BJR

INFECTION

Safety assessment of microsilver-loaded poly(methyl methacrylate) (PMMA) cement spacers in patients with prosthetic hip infections

RESULTS OF A PROSPECTIVE COHORT STUDY

V. Alt,
M. Rupp,
K. Lemberger,
T. Bechert,
T. Konradt,
P. Steinrücke,
R. Schnettler,
S. Söder,
R. Ascherl

Department of Trauma Surgery, University Hospital Regensburg

Correspondence should be sent to V. Alt; email: volker.alt@ukr.de

Objectives

Preclinical data showed poly(methyl methacrylate) (PMMA) loaded with microsilver to be effective against a variety of bacteria. The purpose of this study was to assess patient safety of PMMA spacers with microsilver in prosthetic hip infections in a prospective cohort study.

Methods

A total of 12 patients with prosthetic hip infections were included for a three-stage revision procedure. All patients received either a gentamicin-PMMA spacer (80 g to 160 g PMMA depending on hip joint dimension) with additional loading of 1% (w/w) of microsilver (0.8 g to 1.6 g per spacer) at surgery 1 followed by a gentamicin-PMMA spacer without microsilver at surgery 2 or vice versa. Implantation of the revision prosthesis was carried out at surgery 3.

Results

In total, 11 of the 12 patients completed the study. No argyria or considerable differences in laboratory parameters were detected. Silver blood concentrations were below or around the detection limit of 1 ppb in ten of the 11 patients. A maximum of 5.6 ppb at 48 hours after implantation of the silver spacer, which is below the recommended maximum level of 10 ppb, was found in one patient. No silver was detected in the urine. Drainage fluids showed concentrations between 16.1 ppb and 23.3 ppb at 12 hours after implantation of the silver spacers, and between 16.8 ppb to 25.1 ppb at 48 hours after implantation. Pathohistological assessment of the periprosthetic membrane did not reveal any differences between the two groups.

Conclusion

Microsilver-loaded gentamicin-PMMA spacers showed good biocompatibility and the broad antimicrobial activity warrants further clinical research to assess its effectivity in reducing infection rates in prosthetic joint infection.

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Article focus

- This prospective cohort study assesses patient safety in relation to use of poly(methyl methacrylate) (PMMA) spacers with microsilver in prosthetic hip infections.
- The study's hypothesis is that the use of microsilver-loaded PMMA spacers is safe.
- The study includes: an analysis of clinical and laboratory parameters, measurement of silver concentrations in blood, urine, and drainage fluids; and a histopathological evaluation of the periprosthetic membrane around the spacers.

Key messages

- Microsilver-loaded gentamicin-PMMA spacers showed good biocompatibility, with very low blood and urine silver concentrations.

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- Drainage fluids showed concentrations between 16.1 ppb and 23.3 ppb at 12 hours after implantation of the silver spacers, and between 16.8 ppb and 25.1 ppb at 48 hours after implantation, which is in line with the concept of local application of antimicrobial agents.

Strengths and limitations

- This study includes prospectively collected data in the target population of patients with a prosthetic hip infection.
- Limitations of this study included low patient numbers and a limited follow-up period.

Introduction

Prosthetic joint infections (PJIs) remain a challenge and have a tremendous negative impact both on the quality of life of patients and on healthcare systems.[1] Surgical treatment for PJIs can be divided into implant-retaining strategies, such as irrigation and debridement, and surgical options with implant removal.[2] The latter includes one-stage or two-stage revision procedures, which differ in the timepoint of implantation of the revision implant. For one-stage revision procedures, implantation of the revision prosthesis is performed during the same surgery as the removal of the infected prosthesis. The two-stage concept consists of two interventions, starting with the removal of the prosthesis at surgery 1, followed by insertion of a new prosthesis during the second procedure (surgery 2), which is usually performed a few weeks later. Antibiotic-loaded spacers are frequently inserted during surgery 1, acting both as a drug delivery system and void filler to facilitate implantation of the revision prosthesis.[3] However, after an initial burst from the poly(methyl methacrylate) (PMMA), subinhibitory levels of the released antibiotics have been described, with the risk of bacterial recolonization of the PMMA surface with antibiotic-resistant bacterial strains.[4-6]

While an increasing prevalence of infections in revision arthroplasty with resistant microbes has been reported, sobering clinical results were noted by Gomez et al[7] for two-stage PJI revisions, the 'gold standard' of revision arthroplasty in knee and hip PJIs.[8] In 504 cases, the following were reported: an interstage mortality of 7.5%, an inability to reimplant the prosthesis in 20% of cases; and a failure rate of nearly 20% in revision prosthesis implantation.[7] In this context, the need to improve treatment of PJI is obvious.

Silver has been known and used as an antimicrobial substance in medicine for many decades, and has recently gained interest for the coating of tumour endoprostheses in orthopaedic oncology with convincing clinical results.[9,10] The major benefit of silver is its antimicrobial activity, with preclinical data suggesting that PMMA bone cement loaded with microsilver particles is highly effective against a variety of bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA).[11] Furthermore, it is uncommon for bacteria to develop resistance against silver, most likely due to the multiple mechanisms of antimicrobial action exploited by silver ions.[12] The addition of silver to antibiotic-loaded PMMA spacers therefore offers the potential benefit of broadening and prolonging the antimicrobial protection of the spacer surface due to its wide-ranging antimicrobial properties and favourable release kinetics from PMMA.

However, adverse events due to silver-coated orthopaedic prostheses have also been reported, such as argyria with grey discolouration of the skin next to the implantation site or elevation of serum silver levels,[13,14] emphasizing the need for cautious clinical evaluation of silver-loaded orthopaedic implants. Interestingly, argyria was not found to be associated with elevated serum silver concentrations or significant changes in laboratory parameters in these cases *versus* in patients without the development of argyria who were treated with the same type of silver-coated megaprostheses.[13,14]

We hypothesized that the application of microsilver-loaded PMMA spacers in PJI after total hip arthroplasty (THA) is safe. Therefore, the purpose of the current prospective case series was to assess patient safety after silver-loaded PMMA spacer implantation in patients undergoing infection revision procedures for infected THA. The study included: clinical observation of the patient; silver concentration analysis of the serum, urine, and drainage fluids; and histological analysis of the periimplant membrane.

Materials and Methods

Study design and patients. The study was officially approved by the Ethical Committee of the University Erlangen-Nürnberg, Erlangen, Germany, and all patients gave informed consent for inclusion into the study. For inclusion in the study, patients with prosthetic total hip infections had to be aged 18 years or older and had to be able to give informed consent before surgery. Prosthetic hip infection was diagnosed by positive culture growth of puncture of the hip joint before surgery. Exclusion criteria were allergies to silver or bone cement, severe liver or renal insufficiency, or the participation in any other ongoing clinical trial.

Overall, 12 patients with prosthetic total hip infection treated with silver-loaded gentamicin-PMMA spacers were included in a prospective cohort study. The surgical protocol for the treatment of the infected THA consisted of a modified two-stage procedure with removal of the infected hip prosthesis and implantation of a PMMA spacer during the first revision procedure. During this surgery 1 intervention, the infected hip prosthesis was removed and a thorough debridement was performed, followed by the implantation of either a silver-loaded

gentamicin-PMMA or silver-free gentamicin spacer. In contrast to a 'classical' two-stage procedure, in which a new prosthesis is implanted during surgery 2, the spacers were exchanged 14 days after the index procedure. Patients who had received a silver-loaded gentamicin-PMMA spacer were further treated with a silver-free gentamicin spacer, and vice versa. This second procedure was then followed by a third surgery with removal of the spacer and implantation of the revision prosthesis. Thus, this protocol can be seen as a modification of the 'classical' two-stage procedure to a three-stage procedure with exchange of the spacer in the prosthesis-free interim interval. This is the preferred protocol of the senior author (RA) of this study, with the intention of allowing for a four-week prosthesis-free interval. The aim of this protocol, for use in complex prosthetic total hip infections, is to improve local antibiotic therapy through enhanced release of antibiotics by the exchange of the gentamicin spacers at day 14. The protocol is not specifically designed for the current study with the use of silver-loaded spacers.

All of the patients were operated on by the senior author (RA). This enabled a standardized surgical treatment and PMMA spacer handling. The decision about the sequence of spacer implantation was randomized based on a randomization table generated by drawing lots before the start of the study.

For each intervention, clinical observation of adverse events and silver concentration analysis of blood, urine, and wound drainage samples were performed, as well as histological assessment of the perispacer membrane.

Silver. The microsilver used in this study (MicroSilver BG; Bio-Gate AG, Nuremberg, Germany) consisted of primary silver particles of a nanoparticulate size of 5 nm to 50 nm.[11] The silver nanoparticles form aggregates of 2 µm to 5 µm, which were introduced as silver microparticles into the PMMA cement for the spacers.

Spacers. For silver-free spacers, commercially available gentamicin-loaded PMMA spacers with 0.5 g of gentamicin per 40 g of PMMA (Palacos R+G; Heraeus Medical GmbH, Wehrheim, Germany) were used. The amount of PMMA and the size of the used spacer moulds (StageOne; Biomet, Berlin, Germany) were adapted to the size of the hip joint. The following quantities of PMMA were used: 80 g in two cases; 120 g in nine cases; and 160 g in one case (Table I). In terms of silver spacers, 1% w/w of microsilver (Bio-Gate) was added to the PMMA (0.8 g in one case, 1.2 g in nine cases, and 1.6 g in one case; Table I). The silver was added to the PMMA powder and stirred with a spatula to achieve homogeneous distribution within the powder before the liquid was added. All other steps for the production of the spacers were identical to those for the silver-free spacers. In all cases, vacuum-assisted mixing (Optivac; Biomet, Berlin, Germany) was used.

Preformed hip spacer moulds (StageOne) were then filled with bone cement using a cement gun to create articulating spacers (Fig. 1). After the PMMA spacer hardened, the moulds were cut, the spacers were inserted into the proximal femur, and the hip joint was reduced.

Clinical and laboratory assessment. All side effects of the entire observation period were prospectively assessed, including clinical signs and symptoms, as well as laboratory parameters. Vital signs were measured three times a day and the patient's temperature was measured twice a day. Wounds were checked daily for effusion, swelling, redness, or discolouration. Laboratory parameters tested from collected blood samples were as follows: erythrocyte count, leucocyte count, thrombocyte count, creatinine, urea, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), γ-glutamyl transferase (γ-GT), C-reactive protein (CRP), interleukin (IL)-6, sodium, potassium, and calcium. Laboratory tests were carried out after 12, 48, and 72 hours, as well as on postoperative days 7 and 14.

Silver analysis of blood, urine, and drainage fluids. For silver analysis, 10 ml of blood was collected 12 hours and 48 hours after surgery 1, and at the same timepoints after surgery 2. Urine was collected and pooled for the first 12 hours after surgery. Urine was further collected and pooled between 12 hours and 48 hours after surgery. Urine aliquots of 10 ml from the pooled material were sampled to measure the total silver concentration. Drainage was left in place for 48 hours and drainage fluid was collected for the first 12 hours after surgery, and from 12 hours to 48 hours after surgery as described already for urine, of which 10 ml were used for the analysis.

Urine, blood, drainage fluid, and tissue samples were then analyzed for their total silver concentration. In total, 1 ml of blood, urine, and drainage fluid was suspended in 2 ml nitric acid (65%, Merck Suprapur; Merck, Darmstadt, Germany) and wet-digested in an ultraviolet (UV)-reaction chamber. Inductively coupled plasma mass spectrometry (ICP-MS) was applied according to EN ISO 17294-2 (International Organization for Standardization (ISO), Geneva, Switzerland). A model ELAN 6100 instrument with autosampler AS90 (PerkinElmer, Waltham, Massachusetts) was used for the measurements. Its calibration was carried out with ICP standard solutions (Merck Certipur; Merck). For quality control, the certified reference material, Trace Metals in Drinking Water (TMDW), High-Purity Standards, was analyzed during all measurements. All measurements were performed in triplicate.

Pathohistological assessment of the periprosthetic membrane. For histological evaluation, tissue samples were collected during each surgical procedure. About 5 g of soft tissue was removed from the immediate vicinity of the cement spacers in order to allow for comparable assessment of the periprosthetic membrane. The blinded

Table I. Patient demographics, microbiological findings, comorbidities, poly(methyl methacrylate) (PMMA) bone cement, and microsilver details

Patient	Sex	Age, yrs	Identified bacteria	Relevant comorbidities	Weight of PMMA powder, g	Weight of microsilver added to PMMA powder, g	Surgery 1	Surgery 2		Surgery 3	
							Spacer type	Days between surgeries 1 and 2	Spacer type	Days between surgeries 2 and 3	Implantation of revision THA
1	Male	61	*Staphylococcus aureus*	Diabetes mellitus type II, arterial hypertension, spondylodiscitis	120	1.2	Silver	21	Non-silver	21	Yes
2	Male	45	*Staphylococcus capitis*	None	120	1.2	Non-silver	21	Silver	21	Yes
3	Male	76	*Pseudomonas aeruginosa*, MRSA	AV block grade 1	120	1.2	Silver	16	Non-silver	16	Yes
4	Female	75	Culture-negative	Sacral ulcer, diarrhoea, highly elevated CRP	120	1.2	Silver	15	Non-silver	13	Yes
5	Female	80	*Staph. aureus*	Depression, weakness, osteoporosis, low oxygen saturation	120	1.2	Silver	15	Non-silver	N/A	No*
6	Male	72	*Staphylococcus epidermidis*	Arterial hypertension, CHF, moderate renal insufficiency, diabetes mellitus type II	80	0.8	Non-silver	17	Silver	15	Yes
7	Male	80	MRSE	Pacemaker, diabetes mellitus type II, hypertension	160	1.6	Silver	N/A†	N/A†	N/A†	N/A†
8	Female	75	*Enterococcus faecalis*	Depression, diabetes mellitus type II, arterial hypertension	80	0.8	Non-silver	15	Silver	16	Yes
9	Male	31	*E. faecalis*	None	120	1.2	Silver	15	Non-silver	14	Yes
10	Male	64	*Staphylococcus lugdunensis*	Arterial hypertension	120	1.2	Non-silver	15	Silver	14	Yes
11	Female	77	MRSA	CHF, arterial hypertension, anaemia, atrial fibrillation	120	1.2	Silver	14	Non-silver	14	Yes
12	Female	64	*E. faecalis*	COPD, CHF (NYHA class IV), allergies to chrome and nickel, hypercholesterolemia	120	1.2	Non-silver	15	Silver	14	Yes

*No reimplantation possible due to poor general health status
†Patient chose to withdraw from the study after surgery 1
THA, total hip arthroplasty; MRSA, methicillin-resistant *Staphylococcus aureus*; AV, atrioventricular; CRP, C-reactive protein; CHF, congestive heart failure; MRSE, methicillin-resistant *Staphylococcus epidermidis*; N/A, not applicable; COPD, chronic obstructive pulmonary disease; NYHA, New York Heart Association

samples were then fixed in formalin and embedded in paraffin, and 4 µm thick longitudinal sections were stained with haematoxylin and eosin (H&E). All samples were evaluated by a senior pathologist experienced in orthopaedic pathology (SS) and were categorized according to the classification for periprosthetic membranes suggested by Morawietz et al.[15] Samples with a mean of more than ten neutrophil granulocytes per ten high-power fields (HPFs) were considered to have a high-grade infection. In samples with a mean of between one and ten neutrophil granulocytes, a low-grade infection was diagnosed.[16] Polarized light microscopy was used to detect polyethylene wear particles.

Results

Patients and microbiological findings. A total of 12 patients were included in the study after its official approval from the local ethics committee, and after informed consent was obtained from each patient. Ten of these 12 patients completed the observation period up until reimplantation of the revision prosthesis by surgery 3, according to the study protocol. No adverse events attributable to the silver spacer or other serious adverse events were observed. In one case, reimplantation of a revision prosthesis after surgery 2 was not possible in an 80-year-old female patient (patient 5) due to poor general health including physical weakness, depression, intermittent low oxygen saturation, and severe osteoporosis. One patient asked to be removed from the study for personal reasons without any adverse events detected.

There were six male and five female patients with diagnosed infected THA, with a mean age of 66.7 years (31 to 80) (Table I). Relevant comorbidities included atrial fibrillation, arterial hypertension, congestive heart failure, renal insufficiency, diabetes mellitus type II, depression, and sacral ulcers. All bacteria detected during the first

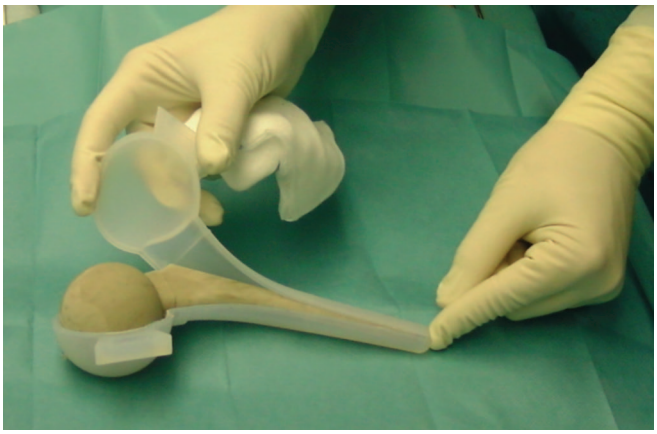

Fig. 1a

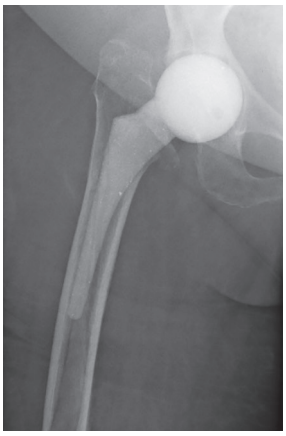

Fig. 1b

a) Microsilver-loaded poly(methyl methacrylate) (PMMA) spacer after hardening in a preformed hip spacer mould (StageOne; Biomet). b) Postoperative radiograph control in anteroposterior view with correct placement of the microsilver-loaded PMMA spacer.

Table II. Microbiological findings

Patient	Sex	Age, yrs	Microbiological findings in surgery 1	Type of spacer surgery 1	Microbiological findings in surgery 2	Type of spacer surgery 2	Microbiological findings in surgery 3
1	Male	61	*Staphylococcus aureus*	Silver	No growth	Non-silver	No growth
2	Male	45	*Staphylococcus capitis*	Non-silver	*Staphylococcus capitis*	Silver	No growth
3	Male	76	*Pseudomonas aeruginosa*, MRSA	Silver	*Enterobacter* spp.	Non-silver	MRSA
4	Female	75	Culture-negative	Silver	No growth	Non-silver	No growth
5	Female	80	*Staph. aureus*	Silver	No growth	Non-silver	N/A*
6	Male	72	*Staphylococcus epidermidis*	Non-silver	*Pseudomonas aeruginosa*	Silver	No growth
7	Male	80	MRSE	Silver	No growth	N/A†	N/A†
8	Female	75	*Enterococcus faecalis*	Non-silver	N/A‡	Silver	N/A‡
9	Male	31	*E. faecalis*	Silver	No growth	Non-silver	No growth
10	Male	64	*Staphylococcus lugdunensis*	Non-silver	*Corynebacterium*	Silver	No growth
11	Female	77	MRSA	Silver	No growth	Non-silver	No growth
12	Female	64	*E. faecalis*	Non-silver	*Enterococcus faecalis*	Silver	*Citrobacter*

*No reimplantation possible due to poor general health
†Patient chose to withdraw from the study after surgery 1
‡Data not collected
MRSA, methicillin-resistant *Staphylococcus aureus*; N/A, not applicable; MRSE, methicillin-resistant *Staphylococcus epidermidis*

surgery are listed in Table II. During the second intervention, five patients were diagnosed with positive culture growth. Four of them had previously been treated with a silver-free spacer and one of them with a silver-loaded spacer during surgery. In the last intervention (surgery 3), two patients were diagnosed with positive culture growth; one had previously been treated with a non-silver spacer and the other had previously been treated with a silver spacer during surgery 1 (Table II).

Clinical observation. At a mean timepoint of day 16 (14 to 21) after surgery 1, all of the 11 patients received their second spacer according to the study protocol. After another mean of 16 days (14 to 21), a revision total hip prosthesis could be implanted in ten of the 11 patients. Six patients initially received the microsilver-loaded spacer, followed by a silver-free spacer at surgery 2 after two weeks, while five patients were first implanted with a silver-free spacer, which was then exchanged for a silver-loaded spacer at surgery 2.

Adverse events included nausea, vomiting, low oxygen saturation, diarrhoea, hypo- and hypertension, renal function disturbances, leg oedema, disorientation, and hallucinations (Table III). Laboratory parameter changes included elevated leucocytes, elevated or decreased thrombocytes, elevated creatinine, or alterations of potassium. All of these changes were deemed to be mild to moderate and none of them was considered to be associated with the silver spacer. No silver-specific adverse events, such as argyria or impairment of the femoral or sciatic nerve, were observed.

Blood analysis. In all but one patient, the detected silver amount was close to or below the detection limit of 1 ppb of blood in all blood samples and thus several times below the reported values of up to 10 ppb in normal subjects.[17] One patient (patient 4) had a silver concentration of 3.0 ppb and 2.8 ppb (3.0 µg and 2.8 µg silver per kilogram of blood) at 12 hours and 48 hours, respectively, after the implantation of the silver spacer during surgery

Table III. Adverse events

Patient	Sex	Age, yrs	Type of spacer surgery 1	Adverse events after surgery 1	Type of spacer surgery 2	Adverse events after surgery 2
1	Male	61	Silver	Redness and itching of the back at day 1, elevated thrombocytes	Non-silver	Elevated leucocytes
2	Male	45	Non-silver	Low oxygen saturation for four days; mild: mouth dryness	Silver	None
3	Male	76	Silver	Leg oedema, nausea, elevated LDH, elevated urea	Non-silver	None
4	Female	75	Silver	Leg oedema, nausea	Non-silver	Nausea, leg oedema
5	Female	80	Silver	Low oxygen saturation for ten days	Non-silver	Diarrhoea
6	Male	72	Non-silver	Nausea, diarrhoea, hypertension, renal insufficiency, disorientation	Silver	Disorientation, diarrhoea
7	Male	80	Silver	Mycosis inguinal area, low potassium, elevated creatinine	N/A*	N/A*
8	Female	75	Non-silver	Low potassium, disorientation, bleeding of haemorrhoids, hypotension, vomiting, urinal mycosis, diarrhoea, high potassium	Silver	Hypotension, vomiting; moderate: urinal tract infection, hyper potassium
9	Male	31	Silver	None	Non-silver	None
10	Male	64	Non-silver	None	Silver	None
11	Female	77	Silver	Hallucinations, bradyarrhythmia; mild: limited renal function	Non-silver	Arterial hypertension, redness neck region, strong sweating, depression; mild: low potassium, limited renal function
12	Female	64	Non-silver	None	Silver	None

*Patient chose to withdraw from the study after surgery 1
LDH, lactate dehydrogenase; N/A, not applicable

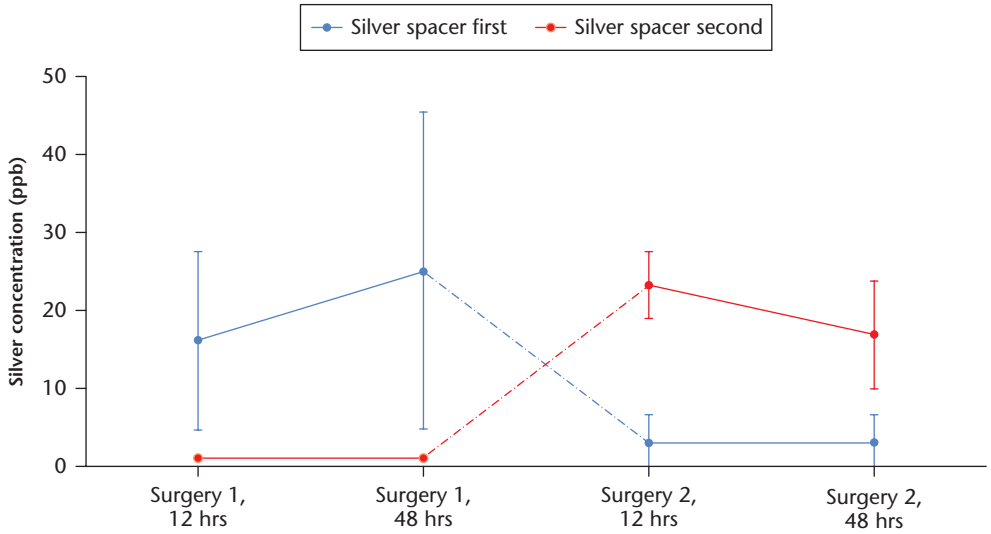

Fig. 2

Chart showing the silver concentration in the drainage fluid.

1 was detected. After exchange of the silver spacer to a silver-free spacer, the silver concentration was 4.1 ppb and 5.3 ppb at 12 hours and 48 hours, respectively, after the intervention.

Urine analysis. The overall urine silver burden in all urine samples was negligible and found to be below the detection limit of 1 ppb.

Drainage fluids. Drainage fluids showed silver concentrations from 6.0 ppb to 38.0 ppb and 5.6 ppb to 68.0 ppb after 12 hours and 48 hours, respectively, following surgical implantation of the silver spacer. In patients who received the silver-loaded spacer first, the mean silver concentration was 16.1 ppb (SD 11.5) at 12 hours after surgery and 25.1 ppb (SD 20.4) at 48 hours after surgery (Fig. 2). The mean silver concentration decreased to 3.1 ppb (SD 3.5) after surgery 2 with the silver-free spacer. The mean silver concentration in the drainage fluid was 1 ppb in all patients at 12 hours and 48 hours after surgery 1 with a silver-free spacer. Following implantation of the silver spacer in these patients, silver concentrations of 23.2 ppb (SD 4.4) at 12 hours after surgery and 16.8 ppb (SD 6.9) at 48 hours after surgery were observed.

In six of the 11 cases, the silver concentrations between 12 hours and 48 hours only changed moderately, within a range of 5 ppb. The maximum concentration of 68.0

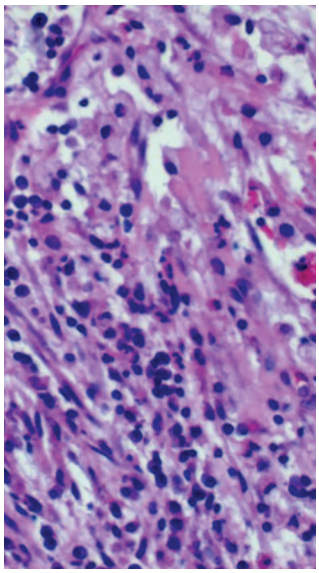

Fig. 3a

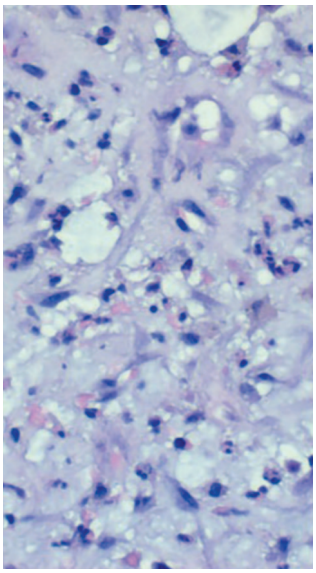

Fig. 3b

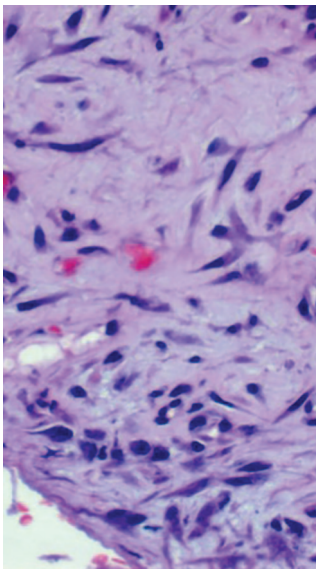

Fig. 3c

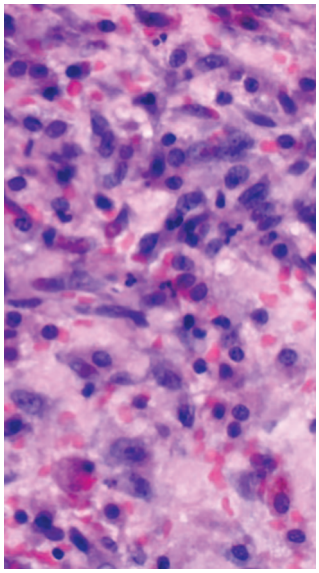

Fig. 3d

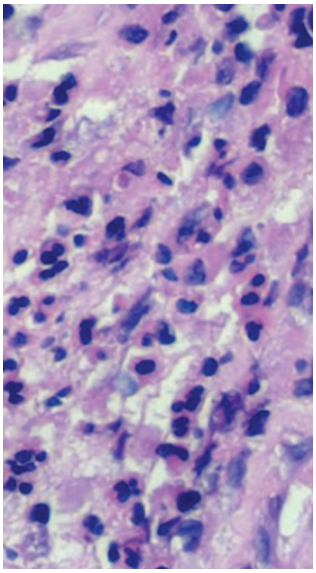

Fig. 3e

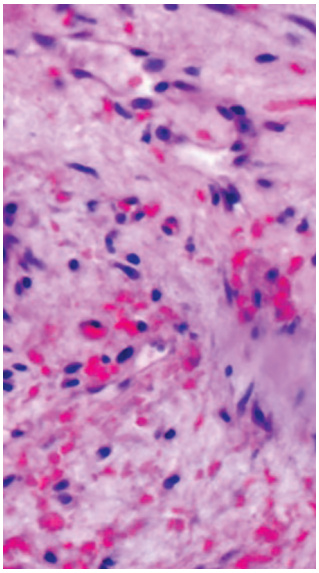

Fig. 3f

Haematoxylin and eosin (H&E) staining and pathohistological assessment of periprosthetic/perispacer membrane in two cases. a) to c) Microsilver poly(methyl methacrylate) (PMMA) spacer first followed by silver-free PMMA spacer. d) to f) Silver-free PMMA spacer first followed by microsilver PMMA spacer. Magnification: 400×.

ppb was seen in one patient, who had already shown the highest concentration of 38.1 ppb after 12 hours.

Histology. In general, no considerable systematic differences between microsilver-loaded and silver-free PMMA spacers were detected and the histomorphological features were similar compared with regular diagnostic samples tested in the same period.

In both cases, the highest inflammatory activity (with a mean of more than ten neutrophil granulocytes per HPF) could be observed in the samples of surgery 1 with explantation of the infected prosthesis (Figs 3a and 3d). Samples taken from surgeries 2 and 3 mostly showed low-grade infection (Fig. 3b) or only a chronic inflammation (Fig. 3c), with a mean of neutrophil granulocytes below the diagnostic threshold of one per HPF (Figs 3b, 3c, 3e, and 3f). No silver particles or indications of toxic effects such as necrosis were found in any of the samples.

Polyethylene wear particles were detected in three of the initial samples and two of the later samples. Since neither of the spacer variants contained polyethylene, these wear particles must have derived from the prosthesis that had already been removed. Light microscopy showed that in seven of the initial samples and five of the later samples, there was fine (< 10 µm) brown (e.g. hemosiderin or iron rich wear particles) or black (e.g. titanium wear particles) pigmented material. There was no association

between the pigmented material and the type of spacer used.

Discussion

To the best of our knowledge, the present study examined for the first time the use of silver-loaded gentamicin-PMMA spacers in a clinical trial. Our group previously showed that PMMA bone cement with 1% w/w of microsilver exhibits promising *in vitro* effects, with high antibacterial effectiveness against multidrug-resistant bacteria.[11] At the same time, no toxic effects on larger eukaryotic cells were seen.[11] Combined, the biocompatibility with eukaryotic cells and cytotoxic effects against multidrug-resistant prokaryotic cells provide a 'therapeutic window' for clinical application.[11] In the present study, 0.8 g microsilver was used in the PMMA spacers in most cases; in one patient, a total amount of 1.6 g microsilver was used. No silver-specific adverse events, such as argyria or impairment of the femoral or sciatic nerve, were observed. Any clinical adverse events detected, such as nausea, vomiting, low oxygen saturation, or laboratory changes (decreased thrombocytes, elevated creatinine, or alterations of potassium) were related to the procedure and to existing comorbidities of the patient, and not to the additional silver loading of the spacer. Serum and urine analysis revealed concentrations around or below the detection limit of 1 ppb in all but one patient. This patient exhibited silver concentrations of 3.0 ppb at 12 hours after the implantation of the silver spacer during surgery 1, and 2.8 ppb after 48 hours. Interestingly, these concentrations even increased up to 5.3 ppb at 48 hours after surgery 2, with the change of spacer from silver-loaded to silver-free PMMA. This patient suffered from multiple comorbidities, such as sacral ulcers, diarrhoea, and highly elevated CRP during admission, whereas creatinine levels were found to be low (0.6 mg/dl at surgery 1 and 0.58 mg/dl at surgery 2). No clear explanation for the marked difference in silver serum levels between this patient and the others could be found. Data from the literature suggest that silver serum levels in silver industry workers are between 0.1 ppb and 23.0 ppb,[18] and around 11.0 ppb[19] without adverse clinical effects, and, therefore, this maximum value of 5.3 ppb blood can be deemed safe for the patient. A limitation of the study is that no preoperative serum silver analysis was performed, which could have helped to explain why the silver levels reached 5.3 ppb blood in this patient.

Drainage fluid levels were between 6.0 ppb and 38.1 ppb after surgery 1 and between 5.6 ppb and 68.0 ppb after surgery 2, which is considerably higher than the measured silver levels in urine and in blood. This is fully in line with the concept of local application of antimicrobial agents, such as in antibiotic-loaded PMMA spacers or beads, to achieve high local concentration and minimize systemic concentrations of the antimicrobial agents at the same time, based on the initial ideas of Buchholz and Engelbrecht[20] and Klemm.[21] Our results confirm a considerable release of silver from the PMMA spacer, which is a prerequisite to fulfil its antimicrobial effects against bacteria in the wound. Due to the fact that no silver-related adverse events were detected, the observed local concentrations of up to 68.0 ppb in the drainage fluid can be considered safe for patients.

With regard to the clinical application of silver in other musculoskeletal contexts, silver poisoning was described following the use of nanoparticulate silver for topical wound dressing (Acticoat; Smith & Nephew, London, United Kingdom).[22] In this case, clinical presentation showed greyish discoloration, fatigue, and lack of appetite.[22] The silver concentration in the blood was determined as 107 ppb.[22] A case series describing silver uptake in patients treated with the same wound dressing reported median maximum silver levels of 56.8 ppb with no haematological or biochemical indicators of toxicity.[23] The maximum level of 5.3 ppb in our study was more than ten times lower than the median maximum levels found in the silver wound dressing group of this case series.[23]

Hardes et al[13] reported on the biocompatibility of silver-coated megaendoprostheses (MUTARS; Implantcast GmbH, Buxtehude, Germany) in a clinical case series of 20 patients with bone tumours. A maximum concentration of 56.4 ppb was found in one patient's serum 15 months after implantation of the silver-coated megaendoprosthesis.[13] Laboratory tests excluded the kidney, liver, and haematological impairment within a follow-up period of 24 months.[13] Again, the maximum measured concentration of 5.3 ppb blood in our study is more than ten times less than the concentration found in the study by Hardes et al.[13] However, different coating techniques and different types of silver (metallic silver, microsilver, silver salts, other silver complexes) result in a significant variation in the release of free silver ions (Ag^+), which are thought to be relevant for silver toxicity.[24] Hence, comparison of toxicological studies should be critically evaluated.

Based on the findings of animal studies, silver and its ionic forms seem to be distributed to all organs.[25] In argyria, the most prominent sign is discolouration of the skin. Local argyria around the microsilver-loaded cement spacers was not detected in any of our patients. The histological findings of tissue samples taken in the immediate vicinity of the spacers showed no difference between groups.

Silver excretion in general is described as urinary and faecal.[24] A urinary-faecal excretion ratio in monkeys is reported as 0.019% to 0.026%.[26] Furthermore, urinary excretion of orally applied silver nanoparticles was found

to be between 0.005% and 0.057%.[25] Hence, it is not surprising that no quantifiable excretion of silver was detectable in the urine of patients in our study.

Accumulation of silver in patients' organs could not be determined directly. However, laboratory tests did not indicate any functional limitation of internal organs due to the deposition of silver. In a rabbit model, Gosheger et al[27] found that no toxicological effects were associated with a silver-coated MUTARS tumour endoprosthesis within a 90-day follow-up period. Accumulation of silver was observed in the liver and spleen with mean values of 90.4 ppb and 27.9 ppb, whereas blood concentrations had a mean of 1.88 ppb.[27]

Our paper has several limitations, including a low number of patients. The case series was planned to test biocompatibility in temporary spacer implantation only. The long-term antimicrobial performance of silver-loaded PMMA cement in reducing reinfection rates after reimplantation of the prosthesis was therefore not evaluated. This will be of the utmost importance for further clinical studies with microsilver-loaded PMMA spacers. An interesting microbiological finding of the current study was the lack of bacterial growth after the use of silver spacers in six of seven cases after surgery 1, compared with proof of bacterial growth in all four cases in which non-silver spacers were used during surgery 1 (Table II). The small sample size and the primary safety study design characteristics do not allow for adequate statistical evaluation in this context.

Furthermore, the short observation period of 28 to 42 days from initial surgery until reimplantation of the revision prosthesis also limits the quality of the study. However, these spacers only serve as temporary implants, and an observation period limited to the duration of the *in situ* stage is acceptable. In the study conducted by Glehr et al,[14] in which silver-coated megaendoprostheses were implanted during bone tumour surgery, patients developed argyria after a median exposure time of 25.7 months. This is substantially longer than the relatively short *in situ* period of silver spacers of only 14 to 21 days.

Bacterial resistance to silver nanoparticles and strategies to overcome this, as recently reported by Panáček et al,[28] have to be addressed in future studies, especially given the large numbers of multidrug-resistant bacteria.[29]

In conclusion, this prospective cohort study on microsilver-loaded gentamicin-PMMA spacers was intended to evaluate the safety of gentamicin-loaded PMMA spacers with the additional loading of microsilver in prosthetic hip infections in a prospective cohort study. The microsilver spacers did not show any adverse clinical events related to silver, such as argyria of the skin. Blood and urine analyses were far below the published silver concentrations of other orthopaedic implants or wound dressings and confirmed the good biocompatibility. Detailed histology of the peri-implant membrane did not reveal any differences between microsilver and silver-free spacers. Therefore, the microsilver PMMA spacers used can be deemed safe, and their broad antimicrobial activity warrants further clinical research to assess their effectivity in reducing infection rates in prosthetic joint infections.

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Author informaton

- V. Alt, MD, PhD, Professor, Chairman and Director, Department of Trauma Surgery, University Hospital Regensburg, Regensburg, Germany.
- M. Rupp, MD, Board Certified Orthopaedic Surgeon, Department of Trauma, Hand and Reconstructive Surgery, University Hospital Giessen-Marburg, Campus Giessen, Giessen, Germany.
- K. Lemberger, Physican Assistance, Department of Orthopaedics, Trauma Surgery and Sports Medicine, Asklepios Klinik Birkenwerder, Birkenwerder, Germany.
- T. Bechert, PhD, Former CEO, Bio-Gate AG, Nuremberg, Germany.
- T. Konradt, MSc, Member of Management Board, Bio-Gate AG, Nuremberg, Germany.
- P. Steinrücke, PhD, Head of R&D, Bio-Gate AG, Nuremberg, Germany.
- R. Schnettler, MD, DVM, Professor, Researcher, Department of Cranio-Maxillo-Facial Surgery, University Hospital Hamburg, Hamburg, Germany.
- S. Söder, MD, Pathologist, Institute of Pathology, Regiomed Hospital, Coburg, Germany.
- R. Ascherl, MD, Professor, Hospital for Traumatology, Special Surgery and Revision Endoprosthetic Surgery, Tirschenreuth, Germany.

Author contributions

- V. Alt: Analyzed the data, Wrote the manuscript.
- M. Rupp: Analyzed the data, Wrote the manuscript.
- K. Lemberger: Collected and analyzed the data.
- T. Bechert: Conceptualized the study, Collected and analyzed the data.
- T. Konradt: Conceptualized the study, Collected and analyzed the data.
- P. Steinrücke: Conceptualized the study, Collected and analyzed the data.
- R. Schnettler: Analyzed the data.
- S. Söder: Analyzed the histopathological samples.
- R. Ascherl: Performed the surgeries, Collected and analyzed the data.
- V. Alt and M. Rupp are joint first authors.

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Conflict of interest statement

- V. Alt reports consultancy fees from Bio-Gate AG related to this study. T. Konradt and P. Steinrücke report employment from Bio-Gate AG, who acted as a sponsor and provided the MicroSilver bone cement.

Ethical review statement

- The study was officially approved by the Ethical Committee of the University Erlangen-Nürnberg, Germany, with the reference #4100 (21.10.2009).