

# Biomaterial-induced inhibition of *Staphylococcus aureus* biofilm formation

## Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademien, Göteborgs universitet kommer att offentligens försvaras i Hörsal Arvid Carlsson, Medicinargatan 3, den 13e September, klockan 13.00.

av **Adam Turner**

## Fakultetsopponent:

Prof. Dr. Martijn Riool, Laboratory of Experimental Trauma Surgery  
University Hospital Regensburg, Regensburg, Germany

## Avhandlingen baseras på följande delarbeten

- I. Turner, A.B†, Gerner, E†, Firdaus, R†, Echeverz, M., Werthén, M., Thomsen, P., Almqvist, S., Trobos, M., 2022. Role of sodium salicylate in *Staphylococcus aureus* quorum sensing, virulence, biofilm formation and antimicrobial susceptibility. *Front Microbiol* 13, 931839.  
<https://doi.org/10.3389/fmicb.2022.931839>
- II. Giraldo-Osorno, P.M†, Turner, A.B†, Mollet Barros, S†, Büscher, R., Gutttau, S., Asa'ad, F., Trobos, M., Palmquist, A. Anodized Ti6Al4V-ELI, electroplated with copper is bactericidal against *Staphylococcus aureus* and enhances macrophage phagocytosis. *Submitted for publication.*
- III. Bartkowska, A†, Turner, A.B†, Blanquer, A., Nicolenco, A., Trobos, M., Nogues, C., Pellicer, E., Sort, J., 2023. Accelerated biodegradation of FeMn porous alloy coated with ZnO: Effect on cytocompatibility and antibiofilm properties. *Surface and Coatings Technology* 471, 129886.  
<https://doi.org/10.1016/j.surfcoat.2023.129886>
- IV. Turner, A.B†, Zermeño-Pérez, D†, Mysior, M.M., Giraldo-Osorno, P.M., O' Gorman, E., Oubih, S., Simpson, J.C., Lasa, I., Ó Cróinín, T., Trobos, M. Biofilm morphology and antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) on poly-D,L-lactide-co-poly(ethylene glycol) (PDLLA-PEG) coated titanium. *Submitted for publication.*
- V. Turner, A.B†, Giraldo-Osorno, P.M†, Douest, Y†, Morales-Laverde, L.A., Bokinge, C.A., Asa'ad, F., Courtois, N., Palmquist, A., Trobos, M. Race for the surface between THP-1 macrophages and *Staphylococcus aureus* on various titanium implants with well-defined topography and wettability. *Submitted for publication.*

† Equal contribution

**SAHLGRENKA AKADEMIN**  
**INSTITUTIONEN FÖR KLINISKA VETENSKAPER**



# Biomaterial-induced inhibition of *Staphylococcus aureus* biofilm formation

Adam Turner

Department of Biomaterials, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Sweden, 2024

## Abstract

Biomaterial-associated infection (BAI) is a severe complication linked to medical device implantation, causing significant patient suffering and high healthcare costs. The pathogenesis of BAI is complex and not fully understood. Infections often occur during the peri-implantation period due to microbial contamination of the implant and surrounding tissues. *Staphylococcus aureus* is the primary causative agent of BAI, due to its array of toxic factors, biofilm formation, and immune evasion mechanisms, which exploit implant-associated local immunocompromise. The ongoing threat of antimicrobial resistance necessitates new strategies to treat and prevent BAI. **This thesis aimed to** evaluate anti-virulence strategies and infection-resistant biomaterials that intend to control BAI through the modulation of *S. aureus* pathogenicity, incorporation of bactericidal metals, prevention of biofilm formation, improvement of the local immune response, and enhancing tissue integration.

Quorum sensing (QS) inhibition of *S. aureus* by sodium salicylate (NaSa) reduced the overall virulence and toxicity of *S. aureus* (**Paper I**). While NaSa did not fully prevent biofilm formation on titanium (Ti), it increased the susceptibility of *S. aureus* biofilms to antimicrobial agents like rifampicin and silver. The effect of NaSa on biofilm formation depended on the QS type (*agr* I-IV) and the material surface (polystyrene, Ti, or 3D collagen wound model). Incorporating metallic ions such as zinc and copper (**Paper II and III**) into an iron-manganese alloy and Ti, respectively, reduced *S. aureus* biofilm formation. Zinc ion release significantly reduced the biovolume and growth rate of *S. aureus* biofilms, while copper ion release was bactericidal towards *S. aureus* lab and clinical strains, achieving complete eradication in some conditions. Copper also increased phagocytosis of heat-killed *S. aureus* by THP-1 macrophages and contributed to their polarisation towards both M1 and M2 phenotypes. In **Paper IV**, PEGylated-PDLLA coatings on Ti proved cytocompatible and significantly reduced biofilm formation of multi-resistant *S. aureus*. This disruption increased the susceptibility of *S. aureus* to several antibiotics, and adding silver provided complementary bactericidal effects without impacting cytocompatibility. In **Paper V**, “race for the surface” experiments with macrophages and *S. aureus* on nano/microscale laser-modified Ti surfaces highlighted the importance of host tissue integration as a preventive strategy against BAI. When *S. aureus* formed a biofilm before the arrival of macrophages, *S. aureus* won the race, secreting virulence factors, eliciting strong cytotoxicity, and promoting biofilm persistence and intracellular survival. When macrophages reached the surface first or simultaneously with *S. aureus*, phagocytic efficacy improved, resulting in fewer viable intracellular *S. aureus* and increased macrophage colonisation.

**In conclusion**, the investigated strategies show potential to enhance biomaterial infection resistance for the clinical management of BAI. Balancing antimicrobial properties with host integration is complex due to the multifaceted pathogenesis of BAI, making it unlikely that any single strategy will resolve the issue. This thesis provides new insights into macrophage-*S. aureus* interactions at the biomaterial surface and represents an *in vitro* proof-of-concept for the potential clinical translation of these antimicrobial strategies.

**Keywords:** Biomaterial-associated infection, *Staphylococcus aureus*, biomaterials, macrophage, titanium, biofilm, antimicrobial, antifouling, immunomodulation, infectious disease, infection control, copper, iron-manganese, PDLLA-PEG, race-for-the-surface.