REVOLUTIONIZING BIOFILM PREVENTION: MICROCALORIMETRIC ANALYSIS OF ANTIMICROBIAL COATINGS AND MATERIALS

INTRODUCTION

Bacterial growth on surfaces is a major problem in many areas of life, from water pipes to medical devices. Efforts are constantly being made to develop materials that will prevent the attachment of bacteria and buildup of biofilms. These efforts include screening and comparing many materials and coatings[1]. Conventional methods rely on the disruption of biofilms from materials and quantifying the bacteria by plating. Not only is this approach time-consuming, but it is also dependent on disruption efficacy. Sonication does not remove all the bacteria from surfaces, and bacterial clumping may lead to an underestimation of the bacterial load[2]. The problems escalate as the material structure becomes more porous and complex.

Microcalorimetry, a method that measures the heat flow changes associated with physical, chemical, or biological processes, offers a unique advantage[3]. Unlike conventional methods that rely on optical measurements, microcalorimetry allows for the direct evaluation of bacterial metabolism and load directly on the material of interest.

RESULTS

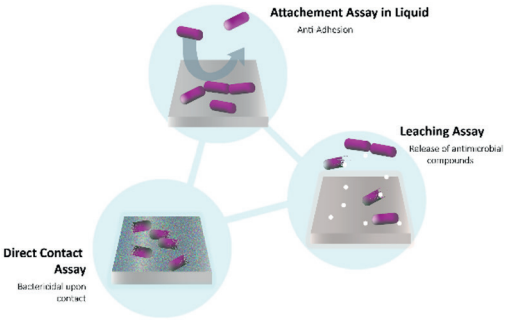

Figure 1 Overview of different material properties and micro-calorimetric assays to study them.

Three basic setups can be used: the study of bacterial attachment, leaching of antimicrobial compounds, and bacterial killing by contact (Figure 1). These setups can be used to develop more complex experimental designs and achieve more *in vivo*-like environments.

Evaluating anti-adhesive surface treatments

The first step of biofilm formation is attachment and if that step can be avoided then downstream issues will be prevented. By comparing the heat flow profiles of untreated versus treated surfaces (e.g., surfaces coated with anti-adhesive polymers), the effectiveness of different surface treatments in preventing bacterial attachment can be evaluated.

The lag time (time to activity) in the heat flow curve is proportional to the initial bacterial load and can be used to estimate the number of attached cells with the help of standard curves.[4] In this assay, the material of interest is incubated with bacteria in PBS for a couple of hours, allowing the bacteria to attach. Thereafter, unattached bacteria are washed away, and the material is placed in broth then into the calScreener (Figure 2A). With high reproducibility, it is observed that coating-A had less bacteria attached after incubation compared to the uncoated material, based on time to activity. Furthermore, coating-A seems to affect the bacterial metabolism and reduces the peak height of the curves (Figure 2B).

Testing antimicrobial compound release

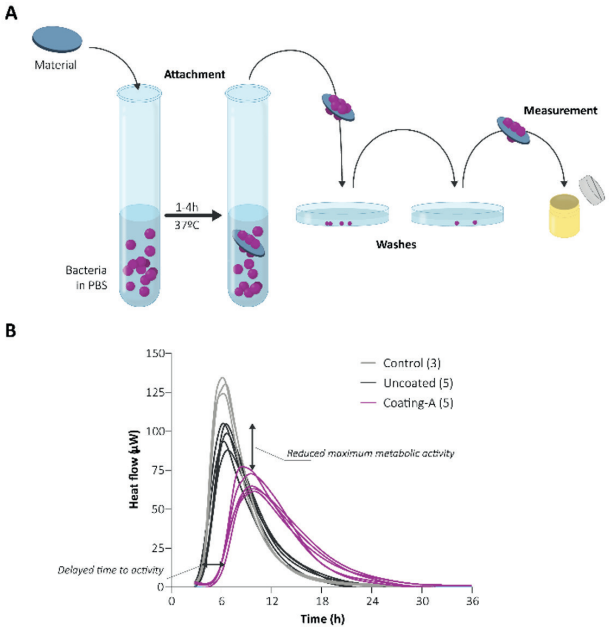

Figure 2A Visual overview of the attachment assay steps. B: Following the step above, rods incubated with S.aureus were placed in TSB, and bacterial load was evaluated in calScreener. Growth control (light grey) shows higher metabolic activity than uncoated rods (dark grey) and rods with coating-A (purple).

The material of interest may be designed to release antimicrobial compounds. To test materials for leached antimicrobial compounds, the basic setup involves incubating the material in a bacterial culture medium to allow any antimicrobial agents to leach out into the media. The media containing the leached antimicrobial com-pounds is transferred to a fresh bacterial culture without the material. The heat flow associated with bacterial growth is then measured, providing insight into the antimicrobial efficacy of the leached compounds. In this example, different metals were incubated in tryptic soy broth for one hour. Thereafter, the broth was inoculated with S.aureus and trans-

ferred to the calScreener. Copper ions leached from brass discs displayed antimicrobial properties, as indicated by the shift of the thermogram showing a longer time to activity (Figure 3).

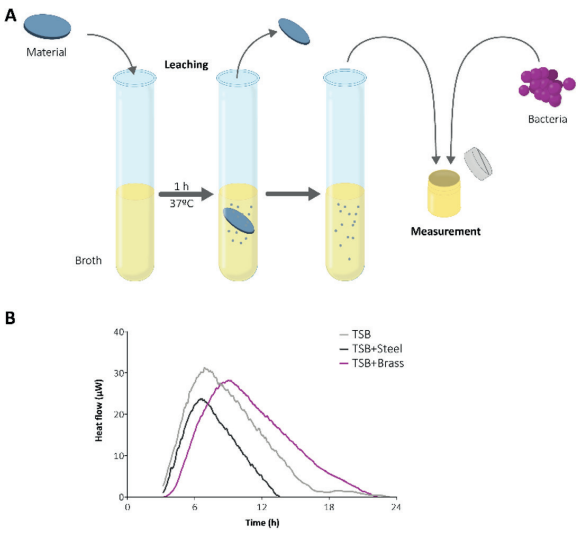

Figure 3A: Visual overview of the leaching assay steps. B: Following the step above, TSB was incubated with discs, inoculated with S.aureus, and evaluated in calScreener. Copper leached from brass discs (purple) increase the lag time compared to growth control (light grey) and steel discs (dark grey).

Contact-dependent killing assay

To test materials for contact-dependent bacterial killing using microcalorimetry, bacteria are directly applied to the surface of the material of interest. The material is then inverted onto an agar cast and into plastic inserts, ensuring that bacteria can only grow where they remain in contact with the material. In this experiment, *S. aureus* was spotted on steel slugs with or without a copper coating and flipped onto agar. Growing in contact with uncoated metal decreases the metabolic output of *S. aureus*. However, the copper coating completely suppresses it (Figure 4).

A modification of this experiment involves embedding the material within semisolid agar, which prevents any planktonic bacterial growth. This setup ensures that any observed bacterial growth and corresponding heat flow changes are due solely to bacteria in direct contact with the material, thus providing a precise measurement of the material's contact-dependent antimicrobial efficacy.

IMPLEMENTATION CASES

Microcalorimetric experiments can be adapted for more advanced applications to mimic *in vivo* environments, providing a closer approximation to clinical scenarios. For example, adding biological fluids such as blood or pus to the experimental setup can simulate conditions found in patient, offering insights into how materials perform under real life conditions. Testing more complex materials, such as catheter tubing or ban-dages, allows for the assessment of antimicrobial efficacy in medical devices. By correlating calorimetric data with surface properties like roughness and hydrophobicity, it is possible to deduce how these factors influence bacterial attach-ment and biofilm formation. This comprehensive approach enables a deeper understanding of material-bacteria interactions and aids in the development of more effective antimicrobial materials for medical applications.

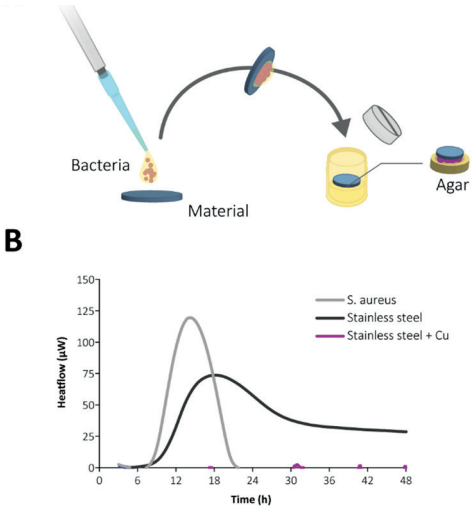

Figure 4A Graphic overview of the contact-killing assay. B: Following the step above, S.aureus was spotted onto metal discs and flipped onto agar casts in inserts. Bacterial activity was monitored using calScreener. Steel coated with copper (purple) inhibits the bacterial activity completely, whereas steel discs (dark grey) only slightly affect the bacterial metabolism compared to growth control (light grey).

CONCLUSIONS

Using the calScreener for material evaluation offers significant advantages in developing antimicrobial surfaces and coatings. This tool provides real-time measurements of heat flow, enabling dynamic moni-toring of bacterial metabolic responses to various materials. Its versatility allows testing under diverse conditions, including complex materials such as porous surfaces, catheter tubing, and bandages, ensuring a comprehensive evaluation of antibacterial properties.

By adopting the calScreener, researchers can accelerate the screening process, reduce costs, and improve the reproducibility of material research. This approach enhances the efficiency of creating antimicrobial sur-faces and supports the development of cost-effective, optimized materials for preventing bacterial growth. Furthermore, the calScreener helps researchers gain a deeper understanding of the fundamental interactions between bacteria and material surfaces, leading to better strategies for preventing bacterial attachment and biofilm formation in various applications, from medical devices to industrial pipelines.

References

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