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THE MOLECULAR AND PATHO-MORPHOLOGICAL RESPOSE TO *STAPHYLOCOCCUS AUREUS* INDUCED IMPLANT ASSOCIATED OSTEOMYELITIS IN A PORCINE MODEL

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Abbreviations: Implant associated osteomyelitis (IAO), interleukin-1 and -6 (IL1 and IL6), peri-implanted pathological bone area (PIBA), Serum amyloid A (SAA)

Background

Implant associated osteomyelitis (IAO) is an invalidating disease where surgical intervention and prolonged antibiotic treatment is most often required[1]. As the number of elderly and joint replacements are increasing, IAO cases are also expected to increase[1]. The most common cause of IAO is *Staphylococcus aureus* and the formation of biofilm is one of the reasons treatment is difficult[1]. This Horizon 2020 project aims to test newly found antimicrobial compounds from marine microalgae in a pig IAO model.

Methods

The study was conducted on 3 month old pigs where a 15 mm steel implant was inserted into the right tibial bone together with 10^4 CFU *S. aureus* S54F9 or saline in control animals[2] (fig. 1). Animals were euthanized 5 days after the surgery. Hereafter, the right tibial bones were split through the implant cavity (fig. 2). One half was used for histology while tissue surrounding the implant cavity from the other half was used for gene expression analysis using high throughput microfluidic qPCR (Fluidigm)[3] (fig. 3).

Insertion of steel implant and injection of 10^4 CFU *S. aureus* in the tibia

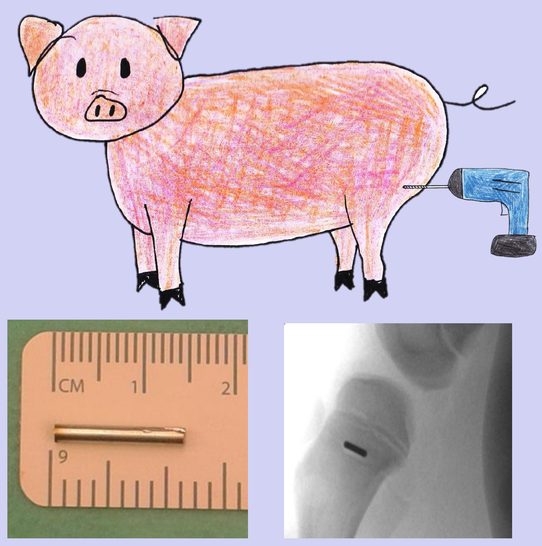


Figure 1: Porcine model

Animals are euthanized 5 days after operation

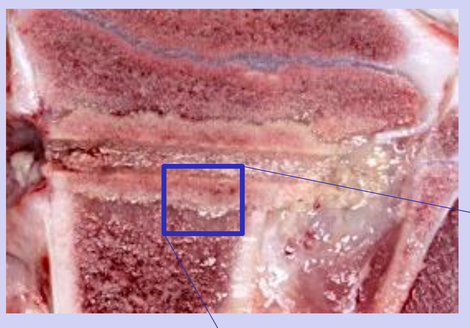
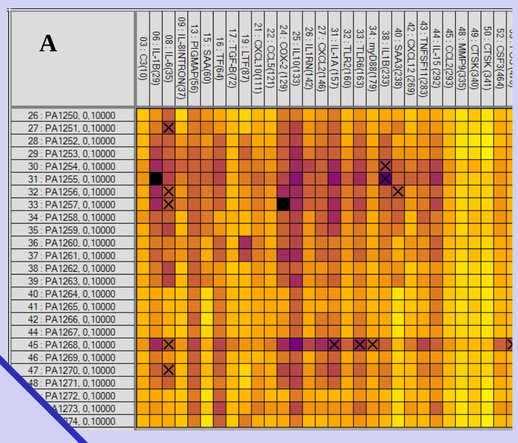


Figure 2: Infected tibia split through the implant cavity.

Quantitative real-time PCR



Histology

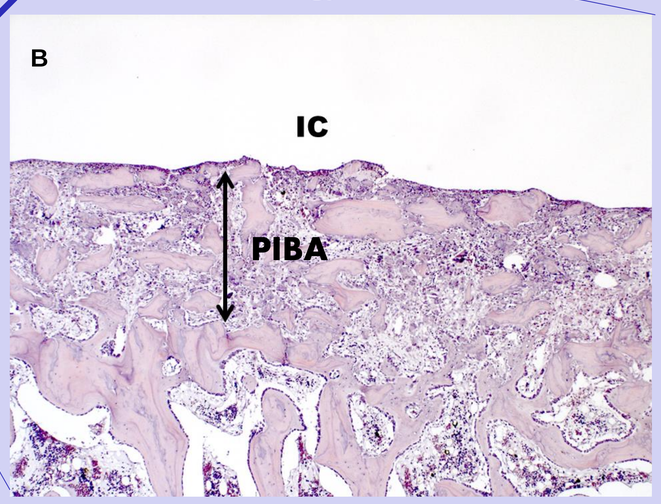


Figure 3 A) Heat map from gene expression analysis and B) overview of the implant cavity and PIBA in the right tibia. Implant cavity (IC), PIBA (dobbelt sided arrow), x4 magnification.

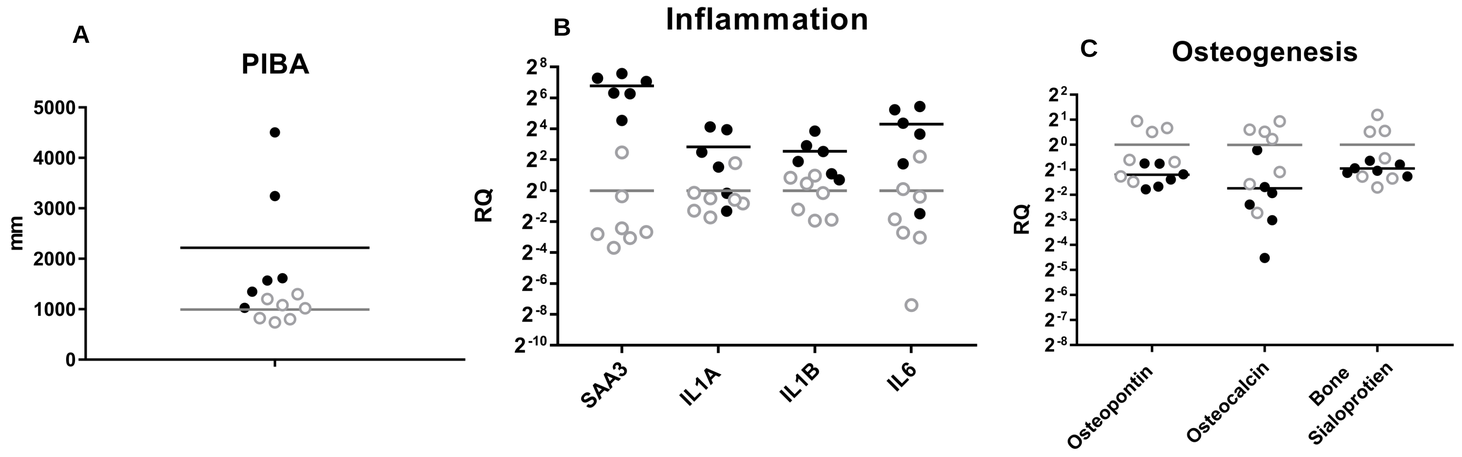


Figure 4 A)Size of the peri-implanted pathological bone area and relative expression (RQ) of genes related to B) inflammation and C) osteogenesis.
Dots represent a single sample and bars represent the mean for the group; infected animals (●/black bar) and controls (○/ grey bar).

Results

All animals receiving *S. aureus* developed an infection while all control animals were negative for bacteria and the PIBAs were significantly different between the two groups (fig. 4A).
Gene expression analysis showed an upregulation of pro-inflammatory cytokines, among others interleukin-1 and -6 (fig 4B). Additionally the acute phase protein serum amyloid a (SAA) was found to be expressed in the bone and was highly upregulated in infected animals (Fig. 4B). In contrast to this, several bone matrix proteins were downregulated (Fig. 4C), indicating decreased bone formation in infected animals.

Conclusion

The described porcine model is a reliable tool to study the immune response to IAO and the effect of different treatments.

Next

In the autumn we will receive newly isolated antimicrobial compounds witch will be tested *in vivo* for the first time in our thoroughly characterized IAO model.

References

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[2]Jensen, L. K. *et al.* Novel Porcine Model of Implant-Associated Osteomyelitis: A Comprehensive Analysis of Local, Regional, and Systemic Response. *J. Orthop. Res.* (2017). doi:10.1002/jor.23505
[3]Lüthje, F. L., Skovgaard, K., Jensen, H. E. & Kruse Jensen, L. Pigs are useful for the molecular study of bone inflammation and regeneration in humans. *Lab. Anim.* (2018). doi:10.1177/0023677218766391

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