THE PREVENTION OF METHICILLIN SENSITIVE STAPHYLOCOCCAL INFECTIONS IN A RABBIT CONTAMINATED IMPLANT BED MODEL USING TOBRAMYCIN OR SILVER PMMA BONE CEMENT

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Introduction
Antibiotic bone cement has proven its value in both experimental and clinical research\textsuperscript{1-2}, but because of the increasing problem of antibiotic resistant bacteria other antimicrobial strategies are investigated. A new type of low-dose metallic silver containing bone cement was shown to be effective in vitro and in vivo against both antibiotic sensitive and resistant bacteria\textsuperscript{3}. As for this silver cement, the main antimicrobial activity is claimed to be a surface phenomenon, which prevents the presence of viable bacteria at the implant surface, we compared in the present study the in vivo efficacy of silver PMMA bone cement in preventing methicillin sensitive Staphylococcal infections with plain and tobramycin containing PMMA bone cement, in a rabbit model of a contaminated local implant bed.

Materials and Methods
The local Animal Care Committee approved this study. In 48 female New Zealand White rabbits 0.6% w/w silver, 1% w/w silver, plain or tobramycin-loaded (tobra) PMMA bone cement (all types: Simplex@P; Howmedica International, Limerick, Ireland) (n=12 per cement type) was injected into the medullary canal of the right femur. Prior to cement injection, the implant bed was contaminated with 10\textsuperscript{5}, 10\textsuperscript{4} or 10\textsuperscript{3} colony forming units (CFU) of Staphylococcus aureus Wood 46 (ATCC 10832) (n=4 per subgroup). All rabbits had a follow-up of 14 days.

Surgery: Surgery was performed under inhalation anesthesia and aseptic conditions. After skin incision, the trochanter tertius was exposed. Using a small drill, the cortex was penetrated and the femoral canal was reamed up to a diameter of 4 mm. Then cooled PMMA bone cement (4°C) was vacuum mixed according to the manufacturers guidelines. After suctioning, 100 µl of bacterial suspension was injected in the medullary canal and subsequently ± 1.2 gram of the cement was injected. Expansion of the cement sealed of the entrance of the canal, thereby preventing spill of bacteria. The cement was allowed to polymerize in situ. Finally, the wound was closed in layers.

Follow-up: Rabbits were checked daily for activity, appetite and wound healing; temperature and weight were recorded as well. Blood samples for ESR and WBC were obtained weekly. Fluoroscopic images were made postoperative and after 14 days (Figure 1).

Sample acquisition and analysis: After 14 days rabbits were killed. Under sterile conditions, bone from the right femur was collected. For bacteriology, the lateral cortex was divided in a proximal sample fully adjacent to the cement and a sample distal from the cement. Bone was weighed and homogenized in 10 ml sterile PBS using a tissue grinder. Serial 10-fold dilutions were plated on blood agar plates. After an overnight incubation at 37°C, the number of viable bacteria was counted and calculated per gram of bone (\textsuperscript{10}logCFU/g). For histopathology, paraffin sections were made of the medial cortex. Slides were stained with haematoxylin and eosin and analyzed for signs of infection.

Statistical analysis: Results are reported as mean±SEM. For the outcome infection (yes or no) we used logistic regression analysis. For all other outcomes we used two-way ANOVA and Tukey-HSD post-hoc testing. P<0.05 was considered significant.

Results
All rabbits recovered well from surgery. ESR after 7 days showed a large increase in the rabbits with plain and both types of silver cement (p=0.987 and p=0.516 vs. plain), whereas it remained normal in the tobra rabbits (p=0.01). WBC had a milder increase and only showed a significant difference between plain and tobra cement (p=0.004). Weight loss after 14 days for plain and tobra cement rabbits was 246±45 and 50±35 gram. Rabbits with 0.6% and 1% silver cement had a weight loss of 213±36 and 202±29 gram (p=0.895 and p=0.786 vs. plain and both p<0.001 vs. tobra).

In the plain and both silver cement groups all rabbits (100%) were infected, whereas with tobra cement only 2 of 12 rabbits (17%) were infected (p=0.001). The number of bacteria cultured from bone adjacent to the cement was 6.4±0.3 and 6.1±0.3 for the 0.6% and 1% silver rabbits (Figure 2). For the rabbits with plain and tobra cement this was 6.2±0.2 (p=0.953 and p=0.991) and 0.0±0.0 (both p<0.001). Although 2 tobra rabbits had a positive result as well, culture of the distal samples showed comparable results to the results of the proximal samples.

Discussion
In this experimental infection model, infections developed in all plain PMMA cement animals, while the tobramycin cement was effective in the prevention of infection development. The silver cements, with both silver concentrations, were not effective with respect to the prevention of methicillin sensitive Staphylococcal infection in comparison to tobramycin-containing bone cement. However, it should be realized that the current model is that of a contaminated implant bed, with bacteria present at the implant surface, but also at a distance from the implant. A PMMA cement releasing an antimicrobial agent, such as tobramycin, will therefore likely be effective against bacteria distant to the implant surface as well. In contrast, the silver cement, predominantly exhibits an antimicrobial effect at the direct cement surface, and not outside the implant materials surface. The results from the present study demonstrate that such a cement could be less useful in situations where there are also bacteria present in the surrounding tissue, like revision surgery. Although S. aureus Wood 46 is often used in infection research\textsuperscript{3}, the difference in infection prevention compared to the previous reports might also be influenced by the virulence of the bacteria used. Whether a PMMA cement with a predominant antimicrobial effect at the direct cement surface has relevance in the prevention of bacterial colonization of PMMA cement, for instance in late haematogenous infections, remains to be seen and should be the topic of further investigations.

References
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Figure 1. Postoperative fluorescent image of right femur with cement in situ. Arrow indicates entry point of cement injection.

Figure 2. Culture results (\textsuperscript{10}logCFU/g) in the proximal bone samples of rabbits contaminated with S. aureus Wood 46.

Figure 3. Images of HE-colored sections of a 1% silver rabbit, showing a severe peristomal reaction (left, 40x) and enlargement of Haversian canals (right, 100x).