

Clinical Device-Related Article

Antibiotics release from cement spacers used for two-stage treatment of implant-associated infections after total joint arthroplasty

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Abstract: Two-stage revision arthroplasty is the treatment of choice for periprosthetic infection, a serious complication after knee or hip arthroplasty. Our prospective clinical trial aimed to investigate the concentrations of gentamicin and vancomycin in wound exudate and tissue in two-stage revision arthroplasty. Wound exudate and periprosthetic membrane samples were collected from 18 patients (10 hip and eight knee patients), who were due for two-stage treatment after a periprosthetic joint infection. Samples were taken during insertion of antibiotic-impregnated spacers and after their removal. The concentrations of gentamicin and vancomycin in wound exudates and adjacent tissue were analyzed using high-performance liquid chromatography mass spectrometry. Average time period of spacer implantation was 13.6 weeks (9.3–22.6 weeks). The concentration of vancomycin in wound exudate decreased from a median of 43.28 µg/mL (0.28–261.22) after implantation to 0.46 µg/mL (0.13–37.47) after the removal of the spacer. In the adjacent tissue, vancomycin concentration

was mainly undetectable prior to spacer implantation (0.003 µg/g [0.003–0.261]) and increased to 0.318 µg/g [0.024–484.16] at the time of spacer removal. This was also observed for gentamicin in the tissue of patients who previously had cement-free implants (0.008 µg/g [0.008–0.087] vs. 0.164 µg/g [0.048–71.75]) while in the tissue of patients with previously cemented prosthesis, baseline concentration was already high (8.451 µg/g [0.152–42.926]). Despite the rapid decrease in antibiotics release from spacer cement observed *in vitro*, *in vivo* antibiotics are much longer detectable, especially in the adjacent soft tissue. © 2018 The Authors. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* published by Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 107B:1587–1597, 2019.

Key Words: antibiotics release, cement spacer, two-stage treatment, periprosthetic joint infection, vancomycin, gentamicin, revision arthroplasty

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INTRODUCTION

Periprosthetic joint infection is the most severe local specific complication associated with total joint replacement (TJR). While the incidence is relatively low, with 0.5–2.0% after primary implantation,^{1,2} it still represents a considerable number of cases, especially given the background of the ever rising implantation rates.³ There are two main treatment regimes: either a single stage revision—removal of the

infected implant, debridement and re-implantation of a TJR—or a long-interval two-stage spacer procedure. The latter is the more commonly used method, in particular for difficult-to-treat pathogens and in patients who had already undergone a previous revision due to infection.^{4,5} In the two stage approach, systemic antibiotic therapy is adjusted according to the intraoperatively detected pathogens and is given for 6 weeks after implant removal in order to

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eradicate the causative pathogens. During this period, spacers made of polymethylmethacrylate (PMMA) cement function as an interposition arthroplasty, which provides mechanical stability, preserves the space needed for the revision implant and when impregnated with antibiotics serves as an antibiotic delivery system.^{6,7} The use of antibiotic-impregnated cement was shown to efficiently prevent implant-associated infections.^{8,9} With the rise of antibiotic-resistant pathogens, often more than one antibiotic is added to the bone cement to enable broadened prophylactic effects. One common supplementation is the combination of gentamicin and vancomycin, which have been shown to act synergistically.^{10,11} While there are a number of studies investigating the *in vitro* and *ex vivo* release of antibiotics from bone cement,^{12–17} less is known about the concentration of the antibiotics in joint fluid and adjacent soft tissue *in vivo*, especially for the duration of the cement spacer treatment.^{18,19}

Therefore, the aim of the present study was to analyze the concentrations of gentamicin and vancomycin in wound exudate and in the adjacent soft tissue (periprosthetic membrane) not only at the time of implantation but also at removal of the PMMA cement spacer. These data were compared to *in vitro* antibiotics release from prepared test specimens of same cement spacer material.

MATERIALS AND METHODS

Study design

Patients from two prospective, multicenter trials investigating cement spacer treatment of implant-associated infections after total hip and knee joint replacement, respectively, were included to analyze antibiotics' release from those spacer implants. Both studies were approved by the Local Ethics Committee of Rostock, Germany (registration numbers: A2011-137 and HV-2011-0011). The study design followed a standardized algorithm of cement spacer treatment as described before.²⁰ Briefly, patients who were preoperatively diagnosed with periprosthetic joint infection due to a positive microbiological joint aspiration and elevated inflammation markers were implanted with a custom-made antibiotic-loaded PMMA cement spacer after complete removal of the infected endoprosthesis and extensive debridement. Two different bone cements were used in the *in vivo* study: PALACOS[®] R+G (Heraeus Medical GmbH, Wehrheim, Germany) a fast-curing, radiopaque, poly(methyl methacrylate)-based bone cement which contains the aminoglycoside antibiotic gentamicin and COPAL[®] spacem, a fast-curing cement-like polymer based on poly(methylacrylate, methyl methacrylate) with no added antibiotics which contains calcium carbonate as an X-ray contrast agent (Heraeus Medical GmbH, Wehrheim, Germany). In all hip patients, PALACOS[®] R+G cement was combined with vancomycin (Vancomycin hydrochloride, Lyomark Pharma, Oberhaching, Germany), while patients with total knee replacements were randomly allocated to either receiving a spacer made from PALACOS[®] R+G cement plus vancomycin or a spacer made from COPAL[®] spacem loaded with gentamicin (gentamicin sulfate) and vancomycin (composition listed in Table I). The

final concentration of the antibiotics in all spacers was 0.5 g gentamicin and 2 g vancomycin per 40 g of spacer cement. The amount of spacer cement differed between 40 and 160 g per patient, depending on the extent of the operation, and was adjusted individually to the patient's joint conditions (Table II). The shape and stability of spacers were ensured by silicon molds with a metal core (StageOne[™] Hip or Knee Cement Spacer Molds, Biomet Inc., Warsaw, IN). After a 4- to 6-week systemic antibiotics therapy and an antibiotics-free time interval of 2 weeks, control samples that is, joint fluid and a punch biopsy of the soft tissue around the spacer were taken without intra-articular local anesthesia in order to avoid interactions with the antiseptic properties of local anesthetics. If clinical and laboratory findings as well as histological and microbiological samples showed no signs of ongoing infection after another 2 weeks, the spacer was explanted and the revision endoprosthesis was implanted. If there were continued signs of inflammation or extended wound secretion, an additional cement spacer exchange was performed according to the treatment scheme. Periprosthetic membrane samples and redon-drainage (i.e., continuous negative drainage) containing wound exudate were taken during implantation as well as during removal of the cement spacers to measure the antibiotic release from the cement spacers.

Study patients

Between July 2012 and January 2015 18 patients (10 total hip replacement (THR) patients and eight for total knee replacements (TKR)) were recruited at the Department of Orthopedics. Inclusion criteria were 45–85 years of age and the ability to understand the scope and importance of the trial. Patients with an active tumor disease, known allergy to the used materials or participation in other medical trials as well as lactating and pregnant women were excluded from this study. All included patients were adequately informed about the study by trained investigators and had signed the informed consent.

From the 18 recruited patients with periprosthetic infection, nine were men and nine were women with an average age of 67.6 (48.6–79.4) with the majority of patients belonging to the 70–79 age group (Table III). Most of the patients (83.3%) were either overweight or obese (Table III). Mean body mass index (BMI) of all 18 patients was 30.4 (19–39). The most common comorbidities were high blood pressure (16 patients), Diabetes mellitus (nine patients) and heart disease including arrhythmia (five patients) followed by metabolic disorders such as hyperlipidaemia (four patients) and hyperuricemia (three patients). Five patients suffered from allergies and two were smokers.

Quantitative determination of gentamicin and vancomycin release *in vivo*

After spacer implantation as well as explantation surgery the redon-drainage containing 24-h wound exudate was collected in order to measure the antibiotic release of gentamicin and vancomycin. Additionally, a piece of periprosthetic membrane (approx. 10 mm × 10 mm × 10 mm) was

TABLE I. Composition of Bone Cements

	PALACOS [®] R+G	COPAL [®] spacem	COPAL [®] G+V
Polymer powder ingredients	<ul style="list-style-type: none"> • Poly(methylacrylate, methyl methacrylate) • Zirconium dioxide • Benzoyl peroxide • Colorant E141 (chlorophyll) • Gentamicin^a (in the form of gentamicin sulfate) 	<ul style="list-style-type: none"> • Poly(methylacrylate, methyl methacrylate) • Calcium carbonate • Benzoyl peroxide • Colorant E141 (chlorophyll) 	<ul style="list-style-type: none"> • Poly(methylacrylate, methyl methacrylate) • Zirconium dioxide • Benzoyl peroxide • Colorant E141 (chlorophyll) • Gentamicin^a (in the form of gentamicin sulfate) • Vancomycin^b (in the form of vancomycin hydrochloride)
Monomer liquid ingredients	<ul style="list-style-type: none"> • Methyl methacrylate • N,N-dimethyl-p-toluidine • Hydroquinone • Colorant E141 (chlorophyll) 	<ul style="list-style-type: none"> • Methyl methacrylate • N,N-dimethyl-p-toluidine • Hydroquinone • Colorant E141 (chlorophyll) 	<ul style="list-style-type: none"> • Methyl methacrylate • N,N-dimethyl-p-toluidine • Hydroquinone • Colorant E141 (chlorophyll)
Ingredients additionally mixed in	<ul style="list-style-type: none"> • Vancomycin^b (in the form of vancomycin hydrochloride) 	<ul style="list-style-type: none"> • Gentamicin^a (in the form of gentamicin sulfate) • Vancomycin^b (in the form of vancomycin hydrochloride) 	

^a 0.5 g (0.84 g gentamicin sulfate) gentamicin per 40 g powder component.

^b 2.0 g vancomycin per 40 g powder component.

acquired during the surgery. The tissue sample was obtained before the implantation of the spacer during initial debridement to eliminate any contact between tissue and spacer. The redon-drainage and the periprosthetic membrane samples were stored at -20°C and weighed. The weight of the membrane samples was 3.2 ± 2.1 g (mean \pm SD) while for the wound exudates an average of 260 ± 161 g (mean \pm SD) were collected after 24 h (Table II). The quantification of antibiotics release was performed by a (GLP)-certified testing facility (Analytisches Zentrum Biopharm GmbH, Berlin, Germany).

Standardized quantities of 1 g of periprosthetic membrane and 0.5 mL wound exudate were deployed for the analysis of gentamicin components C1a, C2/C2a, C1, and vancomycin. Deproteinization was carried out by sonication of tissue and exudate in 4% (v/v) perchloric acid in water followed by centrifugation. The gentamicin components C1a, C2/C2a, and C1 were derivatized with propyl chloroformate in acetone and extracted in ethyl acetate/hexane (liquid/liquid extraction). After evaporation of the organic phase the samples were dissolved in reconstitution solution and separated on a C18 column (Restek Ultra PFPP, 5 μm , 100 \times 2.1 mm, Restek GmbH, Bad Homburg, Germany) under gradient conditions. Vancomycin was separated directly after protein precipitation under gradient conditions using a C18 column (Luna C18(2), 5 μm , 150 \times 2.1 mm, Phenomenex, Aschaffenburg, Germany), too. Analysis was carried out using a high performance liquid chromatography Agilent 1200 system (Agilent Technologies, Waldbronn, Germany) and mass selective detection using an API 4000 QTrap (ABSciex, Darmstadt, Germany). Ionization was carried out with an electrospray interface (ESI, positive polarity) and mass selective detection in the multiple reaction monitoring mode (MRM). The calibration range for gentamicin components C1a/C2 + C2a/C1 in membrane and exudate was 0.00128/0.00160/0.00112 $\mu\text{g}/\text{mL}$ to 1.600/2.000/1.400 $\mu\text{g}/$

mL, respectively. Quality controls with different concentrations showed that accuracy after separation was $99.87 \pm 7.69\%$ (mean \pm SD, coefficient of variation [CV] = 7.70%). The ratio of the gentamicin components to each other varied slightly after elution depending on the concentration (CV = $5.60 \pm 2.49\%$), however, the difference was not significant. The sum of the three components was used for statistical analysis since membrane and exudate samples showed a similar distribution of the different components of gentamicin compared to the calibration samples. Calibration range for vancomycin was 0.0025–2.500 $\mu\text{g}/\text{mL}$. Each batch was controlled using quality control samples at three levels and showed good reproducibility. Lower limit of quantification (LLOQ) for gentamicin were 0.008 $\mu\text{g}/\text{g}$ in periprosthetic membrane samples and 0.004 $\mu\text{g}/\text{mL}$ in wound exudates, LLOQs for vancomycin were 0.0025 $\mu\text{g}/\text{g}$ in periprosthetic membrane samples and 0.125 $\mu\text{g}/\text{mL}$ in wound exudates.

Quantitative determination of gentamicin and vancomycin release *in vitro*

Before production of the test specimens, bone cement powders “COPAL[®] G+V,” “PALACOS[®] R+G” and “COPAL[®] spacem” (Heraeus Medical GmbH, Wehrheim, Germany, Table I) were dried in a desiccator in a vacuum (<20 mbar) for at least 24 h.

COPAL[®] G+V contains gentamicin and vancomycin and did not require further antibiotics addition. PALACOS[®] R+G contains gentamicin but lacks vancomycin, therefore 2 g vancomycin hydrochloride (Lyomark Pharma GmbH, Oberhaching, Germany) were added. COPAL[®] spacem contains neither vancomycin nor gentamicin; accordingly 2 g vancomycin hydrochloride and 0.87 g gentamicin sulfate were added. The final concentrations of antibiotics were 1.17% w/w gentamicin and 4.66% w/w vancomycin, 1.17% w/w gentamicin and 4.67% w/w vancomycin as well as 1.16% w/w

TABLE II. Antibiotics Concentration in Wound Exudate and Tissue Samples and Information About Possible Influencing Factors

Patient ID	Previous Implant Cemented	Weight of 24 h Wound Exudate (g) Collected at	Spacer Characteristics			Antibiotics Concentration in Wound Exudate (µg/mL)				Antibiotics Concentration in Tissue Samples (µg/g)			
			Spacer Cement	Mold Used ^c	Amount (g) of Prepared Cement with [V (g), G (g)]	Vancomycin		Gentamicin		Vancomycin		Gentamicin	
						IMP	EXP	IMP	EXP	IMP	EXP	IMP	EXP
H1 ^a	No	186	Palacos R+G	M1	160 [8.0, 2.0]	n.c.	20.80	n.c.	0.00*	0.09			
H1 2nd spacer			Palacos R+G	n.s.	40 [2.0, 0.5]						0.04	0.16	
H2 ^b	Yes	133	Palacos R+G	M3	160 [8.0, 2.0]	6.69	12.53	0.01	39.49	0.15	1.07		
H3	No	302	Palacos R+G	M3	160 [8.0, 2.0]	3.67	9.44	0.00*	0.26	0.01*	0.13		
H4 ^b	Yes	263	Palacos R+G	M3	160 [8.0, 2.0]	5.47	71.80	0.00*	0.38	25.81	0.05		
H5 ^a	No	n.c.	Palacos R+G	M3	160 [8.0, 2.0]	n.c.	n.c.	0.00*	484.16	0.01*	71.75		
H6	No	167	Palacos R+G	M3	80 [4.0, 1.0]	21.55		0.00*		0.01*			
H6 2nd spacer			Palacos R+G	n.s.	60 [3.0, 0.75]	0.13*	53.87		0.02		0.05		
H7	No	213	Palacos R+G	M1	80 [4.0, 1.0]	37.47	23.01	0.00*		0.01*	0.12		
H8 ^b	No	552	Palacos R+G	n.s.	40 [2.0, 0.5]	19.49		9.36		0.01*			
H8 2nd spacer			Palacos R+G	M3	160 [8.0, 2.0]	0.44	0.01		0.23		0.70		
H9	No	478	Palacos R+G	M3	140 [7.0, 1.75]	189.05	15.63	0.00*	16.31	0.01*	17.98		
H10	No	359	Palacos R+G	M3	160 [8.0, 2.0]	27.82	9.38	0.01	0.29	0.01*	0.87		
K1 ^a	Yes	425	Palacos R+G	T4+F4	160 [8.0, 2.0]	50.19	18.73	n.c.	0.26	0.99	42.93	0.91	
K2 ^a	Yes	384	Copal spacer	T4+F4	160 [8.0, 2.0]	56.18	7.06	n.c.	0.00*	26.20	1.50	3.92	
K3	Yes	151	Palacos R+G	T3+F3	120 [6.0, 1.5]	0.28	52.54	0.01		18.83			
K3 2nd spacer			Palacos R+G	T4+F4	180 [2.25, 9.0]	0.62	104.02		0.37		3.84		
K4	Yes	174	Copal spacer	T2+F2	120 [6.0, 1.5]	93.58	5.82	0.00*	0.03	1.35	0.01*		
K5	No	324	Palacos R+G	T3+F3	120 [6.0, 1.5]	261.22	15.28	0.00*	0.07	0.01*	0.05		
K6	Yes	447	Palacos R+G	T4+F4	160 [8.0, 2.0]	27.61	30.32	0.00*	0.99	31.83	6.24		
K7	Yes	193	Copal spacer	T4+F4	160 [8.0, 2.0]	33.51	14.74	0.00*	0.32	1.70	0.41		
K8	Yes	177	Palacos R+G	T3+F3	160 [8.0, 2.0]	143.09	84.60	0.00*	15.03	8.45	19.13		

All values excluded from the statistical analysis and from depiction in Figures 1 and 2 are underlined in gray.

Abbreviations: H = hip patient; K = knee patient; IMP = spacer implantation; EXP = spacer explantation; V = vancomycin; G = gentamicin; n.c. = not collected.

^a Patient excluded from analysis of wound exudate due to missing sample at spacer explantation.

^b Patient excluded from analysis of vancomycin release due to systemic antibiotics therapy with vancomycin at time point of sample collection.

^c Spacer molds hip with stem size/head size: spacer mold 1 (M1) with 9 × 125 mm/43 mm; spacer mold 2 (M2) 9 × 125 mm/51 mm; spacer mold 3 (M3) 13 × 145 mm/57 mm; spacer mold 4 (M4) 17 × 165 mm/64 mm. Spacer molds knee with tibia and femur: spacer mold tibia 1 (T1) 65 mm M/Lx 39 mm A/P; spacer mold tibia 2 (T2) 70 mm M/Lx 42 mm A/P; spacer mold tibia 3 (T3) 75 mm M/Lx 45 mm A/P; spacer mold tibia 4 (T4) 80 mm M/Lx 48 mm A/P; spacer mold femur 1 (F1) 60 mm M/Lx 43 mm A/P; spacer mold femur 2 (F2) 65 mm M/Lx 48 mm A/P; spacer mold femur 3 (F3) 70 mm M/Lx 52 mm A/P; spacer mold femur 4 (F4) 75 mm M/Lx 57 mm A/P.

* Values below the detection limit. Lower limit of quantification (LLOQ) for gentamicin were 0.008 µg/g in periprosthetic membrane samples and 0.004 µg/mL in wound exudates, LLOQs for vancomycin were 0.0025 µg/g in periprosthetic membrane samples and 0.125 µg/mL in wound exudates. Values below the detection limit were set at the value of the LLOQ for depiction and statistical analysis.

gentamicin and 4.65% w/w vancomycin per weight of powder component for COPAL[®] spacem, PALACOS[®] R+G and COPAL[®] G+V, respectively.

Forty grams of bone cement powder, 20 mL liquid component and antibiotics (in the case of PALACOS[®] R+G and COPAL[®] spacem) were mixed in a porcelain crucible until the mix was no longer adhesive. It was then manually filled into a cylindrical casting form with 25 mm of diameter and 10 mm of height. The mold was covered with polyester foil and condensed with a manually operated hydraulic press at 1–1.5 bar for 30 min. The resulting cylindrical test specimens were removed from the form and dried in a desiccator with orange gel for 2 days. The discs had an average weight of 5.6 g and a surface area of 17.66 cm². The specimens were then transferred to a polyethylene tube containing 20 mL of phosphate-buffered saline solution (PBS) and incubated for 24 h at 37°C. Next, the specimens were removed and transferred into a new polyethylene tube with fresh PBS for a further 24-h incubation period while the supernatants from the test tubes were stored in a fridge at 4°C. This procedure was repeated for up to 5 days. The supernatants from day one, three, and five were analyzed for gentamicin and vancomycin content.

The concentration of vancomycin was measured via separation in a C18 column (XBridge) under gradient conditions using UV-detection. Gentamicin concentration analysis was performed using a commercial kit (COBAS INTEGRA GENT, Roche Diagnostics GmbH, Basel, Switzerland) via automatic biochemistry analyser (COBAS INTEGRA[®] 400, Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

Statistical analysis was performed by GraphPadPRISM version 7.0 (GraphPad Inc. San Diego, CA). In order to include samples with values below the detection limit, these values were set at the value of the LLOQ. Concentrations of vancomycin between implantation and explantation were compared using Wilcoxon signed rank test. Concentrations of gentamicin between implantation and explantation were analyzed with Kruskal-Wallis test followed by Dunn's multiple comparison as post hoc test since here it was also differentiated whether the explanted prosthesis had been cemented or uncemented. The non-parametric tests were chosen as Kolmogorow-Smirnov test revealed that *in vivo* data were not normally distributed. Therefore, *in vivo* data are reported as median with the ranges in square brackets. Correlations analyses were performed using Spearman correlation test.

As the *in vitro* results were normally distributed, they are reported as mean \pm standard deviation and compared using two-way repeated measures ANOVA with time of release and cement type as variables followed by Bonferroni post-tests.

RESULTS

In vivo data

The most common bacterial species found in intraoperative samples at the time point of spacer implantation were

coagulase-negative staphylococci (nine strains) including *Staphylococcus epidermidis* (seven strains), *Staphylococcus lugdunensis* (one strain), and *Staphylococcus capitis* (one strain) followed by *Staphylococcus aureus* (three strains). Furthermore, obligatory pathogenic or opportunistic species such as *Corynebacterium* spp., *Bacillus cereus*, *Finegoldia magna*, *Bacteroides ureolyticus*, *Propionibacterium acnes*, and *Klebsiella pneumoniae* were detected. Of note with respect to the antibiotics in the spacer, in three patients the infection-causing bacteria—three strains of *Staphylococcus epidermidis* and one strain of *Staphylococcus aureus*—were resistant to gentamicin. None of the detected strains were resistant to vancomycin.

In the present study, counting from the previous endoprosthesis implant, the mean time to revision was 76.4 months (1–179 months) with clinical signs recorded slightly earlier (68.8 months, 0–178 months). The majority of cases (55.6%) occurred after 5 years and later (Table I). Following their primary TJR, eight patients (two hips and six knees) had previously undergone revision at the same site. There were no revisions due to septic or aseptic loosening of the primary implant in the hip patients. However, one patient had undergone a debridement of an epifascial cyst and another had an exchange of the acetabular shell due to a fall and resulting breakage within 10 days of primary implantation. Notably, six among eight knee patients (75%) had undergone revision before. Partial or total component exchange surgery was performed in five knee patients; in three of those due to an implant-associated infection. One knee patient had prior wound revisions and debridements only.

The mean period of implantation of the spacer for all patients was 13.6 weeks, in the 14 patients with only one spacer it was 12.1 weeks while in the four patients (three hips and one knee) who had a spacer exchange operation, total period of implantation for both spacers was 18.8 weeks (Table II). Delayed wound healing and continued serous wound secretion following spacer implantation was the main reason for spacer exchange and was indicative of the necessity for an exchange in three cases (two hips and one knee) which was performed shortly after the first spacer implantation after 2 weeks. The decision for the spacer exchange in the fourth patient was based on the histological examination of the control biopsy at week 8, which showed signs of a persistent inflammation.

It is noteworthy that only one of the exchanged first spacers was positive in microbiological tests. Moreover, *Enterobacter cloacae* was only detected in one of the three independent sampling sites of this spacer. None of the patients who had undergone a spacer exchange showed signs of continued infection in the subsequent course of the treatment and up to 1 year follow-up.

Gentamicin and vancomycin release *in vivo*

In vivo release of antibiotics was measured in the subfascial wound exudate of the patients collected for 24 h postoperatively while adjacent membrane tissue samples were taken

TABLE III. Patient Details and Systemic Antibiotic Therapy at the Time Point of Sample Collection

Patient ID	Sex	Age	BMI	Previous Revision of TEP	Months Between Implantation of Previous TEP and		Systemic Antibiotic Therapy Within 48 h After	
					Explantation of TEP	Incipient complaints	Spacer Implantation	Spacer Explantation
H1 ^a	M	73	21	No	21	17	Ce 3 × 1500 mg/d; Mo 1 × 400 mg/d	Ce 3 × 1500 mg/d; Mo 1 × 400 mg/d
H2 ^b	M	68	22	Yes ^c	14	4	Ri 2 × 600 mg/d; Va 2 × 1000 mg/d	Ri 2 × 600 mg/d; Va 2 × 1000 mg/d
H3	M	59	32	No	172	169	Ce 3 × 1500 mg/d	Ce 3 × 1500 mg/d
H4 ^b	F	79	29	Yes ^d	123	117	Ce 3 × 1500 mg/d; Le 1 × 500 mg/d	Ri 1 × 600 mg/d; Va 2 × 1000 mg/d
H5 ^a	M	55	33	No	179	178	Ri 1 × 600 mg/d; Le 1 × 500 mg/d	Ri 2 × 600 mg/d; Le 2 × 500 mg/d
H6	M	77	29	No	72	57	Cl 2 × 600 mg/d	Le 2 × 500 mg/d
H7	F	78	19	No	40	23	Ce 3 × 1500 mg/d; Mo 1 × 400 mg/d	Ce 3 × 1500 mg/d; Le 2 × 500 mg/d
H8 ^b	M	58	34	No	75	74	Ri 2 × 600 mg/d; Va 2 × 1000 mg/d;	Ce 3 × 1500 mg/d; Le 2 × 500 mg/d
H9	M	75	32	No	1	0	Ce 3 × 1500 mg/d; Mo 1 × 400 mg/d	Cl 2 × 600 mg/d; Le 2 × 500 mg/d
H10	M	66	34	No	98	61	Ce 3 × 1500 mg/d; Mo 1 × 400 mg/d	Cl 3 × 600 mg/d;
K1 ^a	F	49	34	Yes ^d	133	118	Ce 3 × 1500 mg/d; Mo 1 × 400 mg/d	Cl 3 × 600 mg/d;
K2 ^a	F	56	39	Yes ^d	7	1	Ce 3 × 1500 mg/d; Cl 2 × 600 mg/d	Ce 3 × 1500 mg/d; Cl 2 × 600 mg/d
K3	F	55	30	Yes ^d	52	52	Ce 3 × 1500 mg/d; Le 2 × 500 mg/d	Ce 3 × 1500 mg/d; Le 2 × 500 mg/d
K4	M	75	37	No	95	81	Ce 3 × 1500 mg/d; Mo 1 × 400 mg/d	Ce 3 × 1500 mg/d; Mo 1 × 400 mg/d
K5	F	73	27	No	178	176	Cl 2 × 600 mg/d	Ce 3 × 1500 mg/d
K6	F	75	27	Yes ^d	29	26	Ce 3 × 1500 mg/d; Mo 1 × 400 mg/d	Ce 3 × 1500 mg/d; Le 2 × 500 mg/d
K7	F	73	31	Yes ^d	8	8	Ce 3 × 1500 mg/d; Le 2 × 500 mg/d	Ce 3 × 1500 mg/d
K8	F	73	37	Yes ^c	78	77	Ri 1 × 600 mg/d; Le 1 × 500 mg/d; Fl 4 × 2000 mg/d	Cl 3 × 600 mg/d; Le 2 × 500 mg/d
Mean/total	9 M/9 F	67.1 ± 9.4	30.4 ± 5.4	8	76.4 ± 58.9	68.8 ± 59.1		

Cumulative data of all patients are either presented as mean ± SD or total number.

H = hip patient; K = knee patient; Antibiotics: Ce = Cefuroxim, Cl = Clindamycin, Fl = Flucloxacillin, Le = Levofloxacin, Mo = Moxifloxacin, Ri = Rifampicin, Va = Vancomycin, all systemic antibiotic treatment within the first 48 h after operation was administered intravenously.

^a Patient excluded from analysis of wound exudate due to missing sample at spacer explantation.

^b Patient excluded from analysis of vancomycin release due to systemic antibiotics therapy with vancomycin at time point of sample collection.

^c Wound revisions and debridements.

^d Partial or total component exchange surgery.

intraoperatively during both spacer implantation and spacer explantation.

Membrane samples were collected from all 18 patients. However, in the case of four patients, wound exudate was only collected from the implantation but not from the explantation of the spacer. In order to allow for pairwise comparison between implantation and explantation samples, those four patients were excluded from the analysis of the wound exudate. Furthermore, patients who received intravenous vancomycin prior and up to 48 h after operations were excluded when analyzing vancomycin release in the wound exudate as well as from the periprosthetic membrane samples. Thus, 11 exudates and 15 membrane samples at each time point were analyzed for vancomycin concentration. As shown in Figure 1(A), vancomycin concentration is significantly reduced in the 24-h wound exudate at explantation compared to during implantation of the spacer (43.28 $\mu\text{g}/\text{mL}$ [0.28–261.22] vs. 0.46 $\mu\text{g}/\text{mL}$ [0.13–37.47], $n = 11$, Wilcoxon signed rank test, $p = 0.0137$). As expected, the concentration of vancomycin was low in the periprosthetic membrane samples before the implantation of the spacer with 10 out of 15 samples being below the LLOQ. The concentration of vancomycin in the membrane was, however, significantly higher at explantation of the spacer (0.003 $\mu\text{g}/\text{g}$ [0.003–0.261] vs. 0.318 $\mu\text{g}/\text{g}$ [0.024–484.16], $n = 15$, Wilcoxon signed rank test, $p < 0.0001$) [Figure 1(B)].

For gentamicin analyses, we differentiated between patients whose previous implant had been cemented (nine patients: two hip and seven knee implants), and those whose removed prosthesis had been uncemented (nine patients: eight hip and one knee implants). There were no differences in the gentamicin concentration in the wound exudates (Kruskal-Wallis test, $p = 0.8814$), neither when comparing implantation and explantation of spacer nor when analyzing the cemented ($n = 7$) and uncemented ($n = 7$) groups [Figure 2(A)]. However, as depicted in Figure 2(B) gentamicin concentrations differed significantly in the periprosthetic membrane samples (Kruskal-Wallis test, $p = 0.0001$). Dunn's post hoc test revealed that even before the implantation of the spacer, the concentration of gentamicin in the periprosthetic membrane samples between the cemented and uncemented group differed significantly (8.451 $\mu\text{g}/\text{g}$

[0.152–42.926, $n = 9$] vs. 0.008 $\mu\text{g}/\text{g}$ [0.008–0.087, $n = 9$], $p < 0.001$). While the concentration of gentamicin increased from implantation to explantation in the uncemented group (0.008 $\mu\text{g}/\text{g}$ [0.008–0.087] vs. 0.164 $\mu\text{g}/\text{g}$ [0.048–71.75], $n = 9$, $p < 0.05$), there was no such change in the cemented group (8.451 $\mu\text{g}/\text{g}$ [0.152–42.926] vs. 1.075 $\mu\text{g}/\text{g}$ [0.008–19.128], $n = 9$).

Spearman analyses of correlation revealed that the differences in antibiotics concentration between implantation and explantation in wound exudate or tissue samples of a patient did not correlate with the period of implantation time of the spacer (data not shown). However, the observed increase in vancomycin concentration in the tissue samples was positively correlated to the amount of cement used to create the spacer implant ($r = 0.6759$; $p = 0.0137$). There were no further correlations between antibiotics concentration and amount of cement. Patients requiring a second spacer were analyzed separately as the amount of cement differed between first and second spacer. The assumption that the necessity of a second spacer was due to a low antibiotics release after implantation could not be confirmed as concentration in the wound exudate 24 h after implantation of the first spacer did not differ significantly in patients with one spacer versus patients requiring a spacer exchange (vancomycin: 50.25 $\mu\text{g}/\text{mL}$ [10.37–261.22, $n = 9$] vs. 21.78 $\mu\text{g}/\text{mL}$ [0.28–43.28, $n = 2$], $p = 0.3273$; gentamicin: 14.74 $\mu\text{g}/\text{mL}$ [3.67–84.60, $n = 11$] vs. 21.56 $\mu\text{g}/\text{mL}$ [19.49–52.54, $n = 3$], $p = 0.2253$; Mann-Whitney test).

Gentamicin and vancomycin release *in vitro*

In order to compare the *in vivo* results from the patient study with the *in vitro* release kinetics of the antibiotics from the spacers, cylindrical specimens were molded from Palacos R+G and Copal Spacem under addition of the respective antibiotics according to the procedures used in the *in vivo* study. Additionally, Copal G+V which already contained both antibiotics of interest, gentamicin and vancomycin, was tested. This cement was not applied in the clinical study as it was not commercially available when the study commenced.

As expected, there was a significant decline of released antibiotics over time for both antibiotics ($p < 0.0001$ for time,

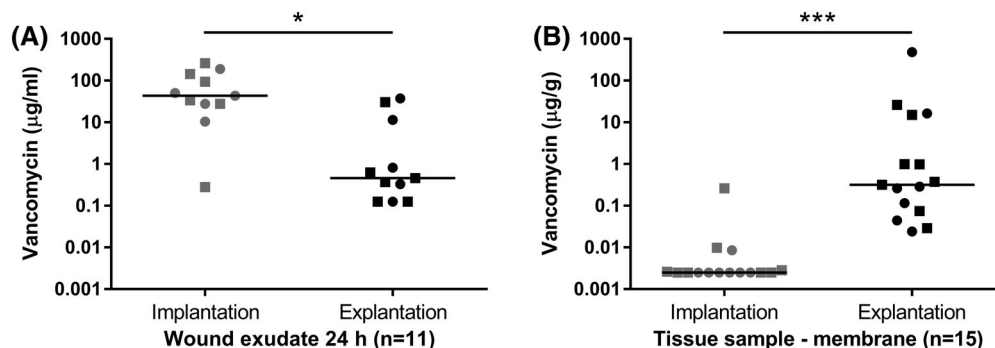


FIGURE 1. Vancomycin concentrations in wound exudate (A) and periprosthetic membrane samples (B) of patients undergoing two stage treatment of periprosthetic joint infection under exclusion of those patients with systemic vancomycin treatment prior and up to 48 h after operation. Single values are presented with the horizontal bar representing the median. Hip patients are represented by dots while knee patients are shown as squares. Time points of samples: spacer implantation and spacer explantation, n : number of patients, * significant $p < 0.05$, *** extremely significant $p < 0.001$ (Wilcoxon signed rank test).

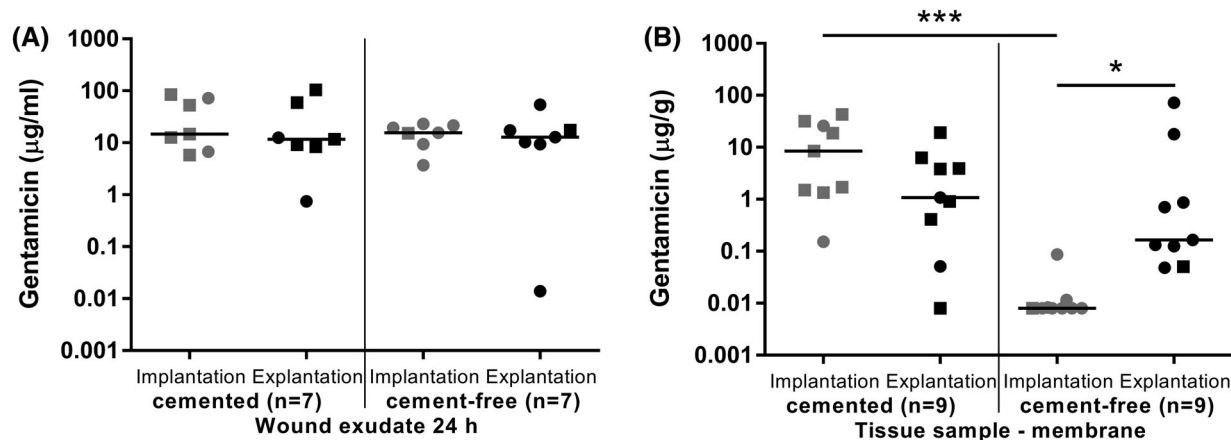


FIGURE 2. Gentamicin concentrations in wound exudate (A) and periprosthetic membrane samples (B) of patients undergoing two stage treatment of periprosthetic joint infection. Patients were divided in two groups depending on whether the removed infected implant was previously cemented or uncemented. Single values are presented with the horizontal bar representing the median. Total hip patients are represented by dots while total knee patients are shown as squares. Time points of samples: spacer implantation and spacer explantation, *n*: number of patients, * significant $p < 0.05$, *** extremely significant $p < 0.001$ (Kruskal-Wallis test).

two-way repeated measures ANOVA for gentamicin and vancomycin, respectively). The interaction of both variables (time \times cement) was also highly significant ($p < 0.0001$ for time \times cement, two-way repeated measures ANOVA for gentamicin and vancomycin, respectively) indicating significant differences in the release kinetics of the cements over time. The differences between the three cements were most pronounced on day 1. The highest release of gentamicin on day 1 was measured for Palacos R+G followed by Copal G+V ($p < 0.001$, Bonferroni post test), while Copal Spacem released the least amount of gentamicin compared to Palacos R+G and Copal G+V ($p < 0.001$ and $p < 0.001$, Bonferroni post tests, respectively). On day 3, only Palacos R+G and Copal Spacem differed significantly from each other with regard to gentamicin release [Figure 3(A)].

Release kinetics of vancomycin from the three types of cement differed from those of gentamicin. On day 1, Copal Spacem released the highest amount of vancomycin with significantly lower amounts set free from Copal G+V followed by Palacos R+G ($p < 0.001$ and $p < 0.001$, Bonferroni post-tests, respectively) [Figure 3(B)].

In general, despite higher concentration of vancomycin in the bone cements the relative release was lower for vancomycin compared to gentamicin (Figure 3).

DISCUSSION

Periprosthetic infection is a severe complication after knee or hip arthroplasty. Over half of the patients in this study were affected by conditions that had been identified as individual risk factors for developing a periprosthetic joint infection such as diabetes, obesity or old age.²¹

The use of antibiotic impregnated cement has been shown to increase the success rate in two-stage revision arthroplasty as a treatment of periprosthetic infection.^{22,23} The assumed reason is the high initial release of antibiotics locally, which prevents a re-infection. A systematic review of

studies investigating the *in vivo* release of antibiotics from bone cements reported considerable differences in the released concentration between, but also within the studies.²⁴ This is in line with our observation of high inter-individual variation for both antibiotics in the 24-h wound exudate after spacer implantation. The authors speculated that the volume of joint fluid surrounding the spacer might be a limiting factor in antibiotics release.²⁴ Thus, the differences in the amount of wound exudate might be one reason for the high inter-individual variation. Additionally, the surface area of the spacer might be an important factor. In a recent randomized controlled trial no difference in the concentration of gentamicin in drain fluid between patients with either a thick or a thin cement mantle was observed, that is, the local concentration of antibiotic was independent from the amount of bone cement.²⁵ While this is contrary to our results, as we observed a correlation between the prepared amount of bone cement and the release of vancomycin, our result could be due to the increased size, and thus an increased surface area, of the cement spacers prepared in the standardized molds. Furthermore, systemic vancomycin administration influences the local antibiotic concentration. Roy et al. reported that already a single intravenous dose of vancomycin resulted in therapeutic concentrations of approximately 6.8 µg/mL vancomycin in joint fluid.²⁶ In order to avoid this bias, only data of vancomycin release after exclusion of patients who had been administered vancomycin intravenously were statistically analyzed. The thus observed significant decline of vancomycin release in the 24-h wound exudate at the time of explantation of the spacer compared to implantation is in agreement with the decreased *in vitro* release. However, given the sharp decrease *in vitro* on day 3 and day 5, it was rather unexpected that there was still a considerable release of vancomycin after an average of 90 days *in vivo*, especially when the spacer had been removed. Such a prolonged release of antibiotics was already described in other studies.²⁷⁻²⁹ The

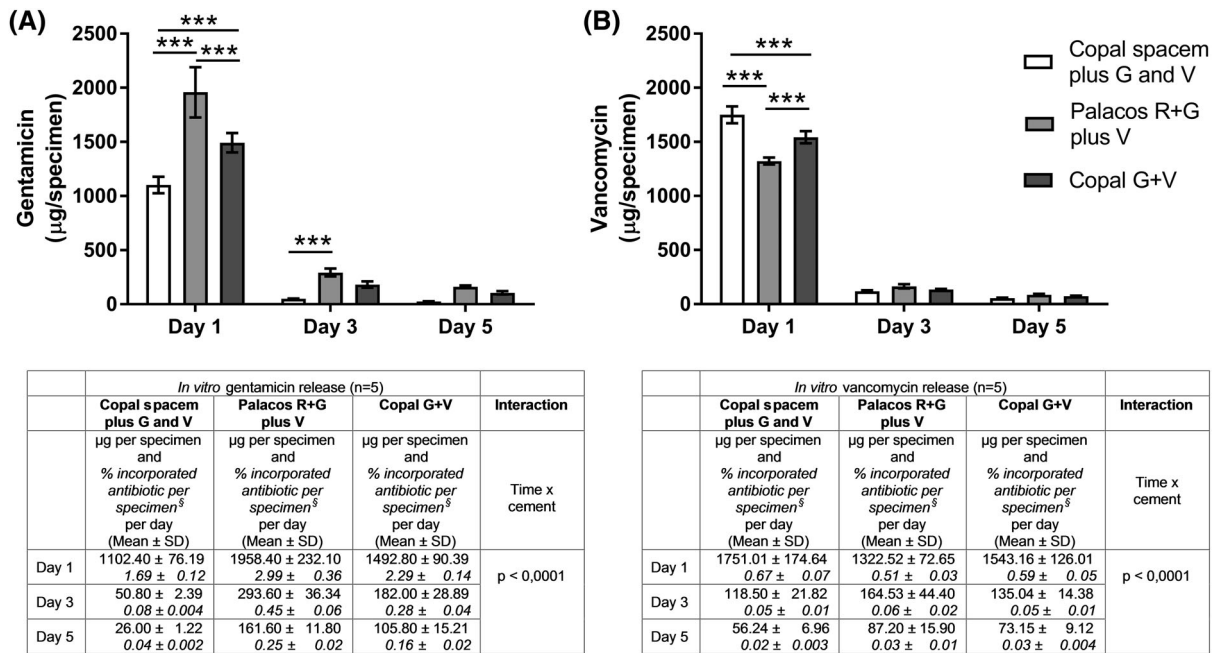


FIGURE 3. Release of the antibiotics gentamicin (A) and vancomycin (B) from cylindrical test specimens of three different cements *in vitro*. Values are mean ± standard deviation of released antibiotic per specimen over 24 h on the indicated days. Percentage of released antibiotics per incorporated antibiotic per specimen per day is presented in Italics. §—Values are calculated based on an average weight of 5.6 g per specimen with 1.17% w/w gentamicin and 4.66% w/w vancomycin, 1.17% w/w gentamicin and 4.67% w/w vancomycin as well as 1.16% w/w gentamicin and 4.65% w/w vancomycin per weight of powder component for COPAL® spacem, PALACOS® R+G and COPAL® G+V, respectively. Results were recorded from five independent experiments. *In vitro* results were compared using two-way repeated measures ANOVA with time and cement as variables followed by Bonferroni post-tests. *** extremely significant *p* < 0.001 (Bonferroni post-tests) G = gentamicin, V = vancomycin.

accumulation of antibiotics in the adjacent tissue and their release after tissue damage due to the second stage procedure could be a possible reason for the detection of antibiotics in the wound exudate after explantation. This hypothesis is supported by the significantly higher vancomycin concentration in the tissue at the time of explantation. The hypothesis could also explain the effects that we observed when analyzing gentamicin. Here the differentiation between patients with cemented and uncemented endoprosthesis prior to spacer implantation also showed that the use of gentamicin-containing cement for fixation of TJRs resulted in considerable accumulation of the antibiotic in the tissue even after several years. We measured significantly higher concentrations of gentamicin in the tissue before the implantation of the spacer in patients with previously cemented compared to uncemented TJRs. Our data suggest that especially gentamicin remains in the tissue, as contrary to vancomycin there was no decline in gentamicin in wound exudates after explantation of the spacer.

However, another hypothesis should not be dismissed here. Powles et al. showed that fracturing old cement during revision surgery led to the release of gentamicin and in turn to the “contamination” of tissue and joint fluid with the antibiotic.³⁰ The disruption of the cement opens new surface areas from which enclosed antibiotics can be released. Indeed, several studies found that a high percentage of the antibiotics remained in the cement.^{18,31} Thus, the detected antibiotics could derive from the cement after disruption during either the explantation of the previous TJR or the explantation of the spacer.

The importance of the surface area for the release kinetics was mentioned, that is, cements with a microporous structure, high porosity or higher surface roughness, which extend the surface area, showed higher and prolonged antibiotic elution.^{15,32} The addition of ingredients such as calcium polyphosphate or mesoporous silica nanoparticles which increase porosity or surface roughness also resulted in improved release kinetics.^{33,34} It was reported that Copal spacem is more effective in preventing biofilm formation compared to Palacos® R+G due to better elution kinetics for gentamicin.³⁵ However, that in our *in vitro* experiments this was only observed for the vancomycin but not for gentamicin could be due to the fact that contrary to our bone cement composition Copal spacem in the aforementioned publication contained clindamycin. Indeed, Boelch et al. reported that the addition of clindamycin improved gentamicin release from test specimens while the addition of vancomycin had no effect.³⁶ In the clindamycin containing Copal cement the weight of gentamicin is with 2.34% higher than in the bone cements tested in our study which have all similar weights of gentamicin and vancomycin. There might be various other reasons why we did not observe the improved release kinetics of Copal spacem for gentamicin. For example, in Palacos R+G and Copal G+V, especially manufactured gentamicin was used, which generally has better release kinetics. Measurement of surface roughness within a previous study³⁷ also showed higher roughness for Palacos® R+G with addition of vancomycin compared to Copal® spacem with gentamicin and vancomycin (*R*_a = 20.89 ± 0.49 µm, *R*_z = 139.54 ± 5.24 µm

and $R_a = 18.93 \pm 0.67 \mu\text{m}$, $R_z = 121.86 \pm 3.85 \mu\text{m}$ for Palacos® R+G plus V and Copal® spacem plus G and V, respectively [mean \pm SD, $n = 3$, unpublished data] which might support the observation that initial release of gentamicin from Palacos® R+G with addition of vancomycin was highest. The importance of the surface was highlighted by the work of Salih et al. who showed that indenting a spacer with a MacDonald dissector more than doubled the gentamicin release.³⁸ In contrast, the test specimens in our *in vitro* investigation had a smooth surface which was ensured by using a polyester foil. While the release kinetics of the combination of gentamicin and vancomycin are relatively well studied *in vitro*, its importance *in vivo* is still not clear. Despite synergistic effects of the combination on the size of the inhibition zone *in vitro*³⁹ a recent *in vivo* study showed that the combination of gentamicin and vancomycin did not offer any advantages regarding clinical outcomes compared to gentamicin alone.⁴⁰ However, especially considering that four of the infection-causing strains in our *in vivo* investigation were identified as gentamicin-resistant highlights the importance of antibiotic combinations to prevent re-infection.

Our inability to replicate *in vivo* the differences that we detected between cements *in vitro* may be due to the relatively low number of patients who received spacers made from Copal spacem and the observed high inter-individual variability. The small sample size is a general limitation in our study. This is due to periprosthetic infection occurring in only approximately 1% of all primary TJR patients. We improved patient recruitment by widening the inclusion criteria to allow recruitment of patients who had undergone revision before but numbers remained low in our general patient population.

CONCLUSIONS

Our data allow the conclusion that while antibiotics release from spacer cement decreases rapidly *in vitro* within the first 24 h, *in vivo* antibiotics are present much longer at similar concentrations, even after an average of 90 days. This was observed especially in the spacer-adjacent soft tissue.

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CONFLICT OF INTEREST

Co-authors Dr. Hans Bösebeck and Mr. Tobias Reichel were employees of Heraeus Medical GmbH at the time of the study. There were no conflicts of interest that affected the design and execution of the study protocol or the analysis of

the data. None of the other authors have any conflicts of interest to declare.

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