Applied and Environmental Microbiology, Apr. 2005, p. 2175–2178 Vol. 71, No. 4
0099-2240/05/$08.00+0 doi:10.1128/AEM.71.4.2175–2178.2005

Viscoelasticity of *Staphylococcus aureus* Biofilms in Response to Fluid Shear Allows Resistance to Detachment and Facilitates Rolling Migration

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Received 10 May 2004/Accepted 5 November 2004

Staphylococcus aureus **is a leading cause of catheter-related bloodstream infections and endocarditis. Both involve (i) biofilm formation, (ii) exposure to fluid shear, and (iii) high rates of dissemination. We found that viscoelasticity allowed *S. aureus* biofilms to resist detachment due to increased fluid shear by deformation, while remaining attached to a surface. Further, we report that *S. aureus* microcolonies moved downstream by rolling along the lumen walls of a glass flow cell, driven by the flow of the overlying fluid. The rolling appeared to be controlled by viscoelastic tethers. This tethered rolling may be important for the surface colonization of medical devices by nonmotile bacteria.**

Biofilm infections are increasingly associated with a variety of medical prostheses (e.g., vascular and orthopedic implants) and delivery devices (e.g., percutaneous catheters), as well as with diseases such as native-valve endocarditis and infectious kidney stones (1, 2, 3, 13). *Staphylococcus aureus* is a key pathogen, feared for its high mortality rate and antibiotic resistance (10). Endovascular biofilm infections caused by *S. aureus* also carry a high risk of metastasis (4, 10). These biofilms are exposed to a broad range of fluid shears, ranging from the continuous, laminar flow in infected infusion lines to the highly variable, turbulent flow in the case of bacterial vegetations on a heart valve. Therefore, the continuous adaptation to mechanical stresses is an important feature of biofilm physiology (7). The responses of biofilms to shear stresses may determine biofilm dissemination in general and the rate of formation and size of biofilm emboli in particular. In previous work, we showed that *S. aureus* biofilm emboli ranged in size from single cells to microcolonies containing thousands of cells in an in vitro catheter infection model (6). Large emboli expressed antibiotic (oxacillin) tolerance similar to that observed in attached biofilms. In the present study, we used a glass capillary system previously used to mimic physiological shear in both catheter (6) and vascular (9) infection models to quantify the viscoelastic response and motion of *S. aureus* biofilm microcolonies in response to varying or steady fluid shear over short (seconds) and sustained (minutes) periods. Biofilms were grown from an *S. aureus* strain (ATTC 25923) on 1/10-strength brain heart infusion broth at 37°C. One milliliter of a 24-h broth culture was inoculated into a 1-mm^2, 140-mm-long glass capillary flow cell (model FC91; BioSurface Technologies, Bozeman, Mont.) integrated into a once-through flow system. Biofilms were monitored with a Cohu (San Diego, Calif.) model 4910 camera mounted on an Olympus BH2 microscope using Scion Image software (Scion Inc., Frederick, Md.). After an attachment period of 30 min, a continuous laminar flow rate (Q) of 60 ml h^{-1} was established to approximate the hydrodynamic conditions typically used in a central venous catheter (6). The average velocity was 1.67 cm s^{-1}, the Reynolds number was 17, and the wall shear stress (τ_w) (at the center line of the flow cell) was 0.125 Pa. The flow rate, and thus the wall shear stress, was controlled with a peristaltic pump. Hydrodynamic parameters were calculated from the flow rate and reactor dimensions as described elsewhere (15). Measurements were made after a 3-day growth period from three independent experiments.

Estimates of the structural responses of the biofilms to rapid fluctuations in τ_w and of the shear modulus of elasticity (G) were made from stress-strain curves by measuring the deformations of individual microcolonies caused by increasing and decreasing τ_w (approximately 5 s for each increment) with the equation $G = \tau_w/\alpha$, where α is the shear angle (defined here as the change in the angle between the upstream edge of the microcolony and the substratum) in response to a change in τ_w (Fig. 1) (15, 16). When τ_w was increased from 0 to 1.8 Pa, the microcolonies stretched downstream and flattened out (Fig. 1A and B). When the shear was removed, the microcolonies returned to approximations of their original shapes. The stress-strain curves (Fig. 1C) were "J" shaped and had a hysteresis loop, which is characteristic of curves for connective and other soft biological tissues (5). Such hysteresis indicates that viscous flow had occurred. G ranged from 0.9 to 5 Pa, which was comparable to values found for other biofilms (14, 18) and was also similar to the values found from the creep experiments, which measured the structural responses of the biofilms to sustained changes in shear stress and allowed quantification of viscous as well as elastic parameters. In these tests, the deformation of individual biofilm microcolonies was measured for 300 s during, and 300 s after, exposure to a τ_w of either 0.46 or 1.125 Pa (flow rates, 3.6 and 9 ml min^{-1}, respectively). The

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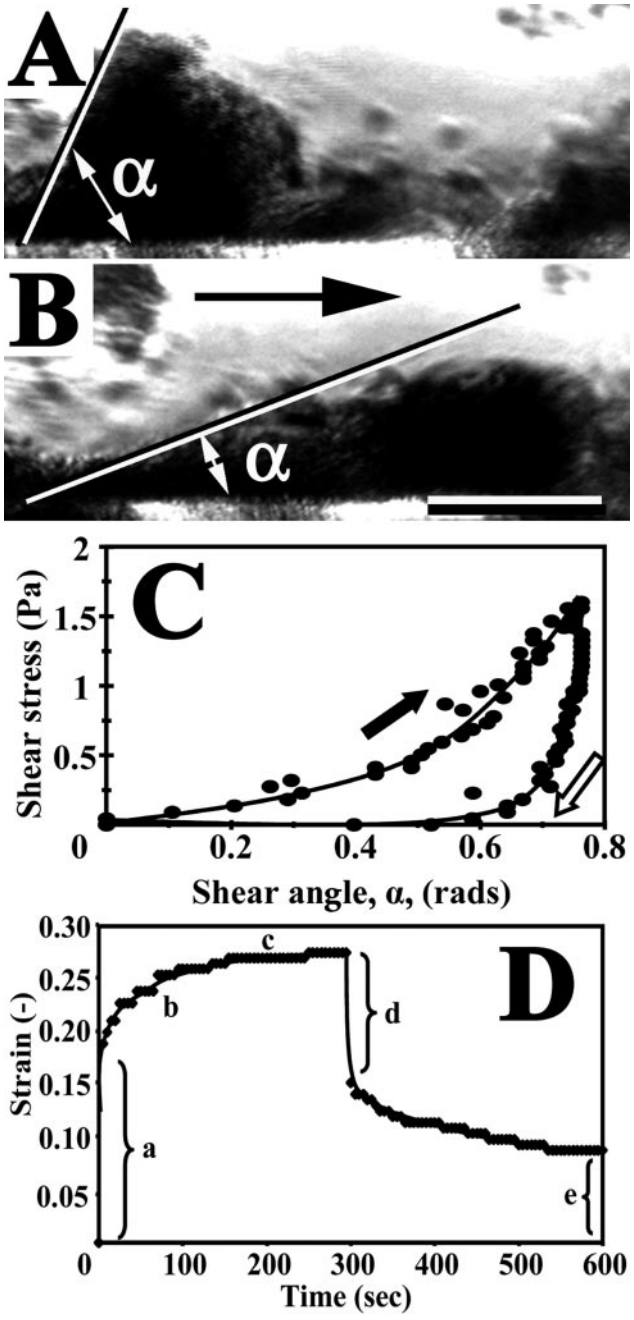

FIG. 1. Side views of an *S. aureus* biofilm microcolony with no fluid shear (A) and after exposure to a fluid shear stress of 1.6 Pa (B). Deformation was quantified by measuring changes in the shear angle α. The black arrow shows the flow direction. Scale bar = 50 μm. (C) Representative stress-strain curve of an *S. aureus* biofilm microcolony exhibiting a characteristic "J" shape, indicating that the biofilm became stiffer as the fluid stress increased. The filled arrow indicates the loading portion of the curve, and the open arrow indicates the unloading portion. The hysteresis loop revealed that, during the test, mechanical energy was dissipated in the biofilm through viscoelasticity. The solid line is the trend line. (D) Creep curve of an *S. aureus* biofilm microcolony. The shear stress was increased from 0 to 0.46 Pa at 0 s and then reduced back to 0 Pa at 300 s. The characteristic viscoelastic curve can be split into five sections: (a) instantaneous elastic response, (b) transient viscoelastic response, (c) viscous flow, (d) instantaneous elastic recoil, and (e) residual strain caused by nonrecoverable viscous flow.

change in length of the biofilm cluster per original length exhibited by the microcolony was recorded at 5-s intervals throughout the test. Creep curves (Fig. 1D) were constructed from these data to calculate G and viscosity (η) as described elsewhere (15). After immediate elastic deformations, the biofilm microcolonies continued to stretch (creep). Upon stress removal, some of the deformation was recovered immediately through elastic contraction. Further contraction through creep recovery occurred over the remaining 5 min of the test; however, a significant residual deformation remained. The curves yielded a G of 4.9 ± 3.7 Pa and an η of 3,500 ± 2,900 Pa/s.

The stress-strain and creep curves clearly demonstrated that the *S. aureus* biofilm microcolonies were viscoelastic (for reference purposes, note that these properties were similar to those of human respiratory mucus, which, like biofilm, is a complex multicomponent material [3, 5]). Although the magnitudes of G and η vary considerably among gram-positive, gram-negative, and mixed-species biofilms, the qualitative viscoelastic responses are consistent (14, 15, 16, 18). This finding suggests that viscoelasticity may be a general mechanism of biofilm adaptation to fluctuating shear stresses. Short-term exposure to elevated shear can be absorbed elastically, while the redistribution of internal physical stresses caused by sustained exposure can be dissipated through viscous flow, reducing the likelihood of structural failure and uncontrolled detachment. Remarkably, these viscoelastic properties of biofilms can also be influenced by growth at different flow rates, suggesting that a phenotypic adaptation can occur over longer time periods (16). *Pseudomonas aeruginosa* biofilms grown under high flow rates, for example, showed stronger cohesiveness than those grown under low shear, resulting in reduced embolization rates in response to higher flow (16).

Time-lapse microscopy showed bacterial microcolonies rolling under steady shear along the sidewalls of the glass capillary (Fig. 2; for online imaging, see www.erc.montana.edu/Res-Lib99-SW/Movies/2003/03-M003_4.htm). The rolling motion was modeled to that of a cycloid curve (Fig. 2C), which traces the trajectory of a point on the perimeter of a rolling circle. The measured data sets fitted the predicted data with an R^2 of 0.971. The mean downstream velocities of three monitored microcolonies were 19, 25, and 55 μm h^{-1} and were linearly proportional to the diameters of the microcolonies (65, 78, and 156 μm, respectively), with an R^2 of 0.999. This finding is consistent with a predicted linear relationship (R^2 = 0.998) between spheres with these diameters and the theoretical drag force (6.2×10^{-10}, 8.8×10^{-10}, and 32.3×10^{-10} N, respectively) based on the simplified assumption of a Stokes Law settling velocity relationship. For our calculations we used the flow velocity at the midline of the microcolonies predicted from the laminar velocity profile. For further details concerning these hydrodynamics, refer to reference 5. The rolling microcolonies were attached to the underlying biofilm layer or the glass surface by "tethers," which were detected by the individual cocci associated with them. The tethers, which measured up to a few micrometers in diameter, initially formed at the leading edge of the microcolony and were gradually stretched to lengths of between 70 and 180 μm over periods of 1 to 3.5 h before finally breaking, allowing a jerky forward motion (Fig. 2). The total bond life, as measured from the time of initial contact to breaking, was 5.6 ±

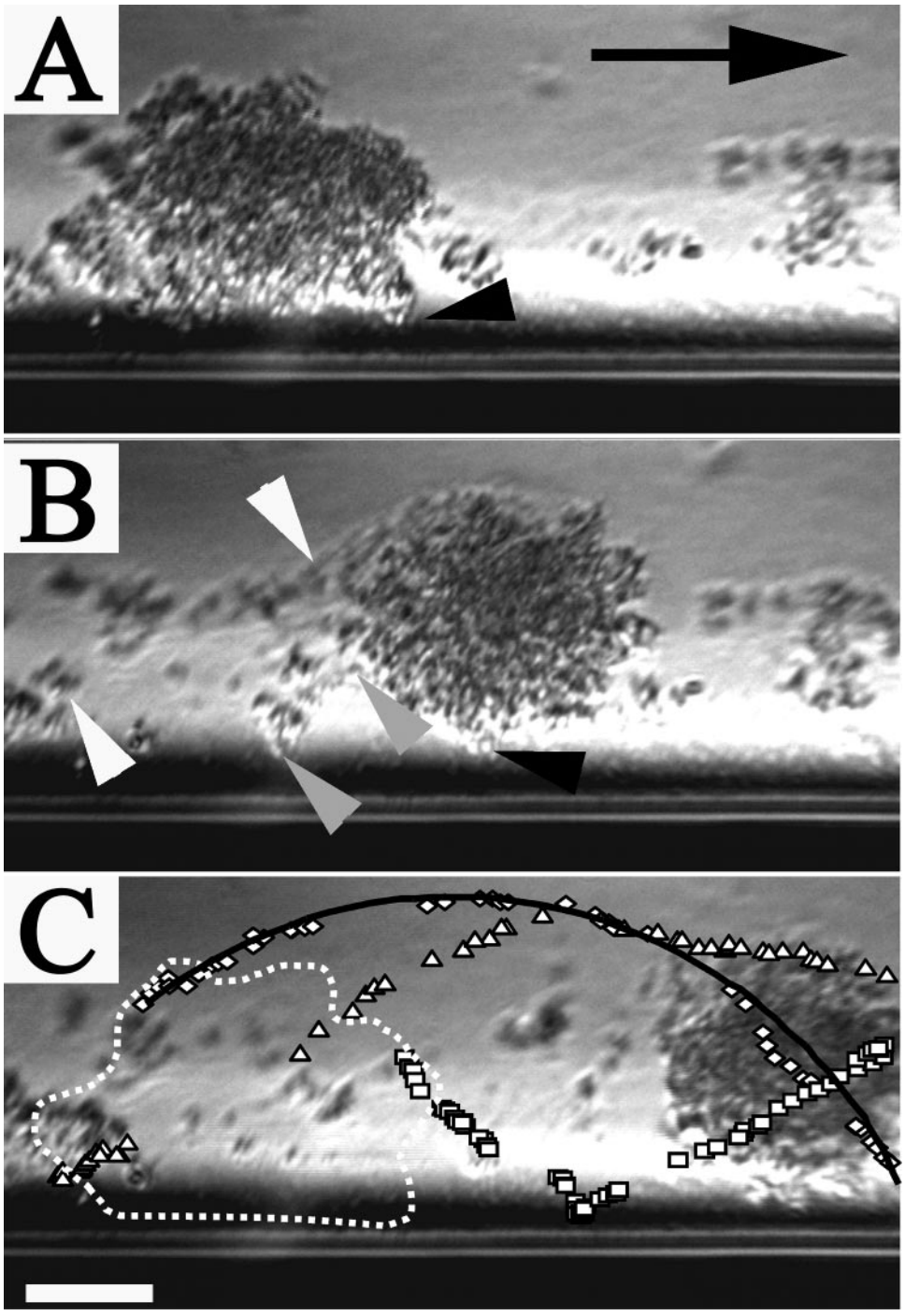

FIG. 2. Shear-mediated rolling migration of an *S. aureus* microcolony in a 5-day-old biofilm attached to the side wall of a glass flow cell lumen at intervals of 0 (A), 6 (B), and 11.5 (C) h. The rolling appeared to be controlled by tethers of biofilm material which formed at the downstream edge (black arrowheads). The tethers became progressively more stretched (an intermediate tether is shown between the gray arrowheads) before breaking (a tether just prior to breaking is shown between the white arrowheads). (C) The trajectory was found by tracking the x and y coordinates of three fiducial points (open symbols) from the original position (indicated by dashed line) at 15-min intervals. The solid black line is a cycloid curve with a radius equivalent to that of the microcolony and with an angular velocity of 8×10^{-5} rad s^{-1} fitted to the data (open diamonds) with an R^2 value of 0.971. Scale bar = 50 μm.

0.8 h. From the ratio of viscosity to elasticity, we derived a relaxation time (5) of approximately 12 min. Simply put, with times of less than 12 min, the biofilm will behave like a solid, but over longer times, it will behave like a fluid. These facts may help explain how the tethers control the rolling motion. Over short periods, the tethers can absorb shear variations elastically by deformation, but over longer periods, they will flow and eventually break, allowing the forward rolling motion.

Interestingly, a similar rolling motion is employed by leukocytes moving along the endothelium, in which forward motion is driven by blood shear, and controlled through the expression of adhesive and receptor selectins which form molecular tethers that continually break and reform between the surfaces of the leukocytes and the endothelial layer (11). Bacteria have developed elaborate strategies to utilize shear forces for the movement of single cells over surfaces. The nonmotile bacterium *Deinococcus geothermalis* can slide along stainless steel surfaces, resisting detachment, whereby molecular tethers connect cells to each other and the substratum (8). In *Escherichia coli*, FimH, a common bacterial adhesin, expresses catch-bond (a bond which is strengthened in response to shear) characteristics in response to shear (17). Increasing shear stress reversibly decreases cell detachment, slowing down or entirely stopping cells rolling along a surface. We have previously documented that biofilm microcolonies may utilize fluid shear for transport. Whole *P. aeruginosa* biofilms can move along surfaces as migrating ripples when they are exposed to shear forces (12). These observations challenge the assumptions that biofilms are sessile (fixed in place) and that their dissemination occurs exclusively through growth or detachment. The rolling of biofilm microcolonies described here may be particularly important for nonmotile bacteria, allowing controlled dispersal along surfaces in the protected biofilm state, particularly in the clinical context of catheter-related and endovascular infections.

This work was supported by National Institutes of Health RO1 grant GM60052-02 (P.S.), Swiss National Science Foundation grant 81BE-69256 (C.A.F.), and the W. M. Keck Foundation (C.J.R.).

We thank S. Wilson from the Center for Biofilm Engineering for experimental assistance.

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