Microbial interactions and treatment strategies in *Staphylococcus aureus*–*Candida albicans* dual-species biofilms

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INTRODUCTION: Virulence, drug tolerance and ultimately the outcome of polymicrobial biofilm infections are greatly influenced by interspecies interactions. Polymicrobial infections caused by a mixture of fungi and bacteria is increasingly recognized in medical settings with having *Candida albicans* and *Staphylococcus* species co-isolated. These cases mostly include infections of intracorporal devices such as infections of implants, central venous catheters infections, as well as periodontal and wound infections. In this study, we aimed to illuminate the synergistic interaction between *C. albicans* and *S. aureus* by using one of the key elements of the interaction, which is excessive oxygen consumption by *C. albicans* and the consequences thereof for *S. aureus*. Also, developing an effective treatment measure with antibiotics and the antimicrobial peptide (AMP) SAAP-148 and susceptibility changes of mixed biofilms was investigated.

METHODS: *C. albicans* SC5314 and *S. aureus* RN4220 strains were used in this study. *C. albicans* and *S. aureus* were cultured at 120 rpm at 37°C for 18 h in Sabouraud dextrose medium and in tryptic soy broth, respectively. Susceptibility to antibiotics was determined by VITEK according to CLSI recommendations. Antifungal susceptibility testing was conducted according to EUCAST. Monospecies biofilms of *S. aureus* with 1×10^8 CFU/mL and *C. albicans* with 1×10^6 CFU/mL inoculated in RPMI 1% glucose were grown at 37°C for 24 hours in flat bottom 96-well polystyrene plates. Mixed species biofilm assays were performed in FBS coated plates. First, 1×10^6 CFU/mL *C. albicans* in RPMI were added and adhesion and germ tube formation of the *C. albicans* was allowed for 90 min at 37°C. Subsequently, the supernatant was removed and 1×10^7 CFU/mL *S. aureus* were added to the wells and incubated at 37°C for 24 h. Experiments were performed under aerobic conditions, and under anaerobic conditions using Oxoid™ CO_2 Gen™ sachets. The treatment with antibiotics and SAAP-148 were applied under the same conditions.

RESULTS: Planktonic monocultures LC99.9% concentration of SAAP-148 against *S. aureus* were 8-fold less susceptible when its co-cultured with *C. albicans* in aerobic (see Table 1). This susceptibility was affected by the environmental O_2 by 2-fold in mono culture.

Table 1. Lethal concentration (LC99.9) of SAAP-148 against planktonic S. aureus with and w/o planktonic C. albicans in aerobic and anaerobic conditions.

	Mono		Mixed	
SAAP 148 (µM)	*Aerobic*	*Anaerobic*	*Aerobic*	*Anaerobic*
	7.5	15	60	120

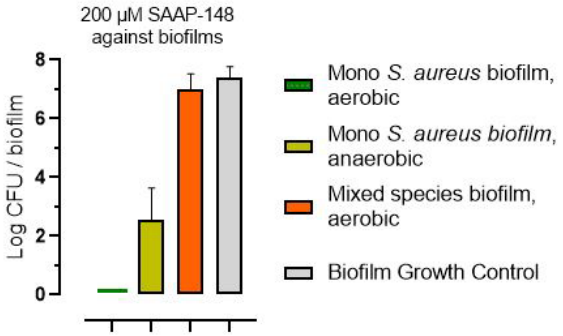

Figure 1: Log CFU per mono S. aureus and mixed biofilms which treated with 200 µM of SAAP-148 and biofilm growth control.

While 200 µM SAAP-148 caused complete killing of mono *S. aureus* biofilms under aerobic conditions, the same concentration showed only a 4.8-log reduction of mono *S. aureus* biofilms in an anaerobic environment (Figure 1). SAAP-148 did not cause a significant reduction of *S. aureus* when it was co-cultured with *C. albicans* in a biofilm (Figure 1).

DISCUSSION & CONCLUSIONS: Susceptibility of *S. aureus* to the AMP SAAP-148 is slightly reduced under anaerobic conditions in mono species biofilm, but becomes even less susceptible when cocultured with *C. albicans*, even under aerobic conditions. Since *C. albicans* has a high rate of oxygen consumption, the coculture with *S. aureus* may be oxygen-limited, causing the reduced AMP susceptibility of *S. aureus*.

Neutralising antibody response against locally applied bacteriophages in patients with musculoskeletal infections

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INTRODUCTION: Given the increasing threat of antimicrobial resistance (AMR) scientists are urgently searching for alternative antimicrobial strategies. Over the past decades, phage therapy (PT) has regained interest. Although recent reports show promising results for treating musculoskeletal infections (MSIs) with PT, questions remain regarding the optimal treatment protocol and the impact of the immune response (and anti-phage neutralizing antibodies) on treatment outcome. With this study, we investigate the induction of neutralizing anti-phage antibodies after local application (10 days, 3x/day) of phages in MSI patients (1). In our treatment protocol, an effective therapeutic threshold (ETT) of 10^7 PFU/mL was defined to ensure sufficiently high phage levels.

METHODS: Phage neutralization tests were performed on serum samples from each MSI patient who was treated locally with phage 'ISP', 'Prima HS', 'PNM' and/or '14_1' according to a modified Adams protocol (2). Serum samples were collected from baseline (prior to the start of PT), during treatment and every two weeks until 12 weeks after stopping PT. The samples were diluted 1:100 in normal saline. 900 µl of diluted serum was incubated with 100 µl of phage (titer 107 PFU/ml) for 30 min at 37°C. After this time interval this mixture was diluted 1.000 and 10.000 times in cold normal saline and phage titration was performed using the double-agar overlay method. Differences in neutralization before PT and the predefined timepoints during and after PT were assessed using a paired *t* test or Wilcoxon test. Normality was determined using the Shapiro-Wilk test. P values <0.05 were considered statistically significant.

RESULTS: The sera of seven patients who underwent PT were evaluated. Two patients (Patients 1 and 7) received a phage cocktail, of which one covered multiple bacterial species (Patient 1). Phage neutralization was observed in all patient samples, albeit its occurrence varied between patients and phages. In five out of seven patients, phage neutralization was statistically significant starting from two weeks after treatment. Furthermore, in one patients (Patient 7), this disparity was evident as soon as day 7 of PT. Of clinical significance, neutralization was deemed relevant when the titer dropped below 10^7 PFU/mL. Such a decline below this therapeutic threshold was observed in six out of the seven patients.

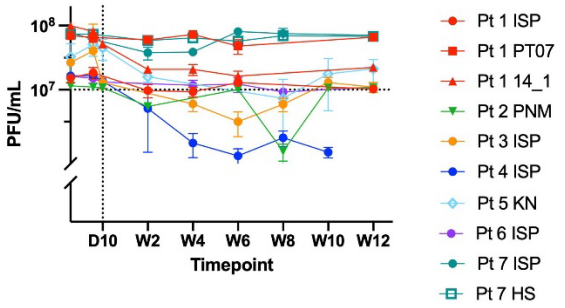

Fig. 1: Repeated phage titers across patients with mean and standard deviation for each phage used

DISCUSSION & CONCLUSIONS: The adaptive immune system has been associated with early depletion of phages and subsequent impairment of PT efficacy. Patients with musculoskeletal infections are treated with PT for a maximum duration of 10 days. Our findings show considerable variability in immune responses among patients and phages. However, there is evidence that repeating treatment within a 12-week period is likely to magnify the impact of phage neutralization. Beyond 12 weeks, phage-neutralizing effects tend to normalize. Further research into phage cocktails or application strategies with reduced immune response is needed as well as research into a possible correlation between phage neutralization and treatment outcome.

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Gentamicin fails to eradicate *Staphylococcus aureus* biofilm *in vitro*, even in combination with rifampin

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INTRODUCTION: Orthopaedic device-related infections (ODRI) are difficult to treat with antibiotic therapy alone, due in large part to the involvement of antibiotic-tolerant biofilms. Determining the time and concentration thresholds necessary for biofilm eradication is essential for optimization of local antibiotic treatment. Aminoglycosides, such as gentamicin, are amongst the most frequently used antibiotics applied locally for ODRI treatment. Rifampin is a systemically administered key antibiotic. One of the pharmacodynamic principles of aminoglycoside therapy is their concentration-dependent activity, leading to a preference for burst-release formulations. In this study, various time and concentration profiles of gentamicin exposure were evaluated, with and without concomitant administration of rifampin, in the treatment of mature *Staphylococcus aureus* biofilm *in vitro*.

METHODS: *S. aureus* biofilms were grown for 5 days on polystyrene pegs in TSB with 1% human plasma, in a 96-well format. Antibiotic challenges were done for 28 days (except for burst release: 14 days), with medium changes every other day. The following regimens were tested: continuous gentamicin (range: 15 to 2000 mg/L), 2 hours gentamicin two times per day (15, 250, 2000 mg/L), burst release (2000 mg/L at day 1 reduced in steps to 2 mg/L at day 14), all with and without a continuous rifampin concentration of 3.3 mg/L. For bacteriology, pegs were broken off, sonicated, and total colony forming unit (CFU) counts per peg were determined.

RESULTS: The 5-day-old biofilm contained mean $6x10^8$ CFU. Continuous gentamicin challenge at a high concentration of 2000 mg/L was most successful, reducing CFU to a mean of 10 CFU per peg (range 0-60 CFU, SD 24, 67% pegs culture negative (CN)). The least successful regimen was the burst release with mean $4x10^8$ CFU at day 14 (0% CN). Gentamicin twice daily challenges (2000 mg/L) resulted in mean $2x10^6$ CFU at day 28 (0% CN). Adding a continuous concentration of rifampin resulted in $2x10^3$ (33% CN), $2x10^4$ (72% CN), and $2x10^6$ (33% CN) CFU, respectively.

DISCUSSION & CONCLUSIONS: While a reduction of living bacteria could be observed over time, all regimens failed to fully eradicate biofilm. The most successful regimen was a continuous exposure to 2'000 mg/L of gentamicin for 28 days, but such concentrations are above known cell toxicity thresholds. The addition of rifampicin did not reduce bacterial counts more in the continuous gentamicin challenge but was more effective in combination with gentamicin burst release and twice a day challenges. Other antibiotics than aminoglycosides may be better for local administration.

Tracking the Pre-Hospital Course of Open Fracture Patients

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INTRODUCTION: It is known that rapid administration of intravenous (IV) antibiotic prophylaxis in patients with open fractures decreases infection risk.[1] While there have been prior studies examining the need for prompt administration of IV antibiotics for open fracture patients presenting to the hospital, these only measure time from arrival at the emergency department to IV antibiotic administration.[2] No studies have reported the prehospital time course of open fracture patients. Determining the time course of open fracture patients prior to presentation to the emergency department could inform the creation of revised emergency medical services protocols, particularly those permitting the prehospital administration of IV antibiotics for open fractures.

METHODS: This study was approved by our institutional review board (STUDY0006920). Patients were identified using current procedural terminology (CPT) codes for open fracture debridement. Patients at least 18 years of age with open fractures, complete in hospital documentation, and complete emergency medical services (EMS) prehospital documentation were included. Pediatric patients, patients with open facial fractures, and those with incomplete documentation were excluded. Data collected included demographic information, fracture description, Gustilo-Anderson classification, dispatch time, on scene time, enroute to hospital time, arrival at hospital time, and transfer of care. We also recorded modality of transport to the emergency department, as well as if IV antibiotics were administered prior to arrival at the hospital.

RESULTS: Seven hundred patient charts were reviewed. 73 patients were brought to the hospital via private vehicle, 44 patients were transferred from an outside hospital, 23 patients had open facial fractures, and 106 patients did not have EMS prehospital documentation available and were excluded. This left 454 patients for analysis in the study. All patients were transported to an urban level I trauma center.

The average time to arrival on scene was 13.2 ± 12.4 minutes. The average time on scene was 20.3 ± 12.2 minutes. The average time transporting to the hospital was 24.1 ± 16.3 minutes. The average total time from dispatch to transfer of care was 68.7 ± 28.9 minutes.

239 patients (52.6%) had transfer of care greater than one hour after time of dispatch. 206 of the 239 patients (86.2%) were injured outside of the urban environment. There was a significant difference in time from dispatch to transfer of care at the hospital between patients originating within the city in which the level I trauma center was located, and those who were injured outside of the city (49.4 ± 17.3 vs. 82.8 ± 27.2 minutes respectively, $p < 0.001$).

Fifty-five patients were transported via helicopter, of which 17 (30.9%) received IV antibiotics. This represented only 3.7% of all open fracture patients included in the study.

DISCUSSION & CONCLUSIONS: A substantial portion of patients with open fractures arrive at the hospital greater than one hour after the time of dispatch. This delay in care for open fracture patients has not been previously described. Routine prehospital administration of IV antibiotics represents an underutilized opportunity to improve care for open fracture patients and deserves further attention.

ACKNOWLEDGEMENTS: The project described in this publication was supported by the University of Rochester CTSA award number UL1 TR002001 from the National Center for Advancing Translational Sciences of the National Institutes of Health.

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Portable Spray Device For Applying An Antimicrobial Coating During Surgery To Surgical Tools And Implants

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INTRODUCTION: The development of infections is a significant problem accompanying orthopaedic implant surgeries. Many of these implants have a complex geometry and are made of a variety of materials. Our group is developing multimodal bioactive coatings made up of polypeptides and polysaccharides, with antimicrobial properties without the use of antibiotics. Layer-by-layer films of poly-L-arginine (PAR) and hyaluronic acid (HA) (PAR/HA) are thin films that are easy to build and have good antimicrobial properties. To apply this antimicrobial polyelectrolyte coating more conveniently, especially during surgery, we have started to develop a portable spray coating system for healthcare professionals.

METHODS: Materials used in orthopaedic implants were studied: metals, such as titanium (Ti) and cobalt-chrome (CoCr) and plastics such as polyether ether ketone (PEEK) and ultra-high molecular weight polyethylene (UHMWPE).
The coating is first built up using a dipping robot as a control: the film is built up by alternative dipping in PAR and HA solutions until 24 layers are obtained. The same coating is then deposited using the portable spray coating system. The spray device prototype has two reservoirs which contain PAR solution and HA solution, and allows the delivery of both solutions in the form of a fine mist. The deposition of the coating is observed by fluorescent microscopy after staining. The antimicrobial activity of the coating against *Staphylococcus aureus* is then assessed.

RESULTS: (PAR/HA) coatings can be successfully constructed on different materials using both the dipping robot and the portable spray device (Figure 1). Antimicrobial activity was observed for all the coatings constructed with the dipping robot. Initial results have been obtained with spray coatings on Ti and UHMWPE. A good antimicrobial activity (>2 log reduction in CFU/mL) was found on both tested coatings (Table 1).

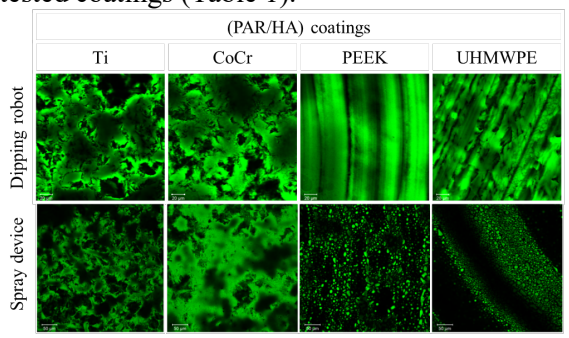

Figure 1: Fluorescent microscopy images of film deposition on four materials with the dipping robot (top) and the spray device (bottom) after PAR-FITC (green) staining

Table 1: Staphylococcus aureus count after contact with (PAR/HA) spray coating on Ti and UHMWPE

	Planktonic bacteria count (control without coating to 100%)	Adherent bacteria count (log reduction vs control without coating)
Ti	14.0 % (±2.9)	2.4 log (±0.4)
UHMWPE	5.0 % (±1.3)	3.2 log (±0.6)

DISCUSSION & CONCLUSIONS: These results show the capacity of (PAR/HA) films to ensure antimicrobial activity when spray coated on different substrates, such as surgical tools and implants. (PAR/HA) spray coating appears to be a simple and powerful system, easy to produce and scale up, without a chemistry process and suitable on all surfaces.

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Evaluating the Safety and Pharmacokinetics of Systemic Versus Local Phage Therapy in Health Sheep

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INTRODUCTION: With the growing concern over antibiotic resistance, especially in treating fracture-related infections (FRI), phage therapy emerges as a potential alternative. However, its safety and distribution remain largely unexplored. This study aims to evaluate the safety and pharmacokinetics of phage therapy administered systemically versus locally in healthy sheep.

METHODS: The sheep will not have any fractures but will have filtration probes in the intramedullary (IM) canal of the tibia. Phage will be administered in two periods using clinically available phage therapy regimens: 3 times per day for 10 days with a latency phase of four weeks between the treatments. Group 1 will receive phage ISP intravenously (IV); group 2 will receive phage ISP by instillation through a draining tube. Animals will be housed for 2 days after cessation of the second phage therapy to continue to draw serum samples and to measure residual phage, distribution patterns, and production of neutralized phage particles after euthanasia.

RESULTS: Initially, a rapid decrease in active phage population was noted: a 5 $\log^{10}$ PFU/mL reduction just 5 minutes after IV administration, with a consistent drop over time. During both IV phage administration cycles in sheep, active titer in plasma following repeated doses after 5 and 10 days decreased at 1 h, 2 h, and 4 h compared to the initial dose on Day 1. Moreover, phage titer in serum was lower in the second than the first cycle. Interestingly, sheep receiving phages locally showed no active phages in blood. The study also measured phage neutralization over time. By day 10 of the first cycle, 60% of phages were neutralized. For local versus IV administration, by day 38, neutralization rates were 25% and 55%, respectively. By day 50 in the second cycle, phage neutralization reached 99.9%. No active phage was detected in bone or tissue post-euthanasia for both routes. However, inactive phage was found in the liver, spleen, and popliteal lymph nodes of sheep with IV therapy, confirmed by PCR at euthanasia.

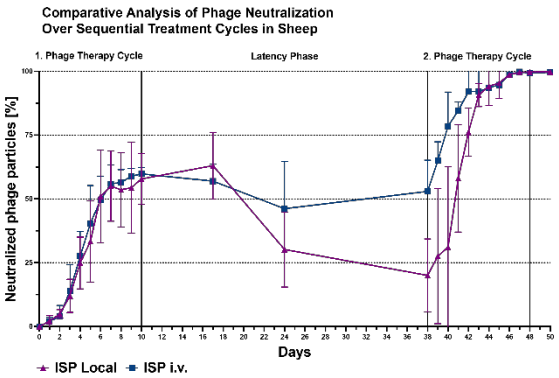

Figure 1. Comparative analysis of phage neutralization over sequential treatment cycles in sheep. The percentage of phage neutralization observed in sheep during the first and second cycles of treatment, with a follow-up period extending from day 10 to day 38 without any treatment. ISP Local: Local application. ISP: Intravenous application

DISCUSSION & CONCLUSIONS: The study reveals rapid clearance and diminished efficacy of intravenously administered phages in sheep, showing initial sharp declines in activity and progressive reductions over time and treatments. The repeat administration increased the clearance of phage. The body effectively neutralized phages, especially by the second treatment cycle, with inactive phages detected in vital organs. These results stress the need for strategic phage therapy approaches, recommending future research in infection models for practical insights.

Targeted poly (D-amino acids) nanoparticles loaded with sitafloxacin for staphylococcal biofilm eradication

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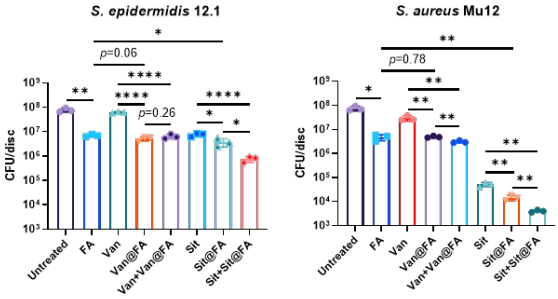
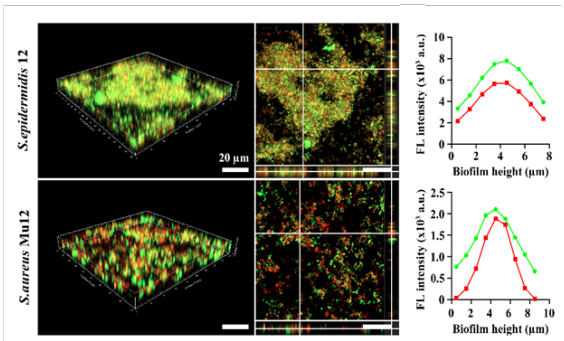
INTRODUCTION: Orthopaedic device-related infections (ODRI) are characterized in large part by antibiotic-tolerant biofilms forming on implants. Despite good clinical practices, biofilms are extremely difficult to eradicate due to mechanism such as tolerance, different metabolic state of the bacterial cells and physical barriers which are self-produced by the bacteria. Addressing this issue necessitates the development of treatments capable of penetrating and disassembling biofilms. The objective of this study was to assemble antibiotic-loaded nanoparticles (NPs) that display DAA on their surface.

METHODS: D-Amino acid nanoparticles were synthesized by dissolving 10mg of poly(α-N-acryloylphenylalanine)-block-poly(β-N-acryloyl-D-aminoalanine) polymers into 1 mL of Dimethyl sulfoxide (DMSO) reaching a final volume of 10 mL with water. Loaded nanoparticles were prepared by adding 1 mg Sitafloxacin powder to the polymers. The activity of NPs and loaded NPs was evaluated with colony-forming units (CFU), confocal laser scanning microscopy (CLSM) and Transmission Electron Microscopy (TEM) against *S. aureus* biofilms grown on titanium disks in Tryptic Soy Broth (TSB) medium for 48hrs, with media changes every 24hrs. The cytotoxicity of the nanoparticles was also assessed against human embryonic kidney 293T cells and human monocytic-leukemia cells after 24hrs of co-incubation.

RESULTS: TEM revealed a diameter of 100 nm. 250 ug/ml of unloaded NPs showed a 1-log reduction on *S. aureus* biofilm. Microscopy analysis showed that the NPs dispersed and created more gaps in the biofilm and reduced its thickness. Sitafloxacin loaded NPs resulted in a further 1-log reduction but the most effective outcome with a 4-log reduction was achieved when sitafloxacin solution was combined with sitafloxacin loaded NPs. Cell viability assay revealed that viability greater than 80% was observed at a concentration of 250 ug/ml of NPs.



Antimicrobial efficacy against staphylococcal biofilms using antibiotics and antibiotic loaded NPs *CFU quantification from in vitro biofilm samples after 24 hrs treatment exposure with the mentioned treatments.*



CLSM analysis of NPs penetration through staphylococcal biofilm *PI loaded NPs were applied on top of mature biofilm and the signal analysed after 24 hrs exposure*

DISCUSSION & CONCLUSIONS: These results highlight the potential of the NPs particularly when loaded with sitafloxacin, as a promising therapeutic approach for combating Staphylococcal biofilm associated ODRI. Further refinement of the NPs formulation holds great promise for increasing the treatment efficacy potentially reducing the risk of resistance development.

ACKNOWLEDGEMENTS: BUCT for providing the chemical expertise and the polymers. This project was funded by AO Trauma.

Development and Characterization of an Antibacterial Implant Surface – Proof of Concept of Albumin/Tannic Acid-coated 3D Printed Polycaprolactone Scaffolds

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INTRODUCTION: Biofilm-related implant infections are notoriously challenging to treat and can lead to chronic infection and persisting inflammation of surrounding tissue. To date, a large body of research can be reviewed for coatings which potentially prevent bacterial infection while promoting implant integration. Yet only a very small number has been translated from bench to bedside. This study provides an in-depth analysis of the stability, antibacterial mechanism, and biocompatibility of medical grade polycaprolactone (mPCL), a clinically widely applied biodegradable polymer, coated with human serum albumin (HSA), the most abundant protein in blood plasma, and tannic acid (TA), a natural polyphenol with antibacterial, properties.

METHODS: Macro- and microporous mPCL scaffolds were incubated in 1% and 5% HSA solutions overnight, at room temperature and under agitation. Resulting coatings of HSA were subsequently stabilized/crosslinked by incubating with 10% or 1% TA. Physicochemical stability and antibacterial mechanism of coated scaffolds were characterized by scanning electron microscopy (SEM), atomic force microscopy (AFM) height and viscoelasticity mapping, transmission electron microscopy (TEM), and molecular simulations.

RESULTS: Molecular docking studies demonstrated that HSA and TA interact mainly through hydrogen-bonding, ionic and hydrophobic interactions, leading to smooth and regular assemblies. Coated scaffolds showed superior viscoelastic properties, with higher elastic recovery and improved scratch resistance in comparison to control surfaces. Furthermore, coated scaffolds inhibited the growth of *S. aureus* and *P. aeruginosa*, two of the most commonly found bacteria in biomaterial-related infections. Most importantly, they were able to reduce *S. aureus* colonization on the mPCL surface, by 99.7± 0.7% in comparison to the non-coated scaffolds. Particularly, modified surfaces maintained their antibacterial properties for three days, resulting in the inhibition of biofilm formation *in vitro.* Interestingly, AFM and TEM data suggested that HSA/TA-coatings cause morphological and mechanical changes on the outer cell membrane of *S. aureus* leading to membrane disruption and cell death (Fig.1). Finally, HSA/TA-coatings showed no cytotoxicity against *in vitro* cultured human cell sheets. This demonstrates their potential to be investigated in an *in vivo* model aimed at prevention of biofilm-related infections.

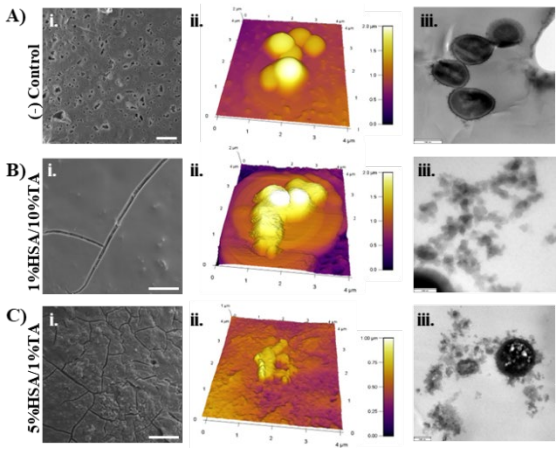

Fig. 1: i. SEM morphology of A) Untreated, B) 1%HSA/10%TA- and C)5%HSA/1%TA-coated mPCL. ii. AFM height, iii. TEM images revealing changes on S. aureus outer membrane when exposed to control and coated scaffolds.

DISCUSSION & CONCLUSIONS: The coated scaffolds prevent microbial growth for up to three days, thereby significantly reducing *S. aureus* colonization and inhibiting biofilm formation. Our findings suggest that the coating damages the outer cell membrane of *S. aureus*, leading to cell death. The HSA/TA coatings showed no signs of cytotoxicity against *in vitro* cultured human cell sheets, reinforcing their potential for further exploration in an *in vivo* model aimed at preventing implant infections caused by biofilms.

Biofilm triggered antimicrobial release coating for implantable devices based on pH-liable boron-diols interactions

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INTRODUCTION: Orthopaedic device-related infections represent a major threat to the success of orthopaedic and trauma surgeries. Controlled drug release offers an appealing solution in the case of implantable devices, as contamination can occur during the operation or in a second moment from contaminated tissues (exogenous infections) or even via hematogenous infection as consequence of septicaemia. Ideally, the release would occur only once biofilm formation starts and therefore exert its action at an early stage of the infection. Biofilm formation is accompanied by acidification of the medium due to the accumulation of by-products of carbohydrate metabolism such as acetic and lactic acid. A pH gradient establishes along the biofilm thickness with the acidity increasing with the depth and reaching pH values as low as 4.5. This work focuses on the development of a pH-dependent biofilm triggered release of modified antibacterial compounds bearing a boronic acid moiety and of antibiotics containing vicinal diols.

METHODS: High-throughput evaluation of the efficacy of the release coating was performed employing a peg-in–well system. The wells were coated with polydopamine (PDA) and the active compound. Gram- bacteria were incubated in static conditions at 25°C for 24h, Gram+ at 37°C for 48h. TSB and TSB 1/20 media were employed for Gram+ and Gram- respectively. The study included six strains (lab and clinical isolates). After incubation, the optical density at 595 nm (OD_{595}) was measured for the planktonic phase. Biofilm formation on the pegs was quantified via crystal violet staining and measure of the optical density at 570 nm (OD_{570}).

RESULTS: A boronic acid derivative of the antibiotic ciprofloxacin was prepared to test our hypothesis. Screening confirmed retention of biocidal activity. The pH-specific release of antimicrobial compound was confirmed via liquid chromatography-mass spectroscopy (LCMS) analysis of samples at pH 7,4 and 5. The coating was able to exert a significant reduction in biofilm formation on both Gram+ and Gram- strains. The PDA coating alone did not significantly affect bacterial growth. Also, no trace of active compound could be found via LCMS in the supernatant solution of a sterile well. This confirmed the dependence of the release on bacterial growth. Moreover, the addition of a buffer at pH=7,4 prevented generalized medium acidification due to planktonic growth, and provided evidence for a localized pH drop within the biofilm layer.

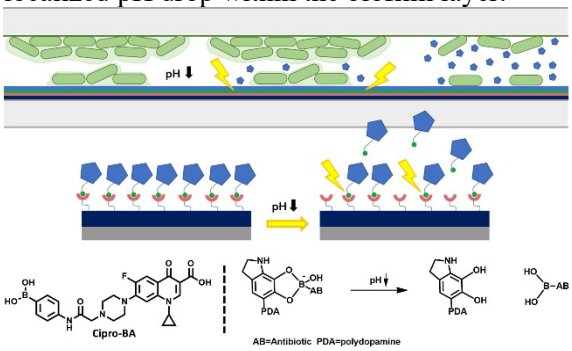

Fig. 1: Graphical representation (top) and schematics (bottom) of the pH-triggered release coating.

DISCUSSION & CONCLUSIONS: An innovative antibiotic release coating based on pH-sensitive boric esters was designed and tested against biofilm formation of a set of relevant strains. Experimental evidence supports the pH trigger and the dependence on biofilm formation of the release. Further optimization is ongoing to aid the translation to the operation theatre.

ACKNOWLEDGEMENTS: This work was supported by PDM post-doctoral scholarship (KU Leuven).

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Carrier-Free Nanodrugs for the Treatment of Multidrug-Resistant Infection

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INTRODUCTION: Multidrug resistant (MDR) bacterial infections pose a serious threat to human life[1]. Antibiotic resistance is largely due to changes in bacterial membrane permeability or efflux[2], which is a huge challenge to overcome. Recently, carrier-free nanodrugs emerge as nanodrug delivery platforms without or with minimum use of inert carrier materials. This endows them with some desirable advantages, including simple preparation, high drug loading capacity, synergistic therapy, and avoiding carrier-induced toxicity[3]. However, still few carrier-free nanodrugs are rationally designed to treat MDR bacterial infection.

METHODS: Herein, taking advantage of dynamic Schiff base reactions, antibiotic tobramycin (Tob) and antimicrobial borneol 4-formylbenzoate (BF) were directly assembled into carrier-free Tob-BF nanodrugs (TB NDs) for reliable eradication of MDR bacteria.

RESULTS: Since some amino groups of Tob were exposed on the TB NDs surface to realize positive charge property of nanocarriers, TB NDs achieved self-loading and self-delivery. In-depth analysis revealed that TB NDs could increase bacterial uptake of nanodrugs and their intracellular accumulation by enhancing permeability. TB NDs showed distinct synergistic bactericidal efficacy in both *in vitro* and *in vivo* assays, particularly in promoting the recovery of clinical xenograft MDR *Staphylococcus aureus* infection in mice (Fig. 1), clearly superior to Tob, BF and physically mixed Tob+BF (with equal dose to TB NDs).

DISCUSSION & CONCLUSIONS: Overall, this work highlights the tremendous potential of carrier-free nanodrugs for overcoming bacterial drug resistance and eradicating MDR bacterial infection, indicating combinational designed strategy of bactericides holds great promise in clinical translations.

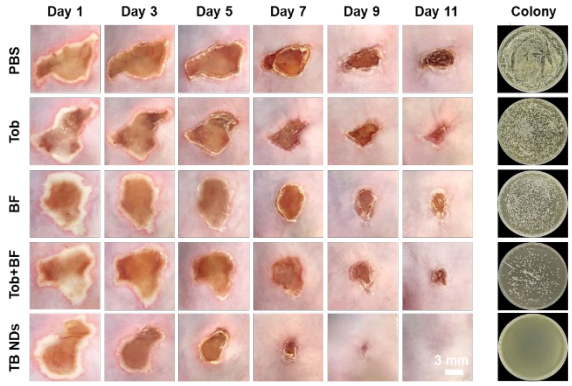

Fig. 1: In vivo antibacterial activity of TB NDs. a) Photographs of MDRSA-infected mice during treatment, b) plated bacterial colonies.

ACKNOWLEDGEMENTS: The authors thank the National Natural Science Foundation of China (52273118 and 22275013) for financial support.

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Electrophoretic Deposition of Gentamicin into Titanium Nanotubes Prevents Prosthetic Joint Infection

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INTRODUCTION: Prosthetic joint infection (PJI) is the leading cause of failure of modern joint replacements; successful treatment for PJI over two years can be less than 50%, with a 5-year mortality over 25%. We created titanium nanotubes (TNTs) on the surface of titanium (Ti) implants and loaded them with antibiotic gentamicin and controlled release agent chitosan through electrophoretic deposition (EPD) as previously performed[1]. We tested these implants in a mouse PJI model.

METHODS: Ti wires underwent TNT surface modification[1]. EPD was used to load gentamicin and/or chitosan onto the wire with surface TNTs (**Fig 1.**). These wires were utilized as femoral implants in our mouse PJI model. Mice (12-week-old male C57BL/6J) were divided into two implant groups: *i)* Chitosan + Gentamicin Group = Ti implants with TNTs loaded with gentamicin and chitosan (n=15; experimental group); *ii)* Chitosan Group = Ti implants with TNTs loaded with chitosan (n=15; control group). Mice received a right femoral intramedullary implant followed by inoculation at the knee joint surgical site with $1x10^3$ CFU of bioluminescent Xen36 *Staphylococcus aureus* (*S. aureus*). Assessments included bioluminescence imaging for Xen36 *S. aureus* over 14 days and CFU analysis at the peri-implant tissue and implant at day 14. Statistical analysis explained in **Figure 1** legend.

RESULTS: Over 14 days assessment following implant placement and inoculation with *S. aureus,* the Gentamicin + Chitosan Group had no evidence of infection based on *i)* no increased Xen36 *S. aureus* bioluminescence signal at the knee joint surgical site at day 1, 3, 5, 7 ,10, and 14) (**Figure 1A and 1B**) and *ii)* no CFUs present at the implant and surrounding tissue at day 14 (**Figure 1C**). All control mice (Chitosan Group) had increased bioluminescence signal, above baseline, at all time-points over 14 days (**Figure 1A and 1B**) and presence of bacteria at the implant and surrounding tissue at day 14 on CFU analysis (**Figure 1C**).

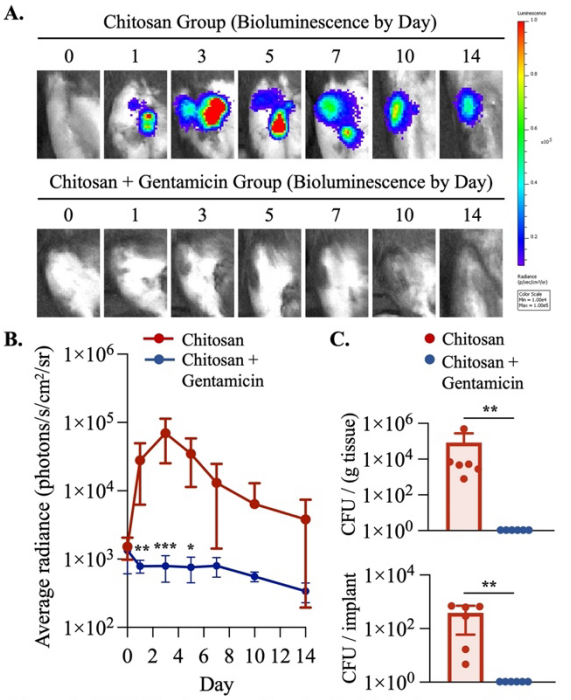

Figure 1. (A) Bioluminescence imaging for bioluminescent Xen36 *S. aureus*. **(B)** Quantification of bioluminescent signal at the region of interest at the knee joint. **(C)** CFU analysis at the peri-implant tissue and implant at day 14 following overnight culture. Data represented by mean ± SD; *p<0.05, **p<0.01, ***p<0.001. For (B), mixed effects analysis with Bonferroni correction for multiple comparisons was performed. For (C), differences in CFU counts were assessed by testing for normality with a Shapiro-Wilk test. Normally distributed data was assessed with an unpaired t-test, and non-normally distributed data was assessed with a Mann-Whitney U test.

DISCUSSION & CONCLUSIONS: Implants with TNTs coated with chitosan + gentamicin through EPD prevented evidence of infection in all mice. This study provides preclinical proof of concept of a highly effective implant coating and local antimicrobial delivery system to prevent PJI, which could also be utilized for PJI treatment, such as during revision surgeries for PJI.

ACKNOWLEDGEMENTS:
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DISC PENETRATION SIGN: A DISTINCTIVE MRI SIGN INDICATING THE SEVERITY OF PYOGENIC SPONDYLITIS

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INTRODUCTION: Pyogenic spondylitis (PS) is a prevalent type of spinal infection characterized by bacterial inflammation of the vertebrae. Magnetic Resonance Imaging (MRI) is crucial for early detection of spinal infections, particularly for early marrow abnormalities, and paraspinal and intraspinal involvements.The current diagnostic approach involves a combination of clinical symptoms, laboratory tests, imaging, surgical findings, and pathology. However, challenges arise due to specificity issues in clinical signs and laboratory markers (WBC, ESR, CRP), as well as low bacterial culture yield, making early and accurate diagnosis difficult. Our study collected data and laboratory results from previous cases of pyogenic spondylitis, analyzing MRI scans to identify a distinct imaging sign, the 'Disc Penetration' sign(DP), indicating the severity of PS, featuring abscess breach of the intervertebral space impacting vertebral borders and intraspinal structures.

METHODS: This retrospective study included 137 patients treated at the Third Affiliated Hospital of Southern Medical University's Department of Spine Surgery from 2013 to 2023.Criteria included clinical symptoms, laboratory signs of infection (WBC, ESR, CRP, albumin levels), imaging findings of osteolytic lesions or abscesses, confirmed pyogenic spondylitis via microbiological culture, pathogen metagenomic next-generation sequencing (mNGS) or pathological examination, and complete clinical data. Patients with earlier spinal operations, multiple infections, or immunodeficiency were excluded. The presence of a Disc Penetration sign (DP) was considered when abscess transversed the intervertebral space and affected anterior vertebral and intraspinal structures.

RESULTS: 56 patients presented with the Disc Penetration sign (DP) and 81 did not. In both groups, there was no significant difference in gender ratio or age between the two groups ($p > 0.05$). However, significant differences were observed in the presence of comorbid diabetes and chronic kidney disease ($p < 0.05$). Laboratory results indicated higher inflammatory indicators (ESR, CRP) and lower albumin levels in the DP group ($p<0.05$). Imaging results showed no significant differences in affected spinal segments or parts ($p>0.05$), but the DP group exhibited more frequent paraspinal abscess formation($p<0.05$).

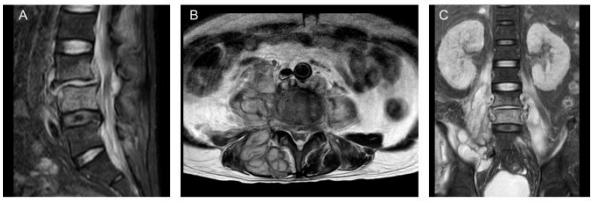

Fig. 1: Typical MRI of Disc Penetration sign.(A)sagittal position on T2WI shows abscess penetrating the anterior and posterior structures of the vertebral body(B)horizontal position shows abscess invading paravertebral structure(C) coronal position shows the psoas abscess

Table 1. Primary laboratory test results

	DP (n=56)	Non-DP (n=81)	P value
WBC($\times10^9$/L)	8.94(6.52-11.90)*	7.65(5.80-9.91)*	0.097
ESR(mm/h)	74.30±33.79	51.46±30.46	<0.001
CRP(mg/L)	47.28(17.16-131.92)*	26.18(4.85-73.57)*	0.003
Hb(g/L)	100.66±19.82	116.99±19.99	<0.001
ALB(g/L)	28.81±6.59	34.09±6.17	<0.001

**Median (25% quartile -75% quartile)*

DISCUSSION & CONCLUSIONS: The study suggests that the Disc Penetration sign in pyogenic spondylitis patients correlates with more severe inflammation and higher incidence of paraspinal abscess, pointing to worse stability of the spine, longer bone restructuring time, and potentially poorer prognosis. This finding helps clinicians rapidly assess the disease's severity and prognosticate outcomes. We also reaffirm the need for early pathogen-specific diagnosis and treatment, with surgical intervention considered for patients with substantial paraspinal abscesses or spinal instability.

Combining sitafloxacin and sugars in a nanodrug to targeting stationary phase Staphylococcus aureus

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INTRODUCTION: The sugar fructose has previously been shown to increase efficacy of aminoglycoside antibiotics against metabolically inactive bacteria. The sugar may achieve this by activating metabolism, membrane potential and growth rate. Combining sugar and antibiotics into a carrier-free nanodrug (ND) may further increase activity, however, the optimal combination, their efficacy against Staphylococcus aureus, and ability to assemble into a ND has not been previously described.

METHODS: *S. aureus* USA300 was grown to logarithmic or stationary growth phase, determined by optical density (OD) measurements, and treated with different antibiotics for 24 hours at 100x minimal inhibitory concentration (MIC), alone or in combination with glucose, fructose, mannose (at 0.2%), xylose and lactose (at 1.25%). Surviving bacteria were quantified by colony forming unit (CFU). Sugar-antibiotic combinations were assembled into NDs using sequential precipitation in a mixed solvent (Dimethylsulfoxid, Dimethylformamide and ethanol) and water. The reaction was confirmed by nuclear magnetic resonance (NMR) and ND stability observed using dynamic light scattering (DLS). The ND size and morphology was determined by scanning electron microscopy (SEM).

RESULTS: Sitafloxacin was shown to be the most effective antibiotic against stationary phase *S. aureus* with a 3 log reduction in CFU. Its efficacy was increased by at least 1 log when combined with 0.2 % glucose, fructose or mannose. ND composed of sitafloxacin and fructose were successfully synthesised and tested with NMR and SEM, which showed an average size of 200 nm and spherical morphology. DLS confirmed stability of the ND for up to 4 days at room temperature.

DISCUSSION & CONCLUSIONS: To target stationary phase *S. aureus*, we combined a potent antibiotic with a sugar that achieves increased bacterial killing activity.

Floating Disc: A Distinctive MR Imaging Manifestation of Native Veterbral Osteomyelitis

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INTRODUCTION: Native vertebral osteomyelitis (NVO) is a chronic infectious disease involving vertebral bodies and intervertebral discs, and is potentially accompanied by paravertebral soft tissue infection and epidural abscess. It has a rather long course and no specific symptoms in the early stage. When recurrent fever or localized pain finally prompt the patients to seek medical attention, the situation may have become life-threatening for lack of proper medical intervention. Staphylococcus aureus indicates as the most common pathogenic microorganism of vertebral osteomyelitis, and it is more prevalent in regions with a low incidence of pulmonary tuberculosis [1]. Magnetic resonance imaging (MRI) is currently a preferred diagnostic method for vertebral and paravertebral infections, with a sensitivity ranging from 91% to 100% [2]. During the diagnosis and treatment, we identified a special radiographic sign on T2-weighted images, which we tentatively termed as "floating intervertebral disc". Infected intervertebral discs demonstrate high signal. The vertebral endplates detach from vertebral bodies, resulting in smaller floating discs within the intervertebral space. Subsequently, the intervertebral space is filled with pus, and the pus surrounds the floating intervertebral discs, which leads to above-mentioned distinct radiographic sign. No previous literature has provided a comprehensive report on this sign. Therefore, this study aims to investigate the differences in clinical manifestations between NVO patients with and without floating discs based the collected clinical and imaging data of NVO patients from T2-weighted images.

METHODS: 57 NVO patients treated at the Department of Spinal Surgery from January 2013 to June 2023 were included in a retrospective study according to inclusion and exclusion criteria.

RESULTS: NVO tend to occur in the lumbar spine, with a higher incidence among males, and staphylococcus aureus is indicated as the most common pathogenic microorganism. Among all the indicators, albumin (37.42±5.381 VS 32.90±6.556, P=0.013) and VAS scores (3.06±0.938 vs 4.28±1.589, P=0.004) differ significantly between NVO patients without and with disc floating, but the inflammatory indexes are not significantly different. The MRI images of NVO patients with floating intervertebral discs show intervertebral space abscesses, with a wider range of infection and a significant decrease in the height of the intervertebral space. Additionally, their CT images indicate more severe vertebral bone destruction and increased air signs in the vertebral body and intervertebral discs.

DISCUSSION & CONCLUSIONS: Combined with the symptoms, imaging signs and healing of NVO. We can tentatively conclude that the floating disc may represent the final stage of native vertebral osteomyelitis. To substantiate this tentative conclusion and thoroughly investigate floating disc pathogenesis, further studies with larger sample sizes are needed, which will ultimately provide a reference basis and guidance for the clinical management of floating discs in the future.

ACKNOWLEDGEMENTS: We would like to acknowledge our colleagues for advising the current subject.

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Towards Biocompatible Immobilized Antibacterial Coatings on Titanium which kill bacteria on contact by Electrostatic Forces

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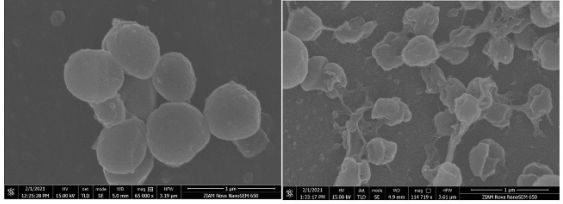
INTRODUCTION: The major cause of failure of biomedical implants is infections caused by bacteria that invaded in wounds during the implantation operations. Infections occur 1-5% in most cases up to more than 50% for external fixation rods. It has become increasingly difficult to combat pathogenic bacteria, because a substantial number of them has become resistant against antibiotics. As it is now and in the future impossible to remove all pathogenic bacteria in surgery rooms new approaches have to be explored to combat bacteria. A promising approach to prevent infections is by coating implants with an antibacterial coating. Indeed, antibacterial coatings containing *leachable* biocides have been developed, but they contaminate the body fluids and are exhausted in due time.

METHODS: More recent *immobilized* antibacterial coatings have attracted much more the attention to overcome the disadvantage of leachable biocides. Inspired by anti-bacterial peptides our coatings contain quaternary ammonium compounds (QACs), which don't cause bacterial resistance. *Soluble* QACs, must have hydrophobic moieties to penetrate in the bacterial wall to destroy the cytoplasmic membrane. However, hydrophobicity can cause haemolysis.

RESULTS: Here we show that for *immobilized* QACs have a different killing mechanisms, based on strong electrostatic forces (see image).



SEM image of *S.Epidermidis* on titanium (left) and on coated titanium

Although hydrophobic moieties are undesired, as it causes haemolysis, all immobilized QACs coatings studied so far were provided with hydrophobic moieties, obtained by alkylation of amines, to acquire high charge densities, which are needed to kill bacteria. We discovered that such high charge densities can also be obtained by adding highly polarizable salts (e.g. NaI) to the coating, resulting in more hydrophilic coatings with excellent antibacterial properties.

DISCUSSION & CONCLUSIONS: The balance between biocompatibility or toxicity of biomedical materials depends on the balance of hydrophilicity and hydrophobicity. In contrast to *soluble* QACs, our *immobilized* QAC-coatings don't need hydrophobicity (haemolysis risk), while still giving excellent anti-bacterial properties, because of the different killing mechanism. Our initial histology results showed indeed that the lower hydrophobicity is beneficial for the biocompatibility. Furthermore, it is important for biomedical implants that our coatings have excellent mechanical properties.

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Antibacterial properties of nisin layer-by-layer based coating on titanium K-wires in *Galleria mellonella* implant-associated infection model

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INTRODUCTION: Orthopaedic implants play a tremendous role in fixing bone damages due to aging as well as fractures. However, these implants tend to get colonized by bacteria on the surface, leading to infections and subsequently prevention of healing and osteointegration. Recently, Roupie *et al.* showed that a nisin layer-by-layer based coating applied on biomaterials has both osteogenic and antibacterial properties. The *Galleria mellonella* larva is a well-known insect infection model that has been used to test the virulence of bacterial and fungal strains as well as for the high throughput screening of antimicrobial compounds against infections. Recently, we have developed an insect infection model with *G. mellonella* larvae to study implant-associated biofilm infections using Kirschner (K)-wires as implant material. Here, we would like to test the antibacterial capacity of nisin layer-by-layer based coatings on K-wires against *Staphylococcus aureus* in the *G. mellonella* larva implant infection model.

METHODS: Prior to the implantation procedure, *G. mellonella* larvae are maintained at room temperature on wheat germ in an incubator. The larvae received bare titanium K-wires (uncoated), or either control-coated or nisin-coated K-wires. After one hour, the larvae were injected with 5×10^5 CFU *S. aureus* bacteria per larva (*i.e.*, hematogenous implant infection model). Next, the larvae were incubated at 37◦C in an incubator and the survival of the larvae was monitored for five days. Moreover, the number of bacteria on the implant surface and in the surrounding tissue was determined after 24h of incubation. Further, scanning electron microscopy (SEM) analyses were performed to study the effect of nisin on biofilm formation.

RESULTS: The larvae receiving the nisin-coated K-wires showed significantly higher survival rates compared to uncoated titanium K-wires, although not when compared to control-coated K-wires. A more than 1-log reduction in number of bacteria on the implant surface and in the surrounding tissue was observed in larvae receiving the nisin-coated K-wires, when compared to uncoated titanium K-wires. SEM analysis showed reduced colonization of the bacteria nisin-coated K-wires compared to the controls.

DISCUSSION & CONCLUSIONS: In conclusion, the antimicrobial nisin layer-by-layer based coating applied on titanium surfaces is able to prevent implant-related *S. aureus* biofilm infection in *G. mellonella* and is a promising antimicrobial strategy to prevent implant-related infections.

In vitro and *in vivo* evaluation of a gentamycin-vancomycin loaded emulsion-based hydrogel for orthopedic device-related infection.

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INTRODUCTION: Fracture related infections (FRI) are one of the major complications in orthopedic trauma surgery, leading to prolonged treatment and increased socioeconomic costs. Infection treatment, via systemic antibiotic administration, may be compromised due to tissue and vascular damage and may not reach sufficiently high concentrations. Local antibiotic delivery via biomaterials such as bone cements can increase local concentrations, however, revision surgery is required to remove them causing additional patient burden, costs and further infection risk.

METHODS: A bioresorbable, injectable, emulsion-based hydrogel (EBH) was developed and evaluated for the treatment of FRI. The EBH is based on readily available raw materials and is manufactured with industrially relevant methods. Viscoelastic properties were evaluated by rheology.

In vitro release of gentamicin sulfate (G) and vancomycin (V) from EBH-GV (0.8% GS and 3.2% V) was done by weighing 500 mg of EBH-GV (n=4) into dialysis tubes. Each gel was immersed into 13 ml of PBS and gently shaken at 37 °C. At each time point, the entire volume of solution was withdrawn and stored until analysis. The quantification was done by HPLC-UV.

Cell viability assay. CellTiter-Blue® assay was performed on fibroblasts (hTERT-BJ1) and on human bone marrow stromal cells (hBMSCs) over 14 days, with time point at day 3, 7 and 14. hBMSCs and hTERT-BJ1 were seeded in a 6-well plate at a density of $1.5x10^4$ cells/cm^2 and grown in Dulbecco's modified Eagle's Medium (DMEM) with 1 g/l glucose and 10% FBS. For hTERT-BJ1. After overnight cell attachment, a 40 µm pore size cell strainer was inserted in each well of the 6-well plate and 1ml of EBH, EBH-G or EBH-GV was pipetted on the cell strainer. Control cultures were carried out in DMEM + 10% FBS without EBH. The medium was refreshed on alternate days. On day 3, 7 or 14, cells were first washed with PBS and then imaged using an inverted microscope. Cells for negative control were incubated for 5 min with Dimethyl sulfoxide (DMSO). All the conditions were incubated with fresh media and Cell Titer Blue reagent. Absorbance was measured at 570nm using 600nm as a reference wavelength with a plate reader.

Finally, the antibiotic-loaded EBH was tested for treatment in an intramedullary nail-related methicillin resistant *Staphylococcus aureus* (MRSA) infection model in the tibia of sheep.

RESULTS: Rheological evaluation of EBH revealed a damping factor of tanδ = G"/G' = 0.54±0.02 and compression testing determined an injection force of 15±2 N. Rheological tack tests displayed higher adhesion energy to soft tissues and metal than to gloves (1.26±0.19, 0.94±0.02 and 0.478±0.12 mJ respectively). Dissolution studies indicated that the gel prolongs the release of antibiotics compared to antibiotic solution. *In vitro* assays with EBH showed no adverse effects on fibroblasts or hBMSCs. Bacteriology results from the *in vivo* study showed complete clearance of the infection in all tissues (n=10) retrieved from all EBH-treated animals (n=4) compared to controls that received only systemic antibiotic treatment where single tissue biopsies of two out of three animals were culture positive at euthanasia, while the third animal showed bacterial contamination only in the bone marrow.

DISCUSSION & CONCLUSIONS: The rheological evaluation of the gel show suitable handling properties such as injectability and low tackiness to gloves while high tackiness to metal, facilitating intraoperative application. Drug release tests showed a prolonged release compared to the control with PBS. The gel was used successfully as a treatment in a challenging infection model with MRSA.

Novel bioactive glass S53P4 cream to prevent orthopedic implant-associated infection

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INTRODUCTION: Multidrug resistance (MDR) poses an alarming challenge in the treatment of implant-associated infections often caused by *Staphylococcus aureus*. Biofilm formation on the implant surface contributes to phenotypic tolerance and persistent infection, which raises the danger of unsuccessful treatment and recurrent infection. In order to prevent these infections we employed the non-antibiotic antimicrobial agent Bioactive Glass (BAG) S53P4, known for its osteogenic and antimicrobial properties. BAG granules (500-800 µm) are used as a bone filler and they are approved for local treatment of bone infections. The antimicrobial mechanism of action of BAG is suggested to be related to ion release, increase in pH and osmotic pressure upon contact with body fluids. These changes would result in a local alkaline environment which is unfavorable for bacterial survival. Recently a novel formulation of BAG has been developed, a cream consisting of BAG powder (≤ 25 µm) and binder. Application of novel BAG cream on implant surfaces may therefore provide a way to prevent implant associated infections. In this study, we investigate the bactericidal effectiveness of BAG S53P4 cream composed of 50% BAG powder and 50% binder in comparison to free BAG powder or granules Additionally, we assessed the potential of BAG cream to be applied on implant materials.

METHODS: The bactericidal activity of BAG granules versus powder against *S. aureus* was first assessed. Then, bactericidal activity of equivalent amounts of BAG cream, powder and binder was determined by applying on Titanium Aluminum Niobium (TAN) discs and allowing elution of ions and/or other substances into solution for 2h, 4h, 8h and 24h. Eluates were collected at each time point and used for bactericidal activity testing and pH measurements. Parallel experiments were performed with BAG granules, for comparison.

RESULTS: The bactericidal activity and pH of BAG powder eluates was higher than of eluates of granules. When comparing the bactericidal activity of BAG cream and equivalent amounts of its components, BAG cream and powder eluates had bactericidal activity but binder did not. It was also noted that the pH was slightly higher at 2h compared to other time points, for both BAG cream and powder eluates.

DISCUSSION & CONCLUSIONS: BAG cream and powder show higher eluted bactericidal activity than granules. BAG cream applied on TAN discs adheres well and remains on the discs after submersion in medium for 24h. The cream thus has favourable characteristics for local application as an antimicrobial substance. To gain a deeper understanding we aim to perform detailed analysis of released ions and changes in osmolality, and the direct influence of the released ions on the bacterial cell.

ACKNOWLEDGEMENTS: The publication is part of the project DARTBAC NWA.1292.190.354 of the research program NWA-ORC which is financed by the Dutch Research Council (NWA).

3D-Printing of Matrix-Based Wound Bioarrays: a Means for High Throuput Evaluation of Antimicrobial Biomaterials

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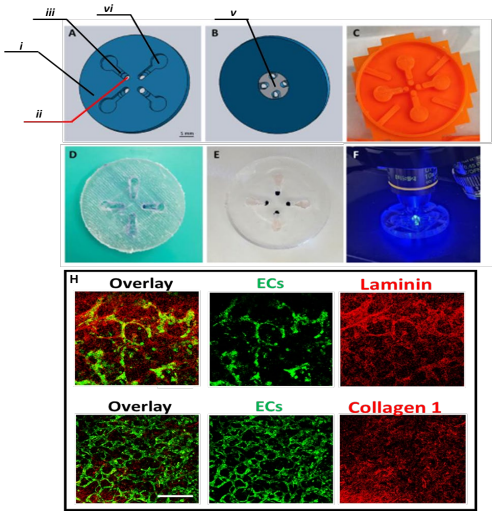
INTRODUCTION: Both surgeons and bioengineers are constantly challenged with reestablishing significant tissue defects in a poly-microbial environment. These defects necessitate approaches that promote wound healing, construct engraftment, and vascularization while possessing an antimicrobial effect and avoiding a harsh immune reaction from hosts. Evaluating and analyzing the ability of tissue replacements to bridge defects, support neovascularization, and prevent a severe inflammatory response is challenging. Moreover, evaluating antimicrobial properties in this setting makes this goal even more complex. Herein, we propose a 3D-based methodology to assess the wound-healing effect of biomaterials for craniofacial repair coupled with antimicrobial agents, including crystalline nano cellulose. Biomaterials and tissues are evaluated for their impact on cells and tissue matrix at the engraftment and for their antimicrobial effect as part of a 3D-printed assay for the evaluation of these attributes.

METHODS: Several autologous tissue components are used in the current study for modeling infected wounds within bioarrays. These include endothelial and adipose-derived stromal cells (ECs and MSCs) combined with biologically inspired hydrogels to form modeled tissue phases. A polydimethylsiloxane (PDMS) array is fabricated based on a 3D design to use as an encasing chamber for bioassays. After the 3D deposition of hydrogels loaded with either MSC monocultures or vascularized co-cultures, an accurate wound is established, followed by structural rehabilitation with the antimicrobial polymer. Neomatrix deposition, neovascularization, and microbial growth are evaluated using confocal microscopy, viability assays, and paracrine assays.

RESULTS: Wounds within bioarrays were successfully monitored for tissue organization and neovascularization, and polymers combined with selected antimicrobial molecules could support wound healing within an infectious environment. These included the deposition of collagens and laminin within the bridging material and the penetration of neo-capillaries into the biomaterial.



Fabrication of 3D printed bioarrays: *(A-B) 3D design of the (PDMS) chip (A, i) for evaluation of biomaterials effect on wound healing and microbial colonies. The design outlines tissue-modelling chambers (ii), microbial migration barriers (iii), media reservoirs for cell-based treatments or infection (vi), and the 10mm coverslip for live imaging (B, v). A – front view, B – rear view. (C) 3D-printed PLA stamp for initial fabrication of devices. (D-E) Fabricated PDMS devices without (D) and (E) pretreatment with pluronic127. Phenol-red and blue dyes indicate semi-diffusion of solutions after coverslips were attached. Images were taken 12 hours after the solution was introduced into chambers and reservoirs, mimicking bacterial inoculation. (F) A fabricated device during live fluorescent imaging. (H) Endothelial capillary organization (green) and deposition of matrix components (red) within tissue models. Scale bar: 100 µm (upper row), 200 µm (lower row).*

DISCUSSION & CONCLUSIONS: The proposed methodology may aid in screening biomaterials for tissue repair, able to sustain wound healing in a highly infections environment before they are cleared for clinical use.

Definition of treatment success and failure in fracture-related infections: a scoping review

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INTRODUCTION: Fracture-related infections (FRIs) pose significant clinical and socio-economic challenges. The establishment of a consensus definition in 2018 marked a crucial advancement for clinical practice and research, enabling treatment standardization and comparison of study results. However, a lack of clear, standardized outcome parameters impedes the evaluation of treatment success, potentially leading to underreporting of treatment failure in current literature. This scoping review aims to provide an overview of outcome parameters in terms of infection, bone healing and clinical outcome to define treatment success or failure in the treatment of FRI.

METHODS: A comprehensive literature search across four databases (PubMed, Embase, Scopus and Web of Science) was performed to identify relevant studies published between 2018 and 2023. Our methodology followed the PRISMA extension for scoping reviews. Studies that reported on treatment outcome in adults with long bone FRI were eligible for inclusion. The primary outcome is the reporting of the persistence, eradication or recurrence of infection as well as radiological and functional outcome. Secondary outcomes are the definition of follow-up intervals and the use of the FRI consensus definition, since its introduction in 2018.

RESULTS: After applying predefined inclusion and exclusion criteria to 5980 retrieved articles, we included a total of 111 studies for analysis and synthesis. Only 20.7% (23/111) of these used a clear definition of treatment success and/or failure in their methodology. In current literature various different approaches to define outcome with respect to infection control, bone healing and quality of life could be found. In terms of infection recurrence, only a few articles specified a change in recurrent culture results to differentiate between infection relapse, persistence or new infection. Furthermore, no standardized follow-up interval could be identified.

Data from this review showed that, despite the introduction of the FRI consensus definition, only 21.6% (24/111) of studies defined infection accordingly. In contrast, 26.1% (29/111) of studies described their own definitions, and 52.3% (58/111) did not provide any specific definition for infection.

DISCUSSION & CONCLUSIONS: This scoping review highlights the lack of standardized outcome reporting in FRI. A clear consensus definition on outcome reporting in FRIs is urgently needed to promote comparability and transparency in clinical research.

Real-time fluorescence imaging of porphyrin-producing bacterial biofilms on orthopaedic material *in vitro*

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INTRODUCTION: Bacterial biofilms formed on implants during periprosthetic joint infections (PJIs) are inhomogeneously distributed and macroscopically invisible. Knowledge about the location of bacterial biofilms on implants could enhance diagnosis and treatment as well as inform prosthesis design aiming at reducing infections. However, most visualization techniques are invasive, usually requiring end-point measurements with biofilm fixation instead of real-time biofilm characterization, and are expensive (e.g. using antibodies). The wound imaging device Moleculight i:X™ (MolecuLight Inc., Canada) has been shown to accurately detect porphyrin-producing bacteria in real-time, allowing for non-invasive biofilm imaging. Therefore, we wanted to explore whether the imaging device would visualize bacterial biofilms on *in vitro* infected orthopaedic implants supplemented with the porphyrin-precursor hexyl aminolevulinate (HAL).

METHODS: Discs (polyethylene, titanium alloy Ti-6AI-4V, cobalt-chromium-molybdenum) of 1 cm diameter were incubated with a defined inoculum of clinical PJI isolates of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* (for 2 and 6 days), or *Cutibacterium acnes* (for 8 and 11 days). In addition, biofilms of *S. aureus* were also grown on hip implant material for 6 days. The biofilms were supplemented with HAL 24h prior to imaging with the Moleculight i:X™ device. The presence of red fluorescence, indicative of bacterial biofilm, the red fluorescence intensity (RFI), as well as the amount of colony forming units (CFU) bacteria recovered from sonicated material (discs) as well as with eSwabs were determined.

RESULTS: Supplementation of infected orthopaedic material in combination with the Moleculight i:X™ device allowed detection of biofilms of all tested strains and on all evaluated materials. RFI varied across the different strains with signal intensities hierarchy of *E. coli* > *S. aureus* > *S. epidermidis* = *C. acnes*. Mature biofilms showed increased RFI (1.2- to 1.8-fold) as compared to early biofilms for all tested strains. HAL supplementation did not interfere with bacterial viability. Targeted swab-sampling of presumed biofilm-positive (red) areas on hip implant material resulted in higher CFU recovery than presumed biofilm-negative (non-red) areas (**Fig. 1**).

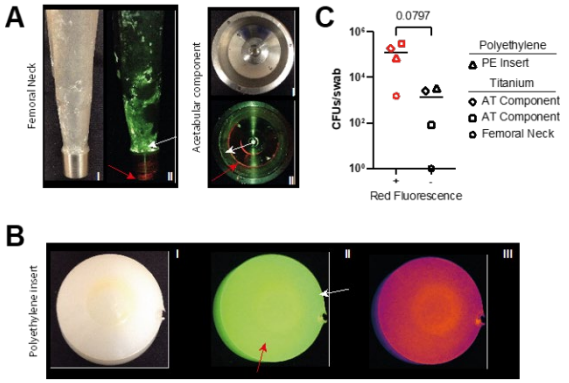

Fig. 1: (A, B) Visualisation and sampling of hip femoral stem, acetabular components and polyethylene insert implant material. (C) Recovered CFUs per swab sample from presumed biofilm-positive (red arrow) and -negative (white arrow) areas.

DISCUSSION & CONCLUSIONS: Our findings show that real-time fluorescence imaging by using the Moleculight i:X™ device and supplementation with a porphyrin-precursor can detect bacterial biofilms on various orthopaedic materials. These findings highlight that exploitation of bacterial auto-fluorescence might provide a rapid and non-destructive method for biofilm localization on orthopaedic hardware. Future studies are required to confirm the potential utility of the proposed technique for biofilm detection on orthopaedic hardware explanted from patients suffering from PJI.

ACKNOWLEDGEMENTS: We would like to thank the company Moleculight Inc. (Canada), for providing the Moleculight iX™ device.

Biosurfactants improve detachment and recovery of bacteria from orthopaedic implants – An *in vitro* study

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INTRODUCTION: The diagnosis of periprosthetic joint infections (PJIs) is a challenge due to microbial biofilm formation on the implant surface. Sonication of explanted devices was shown to improve the microbiological diagnosis, due to physical biofilm detachment. However, sensitivity is low and time-to-positivity (TTP) for sonication culture is extended. Therefore, we asked whether bacterial recovery from implants could be enhanced by using the biosurfactant saponin.

METHODS: Discs (polyethylene [PE], titanium alloy Ti-6AI-4V [TAV], cobalt-chromium-molybdenum [CCM] and polymethyl methacrylate (PMMA)-based bone cement [PMMA]) of 1 cm diameter were incubated with a defined inoculum of different bacterial strains which were isolated from patients with a PJI. *Staphylococcus epidermidis* (3 isolates), *Staphylococcus aureus* (2 isolates), and *Escherichia coli* (2 isolates) biofilms grew for 3 days, while *Cutibacterium avidum* (2 isolates), *Cutibacterium acnes* (2 isolates), and *Cutibacterium granulosum* (1 isolate) grew for 8 days. The discs with biofilms were then treated with either saline solution or various concentrations of saponin. Next, they were either vortexed (1 min) only or sonicated (vortex-sonication-vortex, 1-1-1min). The amount of recovered bacteria was determined by enumeration of colony forming units (CFUs). In addition, TTP of diluted bacterial solutions (1/10) in liquid culture was determined optically.

RESULTS: While sonication with saline solution led to a $1\log_{10}$ increased CFU recovery as compared to vortexing only, saponin ≥ 0.001% led to a $2\text{-}3\log_{10}$ increased CFUs recovery, irrespective of sonication or vortexing only for *S. epidermidis* from PE discs. For TAV, CCM and PMMA discs, 0.01% saponin resulted in 2log10 increased CFUs recovery for *S. epidermidis*. To reach 100% culture regrowth, TTP of liquid cultures of the recovered bacterial solution from sonication and saponin-treated (vortexing only) samples was 4 days and 2 days, respectively. Median CFU enumeration showed $2.2\log_{10}$ (*S. epidermidis*), $0.6\log_{10}$ (*S. aureus*), $0.6\log_{10}$ (*C. avidum*), $1.1\log_{10}$ (*C. acnes*), $0.7\log_{10}$ (*C. granulosum*) and $0.01\log_{10}$ (*E. coli)* increased recovery of saponin-treated (vortexing only) as compared to saline sonicated PE discs (**Fig. 1**).

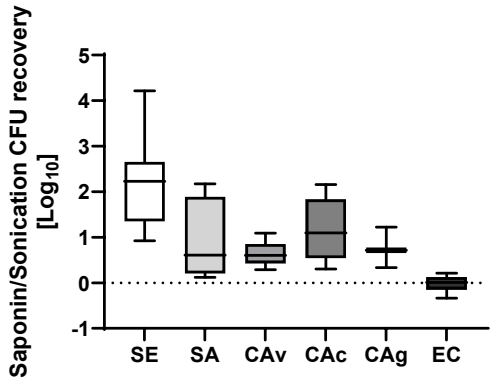

Fig. 1: Box plot showing the relative efficacy (Log_{10}) of saponin treatment (vortexing only) over saline sonication for recovery of CFUs from S. epidermidis (SE, 3 isolates), S. aureus (SA, 2 isolates), C. avidum (CAv, 2 isolates), C. acnes (CAc, 2 isolates), C. granulosum (CAg, 1 isolate) or E. coli (EC, 2 isolates) biofilms grown on PE discs. Shown are Min to Max and median values.

DISCUSSION & CONCLUSIONS: Our findings show that the use of biosurfactants can enhance the recovery of CFUs from biofilms on orthopaedic material as compared to saline sonication for the major PJI causing bacteria. This might enhance the diagnostic value of implant culture, potentially leading to increased sensitivity and decreased TTP. Further clinical studies will be required to evaluate an advantage of saponin alone or in combination with sonication in microbiological laboratories for the diagnosis of PJIs.

Serum metabolite changes in a fracture-related infection model

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INTRODUCTION: The field of immunometabolism has already established the bidirectional ties of metabolites and immune cell function in bacterial infection. However, the metabolic impact of bone fractures and fracture-related infections (FRI) remains largely unexplored. Specifically, FRI, a major and debilitating complication in orthopedic surgery, lacks published metabolomics results.

METHODS: To address this, we used an FRI sheep model to quantify 41 serum metabolites using LC-MS-based targeted metabolomics to characterize the systemic changes over 12 weeks.

RESULTS: During the initial infection, we observed significant changes in only 3 metabolites: A decrease in the redox metabolite cysteine and the energy intermediate octanoylcarnitine, and an increase in the collagen breakdown product hydroxyproline. Importantly, cysteine exhibited only a slow recovery over time. Building on our findings, we developed a classification model to distinguish FRI from healthy sheep, using two metabolite ratios (Hydroxyproline/Glycine, Creatinine/Serotonin) based on logistic regression with a 90.9% predictive power for our samples.

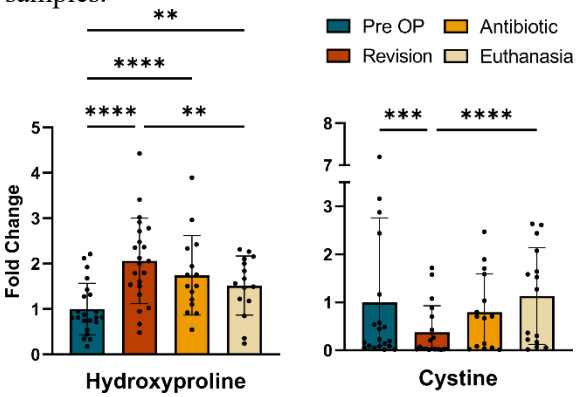

*Fig. 1: Fold changes from pre-OP over all time points. **** p<0.0001, *** p<0.001, p<0.01*

DISCUSSION & CONCLUSIONS: These are the first metabolomics results on FRI, elucidating systemic changes during this debilitating and hard-to-treat complication. Especially the depletion and slow recovery of cysteine opens a new research avenue for metabolic intervention in FRI to improve clinical outcomes.

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Antimicrobial spray-coating using poly-epsilon-lysine and hyaluronic acid to prevent prosthetic joint infection

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INTRODUCTION: Rising antimicrobial resistance to antibiotics will result in an increase in therapeutic insufficiency in prosthetic joint infection. New technologies are needed to protect the implants against bacterial adhesion, attachment and biofilm formation on the implant surface. Many of these implants have a complex geometry, made of various materials. This research aimed to create a coating made up of polypeptides and polysaccharides, with antimicrobial properties and no cytotoxicity. The spray-coating consists of Poly-epsilon-lysine (PEL) and hyaluronic acid (HA) (PEL/HA).

METHODS: To simultaneously apply PEL and HA on Titanium (Ti) and ultra-high-molecular-weight polyethylene (UHMWPE) substrates a single-step spray-coating technology was used. First, the coating deposition was observed by confocal microscopy after staining. Second, biocompatibility and cytotoxicity was assessed *in vitro* according to the ISO 10993-5 test for extracts, using WST-1 and LDH assays and L929 fibroblast cells. Last, *in vitro* antimicrobial activity against S. aureus (ATCC 6538) and E. coli (ATCC 8739) was tested using a combined version of the ISO 22196, ASTM ASTM E2180-18, and JIS Z 2801 test standards. Substrates coated with gentamicin were used as a control.

RESULTS: PEL/HA coatings were successfully constructed on the two substrates. According to ISO 10993-5 materials are non-cytotoxic if the relative cell viability for the 100% extract is ≥70% of the untreated cells. All concentrations of the PEL/HA coated samples extracts demonstrated a biocompatibility of ≥70% using the WST-1 assay. Furthermore, no significant statistical difference between biocompatibility of Ti and UHMWPE uncoated samples as compared to the PEL/HA coated samples was observed. Cytotoxicity measured with the LDH assay also remains <30% for all extracts. For both Ti and UHMWPE substrates, the PEL/HA coating demonstrated high antimicrobial activity against S. aureus and E. coli. Figure 1 shows a clinical relevant 3-to5- log reduction when comparing the uncoated samples to the PEL/HA coated samples after 24h of incubation.

DISCUSSION & CONCLUSIONS: PEL/HA coatings demonstrate high antimicrobial activity when deposited on different substrates, while remaining biocompatible with L929 cells. This coating holds potential for future use in protecting implants from infections by preventing bacterial adherence and attachment.

ACKNOWLEDGEMENTS: This publication is part of the EMILIO project (with project number LSHM21057), co-funded by the PPP Allowance made available by Health-Holland, Top Sector Life Sciences & Health.

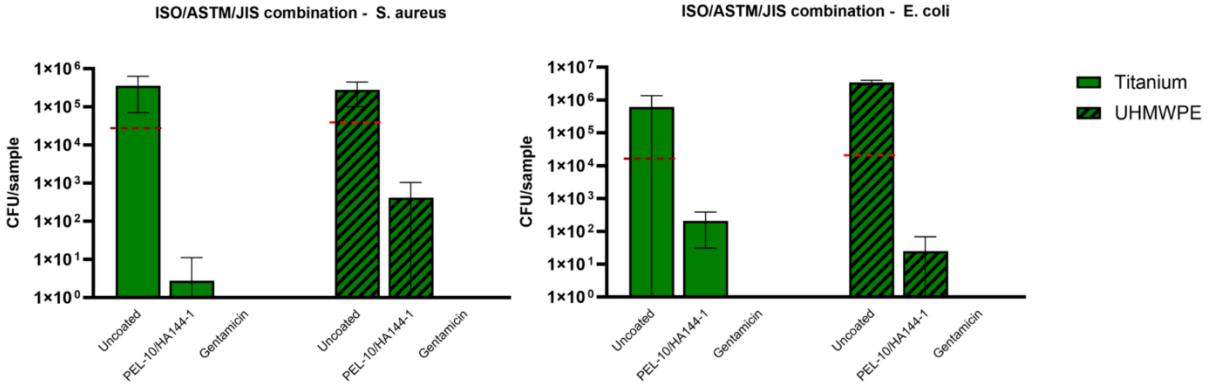

Figure 1 Viable bacteria retrieved from the samples in the ISO 22196, ASTM E2180-18, JIS Z 2801 combination test after 24h of incubation. Titanium substrates in green, and UHMWPE samples in green/black striped. The starting inoculum is represented by the red dashed line.

Prosthetic joint infection research models in NZW rabbits: Opportunities for standardization – a systematic review

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INTRODUCTION: Prosthetic joint infection (PJI) is a major complication following total knee and hip arthroplasty. Rising antimicrobial resistance (AMR) to antibiotics will result in an increase in therapeutic insufficiency in PJI. Numerous new technologies are currently being developed and tested *in vitro* to protect the implants against bacterial adhesion, attachment, and biofilm formation on the implant surface. Preclinical *in vivo* models are still needed to bridge the translational gap to clinical implementation. NZW rabbits have been used most frequently to study PJI *in vivo*. Though rabbit models have been used widely to study PJI and new antimicrobial technologies, there is no consensus about the exact methodology and outcome measures, and many common errors are made in these *in vivo* studies, as recently shown by several ORS-led systematic reviews. Due to discrepancies in methodologies and outcomes, results cannot be compared, and it is unclear when a technique's efficacy is sufficient to progress to a larger animal model or clinical studies. This systematic review will focus on *in vivo* models investigating PJI and new antimicrobial technologies in NZW rabbits. Both similarities and discrepancies will be highlighted between study methodologies, and opportunities for standardization will be defined.

METHODS: This literature search was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. The PubMed, Scopus, and EMBASE databases were searched for literature on prosthetic joint infection in rabbit models. Two independent researchers conducted screening and extraction utilizing the Covidence online platform. Data extraction included bias control, experimental design, and outcome measures of the NZW rabbit models in the articles.

RESULTS: A total of 551 studies were found on the three databases PubMed, Scopus, and Embase, of which 281 remained after duplicate removal. After screening, 57 articles remained that complied with the inclusion criteria. Extraction was divided into three parts: bias control, experimental design, and outcomes. Bias control focused on the blinding and randomization of the studies, the rabbit characteristics, humane endpoints, and caretaking of the rabbits. The aim and duration of the study, information about the inoculum and implant, interventions, and experimental groups, including dropout percentage per group are described in the experimental design. The outcome measurements focus on bacterial culture, health monitoring, hematology, histology, and histological staining. Based on the interventions against PJI observed in the included articles, the articles were divided into six groups: no intervention used against PJI (21%), revision surgery (14%), prevention of PJI with only antibiotics (21%), prevention of PJI with surface modifications (7%), prevention of PJI with coatings (23%), and others (14%).

DISCUSSION & CONCLUSIONS: Despite the current availability of guidelines and recommendations regarding experimental design, bias control, and outcome measures, many articles neglect to report on these matters. Categorization of the studies indicated that 21% used no intervention in their experiments, elucidating the importance and need for standardized guidelines. Furthermore, with the rise of AMR to antibiotics, it is striking that 21% of all articles investigate antibiotics as the sole antimicrobial agent. Ultimately, this analysis aims to assist researchers in determining suitable clinically relevant methodologies and outcome measures for *in vivo* PJI models using NZW rabbits to test new antimicrobial technologies. This analysis encourages future studies to adhere to the ARRIVE guidelines for transparent reporting of animal research.

ACKNOWLEDGEMENTS: This publication is part of the EMILIO project (with project number LSHM21057), co-funded by the PPP Allowance made available by Health-Holland, Top Sector Life Sciences & Health.

Carboxylized graphene quantum dots in a novel antimicrobial coating

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INTRODUCTION: One of the most common complications related to implantation of a biomaterial is biomaterial-associated infection (BAI). These infections may lead to chronic inflammation and in severe cases, loss of implant function and even the need for revision / replacement. Coating of biomaterial surfaces with antimicrobials is an important strategy to prevent infection. However, the use of antibiotics or antifungals is discouraged because of potential resistance development. Graphene quantum dots (GQD) may be an alternative for current prevention strategies. GQD consist of a single layer of carbon atoms in a honeycomb-like structure with photo-activation properties. Upon photo-activation, GQD produce reactive oxygen species (ROS) which can kill bacteria. In this study, we aimed to test colloidal GQD-COOH, as well as a novel GQD-COOH coating for its microbicidal activity against *Staphylococcus aureus*, *Escherichia coli, Candida auris* and *Candida albicans.*

METHODS & RESULTS: To test the microbicidal activity of colloidal GQD-COOH, we used the minimal microbicidal concentration (MMC) assay. After 30 minutes of photo-activation with a 435nm blue light LED, the lowest concentrations of colloidal GQD-COOH which killed 99.9% of the inoculum was 0.8 µg/ml for *S. aureus,* 13 µg/ml for *E. coli*, 6.25 µg/ml for *C. auris* and 12.5 µg/ml for *C. albicans*. Furthermore, we tested a novel GQD-COOH coating for its microbicidal activity using the Japanese Industrial Standard (JIS) assay, where we photo-activated the GQD-COOH coating with 435nm blue light for 30 minutes. The coating consisted of several alternating layers of GQD-COOH and polymer applied on glass slides. It showed strong activity against *S. aureus, E. coli*, *C. auris* and *C. albicans* as photo-activation of the GQD-COOH coating resulted in complete killing of all tested species.

DISCUSSION & CONCLUSIONS: Colloidal GQD-COOH and the GQD-COOH coating show promising microbicidal activity against Gram-positive and Gram-negative bacteria and fungi. Therefore, the GQD-COOH coating has potential for future application in e.g. wound dressings, catheters or external fixators.

ACKNOWLEDGEMENTS: This publication is part of the project DARTBAC (with project number NWA.1292.19.354) of the research programme NWA-ORC which is (partly) financed by the Dutch Research Council (NWO). This project has been supported by European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement no. 955664.

Galleria mellonella larvae: a promising animal model to study biofilm maturation in orthopaedic infections

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INTRODUCTION: In trauma surgery, the development of biomaterial-associated infections (BAI) is one of the most common complications affecting trauma patients, requiring prolonged hospitalization and the intensive use of antibiotics. Following the attachment of bacteria on the surface of the biomaterial, the biofilm-forming bacteria could initiate a chronic implant-related infection. Despite the use of conventional local and systemic antibiotic therapies, persistent biofilms involve various resistance mechanisms that contribute to therapeutic failures. The development of *in vivo* chronic BAI models to optimize antibiofilm treatments is a major challenge. Indeed, the biofilm pathogenicity and the host response need to be finely regulated, and compatible with the animal lifestyle.

Previously, a *Galleria mellonella* larvae model for the formation of an early-stage biofilm on the surface of a Kirschner (K)-wire was established. In the present study, two models of mature biofilm using clinical *Staphylococcus aureus* strains were assessed: one related to contaminated K-wires (*in vitro* biofilm maturation) and the second to hematogenous infections (*in vivo* biofilm maturation). Rifampicin was used as a standard drug for antibiofilm treatment.

METHODS: In the first model, biofilms were formed following an incubation period (up to 7 days) in the CDC Biofilm Reactor (CBR, BioSurface Technologies). Then, after implantation of the pre-incubated K-wire in the larvae, rifampicin (80 mg/kg) was injected and the survival of the larvae was monitored. In the second model, biofilm formation was achieved after an incubation period (up to 7 days) inside the larvae and then, after removing the K-wires from the host, *in vitro* rifampicin susceptibility assays were performed (according to EUCAST).

RESULTS: The first model indicate that *in vitro* biofilm maturation affects the bacterial pathogenicity in the host, depending on the *S. aureus* strain used. Furthermore, the more the biofilm is matured, the more the rifampicin treatment efficiency is compromised. The second model shows that, despite the fast *in vivo* biofilm formation in the host, the number of bacteria, either attached to the surface of the K-wire surface or in surrounding tissue of the larvae, was not increased over time.

DISCUSSION & CONCLUSIONS: Altogether, these results allow the establishment of biofilm models using *G. mellonella* larvae in order to understand the impact of biofilm maturation on both the bacterial pathogenicity and the efficiency of antibiofilm treatments.

Diagnosis of infected and aseptic non-union correlating local gene expression and systemic proteomics, miRNA, and immune cells profiles.

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INTRODUCTION: A critical diagnosis in patients presenting with fracture non-union is the differentiation between infected and aseptic non-union. A preoperative diagnosis, without requiring culture of invasive biopsies, would be preferable as intraoperative decisions largely differ between both scenarios. The aim of this study was to profile preoperative blood samples from patients with non-union and submitted for proteomics, miRNA analyses and peripheral blood mononuclear cells (PBMCs) immunophenotyping and cross-referenced with gene expression data from non-union tissue biopsies to identify potential biomarkers.

METHODS: This prospective multicenter study enrolled patients undergoing revision surgery of femur or tibia non-union. One hundred thirty-seven patients were recruited in the eight level I trauma centers in Germany between January 2019 and April 2022. Patients with implant removal after regular fracture healing (HEAL) were included as a control-group. Preoperative blood samples, intraoperative tissue samples, sonication of osteosynthesis material and 1-year-follow-up questionnaire were taken. Non-union patients were grouped into infected (INF) or aseptic (AS) after assessing bacterial culture and histopathology of intraoperative biopsies. Expression of matrix metalloproteinase-1 (MMP-1) and osteocalcin (OCN) were analyzed using qPCR in intraoperative tissue samples. Targeted proteomics was used to investigate a predefined panel of 45 cytokines in preoperative blood samples. Isolation of miRNA from patients' plasma and miRNA sequencing was performed by Qiagen according to standard protocols. Peripheral blood mononuclear cells (PBMCs) were immunophenotyped using high-dimensional mass cytometry. The study was approved by the Ethics Committee of the Institutional and National Medical Board (Bavarian State Chamber of Physicians, ID 2016-16041).

RESULTS: In total 62 AS, 43 INF, and 32 HEAL patients were recruited. Patients in the two non-union groups (INF and AS) did not differ concerning smoking, diabetes or initial open or closed fracture. Microbiological analyses of intraoperative samples and sonication fluid from the osteosynthesis material revealed a higher occurrence of *Cutibacterium acnes* (*C. acnes*) and Coagulase-negative staphylococci (CoNS) compared to other pathogens.
Proteomics analyses revealed significant increased expression of Macrophage Colony Stimulating Factor 1 (MCSF-1), Hepatocyte Growth Factor (HGF), Interleukin (IL)-6, and Matrix Metalloproteinase 1 (MMP-1) in INF patients compared to AS and HEAL patients. Additionally, *MMP1* was also found to be significantly more expressed in the local tissues of INF patients compared to AS (p=0.0096) and HEAL patients (p<0.0001). MiRNA analyses showed marked differences between HEAL *vs* AS and HEAL *vs* INF patients. One miRNAs was identified as differentially expressed between AS and HEAL: hsa-miR-545-5p. The PBMCs immune profiling showed that AS patients have a significant increase in Treg CD4+ (p=0.0119) compared to HEAL patients and AS and S patients show a significant increase in monocytes (p=0.0097 and p<0.0001) compared to HEAL patients.

DISCUSSION & CONCLUSIONS: This study shows that preoperative blood samples have potential for an earlier detection of infection. Although no single biomarker is sufficient to differentiate these patients preoperatively in isolation, future multivariant analysis of the cytokine data, miRNAs, gene expression and immune cells in combination with clinical characteristics may provide valuable diagnostic insights.

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A novel quaternary ammonium coating of titanium implants prevents murine staphylococcal biomaterial-associated infection

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INTRODUCTION: Infection of implanted medical devices (biomaterials), like titanium orthopaedic implants, can have disastrous consequences, including removal of the device. These so-called biomaterial-associated infections (BAI) are mainly caused by *Staphylococcus aureus* and *Staphylococcus epidermidis*. Formation of biofilms on the biomaterial surface is generally considered one of the main reasons for these persistent infections, although bacteria may also enter the surrounding tissue and become internalized within host cells, or colonize canaliculi in the bone. To prevent biomaterial-associated infection using a non-antibiotic based strategy, we aimed to develop a novel antimicrobial coating for titanium devices based on stable immobilized quaternary ammonium compounds (QACs).

METHODS: Medical grade titanium implants (10×4×1 mm) were dip-coated in a solution of 10% (*w/v*) hyperbranched polymer, subsequently in a solution of 30% (*w/v*) polyethyleneimine and 10 mM sodium iodide, followed by a washing step for 10 min in ethanol. The QAC-coating was characterized using water contact angle measurements, scanning electron microscopy, FTIR, AFM and XPS. The antimicrobial activity of the coating was evaluated against *S. aureus* strain JAR060131 and *S. epidermidis* strain ATCC 12228 using the JIS Z 2801:2000 surface microbicidal assay. Lastly, we assessed the *in vivo* antimicrobial activity in a mouse subcutaneous implant infection model with *S. aureus* administered locally on the QAC-coated implants prior to implantation to mimic contamination during surgery.

RESULTS: Detailed material characterization of the titanium samples showed the presence of a homogenous coating layer at the titanium surface. Moreover, the coating successfully killed *S. aureus* and *S. epidermidis in vitro*. In the murine subcutaneous implant infection model the QAC-coating strongly reduced *S. aureus* colonization of both the implant surface and the surrounding tissue, with no apparent macroscopic signs of toxicity or inflammation in the peri-implant tissue at 1 and 4 days after implantation.

CONCLUSIONS / IMPACT: We developed an antimicrobial coating with quaternary ammonium compounds on titanium which holds promise to prevent BAI. Since the coating is not relying on antibiotics, it is not associated with an increased risks of antibiotic resistance development. As a permanent coating, it is expected to provide long term protection against the much-dreaded infections of orthopaedic implants.

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Probiotic therapy promotes wound healing by modulating PI3K- and TGF-Smad signaling pathways

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INTRODUCTION: Skin wound healing represents a dynamic and intricate biological process involving the coordinated efforts of various cellular and molecular components to restore tissue integrity and functionality[1]. Among the myriad of cellular events orchestrating wound closure, fibroblast migration and the regulation of fibrosis play pivotal roles in determining the outcome of wound healing[2]. In recent years, probiotic therapy has emerged as a promising strategy for modulating wound healing and fibrosis[3], but the underlying mechanisms are still not well understood. Therefore, we here investigate the effect and underlying pathways of a probiotic mixture (BioK) on the promotion of cell migration and anti-fibrotic behavior of human dermal fibroblast (HDFs).

METHODS: Probiotic mixture BioK (*Lactobacillus acidophilus CL1285, Lacticaseibacillus casei LBC80R and Lacticaseibacillus rhamnosus CLR2*) was co-cultured with HDFs in ibidi culture plates with scratch at different multiplicity of infection ratios (MOI 0.01, 0.1 and 1). Bright-field and confocal microscopy (CLSM) were used to record cell migration, and ImageJ software was used to quantify migration rates. RNA-seq analysis was used to further interrogate the potential molecular mechanism underlying the effects of BioK on cell migration and qPCR was employed to confirm identified signalling pathways and key genes involved. The relative expression of Nox-4, α-SMA and Col-I, genes representative for fibroblast to myofibroblast differentiation, was characterised by RT-PCR. Furthermore, protein expression for α-SMA and Col-I was visualised by confocal microscopy. Changes in lactate during cell culture were recorded by pH meter and high-performance liquid chromatography (HPLC).

RESULTS & Conclusion: We observed that BioK effectively promoted the cell migration of human dermal fibroblast in-vitro, which was found to be related to an up-regulation of genes (i.e. Paxillin, PI3K, PKC and ITG-β1) that are involved in the phosphoinositide 3-kinase (PI3K) signaling pathway. Interestingly, we also found that BioK down-regulated gene expression of Nox-4, α-SMA and Col-I, which are involved in TGF-Smad signaling pathway - a major axis in controlling the differentiation of fibroblasts to myofibroblast. Also, protein expression of SMA and and Col-I could be observed, collectively demonstrating BioK's potential to reduce scar formation. One of the mechanisms for this down-regulation was identified to be BioK-produced lactic acid, which lowers the surrounding pH. This is in agreement with previous reports showing that acidic pH plays a major role in fibroblast activity and wound healing.

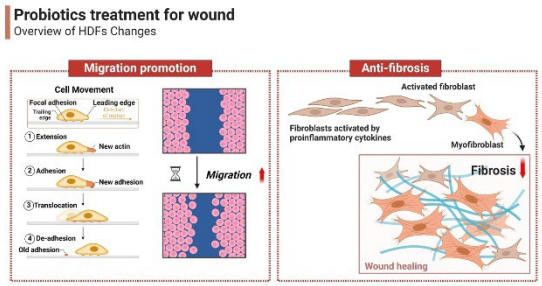

Fig. 1: Schematic showing promoting effect of probiotic BioK upon co-cultured with HDF on cell migration and anti-fibrosis.

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Evaluation of antibiotic-loaded bone cement and cemented K-wires against bacterial infections in *Galleria mellonella*

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INTRODUCTION: Infection of inserted or implanted medical devices can have disastrous consequences to patient health, including removal of the device. Staphylococci are the major cause of implant associated infection (IAI). The placement of cemented implants is often required during surgery for IAI (*e.g.*, PJI and FRI) to eradicate infections. *In vivo* models play a major role in studying the pathogenesis of IAI, biofilm development *in situ*, and the efficacy of (novel) preventive or treatment strategies. However, the majority of these *in vivo* models are performed with a single implant (such as Kirschner (K)-wires), and only a few studies with models employing cemented implants are reported. Here, we aimed to assess the antimicrobial activity of antibiotic-loaded bone cement (ALBC) implants and cemented K-wires.

METHODS: Commercially available bone cement (Palacos R, Palacos R+G, COPAL G+C and COPAL G+V) was purchased from Heraeus GmbH. To prepare ALBC samples as discs, and rod-shaped implants and cemented K-wires for implantation, Teflon molds were used. Firstly, the zone of inhibition of ALBC discs was determined with *Staphylococcus aureus* EDCC 5055. Next, the antimicrobial activity of the ALBC implants and cemented K-wires were analysed in the *Galeria mellonella* larvae using either the hematogenous (*i.e.*, *S. aureus* injection at 60 min after implantation) or the early-stage biofilm (*i.e.*, incubation of implants for 30 min in a *S. aureus* solution prior to implantation) implant infection model. The larvae were incubated at 37°C for five days after implantation and infection, and their survival was monitored. Moreover, the number of bacteria on the implant surface and in the tissue of the larvae after 24h was determined by sonicating the implant and homogenizing the tissue, respectively, followed by quantitative culture of the bacteria. Lastly, scanning electron microscopy (SEM) analyses was performed to visualize biofilm formation on the implant surface.

RESULTS: No adverse effects were observed after implantation of the ALBC implants or cemented K-wires. The larvae receiving Palacos R+G, COPAL G+C or COPAL G+V ALBC implants or cemented K-wires showed higher survival rates compared to the Palacos R control implants without antibiotics. The ALBC implants and cemented K-wires with a combination of antibiotics (*i.e.*, Copal G+C and Copal G+V) demonstrated superior survival over Palacos R+G, containing only gentamicin. A significant reduction in numbers of bacteria in the larval tissue as well as on the surface of the ALBC implants and cemented K-wires was observed. Further, SEM analysis showed the formation of a thick biofilm on the Palacos R control samples and a biofilm with a reduced thickness on the surface of Palacos R+G samples. However, no biofilms were found on the surface of Copal G+C and G+V samples.

DISCUSSION & CONCLUSIONS: In conclusion, the *G. mellonella* larvae infection models with antibiotic-loaded cemented K-wires mimic the patient's situation well.

Characteristics, diagnosis and treatment experience of Brucella spondylitis in non-endemic areas: a retrospective study in Guangdong Province, China

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INTRODUCTION: Brucella spondylitis has been observed to increasingly spread from epidemic areas to non-epidemic areas in recent years. [1] However, misdiagnosis and overlooked cases frequently occur due to clinicians in non-epidemic areas lacking sufficient knowledge about the disease. The objective of this study is to retrospectively analyze the characteristics and clinical results of individuals with Brucella spondylitis in non-epidemic regions, aiming to improve the diagnosis and treatment in such areas.

METHODS: A retrospective study was conducted on 32 patients with Brucella spondylitis between January 2015 and December 2022 at the Third Affiliated Hospital of Southern Medical University in Guangzhou of China. All the data was analyzed including their medical history, clinical manifestations, laboratory indicators, imaging data, bacterial culture results, treatment plans and follow-up outcomes.

RESULTS: A low diagnostic rate of Brucella spondylitis was demonstrated in non-epidemic areas, especially in grassroots hospitals. Patients in these areas shared similarities with those in epidemic areas in terms of age, gender ratio, and urban-rural sources. [2-3] However, there were differences in infection risk factors. In non-epidemic areas, foodborne infections were predominant (71.87%), with a relatively high proportion of non high-risk occupational groups (62.50%). Inflammatory pain (96.88%), neurological symptoms (50.00%), and fever (37.50%) were the most common clinical symptoms, with fewer fever cases and atypical intermittent fever. The positive rates of blood culture (54.5%) and lesion puncture culture (55.56%) were not particularly high. MRI examination is crucial for early diagnosis and differential diagnosis of diseases. The lumbar spine (56.25%) and lumbosacral region (18.75%) were most commonly affected, with a relatively mild degree of intervertebral space stenosis. It has been shown that conservative treatment was not only effective in many cases, but also reduced the economic burden on patients. But surgical intervention was considered necessary, for patients with worsening symptoms of spinal cord/nerve compression as well as spinal instability caused by vertebral destruction.

DISCUSSION & CONCLUSIONS: The characteristics of Brucella spondylitis in the majority of cases in non-epidemic areas is consistent with those in epidemic areas despite some differences in infection factors, occupational population, and clinical manifestations. It is important for clinicians in non-epidemic areas to focus on distinguishing patients with elevated infection indicators from those with spinal degenerative diseases. A comprehensive assessment is recommended by integrating imaging, specificity testing, and bacterial culture results. Confirmed patients are expected to receive long-term combination therapy with antibiotics, while patients with worsening symptoms of spinal cord/nerve compression as well as spinal instability caused by vertebral destruction may need surgical intervention.

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