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Fluorescence-based bacterial overlay method for simultaneous in situ quantification of surface-attached bacteria.

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For quantification of bacterial adherence to biomaterial surfaces or to other surfaces prone to biofouling, there is a need for methods that allow a comparative analysis of small material specimens. A new method for quantification of surface-attached biotinylated bacteria was established by in situ detection with fluorescence-labeled avidin-D. This method was evaluated utilizing a silicon wafer model system to monitor the influences of surface wettability and roughness on bacterial adhesion. Furthermore, the effects of protein preadsorption from serum, saliva, human serum albumin, and fibronectin were investigated. *Streptococcus gordonii*, *Streptococcus mitis*, and *Staphylococcus aureus* were chosen as model organisms because of their differing adhesion properties and their clinical relevance. To verify the results obtained by this new technique, scanning electron microscopy and agar replica plating were employed. Oxidized and poly(ethylene glycol)-modified silicon wafers were found to be more resistant to bacterial adhesion than wafers coated with hydrocarbon and fluorocarbon moieties. Roughening of the chemically modified surfaces resulted in an overall increase in bacterial attachment. Preadsorption of proteins affected bacterial adherence but did not fully abolish the influence of the original surface chemistry. However, in certain instances, mostly with saliva or serum, masking of the underlying surface chemistry became evident. The new bacterial overlay method allowed a reliable quantification of surface-attached bacteria and could hence be employed for measuring bacterial adherence on material specimens in a variety of applications.