Antimicrobial, biocompatible surface coatings for a permanent transcutaneous passage in the concept of osseointegrated limb prosthesis

ABSTRACT INTRODUCTION:
Osseointegration of amputation limb prosthesis appears to be a promising approach to improve quality of life and mobility of amputees in comparison to conventional socket attached devices. However, the clinical implementation of those bone anchored transcutaneous systems is still limited due to infections at the side of the percutaneous passage. In theory a stable adherence of the dermis to the implant is crucial for a permanent aseptic interface. The migration of bacteria into the depth has to be prevented. In our concept of osseointegrated limb prosthesis we address this issue by designing surface coatings with an antimicrobial impact and biocompatible characteristics at the same time.

METHODS:
Different copolymers with surface active phosphonate groups and antimicrobial cationic groups are designed and coated on titanium samples. At first the antimicrobial impact and biocompatibility is characterised in vitro. Coated samples are cultured with S. epidermidis and S. aureus under static and dynamic conditions or with human dermis fibroblasts, respectively (n=10 for each group). Polished blank titanium discs serve as controls. Bacterial adherence is evaluated via live/dead assay at 1 or 5 hours of cultivation. The biocompatibility is displayed by fibroblast attachment at 24h and cell proliferation at 72h via monitoring the amount of cells in a modified LDH assay and cell morphology in electron microscopic analysis. Furthermore, an in vivo animal model with transcutaneous pin samples is developed as shown in figure 1. Coated or blank titanium pins are implanted at the back of hairless mice (n=30 per group). At 1, 2 and 3 weeks after implantation the animals are sacrificed. Initial wound healing at the dermal-implant-interface is analysed regarding the clinical occurrence of infections, the bacterial load and the histomorphology. At the time of explantation the bacteria adhered to the implant surface are displayed with acridin-orange staining in confocal laser scanning microscopy.

RESULTS SECTION:
To date three generations of copolymer surface coatings are developed and characterized. The 1st generation of copolymer coatings already show an antimicrobial capacity with an onset effect and subsequent aggregation of germs but no reduction in the overall bacterial load as shown in figure 2b. The 2nd generation as well as the 3rd generation show a significant reduction of adherent bacteria up to 95% compared to blank titanium samples in vitro [Figure 2a,c+d]. Similar results can be reproduced in static and dynamic culture systems with a variety of species. The adherence and proliferation of human dermis fibroblasts show a slight decrease on polymer coatings compared to the control titanium samples, but is not significant.

For the in vivo test an animal model with percutaneous titanium pin samples is established [Figure 1]. Clinical apparent infection occurred in two mice of the blank titanium group but none in the polymer group. A bacterial migration along the transcutaneous pin is found in three cases – two in the blank titanium group and one in the polymer group. All other samples show no adherent bacteria to the implants. Overall we are not able to demonstrate significant differences in the bacterial load or the morphology of the dermal-implant-interface in the polymer coated group compared to the blank titanium controls.

DISCUSSION:
In our study we were able to design a polymer surface coating for implant materials with antimicrobial and biocompatible properties at the same time. We can demonstrate a high antimicrobial impact to different clinical relevant pathogens in vitro. The biocompatibility to human dermis fibroblasts shows no significant difference to the titanium controls in vitro. Admittedly, our preliminary results of the short term follow up, which was used in this study to evaluate the initial wound healing in vivo, showed no significant benefit of the polymer coatings compared to blank titanium. However, the long term evaluation of the surface characteristics and the dermal-implant-interface in vivo is much more relevant in our clinical setting. This is already addressed in ongoing experiments using the same animal model. Overall we consider the proven basic characteristics of the polymers to be a promising technology for the realisation of a permanent aseptic percutaneous passage as needed for the advancement of osseointegrated limb prosthesis.