Introduction
Bone-associated infections are generally chronic and difficult to eradicate, being often associated with orthopaedic implant procedures. *Staphylococcus aureus* is one of the most relevant etiologic agents due to its ability not only to colonize biomaterial surfaces and bone peri-prosthetic tissues, but also the capability to invade osteoblasts, favouring chronic and recurrent infections. Additionally, the activity of the infected osteoblasts might be modified, thus impairing tissue functionality. When internalization occurs, bacteria are protected from the immune recognition and from antibiotics that may not enter cells. As a result, it is paramount to test new approaches and therapies to prevent intracellular infections and to better understand their implication on bone remodelling and regeneration.

**Aim**
Optimization of two critical parameters for the establishment of an *in vitro* co-culture of *S. aureus* and human osteoblasts: the gentamicin concentration to eliminate the extracellular bacteria after the infection period – granting an intracellular infection model, and the cell lysis conditions to lyse eucaryotic cells and access intracellular bacteria (Fig. 1).

![Figure 1. Representative illustration towards the establishment of a co-culture.](image)

**Methodology**
Mono-cultures of osteoblastic cells (MG63 cell line) and *S. aureus* (ATCC 49230) were exposed to (i) different concentrations of gentamicin (i.e., 0, 50 and 100 µg/ml) for 2 hours and (ii) to different cell lysis conditions (i.e., PBS for 30 min; 0.1% Triton X-100 (Tx) in water for 30 min; 0.2% Tx in water for 20 min and H₂O for 10 min). The effect of these conditions was evaluated through quantification of total protein for MG63 cultures and quantification of colony forming units (CFU) for bacteria.

**Results**

![Figure 2. (A) Total protein quantification of MG63 cell culture after exposure to different concentrations of gentamicin showed no statistic differences between conditions; (B) Total protein quantification of MG63 cell culture submitted to cell lysis presented statistic differences between lysis conditions and control (*p<0.05); (C) No cultivable bacteria (CFU) were detected after exposure to both concentrations of gentamicin and (D) the cell lysis conditions showed no statistic differences between them, and the control, for bacterial cells.](image)

**Conclusion**
- The selected gentamicin concentration for co-culture assays will be 50 µg/mL, as it eliminates extracellular bacteria and does not affect significantly the osteoblastic cells’ functionality.
- The selected condition for cell lysis will be 0.1% Tx for 30 min, given that MG63 cells are lysed and the functionality of bacteria are not significantly affected.

**References**