



EURESTOP WG Meeting | Brussels, Belgium
13 June 2023

Novel synthetic antimicrobial and anti-biofilm peptides
(SAAPs)-containing coatings
to prevent biomaterial-associated infection

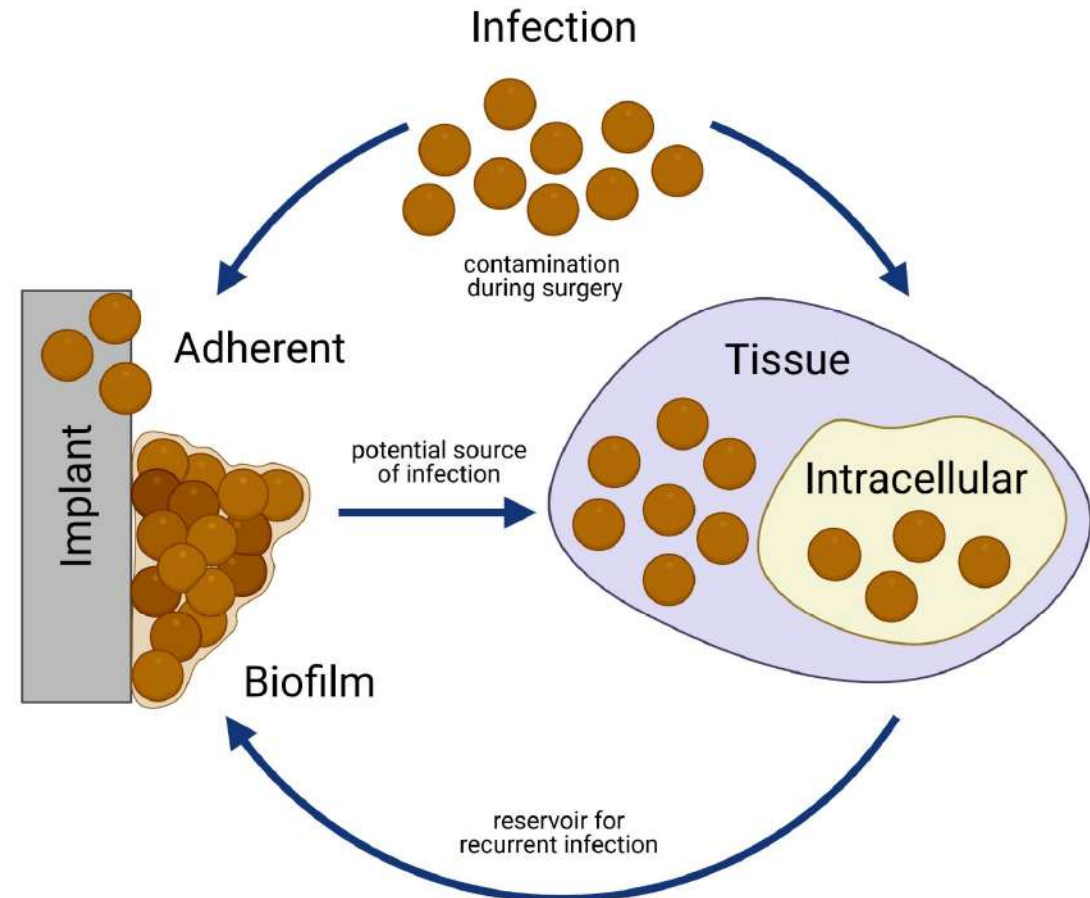
Prof. Dr. Martijn Riool

martijn.riool@ukr.de

Biomaterial-associated infection (BAI)

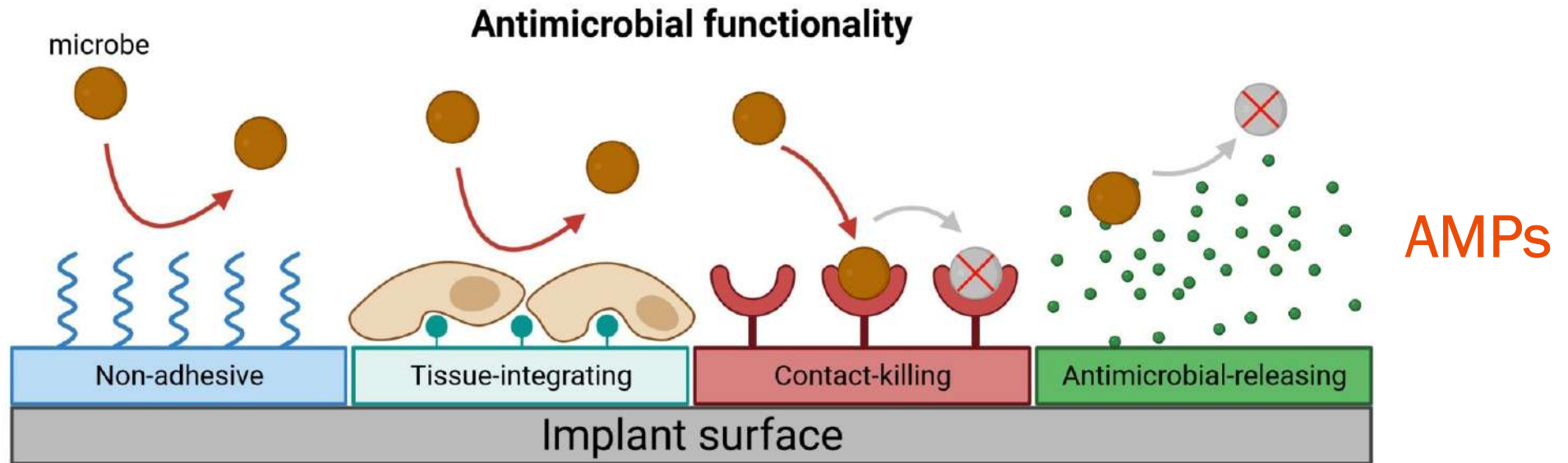
- BAI mostly caused by **staphylococci**
 - Mainly *Staphylococcus aureus*
- **Biofilm formation** on the implant
 - “Race for the surface” (Gristina)
- Colonization of the **peri-implant tissue**
 - **Intracellular** survival
- Increase of multidrug-resistant bacteria

Pathogenesis of BAI



Adapted from Riool et al., Acta Biomater., 2014

Antimicrobial coating strategies to prevent BAI

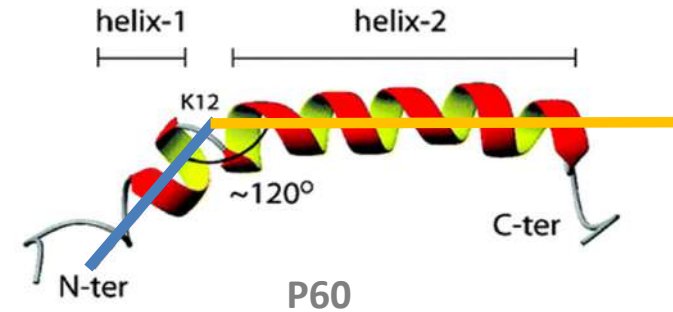


- Ideal situation: **Multifunctional** coating (killing & tissue-integration)

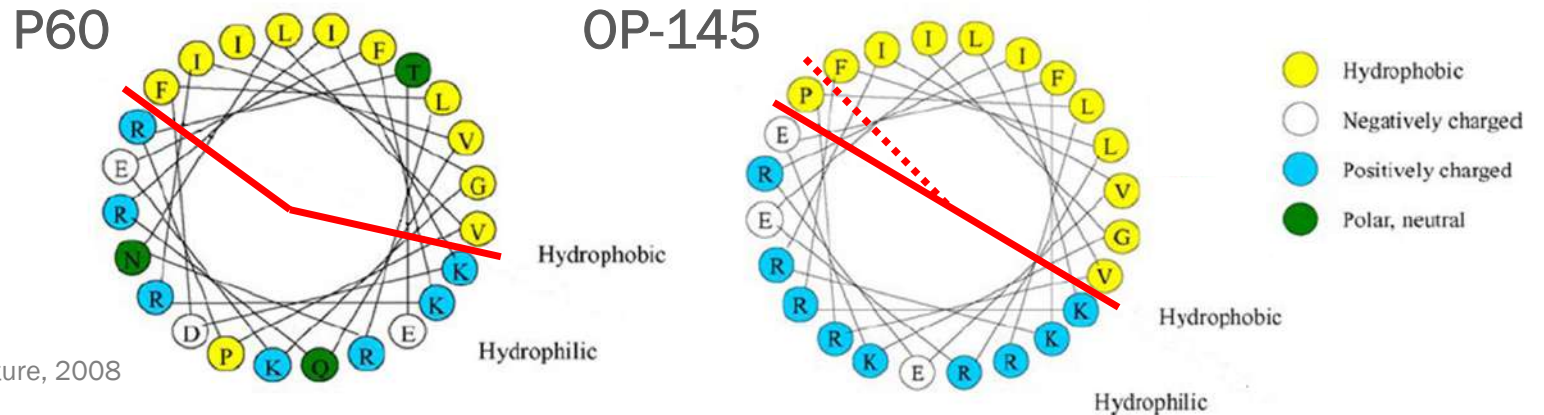
Adapted from Busscher *et al.*, *Sci. Transl. Med.*, 2012

Design of Synthetic Antimicrobial and Antibiofilm Peptides (SAAPs)

- SAAPs derived from the human cathelicidin **LL-37**
- OP-145** (P60.4Ac): 24 amino acid synthetic peptide derived from P60 (LL-37₁₃₋₃₆) with improved helicity and amphipathicity



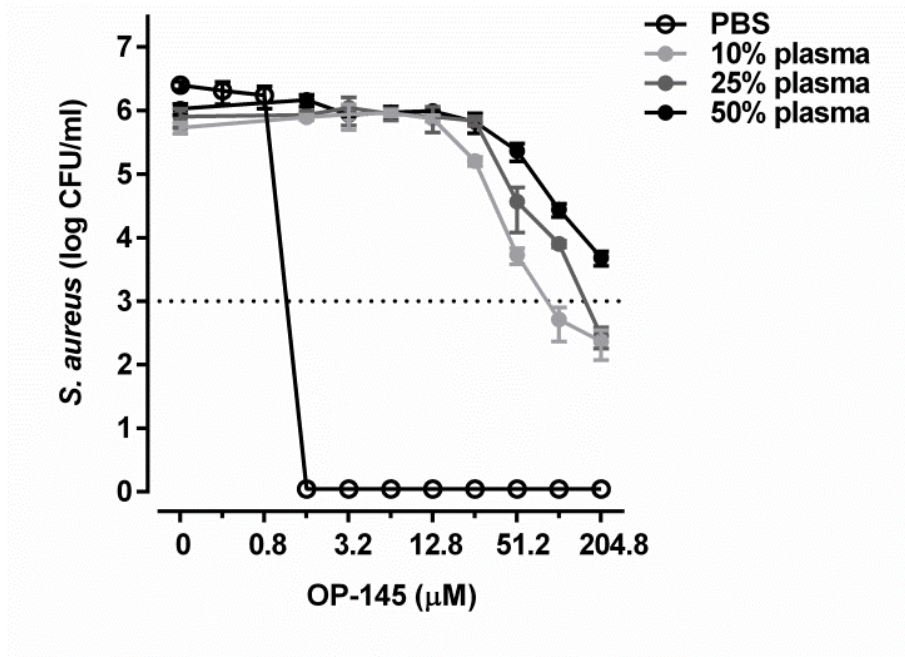
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LL-37	L	L	G	D	F	F	R	K	S	K	E	K	I	G	K	E	F	K	R	I	V	Q	R	I	K	D	F	L	R	N	L	V	P	R	T	E	S
OP-145													I	G	K	E	F	K	R	I	V	E	R	I	K	R	F	L	R	E	L	V	R	P	L	R	



Nell *et al.*, Peptides, 2006; Porcelli *et al.*, Structure, 2008

Antimicrobial and antibiofilm activity of OP-145

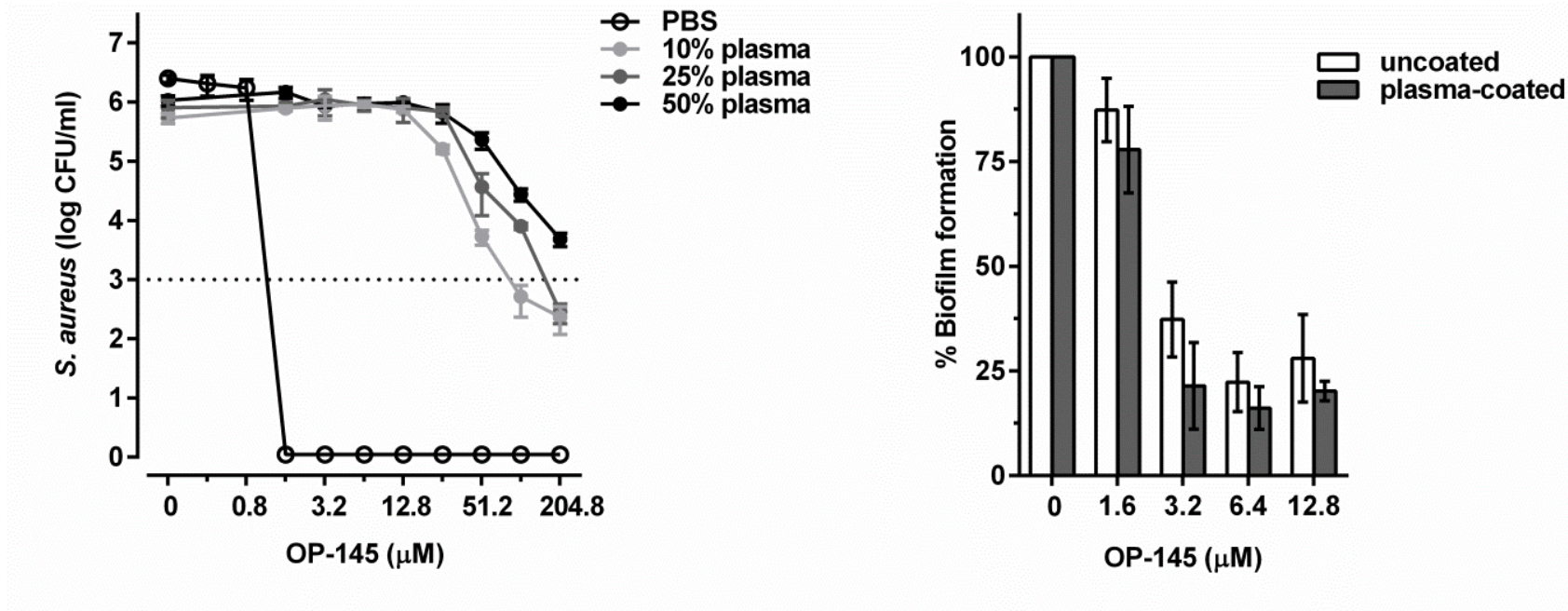
- OP-145 active at low μM concentrations against *S. aureus* JAR060131 (2h, CFUs)
- Plasma inhibits** activity of OP-145



de Breij, Riool et al., J. Control. Release, 2016

Antimicrobial and antibiofilm activity of OP-145

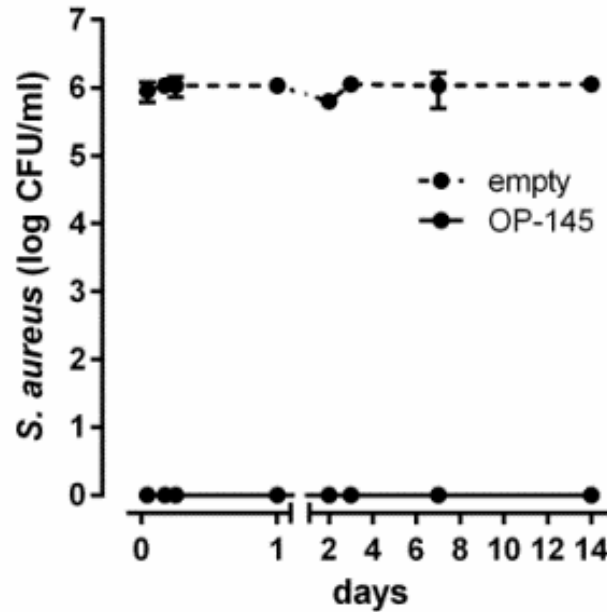
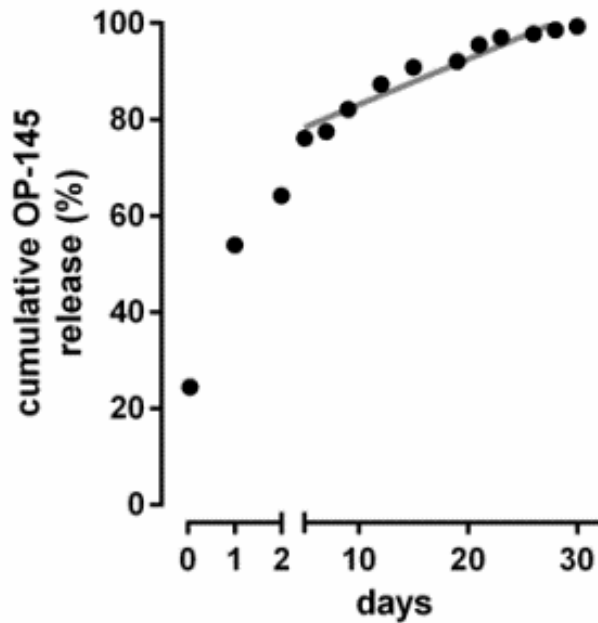
- OP-145 active at low μM concentrations against *S. aureus* JAR060131 (2h, CFUs)
- Plasma inhibits activity of OP-145
- OP-145 prevents biofilm formation (24h, BM2 medium, CV), no influence of plasma (20% pre-coated)



de Breij, Riool et al., J. Control. Release, 2016

PLEX-OP-145 controlled release coating

- Biodegradable Polymer-Lipid Encapsulation Matrix technology (PLEX) coating
- Composed of PLGA / DPPC / DSPC / cholesterol / OP-145 (10 wt%)

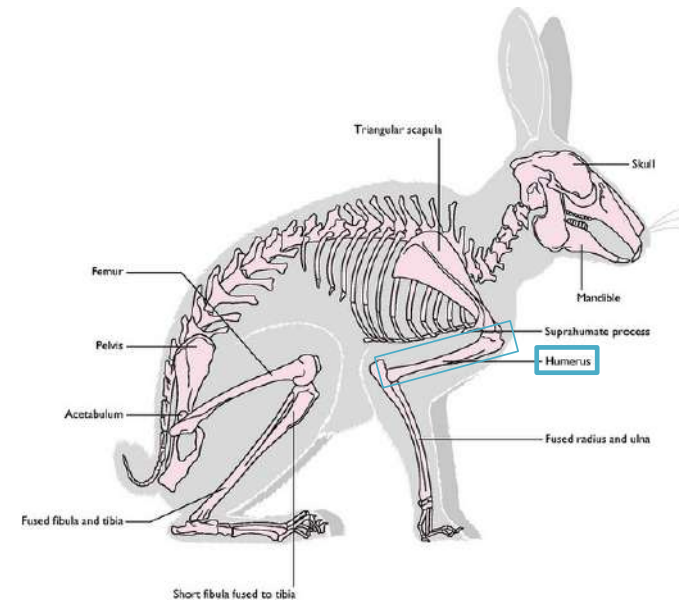


- Initial burst release (55% in first 48h)
- First order kinetic release (~1% per day) for 30 days
- OP-145 released from coating kills *S. aureus*

de Breij, Riool et al., J. Control. Release, 2016

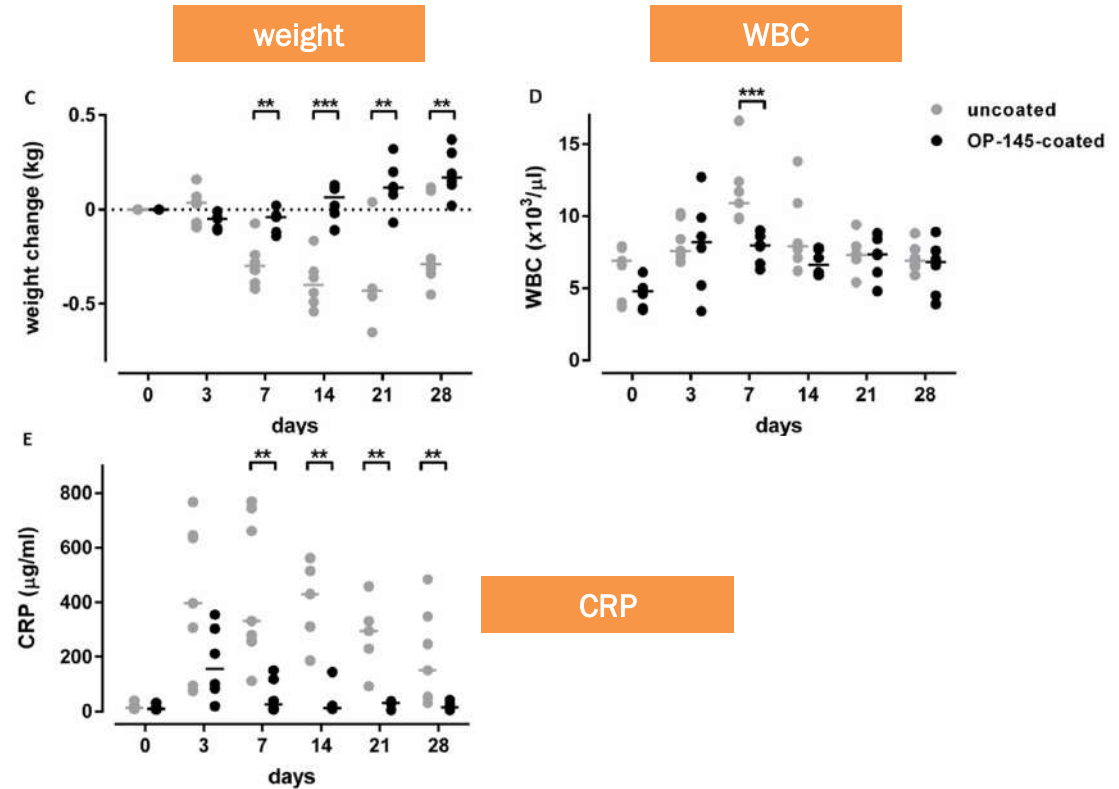
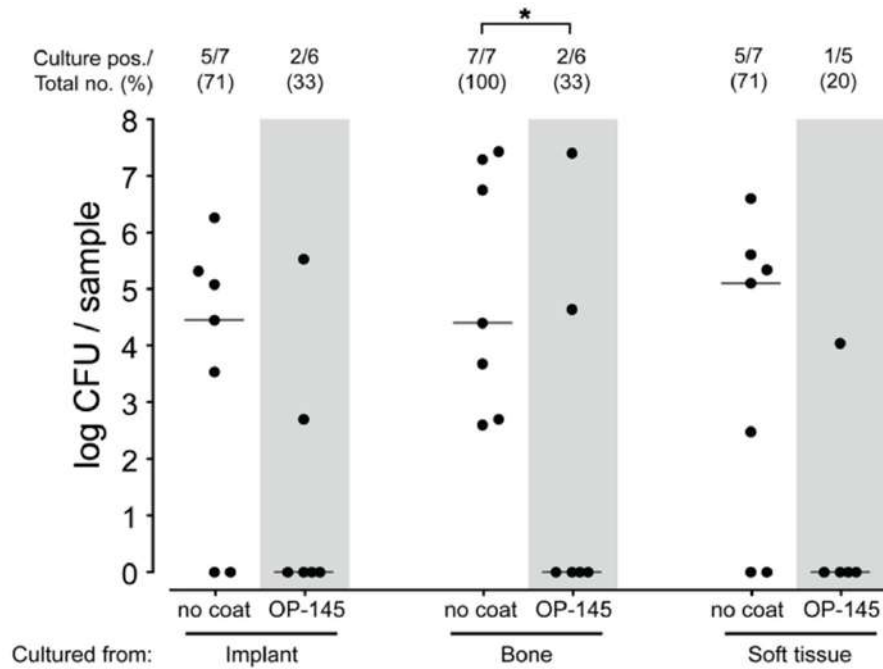
PLEX-OP-145 controlled release coating

- **Intramedullary (IM) nail** infection model
 - New Zealand White rabbits
 - Right humerus
 - 6×10^4 CFU *S. aureus* JAR060131
 - TAN IM nail group 1: No coating
 - TAN IM nail group 2: **OP-145-PLEX** coating
- Evaluation at **28 days**
 - Quantitative culture
 - Clinical parameters
 - Contact radiographs




Research Institute Davos

PLEX-OP-145: prevents rabbit intramedullary nail infection

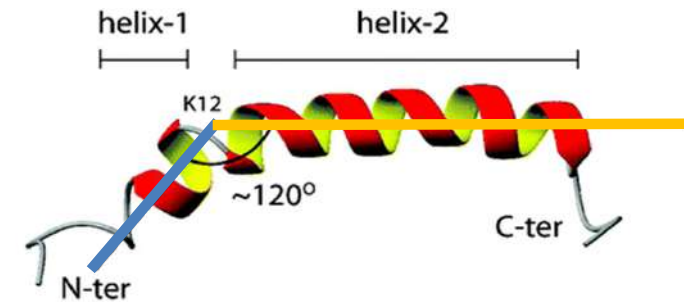


- Trend: reduction in no. of CFU in all samples
- 50% of rabbits infection free
- *Clinical signs of infection prevented with PLEX-OP-145*

de Breij, Riool et al., J. Control. Release, 2016

Design of Synthetic Antimicrobial and Antibiofilm Peptides (SAAPs)

- 24-mer SAAPs derived from the human cathelicidin LL-37
- Random substitutions in P60 (LL-37₁₃₋₃₆)
- α -helical structure intact, increase hydrophobicity and cationic charge
- Screening: improved activity in plasma against *S. aureus*
- Lead peptides: SAAP-145, SAAP-148 and SAAP-276

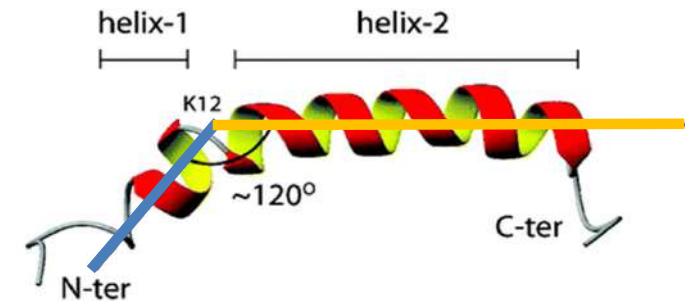


	P60																																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
LL-37	L	L	G	D	F	F	R	K	S	K	E	K	I	G	K	E	F	K	R	I	V	Q	R	I	K	D	F	L	R	N	L	V	P	R	T	E	S
SAAP-145													L	K	R	L	Y	K	R	L	A	K	L	I	K	R	L	Y	R	Y	L	K	K	P	V	R	
SAAP-148													L	K	R	V	W	K	R	V	F	K	L	L	K	R	Y	W	R	Q	L	K	K	P	V	R	
SAAP-276													L	K	R	V	W	K	A	V	F	K	L	L	K	R	Y	W	R	Q	L	K	K	P	V	R	

Porcelli et al., Biochemistry, 2008; de Breij & Riool et al., Sci. Transl. Med., 2018; Riool & de Breij et al., Adv. Funct. Mater., 2017

Design of Synthetic Antimicrobial and Antibiofilm Peptides (SAAPs)

- 24-mer SAAPs derived from the human cathelicidin LL-37
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- Screening: improved activity in plasma against *S. aureus*
- Lead peptides: SAAP-145, SAAP-148 and SAAP-276



Peptide	LC99.9 (μM)	
	PBS	50% plasma
OP-145	1.6	≥ 204.8
SAAP-145	1.6	12.8
SAAP-148	1.6	12.8
SAAP-276	1.6	6.4

Porcelli *et al.*, Biochemistry, 2008; de Breij & Riool *et al.*, Sci. Transl. Med., 2018; Riool & de Breij *et al.*, Adv. Funct. Mater., 2017

Broad antimicrobial activity of SAAP-148

- SAAP-148 kills MDR **ESKAPE** pathogens and colistin-resistant *E. coli* (LC99.9, 24h)

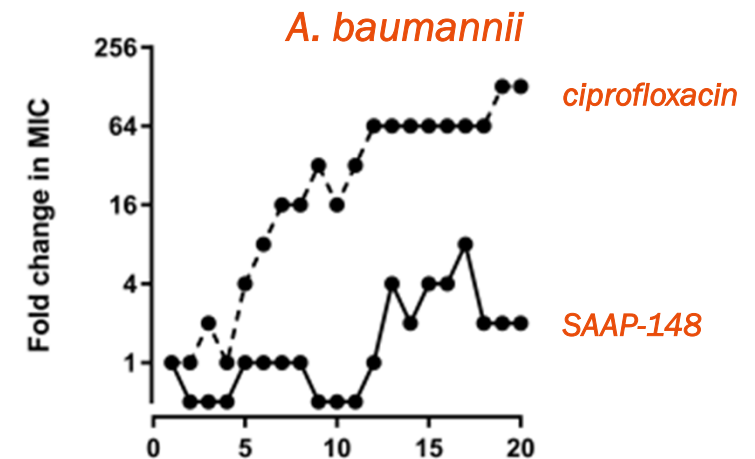
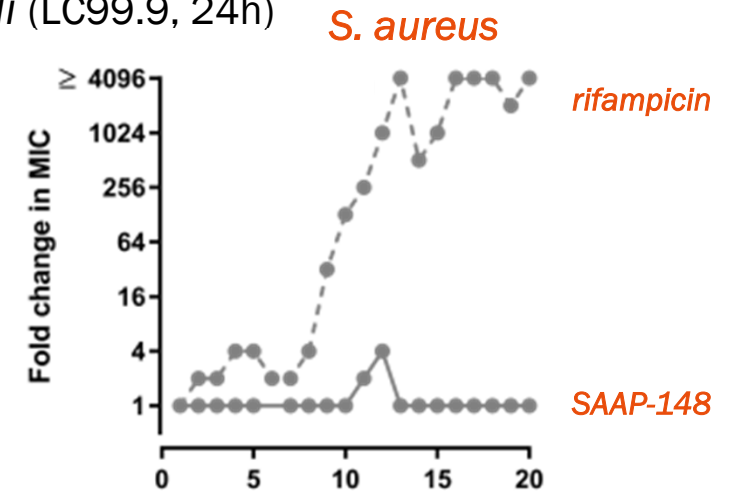
Species	Strain	Antibiotic resistance													LC99.9 P148 (μ M)					
		aminoglycosides	ansamycins	carbapenems	cephalosporins	fluoroquinolones	fusidanes	glycopeptides	glycylcyclines	lincosamides	lipopeptides	macrolides	monoxycarbolic acid	oxazolidinones	penicillins	polymyxins	sulfonamides	tetracyclins	PBS	50% plasma
<i>E. faecium</i>	LUH15122																		1.6	6.4
<i>S. aureus</i>	LUH14616																		1.6	3.2
<i>K. pneumoniae</i>	LUH8995																		0.4	3.2
<i>A. baumannii</i>	RUH875																		0.8	1.6
<i>P. aeruginosa</i>	LUH15103																		6.4	12.8
<i>E. cloacae</i>	LUH15114																		12.8	3.2
<i>E. coli</i>	LUH15117																		0.8	6.4

de Breij & Riool et al., Sci. Trans. Med., 2018

Broad antimicrobial activity of SAAP-148

- SAAP-148 kills MDR **ESKAPE** pathogens and colistin-resistant *E. coli* (LC99.9, 24h)
- No resistance** development

Species	Strain	Antibiotic resistance													LC99.9 P148 (μM)					
		aminoglycosides	ansamycins	carbapenems	cephalosporins	fluoroquinolones	fusidanes	glycopeptides	glycolycines	lincosamides	lipopeptides	macrolides	monoxycarboic acid	oxazolidinones	penicillins	polymyxins	sulfonamides	tetracyclins	PBS	50% plasma
		<i>E. faecium</i>	LUH15122																	1.6
<i>S. aureus</i>	LUH14616																	1.6	3.2	
<i>K. pneumoniae</i>	LUH8995																	0.4	3.2	
<i>A. baumannii</i>	RUH875																	0.8	1.6	
<i>P. aeruginosa</i>	LUH15103																	6.4	12.8	
<i>E. cloacae</i>	LUH15114																	12.8	3.2	
<i>E. coli</i>	LUH15117																	0.8	6.4	

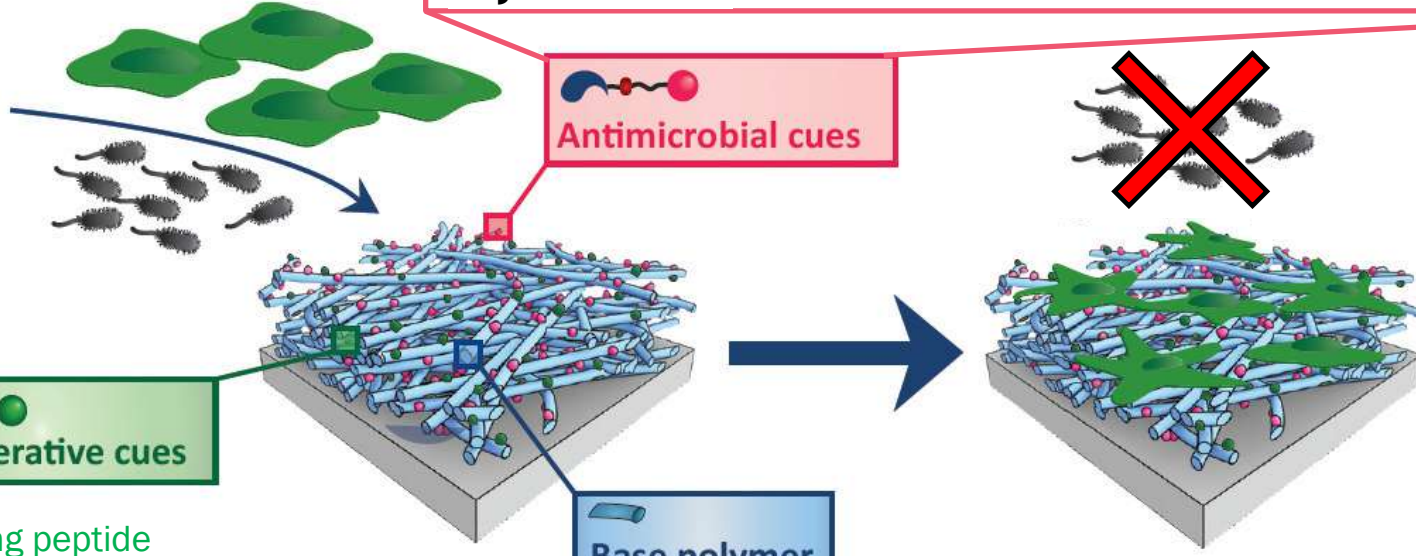
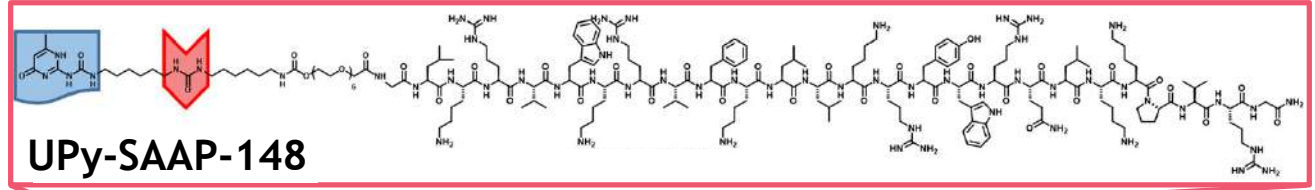
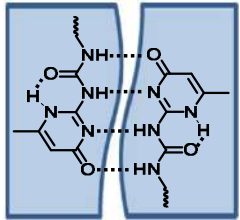


de Breij & Riool et al., Sci. Trans. Med., 2018

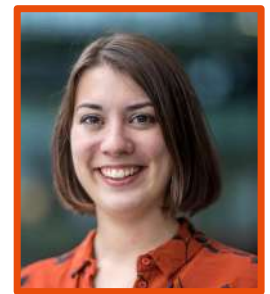
Supramolecular multifunctional biomaterials

with *antimicrobial* and *regenerative* activity

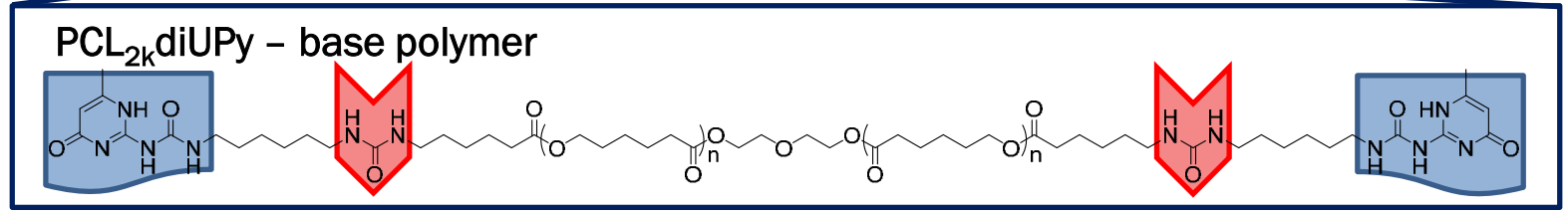
Ureido-pyrimidinone (UPy)



UPy-heparin binding peptide
UPy-cyclic RGD



Dr. Moniek Schmitz
Technical University
Eindhoven



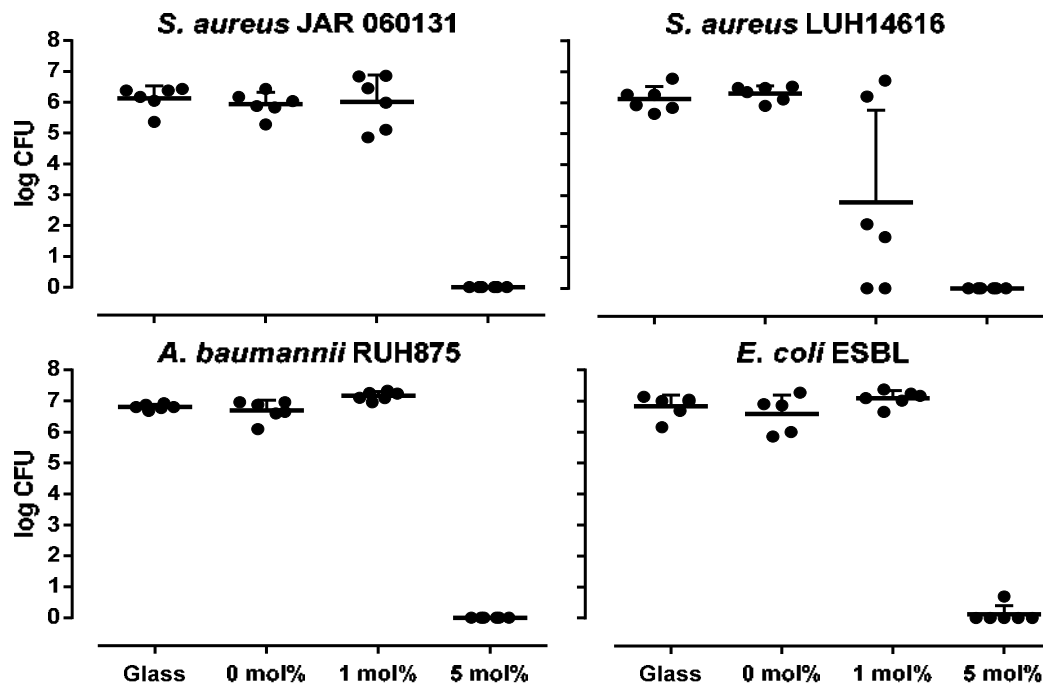
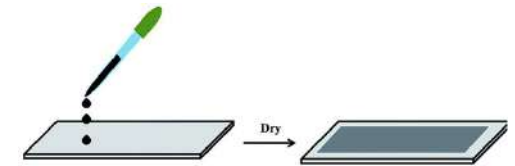
UPy-functionalization of AMPs: SAAP-148 remains active

- UPy-SAAP-148 kills (MDR) *S. aureus*, *E. coli* and *A. baumannii* (LC99.9, RPMI, 2/24h)

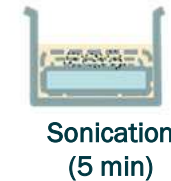
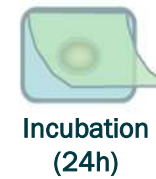
		LC99.9 (μM)			
		MSSA	MRSA	<i>E. coli</i> ESBL	<i>A. baumannii</i>
2 hours	SAAP-148	1.4	0.46	3.75	0.93
	UPy-SAAP-148	0.93	0.93	0.93	0.93
24 hours	SAAP-148	0.93	0.46	1.88	0.93
	UPy-SAAP-148	0.93	0.93	1.88	1.88

Surface antimicrobial activity of UPy-AMP solid samples

- Dropcasting of PCL_{2k}-diUPy films with 1 and 5 mol% UPy-SAAP-148
- Surface antimicrobial activity testing (JIS Z2801, 24h)



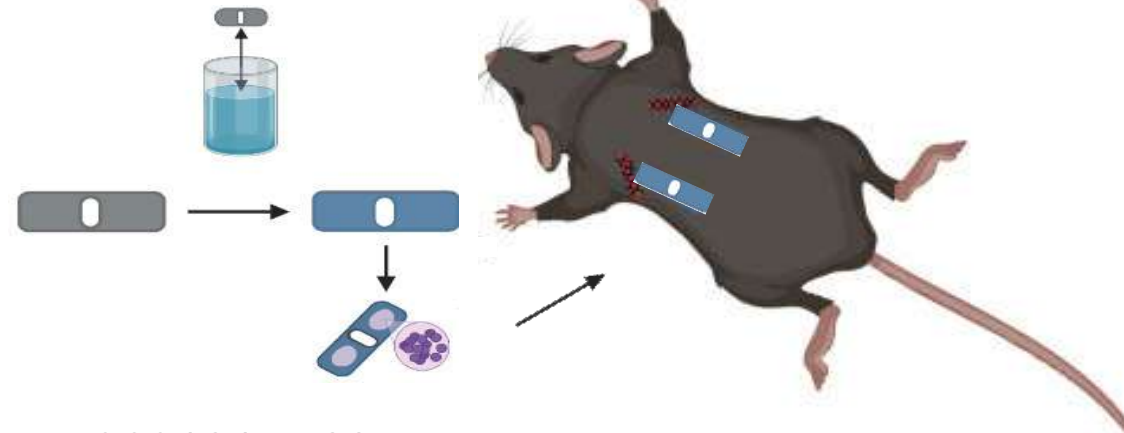
JIS Z2801 assay



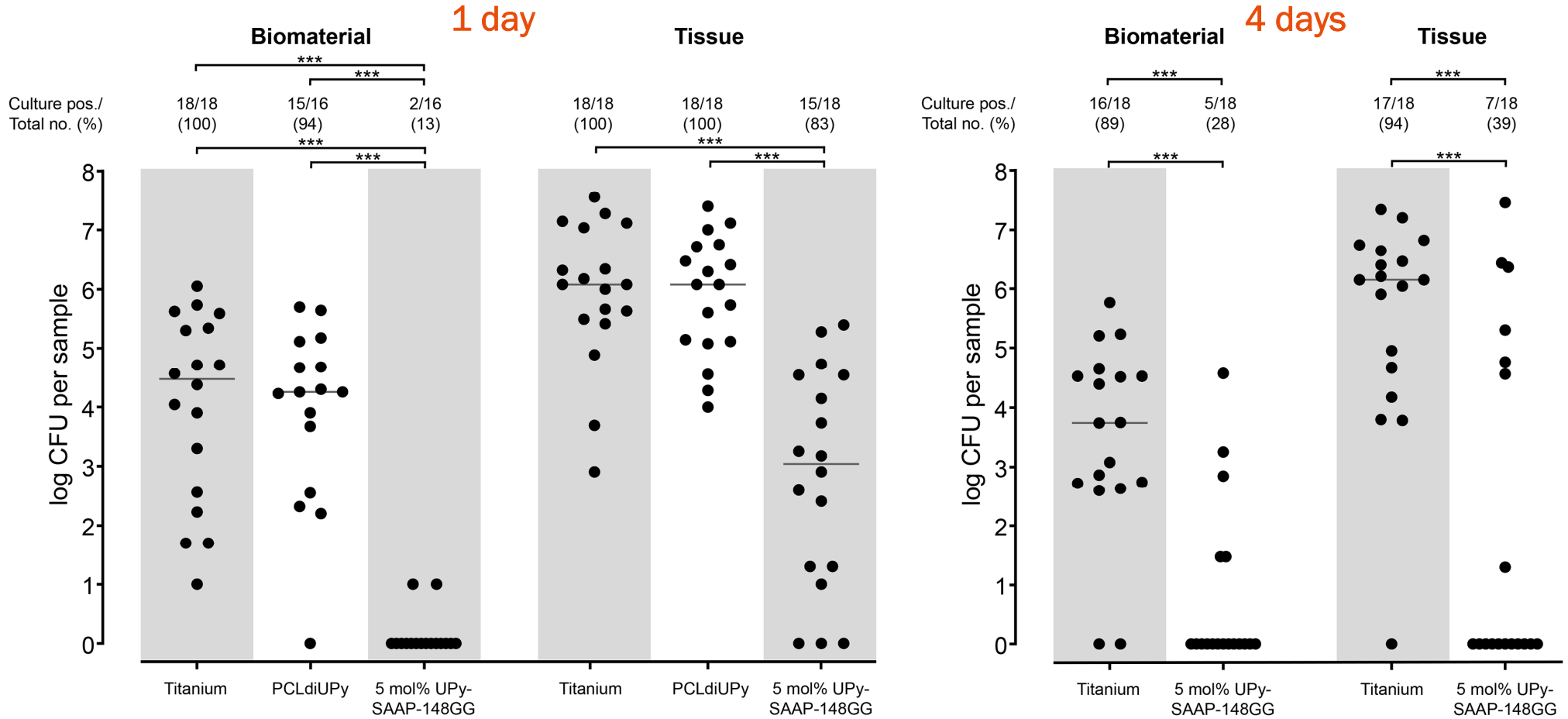
- ✓ Immobilized SAAP-148 (5%) has surface antimicrobial activity

In vivo evaluation in murine subcutaneous BAI model

- Dipcoated in PCL_{2k}-diUPy solution with 5 mol% UPy-SAAP-148
- Controls:
 - No coating (bare titanium)
 - PCL_{2k}-diUPy coating (w/o UPy-SAAP-148)
- Mouse BAI model:
 - 2 (coated) implants per mouse
 - Inoculum of 2×10^5 CFU (in 6.25 μ l) *S. aureus* JAR060131 (MSSA)
 - Inoculum applied on the surface prior to subcutaneous implantation
 - 1 and 4 days incubation
 - Evaluation: quantitative culture of both implant and surrounding tissue



In vivo evaluation in murine subcutaneous BAI model



Schmitz & Riool et al., Adv. Mater. Technol., under review

Antimicrobial peptides prediction using machine-learning (ML)

- Prediction tool to discover novel AMPs: **CalcAMP**
- Accuracy CalcAMP:
 - Gram+: 79%
 - Gram-: 80%



Gizem Babuççu
ESR14



Nikitha Vavilthota
ESR15



Bournez, Riool *et al.*, Antibiotics, 2023

Summary: Novel SAAPs

- ✓ Broad spectrum, including multidrug-resistant strains
 - ✓ Active in PBS and human plasma
 - ✓ Anti-biofilm activity
 - ✓ No resistance development
 - ✓ SAAP-releasing coating prevent *S. aureus* infection *in vivo*
 - ✓ Incorporation of UPy-SAAP-148 into UPy-PCL solid materials, active *in vitro* & *in vivo*
 - ✓ CalcAMP, a new prediction tool for AMPs was developed
- ✓ Promising novel generation of AMPs in the fight against biomaterial-associated infection

Participating in:



DARTBAC prepares the Netherlands for the post-antibiotic era caused by Anti Microbial Resistance (AMR)

nwa-dartbac.nl @dartbac

- Create societal AMR awareness
- Develop new antimicrobial technologies
- Optimize AB efficacy with combination therapy
- Standardize testmodels for faster clinical implementation



Dr. Chris Arts, coordinator

nwa-dartbac.eu



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Not in the picture:
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Tobias Gsottberger



THANK YOU FOR YOUR ATTENTION!



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