Deciphering the indolence of osteomyelitis: Evaluation of the uptake of *Staphylococcus aureus* and silver nanoparticles into osteoblasts

**INTRODUCTION:** Chronic osteomyelitis is a particularly troublesome condition that is extremely difficult to treat. The causative agent of 80% of osteomyelitis cases is *Staphylococcus aureus*. *S. aureus* is able to be incorporated into osteoblasts and to persist in an intracellular environment (Ellington 2003). The intracellular location of *S. aureus* provides a mechanism for the indolence of osteomyelitis. The first objective of this research was to assess the uptake potential of *S. aureus*. Novel therapies are needed to combat the increasing antibiotic resistance of *S. aureus*. Photothermal therapy is a recent technique that uses conjugated nanoparticles and laser energy to target and kill specific cells. Silver nanoparticles have been shown to have direct antimicrobial effects against several pathogens (Kim 2007). A second objective of this research was to assess the uptake of silver nanoparticles into osteoblasts and to examine their possible cytotoxicity.

**METHODS:** Osteoblasts were explanted and grown from human clinical bone samples and rat femurs. The donor bone was crushed, and soft tissue and bone marrow were removed by scraping and vortexing. Bone fragments were then cultured and incubated. *S. aureus* (ATCC strains 49230 or 25923) were grown on Columbia blood agar plates and labelled with 5-(6)-carboxyfluorescein-succinimidyl ester (CFSE). 100 strains were grown on Columbia blood agar plates and labelled with either alkaline phosphatase and propidium iodide (PI) for rat osteoblasts or just PI for human osteoblasts. A Zeiss LSM 510 confocal microscope was used to image the coverglasses. A three channel microscope setting was used to excite anti-alkaline phosphatase or CFSE fluorescence (green) at 488nm, PI at 543 and to irradiate silver nanoparticles at 633 nm.

**Flow Cytometry:** Human osteoblasts were incubated with CFSE-labelled *S. aureus* and rat osteoblasts with nanoparticles in chambered coverglasses for 2 h. Osteoblasts were then fixed and stained with either alkaline phosphatase and propidium iodide (PI) for rat osteoblasts or just PI for human osteoblasts. A Zeiss LSM 510 confocal microscope was used to image the coverglasses. A three channel microscope setting was used to excite anti-alkaline phosphatase or CFSE fluorescence (green) at 488nm, PI at 543 and to irradiate silver nanoparticles at 633 nm.

**Confocal Microscopy:** Human osteoblasts were incubated with CFSE-labelled *S. aureus* and rat osteoblasts with nanoparticles in chambered coverglasses for 2 h. Osteoblasts were then fixed and stained with either alkaline phosphatase and propidium iodide (PI) for rat osteoblasts or just PI for human osteoblasts. A Zeiss LSM 510 confocal microscope was used to image the coverglasses. A three channel microscope setting was used to excite anti-alkaline phosphatase or CFSE fluorescence (green) at 488nm, PI at 543 and to irradiate silver nanoparticles at 633 nm.

**RESULTS:** Confocal microscopy demonstrated nanoparticles located intracellularly in rat osteoblasts. The osteoblast was identified using differential interference contrast (DIC) as well as by an anti-alkaline phosphatase antibody (green). Irradiated nanoparticles appear purple. Similarly, human osteoblasts infected with *S. aureus* show intracellular location using confocal microscopy. Using DIC, the shape of the osteoblast was observed. *S. aureus* were identified intracellularly by CFSE fluorescence (green) as well as DNA staining by PI (red), as shown in Fig. 2.

**REFERENCES:**

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