A Reproducible Implant Infection Model in Rabbits

INTRODUCTION:
Orthopaedic prostheses implantation is an invasive surgical procedure, with an increased risk for post-operative infections. Since the life span and quality of these implants is improving and more prostheses are implanted every year, the incidence of post-operative infections will increase. Most implants are based on titanium or nickel-chrome-molybdenum alloys, while their overall biocompatibility is good, these materials do not possess antimicrobial properties.

For the evaluation of novel antimicrobial implant coatings, in vitro, ex vivo and in vivo testing are essential to establish a reliable coating, suitable for orthopaedic implants. In this study a rabbit osteomyelitis model, reproducibly resembling clinical orthopaedic implant infections, is being developed. The model will be utilized for evaluating the efficacy of novel antimicrobial coatings on titanium substrates for orthopaedic implantation. We here report on a pilot study that evaluates surgical methods, imaging techniques and osteomyelitis outcome.

METHODS:
In the rabbit osteomyelitis model a titanium rod was placed in the proximal metaphysis of the tibia (Fig. 1). Rabbit cadaveric studies were performed to evaluate surgical approaches for the implantation procedure preceding the in vivo experiments.

The implants were contaminated with various inoculum sizes of Staphylococcus aureus (ATCC 49230) to induce osteomyelitis. Bacterial suspensions were applied into the reamed tibial medullary canal before implant positioning. After implant positioning, the entrance was sealed with bone wax. Correct implant positioning and the degree of infection were evaluated throughout the follow-up period by X-ray, μPET and µCT imaging. Health status was monitored by total body weight and, as well as hematological analyses, like C-reactive protein and erythrocyte sedimentation rate determination. After 42 days of follow-up, animals were sacrificed. The tibia and titanium implants were evaluated by histological analysis of bone integration, biofilm formation and clinical signs of infection in the surrounding tissue, as well as total bacterial counting in the bony tissue.

RESULTS:
Rabbit cadaveric experiments showed that surgery through the patella tendon is preferable to the medial parapatellar arthrotomy. Because the parapatellar arthrotomy has an increased risk of damaging the intra articular tissue and causing septic arthritis.

In the in vivo experiments the tibia was reamed by hand drilling at the position where in human fracture treatment the intramedullary nail is inserted. The proximal tibia of rabbits were contaminated with different inocula of exponentially growing S. aureus. Subsequently, the implant was positioned and the entrance to the medullary canal was sealed with bone wax. The wound was closed in layers, no post-operative wound infection occurred.

During follow-up, several imaging techniques were evaluated for their potential to evaluate the infection in this model. μCT analysis provided us with additional post mortem information on implant position, bone remodeling and osteolysis due to the implant infection. Our μCT data show clear differences in the structural bone-remodelling of the infected versus the contaminated tibiae. Cortical thickening, periosteal reactions and osteolysis were observed (Fig. 2). 18F-FDG μPET imaging enabled the differentiation between sterile inflammation (post operative effects) and bacterial infection of the implant area. Where the control animals (uncontaminated implants) showed a low uptake of the radiofarmacon, the infected tibiae showed an increased uptake of the tracer around the implant (Fig. 3). At 7 days after surgery an increased uptake could readily be observed in both groups. Still, standardized uptake values of the implant area of the infected animals were higher at 7 days and continued to be higher (2-fold) than the control animals throughout the experiment. These data, combined with hematological analyses, bacterial culturing of post mortem tibia samples, X-ray imaging and histology, are expected to strengthen this animal model for use in orthopaedic implant related infection studies, as well as antimicrobial coating evaluation.

DISCUSSION & CONCLUSIONS:
Both μCT and μPET show clear signs of infection in the contaminated implant group as compared to the control group. The infections were confirmed by post mortem bacterial culturing. Histological sectioning of the implant area and analysis of the hematological parameters further support the infected status. Although our preliminary data suggest a quantitative relation between the μPET signal and infection grade, further analysis is necessary to confirm this. Together with all its readout parameters, this model is expected to contribute to the evaluation of novel antimicrobial coatings for future use on orthopaedic implants.

SIGNIFICANCE:
Our model presents a reproducible implant infection animal model and is supported by physiological and haematological parameters, in vivo imaging, ex vivo imaging, histology and an extended follow-up period. The model is expected to be used for the evaluation of novel anti-microbial coatings on orthopaedic implants.

ACKNOWLEDGEMENTS:
All performed animal experiments were approved by the institutions animal ethical committee (DEC 2010-089).
This study is a part of the NANTICO project, financed by the BioMedical Materials institute, co-funded by the Dutch Ministry of Economic Affairs, Agriculture and Innovation.