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

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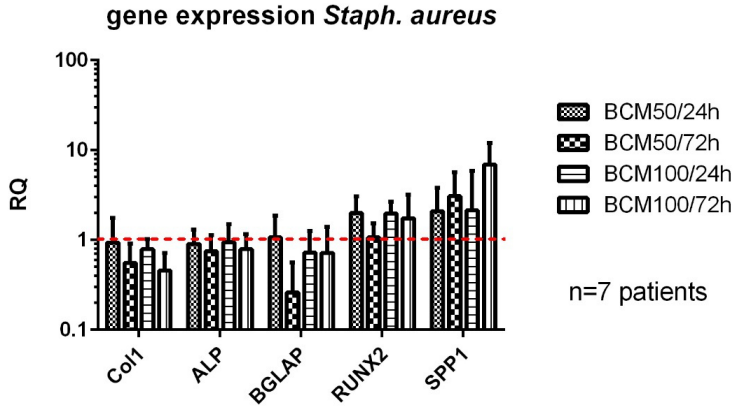
Background: Due to demographic changes, osteomyelitis and implant-associated infections are on the rise. Treatment takes long and often requires multiple surgical procedures. As approx. 70% of all bone infections are caused by *Staph. Aureus* (SA), a common biofilm-producer, it is crucial to understand 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.