The role of macrophages and intracellular survival of *Staphylococcus aureus* – new insights from direct evaluation of human tissue samples

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Background
Staphylococcus aureus (SA) can cause various infections and is associated with high morbidity and mortality rates of up to 40%. Antibiotic treatment often fails to eradicate SA infections even if the causative strain has been tested susceptible *in vitro*. The mechanisms leading to this persistence is still largely unknown. In our work, we hypothesize that interactions with host cells allow SA to persist at the site of infection.

Methods
We established a sampling workflow to receive tissue samples from patients requiring surgical debridement due to SA bone-and joint or soft-tissue infections. We developed a multiplex immunofluorescent staining protocol which allowed us to stain for SA, leukocytes, neutrophils, macrophages, B-cells, T-cells, DAPI and cytoplasmatic marker on the same sample slide. Further, distance of SA to cell nuclei was measured. Interaction of immune cells and SA on a single cell level was investigated with high-resolution 3D microscopy. We then validated our findings applying fluorescence-activated cell sorting (FACS) on digested patient samples. Finally, we aimed to reproduce our *ex vivo* patient results in an *in vitro* co-culture model of primary macrophages and clinical SA strains, where we used live cell microscopy and high-resolution microscopy to visualize SA-immune cell interactions and a gentamicin protection assay to assess viability of SA.

Results
Here, we revealed that CD68+ macrophages were the immune cells closest to SA with a mean distance of 56µm (SD=36.4µm). Counting the amount of SA, we found in total >7000 single SA in nine patients. Two-thirds of SA were located intracellularly. Two-thirds of the affected immune cells with intracellular SA were macrophages. The distribution of intra- to extracellular SA was independent of ongoing antibiotic therapy and underlying infection type. FACS confirmed these findings. In our co-culture model, intracellular SA remained alive for the whole observation period of eight hours and resided in RAB5+ early phagosomes.

Conclusion
Our study suggests an essential role of intracellular survival in macrophages in SA infections. These findings may have major implication for future treatment strategies.