Implant-associated Infections in Total Joint Arthroplasty: In vitro Analysis of Bacterial Colonization and Biofilm Formation on Human Primary Osteoblasts

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Disclosures:

Introduction: Total joint replacement is one of the most frequently done and successful surgeries in recent times. Periprosthetic infections occur in 1 to 7 % after primary joint replacement [1]. Even modern surgical procedures, aseptic conditions as well as perioperative prophylactic antibiotics and special antimicrobial implant-surfaces do not prevent from periprosthetic implant associated infections [2-4]. The relative high infection rates in orthopaedic surgery are caused by the special constellation of implantation of extraneous material and the very small infection-provoking bacterial inocula, which are characterized by a typical pathogenic spectrum. Directly after implantation an initial adhesion of commensals and pathogens takes place at the implant surface [5]. Unfavorable conditions like immunosuppression support the formation of bacterial biofilms on the implant surface. Within these biofilms, each bacterial species is protected from host’s own defense mechanisms and systemic antibiotic therapy [6]. Thus, adhering to biomaterials and forming of biofilms, are important factors in the pathogenicity of microorganisms, which can result in persistent infections despite aggressive antibiotic therapy [6]. Especially staphylococci, including the species Staphylococcus aureus and coagulase-negative staphylococci such as Staphylococcus epidermidis, are counted among implant-associated infection-causing germs [5]. The chronological order of the implant colonization by cells and bacteria may cause another problem of implant-associated infections (“race for the surface”). Eukaryotic cells can be restricted or even disabled in their growth and colonization ability due to initial bacterial colonization of the implant surface. However, even a weak colonization of eukaryotic cells on the implant surface may promote the growth of the implied bacterial species and their associated biofilm formation [6]. The objective of this experimental study was the establishment of an in vitro co-culture test setup of human primary osteoblasts and S. epidermidis to emulate the initiation of implant-associated infections in situ. Furthermore, it was to investigate whether the co-culture model is a suitable method to analyze antimicrobial and bioactive implant surfaces and coatings.

Methods: Co-cultures of primary human osteoblasts and S. epidermidis (RP62A) were performed in 24-well plate format as single-species infections with a MOI of 0.04 (25.000 cells/ml, 1000 CFU/ml). During further procedure the in vitro co-cultures of primary human osteoblasts and S. epidermidis were extended with sterile titanium test samples (Ti6Al4V) to mimic the surface of endoprosthetic implants. Special osteoblasts MEM-Dulbecco’s media (Biochrom AG, Germany) without calcium and antibiotics, containing 10 % fetal calf serum (FCS), was used for both co-culture setups. Osteogenic adjunctions such as ascorbic acid, β-glycerophosphate and dexamethason were added to the media. The co-cultures were incubated over a period of 7 days under aerobic conditions at 37 °C, 5 % CO2. For initial adhesion, human primary osteoblasts were allowed to grow for 24 h prior to infection. Changes of media were performed after 48 h and 4 days of infection. Subsequently, planctonic S. epidermidis was quantified by centrifugation and determination of colony-forming units (CFU) on caso-bullion agar-plates (CB-agar) in both co-culture test setups. The quantification of S. epidermidis within the biofilm on the surface of the test samples was performed by sonification and determination of CFU. For setting-up tests excluding titanium samples, the biofilm was mechanically removed from the 24-well plate with a pipette tip, diluted serially in 1x PBS and subsequently plated on CB-agar plates for assignment of CFU. A quantification of adherent living primary osteoblasts on the test samples, the well-plate bottom and in the media was performed by trypan blue staining and a subsequent counting of living cells using an Abbe-Zeiss counting cell chamber. Scanning electron microscopy (SEM) was used for evaluation of the osteoblast and bacteria surface as well as the topography and composition of the bacterial biofilm.

Results: Preliminary results show an increase in the number of vital planctonic and biofilm-bound S. epidermidis by three and four dimensions respectively after 7 days of infections in both co-culture setups. A distinct decrease of living human primary osteoblasts in simultaneous cultivation with S. epidermidis was observed. For the model excluding titanium, 4.96 % ± 0.7 % (n=3 ± SEM) of living cells were quantified after 7 days prior to infection. After 7 days of infection 44.04 % ± 9.74 % (n=3 ± SEM) of living cells were counted for the titanium containing setup. The SEM analysis showed a high number of S. epidermidis organized in bacterial grapes alone and mostly on top of damaged human primary osteoblasts.

Discussion: The aim of this work was to establish an in vitro co-culture model of human primary osteoblasts and S. epidermidis. The preliminary results demonstrate the possibility of combining the bacterial and cellular elements in one test setup over a period of 7 days, as well as adding an inorganic implant material like titanium. The special osteoblasts MEM-Dulbecco media without calcium and antibiotics containing 10 % FCS and osteogenic adjunctions, which is used for eukaryotic cell culture purposes, is as well suitable for simultaneous cultivation of S. epidermidis and human primary osteoblasts. However, a distinct
negative impact on the cell growth of the primary human osteoblasts due to bacterial proliferation was observed. The established in vitro co-culture test setup, used in this study, provides a clinically relevant testing method for antimicrobial and bioactive surfaces of endoprosthetic implants. An elongation of the incubation time up to 14 days should be performed and other types of implant surfaces may be subject to further investigations using the established co-culture test setup.

**Significance:** The clinical relevance of this research consists in the development of a newly method for screening antimicrobial and bioactive implant surfaces in vitro based on an in vitro co-culture test setup of human primary osteoblasts and S. epidermidis which mimics the situation of an implant-associated infection. Based on the in vitro set-up, new types of antimicrobial implant surfaces might be identified in the future.

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**References:**

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