Cell-to-cell aggregation in *S. epidermidis* and its effect on quantification of total and viable bacteria within biofilms

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Biofilms forming on the surface of indwelling medical devices by microorganisms such as *Staphylococcus epidermidis*, act as a source of acute infections. Since colonization of medical devices represents a serious problem in public healthcare-associated infections, bacteria forming biofilms have been an important issue often studied. Proper quantification of viable bacteria within *S. epidermidis* biofilms can however be challenging. Often, biofilm quantification of *S. epidermidis* is performed with colorimetric methods but these do not provide information regarding viable bacteria. CFU counting is often used but in the case of *S. epidermidis*, a bacteria that normally grows in clusters of cells, sonication is always required in order to obtain individual cells. In older biofilms, the number of dormant bacteria is expected to be higher than in young biofilms. Therefore, disrupting a biofilm structure without damage the cells in older biofilms can be a challenge.

Here, biofilm samples of *Staphylococcus epidermidis* 9142 strain grown for 24, 48 or 72H in TSB supplemented with 0,5% glucose were ressuspended 1 mL of physiological saline solution and sonicated at different cycles. Following sonication biofilms were quantified using three different approaches: colorimetric methods, CFU counting, and microscopic evaluation using a Neübauer chamber coupled with staining with fluorescence dye *LIVE*/*DEAD*® BacLight ™ Bacterial Viability Kit (Molecular Probes Inc). Cell counting was optimized using Sigma Scan Pro and validated against manual counting of the images.

In the conditions used, higher numbers of sonication cycles prevented any clustering of cells but were affecting cell viability. On the other hand, lower numbers of sonication cycles were not effective in completely eliminating cell clusters, especially in 72H-old biofilms. The presence of the cell clusters at the lower sonication cycles resulted in high variability of CFU counting. On the other hand, cell counting with a Neübauer chamber was the best way to proper quantify the total and viable bacteria within the biofilms. By using the automatic counting software and validating the methodology, quantification of biofilms was relatively fast and reliable to perform.

Keywords Biofilm; *Staphylococcus epidermidis*; Sonication, automatic cell counting