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Evaluation and testing of polymethylmetacrylic (PMMA) bone cements with admixed Amphotericin B

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Abstract

Background Amphotericin is admixed to Polymethylmethacrylic (PMMA) spacers for fungal periprosthetic joint infections (PJI) during two-stage exchanges. We aimed to analyse the mechanical properties of PMMA cement with admixed Amphotericin B.

Materials and methods We tested Amphotericin in PMMA cement mechanically, its elution properties in vitro and present two cases of fungal PJI treated with Amphotericin B powder in Copal cement in vivo.

Results Sterile Amphotericin B is not available as a pure substance but only as powder for infusions. PMMA mixed with such pharmaceutical Amphotericin B formulations colored the cement orange. Compression strength was slightly decreased, bending and impact strength significantly decreased whereas bending modulus was increased. Drug elution was high within the first 24 h and decreased over time until day 5. Amphotericin B in combination with Copal was successfully used in two cases with *Candida ssp.* infections. No negative side effects, especially no nephrotoxic effects, were observed. Sterile Amphotericin B powder for preparing an infusion solution contains only small amounts of pure drug. In vivo polymicrobial *Candida*-infections with bacterial co-infection were successfully treated using the combination of Copal cements with added Amphotericin B without systemic nephrotoxic impact.

Conclusions The addition of Amphotericin B to PMMA cement affects the cement's properties in vitro whereas in vivo the combination with Copal is clinically successful in treating complex cases of fungal PJI.

Level of evidence Level IV.

Keywords Amphotericin B, PMMA, *Candida*, Fungal PJI, ISO 5833

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Introduction

Even though the scientific interest in periprosthetic joint infections (PJI) has been steadily increasing over the years [1, 2], information regarding fungal PJI remains relatively scarce. Fungal periprosthetic joint infections are rare but serious, accounting for less than 1% of culture positive PJI [3, 4]. *Candida spp*. are the most frequently cultivated fungi [5]. The most common treatment strategy in chronic infections is the two-stage revision [5, 6]. Polymethylmethacrylate (PMMA) spacers are regularly used containing Amphotericin B, Fluconazole or Voriconazole. The primary clinical experience treating In invasive fungal arthritis clinical experience is best for Amphotericin B, with or without flucytosine [7, 8]. Fungal organisms are slowly growing, and infections often occur in tissues which are poorly penetrated by therapeutics. Understanding the pharmacological properties of any antifungal agent is crucial for optimizing patient outcomes [9]. The distribution of Amphotericin B formulations in bone marrow and fat tissue differs [10] as plasma concentrations of liposomal Amphotericin B are 50% higher than bone marrow concentrations. In one US study, liposomal Amphotericin has greater release from antibiotic-loaded bone cement (ALBC) than Amphotericin desoxycholate [11].

Wang [12] staged reimplantations for the treatment of fungal peri-prosthetic joint infections following primary total knee arthroplasty. They compiled the largest case series in which every patient received the same antifungal-loaded bone cement spacer (1 g Vancomycin plus 100 mg Amphotericin B in 40 g PMMA cement). Deelstra at al. (2013) [13] presented a successful treatment strategy for a total hip replacement infected with *Candida albicans* with a staged procedure using an antifungal-loaded cement spacer in an immunocompetent patient after revision surgery.

Local antifungal medication during the primary surgical treatment for fungal PJI was either applied by implanting an impregnated cement spacer, by placing intraarticular powder (100 mg Amphotericin B) or by daily intraarticular lavage (Fluconazole 200 mg/d) [14–16].

Pro-implant Foundation previously published their experiences with dosages for implant fixation in fungal PJI: 100 mg of liposomal Amphotericin B with 40 g PMMA cement for fixation [17]. Amphotericin B is a very effective antifungal agent and liposomal absorption increases its bioavailability [18]. The manual addition of antifungal agents such as Amphotericin did not have any significant impact on compressive strength [18, 19]. Spacers are loaded with therapeutical dosages by manually adding 200 mg of liposomal Amphotericin B. With this concentration the ISO compression strength thresholds over time were no longer met [18]. The question of major importance in this study is how the mixture of PMMA cement with antifungals affects mechanical stability and elution properties for dosages recommended by the Pro-implant Foundation [17].

Due to the low total number of cases and the possible adverse effects of most antifungal substances, no clear-cut consensus exists on the optimal formulation to create spacers with admixed antifungal agents [20].

We thus tested pure Amphotericin powder and sterile AmBisome® added to PMMA in different dosages and their effect on ISO and DIN mechanical and microbiological cement properties. Furthermore, we described two complex cases of PJI involving *Candida ssp.*. demanding two-staged procedures using PMMA spacers with admixed liposomal Amphotericin B. The infections were well-controlled without any adverse effects, with a special focus on renal function.

Materials and methods

The cements used were Palacos R, Palacos R + G and Copal G + C, all manufactured by Heraeus Medical (Wehrheim, Germany). The methodology adheres to the standards set forth by the International Organization for Standardization (ISO 5833 [21]), and the German Institute for Standardization (DIN 53435 [22]),.

Amphotericin B was available in two formulations: AmBisome® (Amphotericin B, liposomal) manufactured by Gilead Science, Inc. and Amphotericin B non-liposomal CAS. 1397-89-3 Bejing Mesochem Technology Co. Ltd, China, non-sterile, AK 860. The standard formulation of AmBisome® consists of 1.33 g powder containing 50 mg pure Amphotericin B (Fig. 1).

Standardized specimen were produced by mixing 40 g of polymethylmethacrylate (PMMA) powder with 20 ml of fluid monomer methylmethacrylate (MMA). Palacos R without antibiotics was used as a reference. Palacos R + G contains 0.5 g of Gentamicin and Copal G + C contains 1.0 g Gentamicin and 1.0 g Clindamycin. 0.2 g of AmBisome® contain 7.52 mg of Amphotericin B (Table 1).

Amphotericin B was either used as AmBisome® (Amphotericin B, liposomal) (powder for preparation of an infusion solution) or as non-liposomal pure Amphotericin B powder.

An overview of all in vitro tests performed is given in Table 2.

Several doses of pure Amphotericin B and liposomal Amphotericin B (AmBisome) were added to the cement during the mixing procedure. AmBisome® was pulverized with a mortar and pestle to create a homogeneous powder. Palacos R, Palacos R + G, and Copal G + C were tested plainly and with variations of Amphotericin B and AmBisome® in three test arrangements.

ISO Compression strength test

The ISO Compression strength test was performed on cylindric specimens, which were created by filling the

Fig. 1 Pure non-sterile non-liposomal Amphotericin B powder (**a**). Sterile AmBisome® powder for infusion (**b**)

Table 1 Formulations of PMMA cement with amphotericin B tested

Cement	Added pure amphotericin B powder (active ingredient)	Added AmBisome® (1,33g contains 50mg pure amphotericin B powder (active ingredient/ total amount of powder)
Palacos R	0.5 g	
	0.8 g	30 mg / 0.8 g
Palacos R + G	0.8 g	30 mg / 0.8 g
	2.66 g	100 mg / 2.66 g
Copal G + C	1.33 g	50 mg / 1.33 g

cement into tubes with an inner diameter of 6 mm. The resulting rods were cut into 12 mm long samples which were placed upright into the test apparatus (Instron modernized by Zwick / Roell). Gradually increasing pressure was applied on the mould body until the imminent destruction of the body resulted. This value was noted, and the compressive strength was automatically calculated. Each formulation (10 different compositions (Table 2)) was tested with 10 samples, resulting in to 100 samples in total.

ISO bending strength and ISO bending modulus

ISO bending strength and ISO bending modulus were tested according to ISO5833. 6 long, rectangular moulded bodies with a thickness of 3 mm and a width of 10 mm were tested for each of the 10 combinations of PMMA and Amphotericin B. The pressure on the body was raised until it broke apart and the flexure modulus and the flexure strength were calculated.

DIN impact strength

Eight Dynstat samples were created for each dose tested (15 mm x 10mmx 3 mm.) The body was placed into the apparatus and a pendulum bob was impacted the body with a standardised energy of 0,5 J. Afterwards the amplitude of the pendulum post impact was noted and the impact strength was calculated using this value.

Microbiological elution data

Release data of bone cements containing Amphotericin were analyzed by 'Analytisches Zentrum Biopharm GmbH Berlin' (Berlin, Germany). The prepared bone cement samples ($9 \times 5 \times 2$ mm) were diluted in 20 ml freshly prepared dissolution medium (0.1 M phosphate buffer, pH 7.4). The closed sample vessels were stored at 37 °C for 24 h (first timepoint). After storing, the release solution was transferred into another sample vessel and stored at -20 °C. The sample vessel was refilled with further dissolution medium and was stored again until the next timepoint and the procedure was repeated. The collected samples were analyzed with high performance liquid chromatography (HPLC) with MS/MS detection (calibration standard: Amphotericin B, internal standard Rifaximin) for 5 days and measurements were made in µg per sample.

Creatinine

Creatinine was tested using an enzymatic test for creatinine in at least 0.5mL of blood plasma in containers using Li-Heparin-gel additives.

Table 2 Methodology of in-vitro testing

Cement formulation	Test / Limit				
	Compressive Strength [MPa] <70 Mpa	Bending Modulus [MPa] < 1800 Mpa	Bending Strength [MPa] < 50 Mpa	Impact/Strength [kJ/m^2] < 2.5 kJ/m2	Elution in µg per sample
Palacos R plain	☑	☑	☑	☑	
Palacos R + 0.5 g pure Amphothericin B					☑
Palacos R + 0.8 g pure Amphothericin B	☑	☑	☑	☑	
Palacos R + 30 mg active ingredient/0,8 g AmBisome®	☑	☑	☑	☑	
Palacos R + G plain	☑	☑	☑	☑	
Palacos R + G + 0.8 g pure Amphothericin B	☑	☑	☑	☑	
Palacos R + G + 30 mg active ingredient/0,8 g AmBisome®	☑	☑	☑	☑	
Palacos R + G + 2.66 g pure Amphothericin B	☑	☑	☑	☑	
Palacos R + G + 100 mg active ingredient 2,66 g AmBisome®	☑	☑	☑	☑	
Copal G + C plain	☑	☑	☑	☑	
Copal G + C + 50 mg active ingredient/1,33 g AmBisome®	☑	☑	☑	☑	

The methodology was configured as an in vitro antifungal study, and the results are presented herein

*pure Amphotericin B - Substance (Beijing Mesochem Technology Co., Ltd.); green = coloured areas are tested properties. Ambisome® with 50mg pure amphotericin B

Fig. 2 Different formulations and appearances of AmBisome®(**a**) AmBisome® as pure powder; (**b**) AmBisome® powder mixed in PMMA powder; (**c**) hardened AmBisome® containing Copal specimens

Results

Appearance

Mixing AmBisome® with PMMA not only changes the consistency of the cement, but also its colour. Orange Amphotericin B powder (Fig. 2a) changed the usually green coloured powder of Palacos and Copal cements used as it turns to yellowish-orange (Fig. 2b). The same was observed with the hardend cement samples (Fig. 2c).

ISO Compression strength

All cements tested containing Amphotericin B met the specification of ISO 5833 [21]. When adding Amphotericin B to plain Palacos R, only slightly lower compression values were detected (82.9 and 82.5 MPa). The same was true for Palacos R + G, with the exception of admixing 2.66 g pure Amphotericin B which produced slightly higher compression values (86.9 MPa). The lowest compression value was observed with Palacos R + G with 2.66 g AmBisome® (77.2 MPa). (Fig. 3)

The ISO compressive strength of the double-ALBC PMMA cement slightly increased by 3%, when 1.33 g Amphotericin B (CAS No 1397-89-3) were admixed to Copal cement (Table 3).

ISO bending strength

Plain Palacos showed higher values in bending strength than Palacos R + G. The addition of Amphotericin B decreased the values in every case whilst all tested

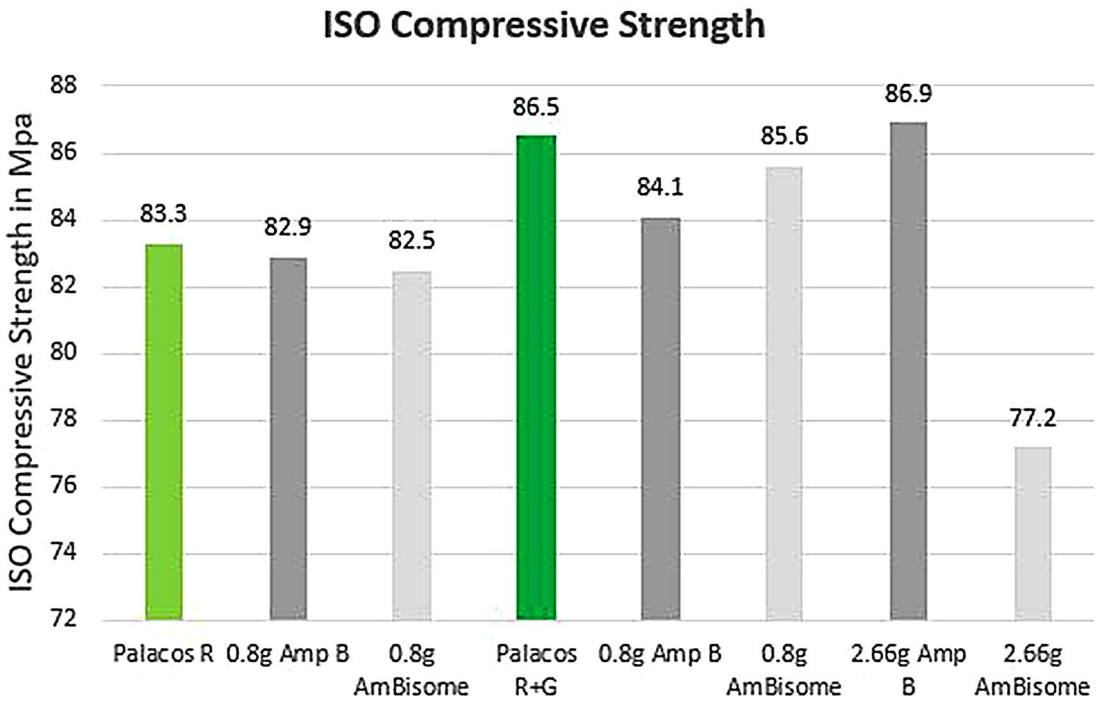

Fig. 3 ISO compressive strength of PMMA Cement samples. Samples were admixed with Amphotericin B (CAS No 1397-89-3, dark grey) non-liposomal, non-sterile and AmBisome® (liposomal, light grey). The dosage range in both from 0.8–2.66 g and compressive strength was measured in MPa (limit 70 MPa). The results of the compressive strength test corresponded to the standard specifications of ISO 5833. References: Palacos R (green) and Palacos R + G (dark green)

Table 3 ISO Compressive strength of double-loaded ALBC

Amphotericin B non-liposomal* (Pure Substance CAS- No.1397-89-3)	ISO Compressive Strength in MPa
Copal	82.2
Copal + 1.33 g (Amphotericin B)	84.6

Copal with an admix of Amphotericin B (CAS No 1397-89-3) with 1.33 g

Amphotericin B containing cement formulations met the bending specification of ISO 5833. Only slightly lower values were observed by Amphotericin added to plain Palacos R (65.8 and 67.9 MPa). When adding Amphotericin B to Palacos R + G, the pure Amphotericin B combination produced the lowest bending strength (59.1 MPa). High dosages of Amphotericin B reduced the bending strength significantly (51.9 and 54 MPa). No significant difference between pure and formulated Amphotericin B was observed (Fig. 4).

The ISO bending strength Copal cement decreased by 19% (65 to 52.7 MPa), when 1.33 g Amphotericin B (CAS No 1397-89-3) were admixed to the PMMA powder. Results were within limits of the standard specifications of ISO 5833 (Table 4).

ISO bending modulus

The bending modulus is not significantly influenced by adding Amphotericin B to Palacos cements. In all cases tested the ISO 5833 specifications were met. The lowest bending modulus was registered for Palacos R + G with 2.66 g AmBisome (2569 MPa). In all other cases the bending modulus increased compared to the plain reference cement formulations. The highest bending modulus (3228 MPa) was observed in Palacos R + G with 2.66 g pure Amphotericin B (Fig. 5).

The ISO bending modulus of Copal slightly increased by 3.2% (2914 to 3008 MPa), when 1.33 g Amphotericin B (CAS No 1397-89-3) were admixed to the PMMA powder. As with ISO bending strength, the results corresponded with the requirements for the standard specifications of ISO 5833 (Table 5).

DIN impact strength (Dynstat)

Dynstat impact strength of Palacos R (w/o Antibiotics) was significantly reduced up to 44% after the addition of Amphotericin B (CAS No 1397-89-3) and up to 23% after the addition of AmBisome®. When added to Palacos R + G (w Gentamicin) Amphotericin B decreased the Dynstat impact strength with increasing dosage up to 44% (. The amount of AmBisome® added caused a similar dose dependent decrease in Dynastat impact strength between 11 and 44%. In all cases the impact strength of the pure Amphotericin B was lower than compared with sterile AmBisome® (Fig. 6).

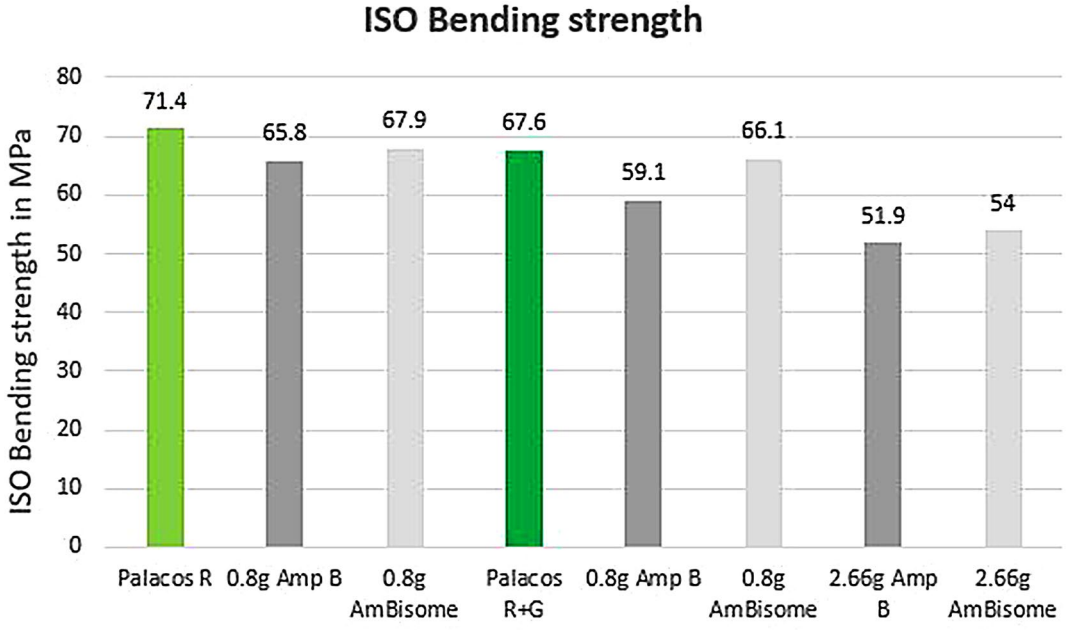

Fig. 4 ISO bending strength of PMMA Cement samples. PMMA samples were admixed with Amphotericin B (CAS No 1397-89-3, dark grey) non-liposomal, non-sterile and AmBisome® (liposomal, light grey). The dosage range in both from 0.8–2.66 g was measured in MPa (limit 50 MPa). The results of the bending strength test corresponded to the standard specifications of ISO 5833. References: Palacos R (green) and Palacos R + G (dark green)

Table 4 Bending strength of double-loaded ALBC

Amphotericin B non-liposomal* (Pure Substance CAS- No.1397-89-3)	ISO Bending Strength in MPa
Copal	65
Copal + 1.33 (Amphotericin B)	52.7

Gentamicin + Clindamycin (Copal G + C) and an admix of Amphotericin B (CAS No 1397-89-3) with 1.33 g

Table 5 Bending modulus of double-loaded ALBC

Amphotericin B non-liposomal* (Pure Substance CAS- No.1397-89-3)	ISO Bending Modulus in MPa
Copal	2914
Copal + 1.33 (Amphotericin B)	3008

Copal G&V and an admix of Amphotericin B (CAS No 1397-89-3) with 1.33 g

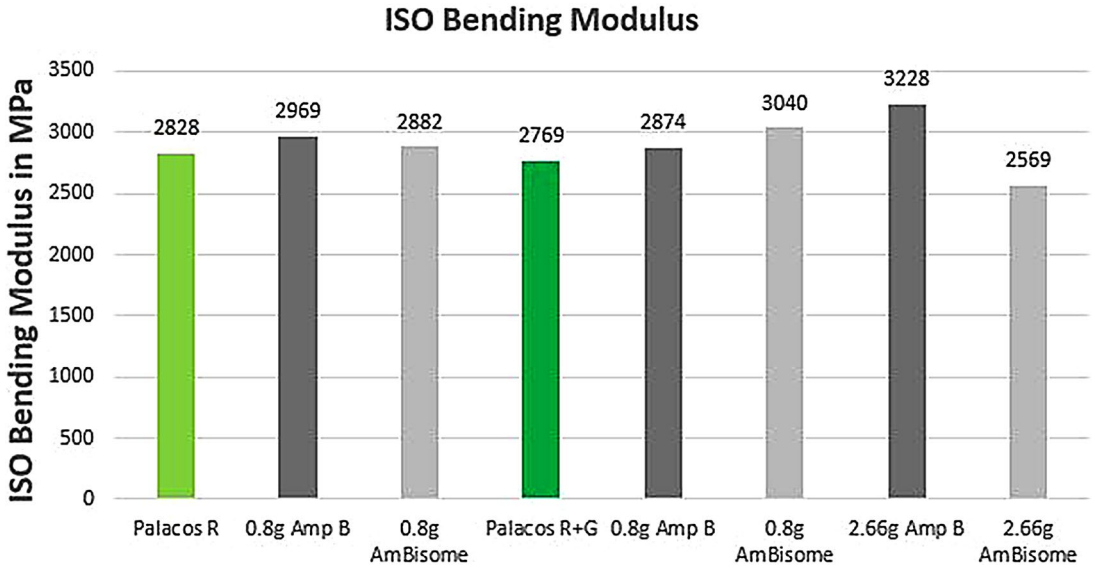

Fig. 5 ISO bending modulus of PMMA Cement samples. Samples were admixed with Amphotericin B (CAS No 1397-89-3, dark grey) non-liposomal, non-sterile and AmBisome® (liposomal, light grey). The dosage range in both from 0.8–2.66 g was measured in MPa. The results of the bending modulus test corresponded to the standard specifications of ISO 5833. References: Palacos R (green) and Palacos R + G (dark green)

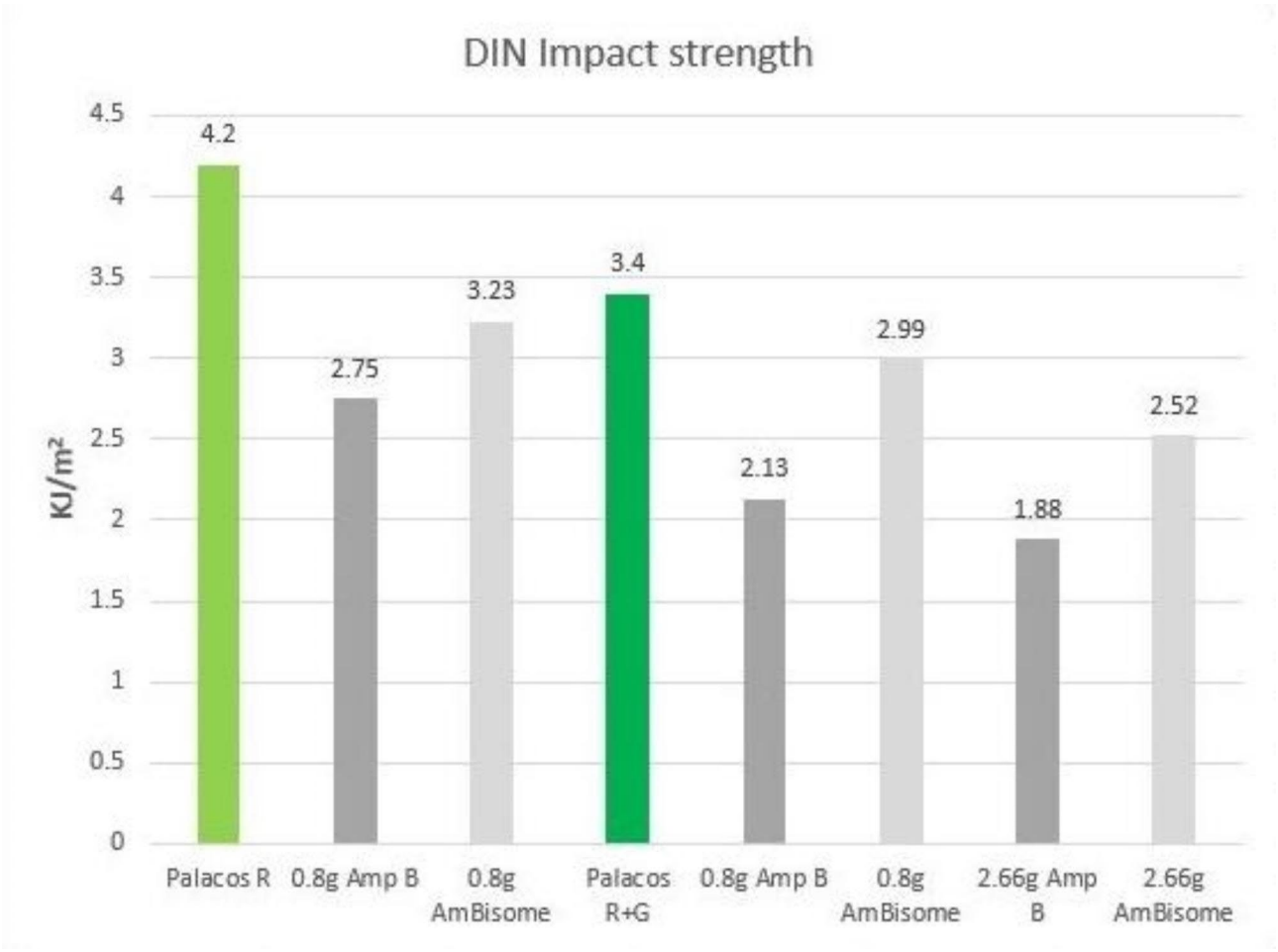

Fig. 6 DIN Impact Strength

Table 6 Dynstat impact strength

	Dynstat in KJ/m2	relative change to reference
Palacos R	**4.2**	
+ 0.8 g Amphotericin B	2.75	-34.52%
+ 0.8 g AmBisome	3.23	-23.90%
Palacos R + G	**3.4**	
+ 0.8 g Amphotericin B	2.13	-37.36%
+ 0.8 g AmBisome	2.99	-12.06%
+ 2.66 g Amphotericin B	1.88	-44.70%
+ 2.66 g AmBisome	2.52	-25.88%
Copal	**3.08**	
+ 1.33 g Amphotericin B	2.01	-33%

Percentages are in comparison with the references, which are the respective PMMA cements without admixed drug

Impact strength of Palacos R 4.2 kJ/m^2 was superior to Palacos R + G 3.4 kJ/m^2 and Copal, 3.08 kJ/m^2 (Table 6).

Elution

Amphotericin B release was highest on day 1 and remained detectable for 4 additional days (days 2–5). The release on day 1 is very dominant and decreases significantly within the next 48 h (Fig. 7).

Case Presentation 1

A 50-year-old paraplegic patient (male) developed an infection with *Candida albicans* after numerous procedures to treat the instability of his right total hip replacement (THR). He was paraplegic sub Th8 resulting from a gunshot injury in 1991 with concomitant fracture of the thoracic vertebrae 10 and 11. The THR was primarily implanted for a neck of femur fracture after a minor fall in 2011. The first 6 years were uneventful, but the patient developed instability and needed three surgical interventions for recurrent dislocations in September 2017. He developed urosepsis with *Klebsiella pneumoniae* during the same inpatient stay. The following year a sinus formed over the hip which was treated with open biopsy. Samples turned positive for *Staphylococcus epidermidis*. No further treatment was initiated complying with the patient's wish at that time.

In 2021 the THR became symptomatic as the inlay had fractured and a two-staged excision arthroplasty was

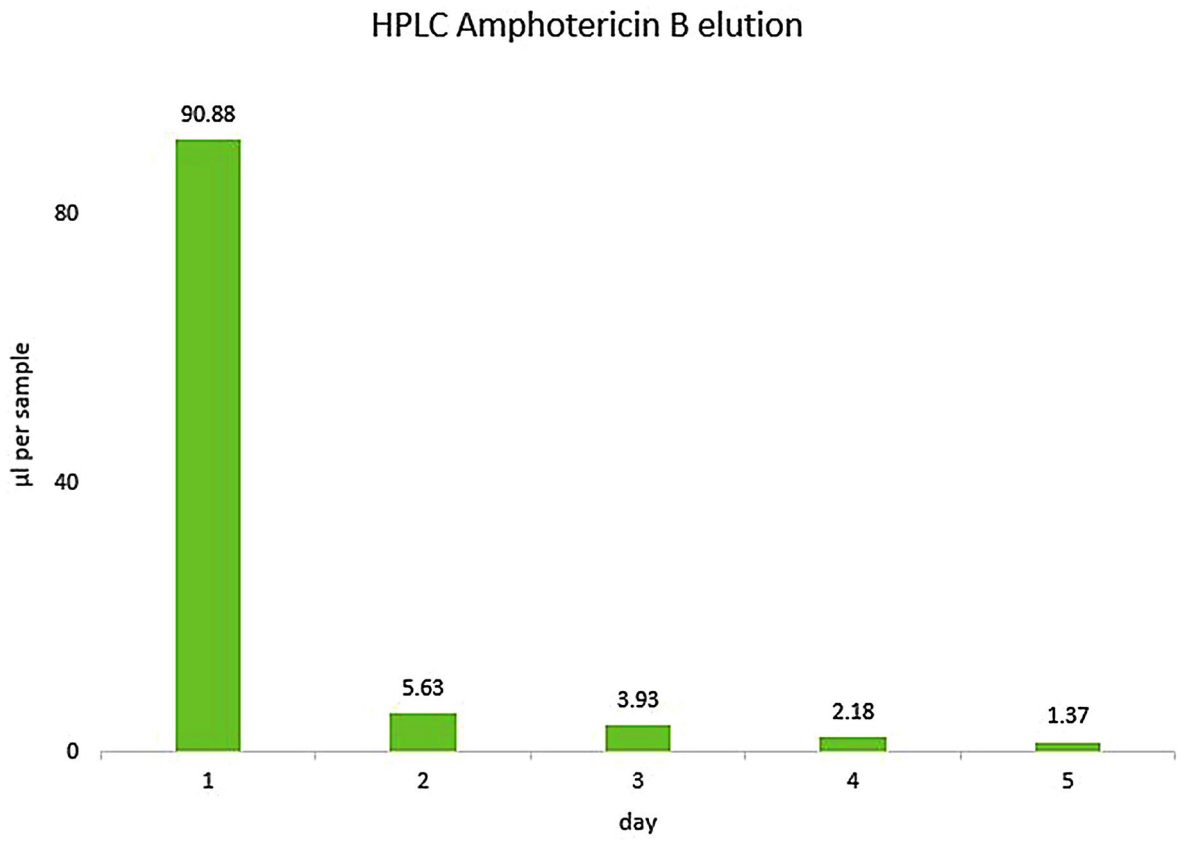

Fig. 7 HPLC elution data of Amphotericin B from PMMA cement. Elution was recorded over a period of 5d and measured in µl per sample (specimens: 9 × 5 × 2 mm). HPLC with MS/MS detection (calibration standard: Amphotericin B, internal standard Rifaximin, AZB, Berlin, Germany)

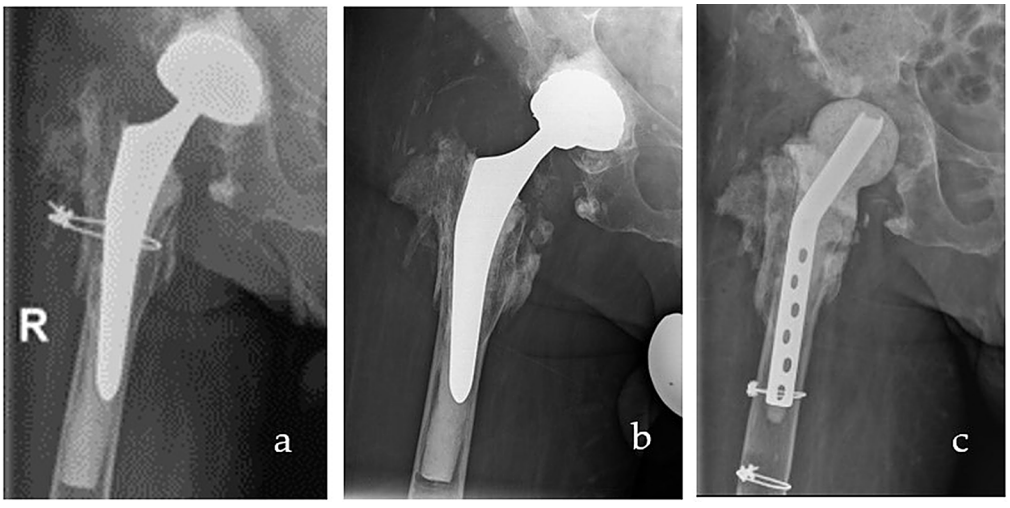

Fig. 8 Right hip x-rays in ap views. (**a**)Cemented THR prior to debridement and sampling; (**b**) Cemented right THR 3 years after the debridement with the fractured inlay; (**c**) handformed PMMA spacer over a blade plate

performed (Fig. 8a, b). During the 1st stage the THR was removed and a preformed size 54 Spacer (Vancogenx®-Space Hip, Tecres, Sommacampagna, Italy) was inserted and fixed with 40 g of Copal® G + V. Once daily intravenous Daptomycin 850 mg/d (Cubicin®, MSD Merck Sharp&Dohme AG, Lucerne, Switzerland) was started as systemic treatment.

Intraoperative cultures grew *Candida albicans* in deep tissues and sonication. After a loading dose of 800 mg of oral Fluconazole (Diflucan®, Pfizer PHARMA GmbH,

Berlin, Germany), a daily 400 mg of oral Fluconazole were given as systemic antimycotic therapy. As the infection was not well controlled with the spacer previously inserted, another 1st stage with exchange of the spacer was performed. A handmade and -mixed antifungal articulating spacer with 200 mg Amphotericin B (4 vials of AmBisome®) and 40 mg of Copal® G + V was formed over a blade plate (Fig. 8c). Those latter samples were positive for *Pseudomonas aeruginosa* in 2 specimens. Therefore, 750 mg of Ciprofloxacin (Ciproxin®, Bayer AG, Zurich, Switzerland) BDS were added to the antimicrobial regimen.

After another 6 weeks of treatment with local and systemic antimicrobial therapy, the infection was well-controlled. Thus, the spacer was removed and a situation with resection arthroplasty plus an interposed pedicled vastus lateralis/vastus intermedius muscle flap was created. The total systemic antimicrobial therapy was continued for 3 months.

As the patient was non-ambulating since the gunshot injury, satisfactory outcome was achieved. He did not show any signs of local or systemic infection recurrence with a follow up of 4 years.

Case presentation 2

A 63-year-old female patient suffered from a long standing painful left total knee replacement (TKR) (Fig. 8a). She was ambulating for short distances using crutches. Over a period of 6 months prior to presentation, she experienced significant swelling and erythema. During the standard workup for a painful joint replacement, an aspiration showed a leucocyte count of 9'130 with 73% PMN (polymorphonuclear cells). Cultures were positive for *Candida parapsilosis*. Thus, a two-stage exchange with an antimycotic spacer was planned.

The patient previously had multiple surgeries for her left knee following the primary TKR for avascular necrosis of the medial condyle. After secondary patella resurfacing in 2014, she suffered from a culture-negative infection which was treated with a one-stage exchange the following year. She has a medical record with significant comorbidities such as obstructive sleep apnoea syndrome, hypertension, bleeding disorder (hereditary hypofibrinogenaemia), recurrent seizures and occlusive peripheral vascular disease.

During 1st stage surgery a PMMA spacer (Copal® G + V) was formed using the preformed "knee-moulds" by Heraeus Medical (Fig. 9b). A total of 120 g of Copal® G + V were hand mixed with 600 mg of Amphotericin B (AmBisome®). After three months the second stage surgery was done, the spacer was removed and a revision TKR implanted (Fig. 9c). Systemically, intravenous 50 mg once daily of Caspofungin (Caspofungin Sandoz eco®, Sandoz Pharmaceuticals, Rotkreuz, Switzerland) was given the first week after the 1st and 2nd stage which was then exchanged for oral Fluconazole 400 mg/d (Diflucan®) which was given between surgeries and for a total of 6 months after reimplantation.

After 1.5 years of follow-up the implant remains stable and neither local nor systemic signs of persistent infection are present. Due to multiple surgeries and recurrent falls attributed to her comorbidities the patient remains impaired in function and is limited to walking with crutches within the limits of her room.

Potential adverse effects

The risk profile of AmBisome® comes with a few significant potential adverse effects. Common side effects are fevers, shivers, anaphylactic reactions, low serum potassium, nausea, vomiting, impaired kidney function and head- or backpain, No adverse event was documented for either patient. Both patients maintained normal serum creatinine levels during their treatment with Amphotericin B. Preoperative kidney function was normal and serum creatinine levels stayed well below upper thresholds after implantation of, during the treatment with and after removal of the spacer (Fig. 10).

Discussion

The change of colouration of the usually greenish cement to orange is normal in mixtures containing Amphotericin B as AmBisome® is an orange powder by itself. There is

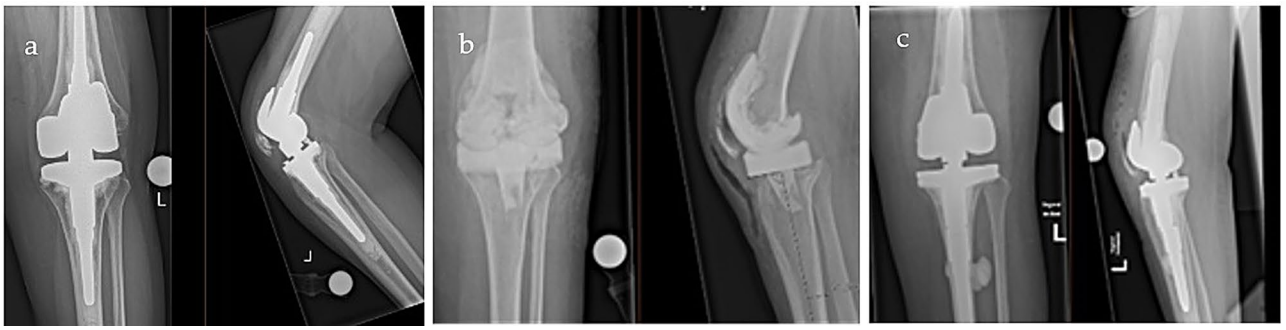

Fig. 9 Left knee x-rays on ap and lateral views. (**a**) Preoperative Imaging left TKR; (**b**) handmixed "mould"-made knee spacer with Copal G + V and Amphotericin B; (**c**) Post left revision TKR after 2nd stage surgery

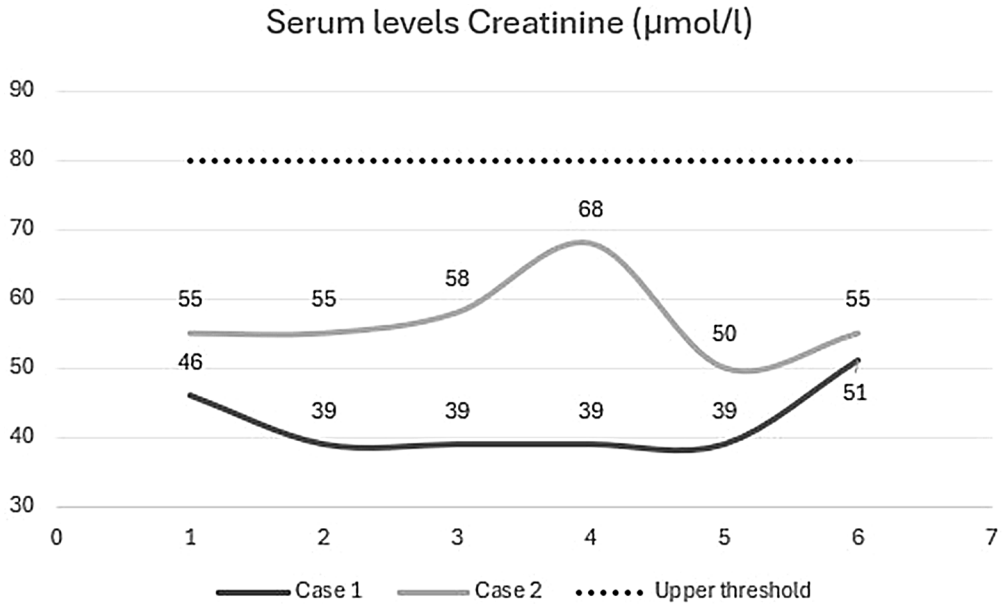

Fig. 10 Serum creatinine levels (µmol/l) tested during treatment

Table 7 Comparison of sterile commercially available amphotericin powder preparations

Amphotericin B	Dosage form	Manufacturer/ Product name	Amphotericin B in mg active substance	Total weight
l-Amphotericin B	Powder for infusion+	Gilead science GmbH AmBisome®	50 mg	1.33 g
c-Amphotericin B	Powder for infusion	Cephlapharm (before Bristol-Myers Squibb) Amphotericin B	52 mg	114 mg
Amphotericin B colloidal dispersion (c-Amphotericin B)	Powder lyophilized	InterMune, Inc. Amophotec®	50 mg	1.03 g
Amphotericin B lipid-complex	Concentrate for Suspension for Infusion	Cephalon Abelcet® (no longer on the market)	5 mg/ml a vial containing 20 ml (100 mg Amphotericin B	N/A
c-Amphotericin B	Powder for sterile concentrate	Neon Healthcare Limited Fungizone®	50 mg	N/A Inquired with the manufacturer

l-Amphotericin B = liposomal Amphotericin B, c-Amphotericin B = conventional (non-liposomal) Amphotericin B

no chemical interaction between the Amphotericin powder and the polymer powder of PMMA cement.

Due to the high amount of powder creating a homogenous mixture proved to be challenging. Just as in adding high doses of Voriconazole, white specks appeared on the surface [7, 8]. Higher amounts of solid components in mixing cement could lead to higher release rates but are detrimental to the cement's mechanical properties such as compressive and bending strength [8, 23–25]. The inhomogeneous structure of the cement might help to release the active drug, but if a homogenous mixture is desired, low viscosity cement or high viscosity cement with actively cooled components should be used [26] (Table 7).

In order to achieve the presumed effective local dosages, it is necessary to consider the content of Amphotericin in the dosage form for infusion. The effective dosage is typically administered at a concentration of 200–600 mg of the active ingredient [17, 27]. A total weight of 1.33 g of Amphotericin B in powder for infusion contains 50 mg of the active ingredient (Tabl.5). In order to achieve the aforementioned recommendations, a dosage of 5.32 g to 15.96 g in total weight is required, administered in 40 g of bone cement. Given the mechanical stability of the material, the maximum recommended antimicrobial dose of 10% of the PMMA powder amount (equivalent to 4 g per 40 g) is exceeded by a factor of almost 4.To our knowledge, no study has been published for neither Amphotericin B nor Fluconazole studying

the effect the amount of powder has on the mechanical properties [7]. Marra and Houdek stated how much active drug of Amphotericin B was mixed to PMMA, but no data was published on the amount of powder added, thus, every statement made about mechanical stability has to be interpreted with caution [28, 29].

Concerning ISO compression strength, we found no significant effect of adding Amphotericin to PMMA. The lowest compression strength was found with Palacos R + G and 2.66 g AmBisome® (77.2 MPa). Czuban et al. tested lower dosages and were able to show that independent from the Amphotericin B formulation used, the compressive strength of PMMA spacers stayed within the thresholds for implant fixation [18]. This was in keeping with the results of Chang [19]. Additionally, we found similar results with Amphotericin B in Copal cement and by adding additional antibiotics to Copal which is used for revision surgeries [30, 31].

According to the ISO bending and DIN impact strength the addition of Amphotericin B decreased the values in every case whereas the ISO bending modulus was increased. These results are very similar to those when manually adding Voriconazole to PMMA [8].

It is not advisable to aim for either a lower or a higher compressive strength. For example, high compressive strength combined with a high modulus of elasticity is disadvantageous in surgery. The cement's purpose is to provide an elastic buffering interface. The standard must include an upper limit, mainly for the modulus of elasticity but also for compressive strength. In comparison to highly viscous orthopedic cement, most low-viscosity PMMA cements show the highest compressive strength, which must be taken into consideration [32].

In our study the release of Amphotericin B peaked on day 1 and the substance was still detectable on day 5 tested by HPLC. Spectrometric methods have been demonstrated to yield superior results, particularly in the case of Amphotericin B liposomal/non-liposomal analysis within the UV-VIS range [18]. Amphotericin B provided consistent supernatant concentrations (release), between 1.75 and 2.0 µg/mL over 110 days [13]. Marra et al. (2001) [29] performed an in vivo study of the elution of Amphotericin B from Palacos bone cement (Heraeus, Wehrheim, Germany). They detected a maximum wound fluid concentration of 3.2 mg/L and an undetectable serum concentration 50 h post implantation. In addition to systemic therapy postoperative, adjunctive antifungal-loaded bone cement has been utilized to successfully treat these infections. Amphotericin B is most commonly used, but cases utilizing Voriconazole, Fluconazole, and Itraconazole have been reported as well. In vitro data suggest a minimal elution of Amphotericin B [24].

In vivo both patients presented in this study were successfully treated for their PJI with *Candida spp* with Amphotericin B admixed to Copal spacers, due to its efficacy on *Candida* infections and its compatibility with PMMA cement in terms of heat stability during polymerization and release from the cement [13, 18, 28].

Systemically administered mixtures containing Amphotericin B are known to be nephrotoxic, an effect which is supposed to be smaller in liposomal Amphotericin B formulations such as AmBisome®. As they contain unusually high amounts of the drug, long-standing PMMA spacers could potentially be nephrotoxic [33, 34]. Neither patient presented showed any relevant changes in kidney function or other adverse effects during the treatment with Amphotericin B. The literature on PMMA spacers containing Amphotericin B in PJI with *Candida spp*. is scarce but suggests good tolerance and favorable clinical results [13, 15, 26, 35–37].

A recent review from 2022 did not find any consensus in the usage of antifungal drugs in PMMA spacers. Amphotericin B, Fluconazole and Voriconazole were the three most common substances used [7].

In fungal PJI in hips and knees Fluconazole is the antimycotic agent most regularly added to form PMMA spacers followed by Voriconazole. Because of its potential nephrotoxicity Amphotericin B was less frequently used. On the other hand, Amphotericin B reliably inhibits fungal growth and biofilm formation when released from PMMA cement [28, 34], liposomal formations showed better drug release than standard recipes [18]. Marra et al. detected relevant drug levels in blood serum and drain fluid whilst successfully treating *Candida* infections with Amphotericin B loaded PMMA spacers [29].

The safety profile of liposomal Amphotericin B formulas is much more favorable, especially in terms of nephrotoxicity, than the "old" Amphotericin B desoxycholate. Voriconazole and Fluconazole can have systemic side effects such as hallucinations, low serum potassium or sodium, cardiac side effects, nausea and vomiting as well as hepatotoxicity. Thus, every antifungal drug administered needs close monitoring [38, 39].

Gentamicin is another drug known for its nephrotoxicity. Muir et al. did show, that even high local doses of Gentamicin did not have systemic side effects nor did they affect kidney function [33].

According to local recommendations 200 mg of Amphotericin B (AmBisome®, Gilead Sciences Switzerland Sàrl, Zug, Switzerland) were mixed with every 40 g of Copal® G + V (Heraeus Medical, Wehrheim, Germany). Liposomal Amphotericin B is not available as a pure sterile substance, and the formula available, AmBisome® contains additives to enhance resorption as an infusion. Thus, 1.33 g of powder are needed to administer 50 mg of the active drug (3.8%). 40 g of Copal G + V contain 2.5 g of antibiotic substance, i.e. 0.5 g of Gentamicin and 2 g of Vancomycin. When adding 200 mg of Amphotericin B

via AmBisome® to 40 g of Copal G + V, a total of 7.82 g of powder are used with the PMMA cement.

Krampitz et al. tested the mechanical properties of different formulas of Voriconazole spacers (200 mg and 600 mg). 44–51 g of powder were used to create these formulas. Even though it remains unclear if the mechanical properties are the same, it seems it is more the amount of powder than the substance itself [8]. In our cases, the active substance was 16% of the total 47.82 g of powder used which is more than the recommended 10% but well below the 26% of the 600 mg Voriconazole spacers.

Furthermore, the 10% rule is used for creating PMMA cement suitable for implant fixation. The high mass and diameter of any spacer reduce the demands in terms of mechanical stability for the formula. Moreover, the second patient was kept to protected weight bearing until the definitive implant was inserted while the first patient was paraplegic and thus non-weightbearing.

There are some concerns regarding systemic toxicity, such as nephrotoxicity, due to the uncontrolled elution of antibiotic-containing PMMA spacers [40–42], but multiple studies have recently called this paradigm into question [36, 43–46]. In summary, systemic toxicity due to the antibiotics and antimycotic released from cement spacers seems unlikely, and the clinical practice guidelines from the Infectious Disease Society of America agree that systemic toxicity from antibiotics used in cement is exceedingly rare [36, 44].

Chaudhry et al. found in a recent review, that the use of nephrotoxic substances was one of the major risk factors for acute kidney injury in two-stage revisions of PJI [36]. We were unable to identify a similar effect in our group, as both patients maintained normal renal function, even though high amounts of Amphotericin B were used.

The small sample size is surely a limitation to this study, but as mentioned above, cases of PJI involving PMMA spacers with admixed antimycotic drugs are exceptionally rare. We strongly advocate for conduction of clinical trials with long-term follow-up to evaluate our findings.

Conclusion

The orange colouration of PMMA spacers with AmBisome® is nothing to worry about as the colour stems from the orange powder itself. The mechanical properties of PMMA cement with admixed Amphotericin B remained well within the limits required by the ISO testing and thus to support spacers of the hip and/or the knee, even though the amount of powder used was almost double the recommended 10% threshold. Sterile Amphotericin B is available as powder for preparing an infusion solution and contains only small amounts of pure drug. In vivo polymicrobial *Candida* infections complicated with bacterial co-infections were successfully treated using the combination of Copal cement with added Amphotericin B without systemic nephrotoxic impact.

Abbreviations

ALBC	Antibiotic-loaded Bone Cement
DIN	German Institute for Standardization
HPLC	High performance liquid chromatography
ISO	International Organization for Standardization
PJI	Periprosthetic Joint Infection
PMMA	Polymethylmethacrylate

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Data availability
The data generated and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Written informed consent has been obtained from the patients to publish this paper.

Competing interests
F.F., M.M. and J.S. declare they have no competing interests. M.C. received a grant from Heraeus Medical unrelated to this research. B.K., R.S. and K.-D.K. are employed by Heraeus Medical.

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