P2302 Effects of soluble factors of *Staphylococcus aureus* on eukaryotic bone cells (primary human osteoblasts)

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Background: Due to demographic changes, osteomyelitis and implant-associated infections are on the rise. Treatment takes long and often requires multiple surgical procedures. As approx. 70% of all bone infections are caused by *Staph. Aureus* (SA), a common biofilm-producer, it is crucial to understand the interaction, e.g. prosthesis loosening, between prokaryotes and eukaryotes. Bacteria in non-planktonic form secrete a variety of molecules: biofilm forming Polysaccharides, lipids, extracellular DNA as well as toxins. We investigated the impact that biofilm-produced molecules had on metabolism, mineralization and osteogenic differentiation of primary human osteoblasts (phOB).

Materials/methods: Biofilm-conditioned media (BCM) was produced by incubating *Staph. aureus* (strain 24923) over 4 days. The resulting biofilm was incubated in osteogenic media (OM) for 72h and filtered sterile. Parallel we isolated primary human osteoblasts from the cancellous bone of the femoral head from 7 different patients with the Explant-method and cultivated them in OM. For measuring metabolic activity (WST-1-Assay, 7 technical replicates), Mineralization (Alizarin stain) and osteogenic differentiation (Gene expression assay of COL1A1, ALP, BGLAP, SPP1, RUNX2) phOB were seeded at 10000/cm² and incubated 24h respectively 72h with BCM 100% and 50%. Non-treated OM served as control. Statistical analysis was performed using two-way anova.

Results: After incubation with 100%-BCM for 72h metabolic activity decreased to 26% (p≤0,0001) whereas after 24h it was still at 57% (p≤0,01). 50%-BCM showed similar results, but on a smaller scale, by reducing metabolic activity to 49% (BCM50/72h, p≤0,001) and 74% (BCM50/24h). Mineralization did not show a clear proposition. As for osteogenic differentiation, upregulated genes were *RUNX2* (2-fold) and *SPP1* (2-fold (24h) to 7-fold (BCM100/72h). Downregulated genes were *COL1A1* to 0,4-fold (BCM100/72h), and *BGLAP* to 0,3-fold (BCM50/72h). *ALP* did not change significantly.

Conclusions: Soluble biofilm-factors of SA significantly inhibit osteoblastic cell metabolism, time and concentration dependent. Through downregulation of
COL1A1 and BGLAP they may account for less bone stability and mineralization. With upregulation of SPP1 and partially RUNX2 they may impact osteoclast and cell cycle regulation. Concluding this data suggests that soluble, potentially far-reaching, biofilm-factors influence osteogenic processes similar substantial to direct attachment of bacteria.