**Background**

A two-stage procedure can be proposed to manage prosthetic joint infections (PJI): after the infected tissue is removed, a bone cement spacer is inserted to avoid the filling by fibrous tissue. Following an adapted effective antibiotic therapy, the spacer is retrieved and a new prosthetic implant is implanted. Antibiotic-loaded bone cements (ALBCs) are frequently used in 2-stage procedures. They provide a local release of antibiotics to prevent the neodermation of fibrous tissue. Here we propose to study in vitro the prophylactic effect of plain cement (no antibiotic), G cement (gentamicin), G+V cement (gentamicin+vancomycin) and G+C cement (gentamicin+clindamycin) in order to assess the interest of combining antibiotics in ALBCs to prevent biofilm formation.

**MATERIAL & METHODS**

Standardized cylinders made from commercially available plain cement (no antibiotic) G cement (gentamicin), G+V cement (gentamicin+vancomycin) and G+C cement (gentamicin+clindamycin) were included in Tryptic Soy Broth with 1% glucose for 1 to 9 days at 37°C with medium changed each day. Biofilms of 6 clinical strains of Staphylococcus aureus and 2 clinical strains of Staphylococcus epidermidis with specific antibiotic susceptibilities were grown for 24h in a 96-well plate in solution solutions resulting from in vitro release from ALBCs at 1, 3 and 9 days. Biofilms were then softly rinsed using our homemade steam-based method (Tasse et al., 2018) and the number of viable cells in the biofilm was evaluated by plate counting.

**S. aureus**

- **Day 1:** G+V and G+C significantly decrease biofilm formation at D1, D3 and D9 in comparison with plain cement.
- **Day 3:** G significantly decreases biofilm formation at Day 1. Day 3 but not at Day 9.
- **Day 9:** Combinations of antibiotics seem more effective than gentamicin alone against MSSA biofilm formation at Day 7.

**S. epidermidis**

- **Day 1:** G+V and G+C significantly decrease biofilm formation at D1 and D3 in comparison with plain cement.
- **Day 9:** G is not effective against Gentamicin MRSA biofilm formation.
- **Day 9:** Combinations of antibiotics seem more effective than gentamicin alone against Gentamicin MRSA biofilm formation.

**Effect of ALBCs on (A) MSSA biofilm formation, (B) Gentamicin-resistant MRSA biofilm formation, (C) S. aureus biofilm formation (all strains merged), (D) MRSE biofilm formation, (E) Gentamicin-resistant MSSE biofilm formation and (F) S. epidermidis biofilm formation (all strains merged)** Three independent experiments in triplicate for each condition: one strain (9 values) for (A)(B)(D) and (E) and six and five strains (one mean by strain) for (C) and (F) respectively. * means p<0.05 compared to plain cement, Kruskal Wallis test with a Dunn's post hoc test.

**CONCLUSION**

Our in vitro results suggest that combining gentamicin with vancomycin or clindamycin increase the prophylactic anti-biofilm effect compared to ALBCs loaded with gentamicin alone. These findings are especially relevant as G+C and G+V cement are still effective against gentamicin, vancomycin and clindamycin-resistant strains. This study was financially supported by Heresium Medical but the company was not involved in the experimental process and the data analysis.