Validation of a rabbit model of *in vivo* biofilm formation on beads for *ex vivo* evaluation of the antibiofilm activity of antistaphylococcal drugs

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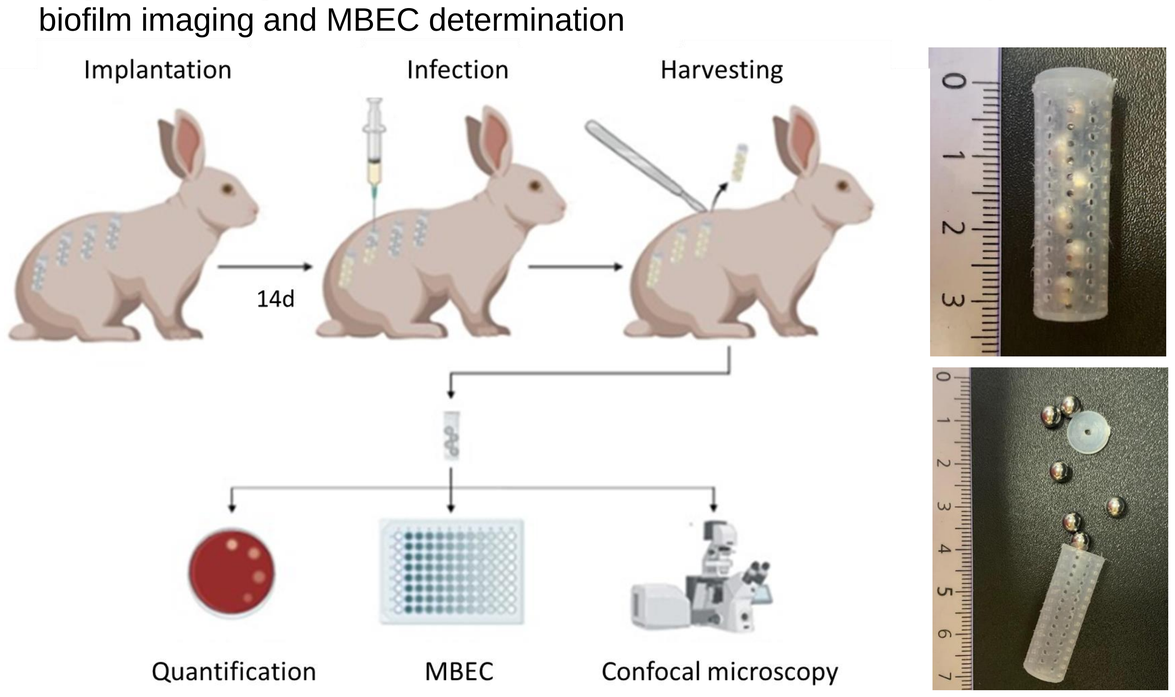
Rational

- Determination of antibiofilm activity of antimicrobials is a prerequisite for treatment of device-associated infections.
- Current *in vitro* models shows important variations upon technical conditions.
- On the other hand, animal models only allow the evaluation of a limited number of conditions.

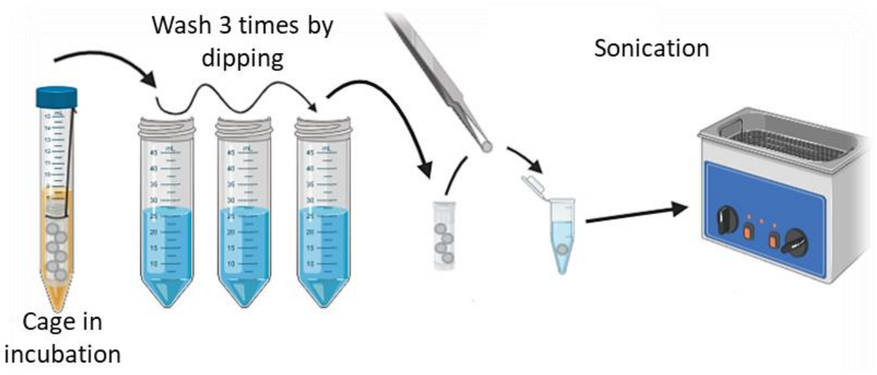
Description of the model

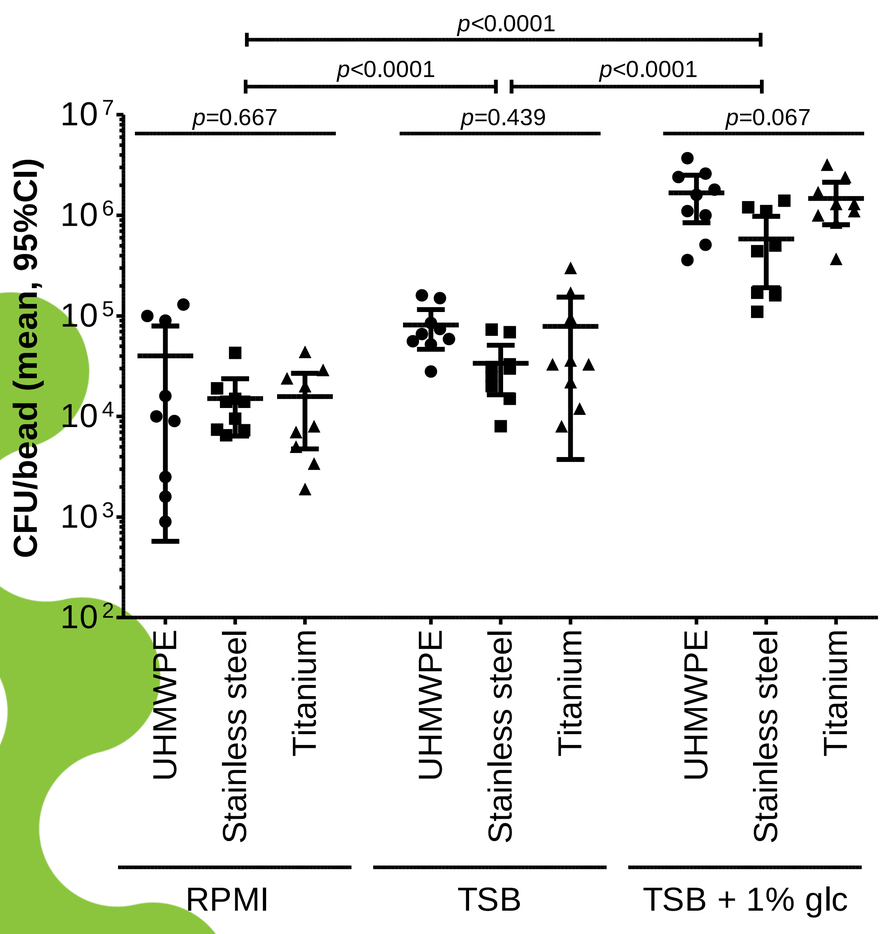
- Implantation of 6 cylindrical (30x10mm) cages *per* rabbit containing 6 beads (Ø 5mm) of polyethylene (UHMWE), titanium or stainless steel
- Infection at D14 by a standardized inoculum of *S. aureus* SH1000
- Cage harvesting after biofilm formation to use beads for bacterial quantification, biofilm imaging and MBEC determination



In vitro biofilm formation on beads

- Incubation of cages for 24h in a standardized bacterial solution in RPMI, TSB and TSB + 1% glucose
- Lavage and sonication of each bead for bacterial quantification





→ Confirmation of a **significant impact of culture condition** on biofilm formation, justifying *in vivo*/*ex vivo* evaluations

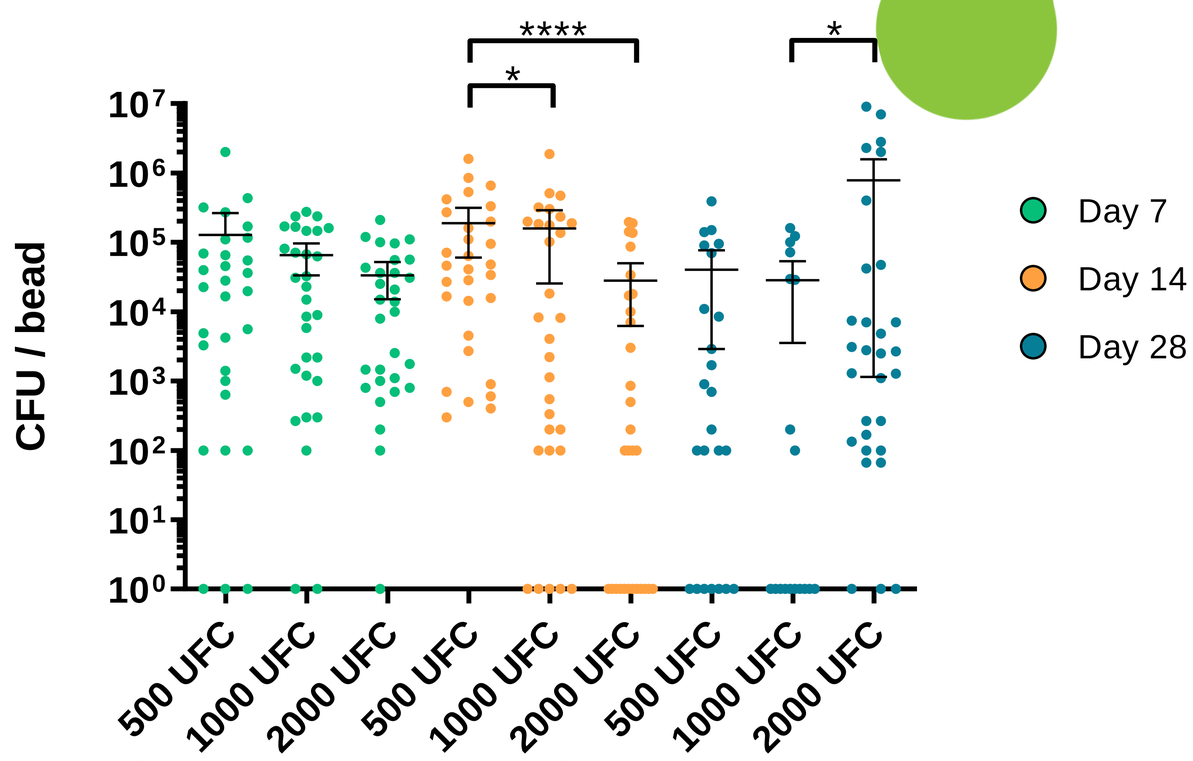
→ **No difference between biomaterials**, allowing choosing UHMWPE for animal experiments, due to its lighter weight

Objectives

Description of a rabbit tissue-cage model allowing *ex vivo* screening of a large panel of molecules against *in vivo*-formed biofilm with a limited number of animals

Characterization of the in vivo model of biofilm formation on UHMWPE beads

- Evaluation of increasing inocula (500/1000/2000 UFC/cage) and harvesting times (7/14/28 days post-infection)

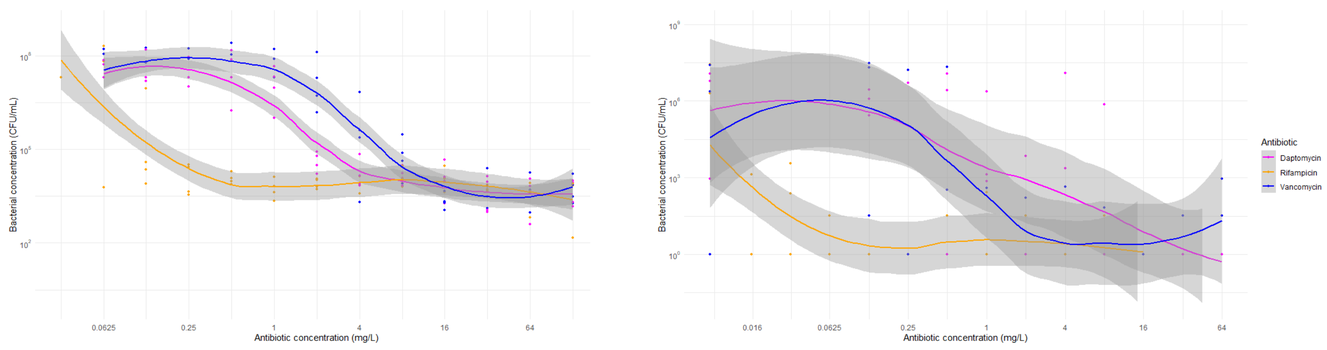


- Number of sterile cages increased significantly with time : 6.7%, 20.0% and 33.3% at day 7, 14 and 28, respectively
- Histopathological analysis of cage-surrounding membranes showing chronic inflammation (collagenous fibrosis, fibrin and granulation tissue)

→ Choice of the minimum infecting inoculum of 500 CFU/cage for 14 days of infection for further experiments, including MBEC determination, due to its best reproducibility/chronicity ratio

Vancomycin, daptomycin and rifampin MBEC determined by the reference in vitro MBEC assay, and on UHMWPE bead-associated in vitro and in vivo formed biofilm

	in vitro MBEC assay®	UHMWPE bead-associated biofilm	
		In vitro (***p*-value for MBEC assay® comparison**)	***Ex vivo*** (***p*-value for *in vitro*-formed bead-associated biofilm comparison**)
Vancomycin (mg/L)	4.50	13.3 (*p*=0.07)	1 (*p*=0.04)
Daptomycin (mg/L)	3.25	4 (*p*=0.88)	1.5 (*p*=0.027)
Rifampin (mg/L)	0.062	0.333 (*p*=0.05)	<0.016 (*p*=0.04)



→ MBEC90 determined on bead-associated biofilms were significantly lower on *in vivo* formed biofilm compared to *in vitro*.

This new rabbit model allows *ex vivo* evaluation of the antibiofilm activity of antistaphylococcal drugs on *in vivo*-formed biofilm with a limited number of animals, showing significant variations compared to the reference method for MBEC determination.

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