

Comparison of different methods to quantify bacterial biofilms on implants

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Today, the use of implants has become a standard treatment procedure and the number of patients is continuously rising. Concurrently, the number of biofilm-associated implant infections is also increasing causing time consuming and cost intensive treatments. Newly developed antibacterial implant materials that aim at avoiding biofilm formation must undergo an exhaustive in vitro evaluation process before obtaining an official approval as a biomaterial for patient treatment. This study compares commonly used microscopic, microbiologic and biochemical methods to quantify bacterial biofilms on implant surfaces to unravel their comparability and applicability.

Material and Methods

Staphylococcus aureus (DSM 20231) and *Aggregatibacter actinomycetemcomitans* (MCCM 2474) were used as model organisms in this study. Microbial adhesion was evaluated after 5 hours of incubation in 50 mM TRIS HCl buffer and biofilm formation was studied after 24 hours of culturing in tryptic soy broth supplemented with 50 mM glucose (*S. aureus*) or Schaedler broth (*A. actinomycetemcomitans*) respectively. The microbial adherence to titanium, a standard implant material, and to copper, as an antibacterial substratum, was evaluated under the described experimental conditions. Bacteria were quantified using the following methods: LIVE/DEAD staining and confocal laser-scanning microscopy, enzymatic detachment or detachment by sonication combined with CFU counting, a resazurin-based assay, the BacTiter Glo™ assay and crystal violet staining.

Results

All assays were suitable to demonstrate a reduced microbial attachment to copper surfaces. Nevertheless, the determined reduction ratios were strongly dependent on the chosen experimental method.

Conclusion

The described assays are based on different (bio-) chemical/ microbiological reference parameters to quantify biofilm formation. Therefore, an appropriate method should be carefully chosen on the basis of the respective scientific question. For some issues it may become necessary to use more than one assay to comprehensively quantify biofilms.