardakas KZ. Fosfomycin. Clin Microbiol Rev [Internet]. 2016 Apr 9 [cited 2019 May 31];29(2):321–47. Available from: http://cmr.asm.org/lookup/doi/10.1128/CMR.00068-15
73. Baleani M, Persson C, Zolezzi C, Andollina A, Borrelli AM, Tigani D. Biological and biomechanical effects of vancomycin and meropenem in acrylic bone cement. J Arthroplasty [Internet]. 2008 Dec [cited 2019 Jun 10];23(8):1232–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0883540307006055
74. Andollina A, Bertoni G, Bertoni F, Zolezzi C, Trentani F, Trentani P, et al. Vancomycin and meropenem in acrylic cement: elution kinetics of in vitro bactericidal action. Chir Organi Mov [Internet]. 2008 Apr 21 [cited 2019 Jun 10];91(3):153–8. Available from: http://link.springer.com/10.1007/s12306-007-0025-0
75. Persson C, Baleani M, Guandalini L, Tigani D, Viceconti M. Mechanical effects of the use of vancomycin and meropenem in acrylic bone cement. Acta Orthop [Internet]. 2006 Aug 8 [cited 2019 Jun 10];77(4):617–21. Available from:

https://www.tandfonline.com/doi/full/10.1080/17453670610012692

76. Charlton-Ouw KM, Kubrusly F, Sandhu HK, Swick MC, Leake SS, Gulbis BE, et al. In vitro efficacy of antibiotic beads in treating abdominal vascular graft infections. J Vasc Surg [Internet]. 2015 Oct [cited 2019 Jun 10];62(4):1048–53. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0741521414006533

77. Gálvez-López R, Peña-Monje A, Antelo-Lorenzo R, Guardia-Olmedo J, Moliz J, Hernández-Quero J, et al. Elution kinetics, antimicrobial activity, and mechanical properties of 11 different antibiotic loaded acrylic bone cement. Diagn Microbiol Infect Dis [Internet]. 2014 Jan [cited 2019 Jun 10];78(1):70–4. Available from: https://linkinghub.elsevier.com/retrieve/pii/S073288931300535X

78. Samuel S, Mathew BS, Veeraraghavan B, Fleming DH, Chittaranjan SB, Prakash JAJ. In vitro study of elution kinetics and bio-activity of meropenem-loaded acrylic bone cement. J Orthop Traumatol [Internet]. 2012 Sep 30 [cited 2019 Jun 10];13(3):131–6. Available from: http://link.springer.com/10.1007/s10195-012-0191-1

79. Cerretani D, Giorgi G, Fornara P, Bocchi L, Neri L, Ceffa R, et al. The in vitro elution characteristics of vancomycin combined with imipenem-cilastatin in acrylic bone-cements: a pharmacokinetic study. J Arthroplasty [Internet]. 2002 Aug [cited 2019 Jun 10];17(5):619–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12168180

80. Chang Y, Tai C-L, Hsieh P-H, Ueng SWN. Gentamicin in bone cement: A potentially more effective prophylactic measure of infectionin joint arthroplasty. Bone Joint Res [Internet]. 2013 Oct [cited 2019 Jun 10];2(10):220–6. Available from: http://online.boneandjoint.org.uk/doi/10.1302/2046-3758.210.2000188

81. Bowyer GW, Cumberland N. Antibiotic release from impregnated pellets and beads. J Trauma [Internet]. 1994 Mar [cited 2019 Jun 10];36(3):331–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8145311

82. Eick S, Hofpeter K, Sculean A, Ender C, Klimas S, Vogt S, et al. Activity of Fosfomycin- and Daptomycin-Containing Bone Cement on Selected Bacterial Species Being Associated with Orthopedic Infections. Biomed Res Int [Internet]. 2017 [cited 2019 Jun 10];2017:2318174. Available from:

https://www.hindawi.com/journals/bmri/2017/2318174/

83. Roth KE, Krause B, Siegel E, Maier G, Schoellner C, Rommens PM. Liquid dextran does not increase the elution rate of different antibiotics from bone cement. Eur J Orthop Surg Traumatol [Internet]. 2015 Jan 11 [cited 2019 Jun 10];25(1):83–9. Available from: http://link.springer.com/10.1007/s00590-013-1408-6

84. Yuenyongviwat V, Ingviya N, Pathaburee P, Tangtrakulwanich B. Inhibitory effects of vancomycin and fosfomycin on methicillin-resistant Staphylococcus aureus from antibiotic-impregnated articulating cement spacers. Bone Joint Res [Internet]. 2017 Mar [cited 2019 Jun 10];6(3):132–6. Available from: http://online.boneandjoint.org.uk/doi/10.1302/2046-3758.63.2000639

85. Holding H. One PALACOS®. Many options. Flexibility at the highest level. [Internet]. 2020 [cited 2020 Jul 21]. Available from: https://www.heraeus.com/us/hme/usa_products_heraeus_medical/usa_palacos/usa_palacos_bone_cement.html

86. Steinmetz I, Suerbaum S, Hahn H, Burchard GD, Kaufmann SHE. Nichtfermentierende Bakterien (Nonfermenter): Pseudomonas, Burkholderia, Stenotrophomonas, Acinetobacter. In: Medizinische Mikrobiologie und Infektiologie. 7th ed. Berlin, Heidelberg: Springer; 2012. p. 265–8.

87. CDC. Pseudomonas aeruginosa gram [Internet]. 1979 [cited 2020 Jul 21]. Available from: https://commons.wikimedia.org/wiki/File:Pseudomonas_aeruginosa_gram.jpg

88. (DBCLS) DC for LS. 201708 Pseudomonas aeruginosa [Internet]. [cited 2020 Jul 21]. Available from: https://commons.wikimedia.org/wiki/File:201708_Pseudomonas_aeruginosa.svg

89. Suerbaum S, Hahn H, Burchard GD, Kaufmann SHE. Enterobakterien. In: Medizinische Mikrobiologie und Infektiologie. 7th ed. Berlin, Heidelberg: Springer; 2012. p. 230–2; 240–1.

90. CDC. Proteus-mirabilis [Internet]. 1976 [cited 2020 Jul 21]. Available from: https://commons.wikimedia.org/wiki/File:Proteus-mirabilis.jpg

91. Rocky Mountain Laboratories, NIAID N. EscherichiaColi NIAID [Internet]. 2005 [cited 2020 Jul 21]. Available from: https://commons.wikimedia.org/wiki/File:EscherichiaColi_NIAID.jpg
92. Y_tambe. Escherichia coli gram [Internet]. 2005 [cited 2020 Jul 21]. Available from: https://commons.wikimedia.org/wiki/File:Escherichia_coli_Gram.jpg
93. Gatermann SG, Suerbaum S, Burchard GD, Kaufmann SHE. Staphylokokken. In: Medizinische Mikrobiologie und Infektiologie. 7th ed. Berlin, Heidelberg: Springer; 2012. p. 187–94.
94. Tambe Y. Staphylococcus aureus Gram [Internet]. 2005 [cited 2020 Jul 21]. Available from: https://commons.wikimedia.org/wiki/File:Staphylococcus_aureus_Gram.jpg
95. CDC. MRSA7820 [Internet]. 2005 [cited 2020 Jul 21]. Available from: https://commons.wikimedia.org/wiki/File:MRSA7820.jpg
96. Lu CL, Liu CY, Huang YT, Liao CH, Teng LJ, Turnidge JD, et al. Antimicrobial susceptibilities of commonly encountered bacterial isolates to fosfomycin determined by agar dilution and disk diffusion methods. Antimicrob Agents Chemother [Internet]. 2011 Sep 1 [cited 2021 Mar 21];55(9):4295–301. Available from: http://www.srga.org
97. Bertazzoni Minelli E, Benini A, Bassi C, Abbas H, Falconi M, Locatelli F, et al. Antimicrobial activity of human pancreatic juice and its interaction with antibiotics. Antimicrob Agents Chemother [Internet]. 1996 [cited 2021 Mar 21];40(9):2099–105. Available from: https://pubmed.ncbi.nlm.nih.gov/8878588/