Bimodal labelling of S. aureus for detection of bacterial colonization in skin infections by MRI

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Introduction: *Staphylococcus aureus* is a versatile human pathogen potentially leading to a diverse array of infections once the natural skin and mucosal barriers are breached (1). Besides minor skin and wound infections it is also a frequent cause of serious and life-threatening diseases such as pneumonia, endocarditis, arthritis, sepsis, peritonitis and osteomyelitis (2, 3). For the understanding of staphylococcal disease mechanisms several mouse models are in use and in order to visualize the course of an infection in real time in vivo imaging techniques have been developed. Due to high spatial resolution and high tissue contrast, especially MRI represents a versatile method to image bacterial infections non-invasively (4). Using the application of iron-oxide particles, cell labelling and tracking methods have been developed recently, and thus monitoring of localization and migration of macrophages and neutrophiles during an infection was achieved. However, the visualization of bacterial colonization within inflamed tissue is still challenging and sources of chronic infections with persistent bacteria have not been directly visualized by MRI. Labelling protocols for mammalian cells such as tumor cells or stem cells (5) are widely established but labelling of bacteria is particularly difficult due to their small size and protective bacterial cell wall. Here, we developed a protocol for labelling bacterial cells with rhodamine-coated VSOPs, which allowed us to visualize bacterial colonies in tissue and to distinguish infection from inflammation both by MRI and FRI (Fluorescence Reflectance imaging).

Experiments: *Bacterial labelling:* A cell suspension of 10^9 CFU/ml of competent *Staphylococcus aureus* cells, strain 6850, was electroporated with rhodamine coated 5 nm iron-oxide particles (VSOPs; Ferropharm, Teltow, Germany) in a concentration of 19.2 µM. *Fluorescence microscopy and FRI:* Electroporated bacterial cell suspensions of a concentration of 10^8 CFU/ml were stained with DAPI (1 µg/ml) for 30 min and visualized by using an AxioImager microscope (Zeiss, Göttingen, Germany). In the mouse model, 24 h post infection, mice were measured by FRI (Kodak, USA). *Mouse model*: A bacterial skin infection was induced at the left flank of nu/nu mice by injecting 100 µl of a suspension of either iron-labelled or unlabelled *S. aureus* bacteria. The bacterial suspensions were prepared in PBS in a concentration of 1 x 10^8 CFU/ml. Within 24 h after inoculation, an ulcerous abscess had developed with septicemic progression of the disease. Both iron-labelled and unlabelled S. aureus bacteria were administered to 5 mice each, and induced infections of identical course. For MRI measurements, the animals were anaesthetized with isofluorane. *MRI*: MR images were obtained on a Bruker BioSpec 94/20 (Ettlingen, Germany) equipped with a 1 T/m gradient system and a 35 mm birdcage coil. Animals were investigated with FLASH sequences and ultra-short echo-time (UTE) MRI to induce both negative and positive iron contrast by using different echo times. (FLASH: TR: 1500 ms, TE: 6 ms, FA: 30 deg, FOV: 3.20 x 3.20 cm, MTX: 256 x 256; UTE: TR: 100 ms, TE: 314 µs (negative contrast), TE: 20 µs (positive contrast) FA: 20 deg, FOV: 3.20 x 3.20 cm, MTX: 256 x 256). To reduce susceptibility artefacts at the air-skin interface the flanks of the mice were covered by alginate gel during measurements (6).

Results: Electroporation proved to be an efficient method to label *S. aureus* with iron-oxide nano-particles. Labelled *S. aureus* suspensions were visualized by fluorescence microscopy detecting rhodamine (Fig. 1). Approximately 50 % of the cell population was labelled. Bacterial growth curves and virulence assays confirmed that viability and virulence of the culture was not compromised after electroporation when compared to unlabelled bacteria. In the mouse model, large subcutaneous areas of high signal intensity at the site of infection were observed by MRI after 24 h indicating edema and inflammation (red circle in Fig. 2 A). *S. aureus* colonies at the site of infection were observed as hypointensities due to the iron label (arrow head in Fig. 2 A). However, despite the use of a susceptibility matching alginate gel, artefacts obscured the bacterial signal (green arrows in Fig. 2) in some areas. In UTE images (Fig. 2 B, C) susceptibility artefacts were absent due to short TE, but bacterial colonies with high concentration of iron were still observed as hypointensities. Further reducing TE to 20 µs, produced positive contrast from iron labelled bacteria (arrow head in Fig. 2 C), showing the typical bright rim as described previously (7). Due to the rhodamine-label localization of the colonies could be confirmed by FRI.

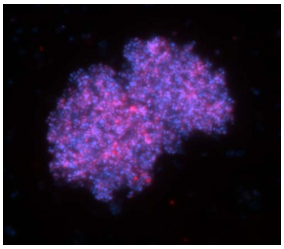

Fig1: Fluorescence microscopy of *S. aureus* cell clusters (magnification: 40x) labelled with DAPI (blue) and rhodamine- coated iron particles (red).

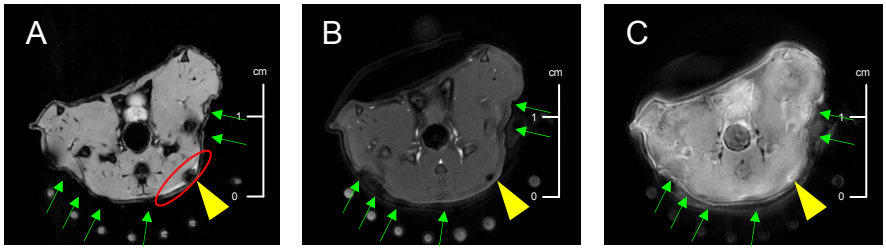

Fig2: Axial FLASH (A) and UTE images (B, negative contrast and C, positive contrast) of the mouse flank showing an ulcerous abscess (red circle) with bacterial colonization (arrow head). Susceptibility artefacts from air-skin interface were only observed by FLASH acquisition (green arrows in Fig. 2 A).

Conclusion: *S. aureus* can be labelled with bimodal contrast agents by electroporation. Thus, in vivo detection of small *S. aureus* colonies in infection models is feasible by MRI. Using different sequences with complementary image contrasts (in particular UTE) bacterial colonies can be unambiguously identified, as was confirmed by fluorescence imaging. The methods presented here may provide a versatile tool to follow bacterial infections in vivo and thus add a novel aspect to the field of molecular MRI.

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