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14. ABSTRACT The microstructure of novel Langmuir-Blodgett (LB) monolayer films prepared from lytic bacteriophage as a biorecognition coating for methicillin-resistant Staphylococcus aureus (MRSA) bacterial biosensors was characterized using scanning imaging ellipsometry (SIE) and scanning electron microscopy (SEM). High lateral resolution SIE appeared to be ideal for the non-destructive analysis of ultra-thin organic LB films and complementary to SEM in comparison to other invasive techniques including atomic force microscopy, scanning tunneling microscopy, scanning force microscopy and X-ray diffraction. Field emission SEM permitted visual examination of monolayer structure. SIE allowed film thickness analysis, 3D mapping and monolayer surface imaging, and charged-coupled device (CCD) biosensing of MRSA.					
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Phage Langmuir-Blodgett Films for Biosensing Applications

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Summary

The microstructure of novel Langmuir-Blodgett (LB) monolayer films prepared from lytic bacteriophage as a biorecognition coating for methicillin-resistant *Staphylococcus aureus* (MRSA) bacterial biosensors was characterized using scanning imaging ellipsometry (SIE) and scanning electron microscopy (SEM). High lateral resolution SIE appeared to be ideal for the non-destructive analysis of ultra-thin organic LB films and complementary to SEM in comparison to other invasive techniques including atomic force microscopy, scanning tunneling microscopy, scanning force microscopy and X-ray diffraction. Field emission SEM permitted visual examination of monolayer structure. SIE allowed film thickness analysis, 3D mapping and monolayer surface imaging, and charged-coupled device (CCD) biosensing of MRSA.

Motivation

The ability to transfer homogeneous, well-controlled nanoscale LB films to a wide variety of substrates such as gold, mica and glass makes the technique suitable for nanotechnology applications, including biosensors^{1,2}. Films are dependent upon subphase pH, molecular orientation, substrate properties^{3,4} and spacing and therefore nanoscale characterization is important to proper self-assembly on substrates. SIE and SEM appear to be good methods for evaluating phage monolayer sufficiency and adequacy of transference to substrates and may allow advancements in the design and fabrication of LB-based biological sensors. Importantly, SIE promotes non-destructive quality control analysis during sensor fabrication. SIE allows biosensing through CCD imaging or ellipsometry, where changes in color intensity profile or thin-film thickness profile are proportional to the amount of bound target analyte, respectively.

Results

Stable monolayers of *S. aureus*-specific lytic phage, phospholipid and stearic acid were formed at an LB air-water interface then transferred onto gold-coated silica substrates at constant surface pressures of 18, 17, and 47 mN/m, respectively. Modeling indicated free-floating phage at the interface, with orientation dependent upon film compression pressure (Fig. 1). SEM revealed nearly homogenous phospholipid and stearic acid monolayers on substrates with no visible discontinuities (Fig. 2). In contrast, phage monolayers were discontinued patches covering ~10% of substrates. Still, the LB method increased substrate surface coverage 100% in comparison to biotinylation⁵. 3-D thickness profiles from delta mapped SIE images revealed average monolayer thicknesses of 49.8 ± 18.3 nm, 21.4 ± 2.1 Å and 18.5 ± 1.6 Å for phage, phospholipid and stearic acid, respectively. Surface substrate profiles showed phospholipid and stearic acid monolayers oriented at $38.9 \pm 2.1^\circ$ and $40.3 \pm 4.1^\circ$ angles, respectively. CCD imaging analysis of post-assayed MRSA-phage substrate yielded an average intensity (arbitrary units) of 159 ± 7 and 194 ± 13 for 10^8 and 10^9 CFU/ml MRSA concentrations, respectively (Fig. 3).

¹R. Guntupalli, et al., "Rapid and sensitive magnetoelastic biosensors for the detection of *Salmonella* Typhimurium in a mixed microbial population," J. Microbiol. Methods, vol 70, nr. 1, p. 112-118, 2007.

²R. Guntupalli, et al., "A magnetoelastic resonance biosensor immobilized with polyclonal antibody for the detection of *Salmonella* Typhimurium," Biosens. Bioelectron., vol. 22, nr. 7, p. 1474-1479, 2007.

³A.H.R. Flood, et al., "Meccano on the nanoscale-a blueprint for making some of the world's tiniest machines," Aust. J. Chem., vol 57, p. 301-322, 2004.

⁴D.R. Talham, "Conducting and magnetic Langmuir-Blodgett films," Chem. Rev., vol 104, p. 5479-5501, 2004.

⁵L. Gervais, et al., "Immobilization of biotinylated bacteriophages on biosensor surfaces," Sens. Actuators B Chem., vol 125, p. 615-621, 2007.

Figures

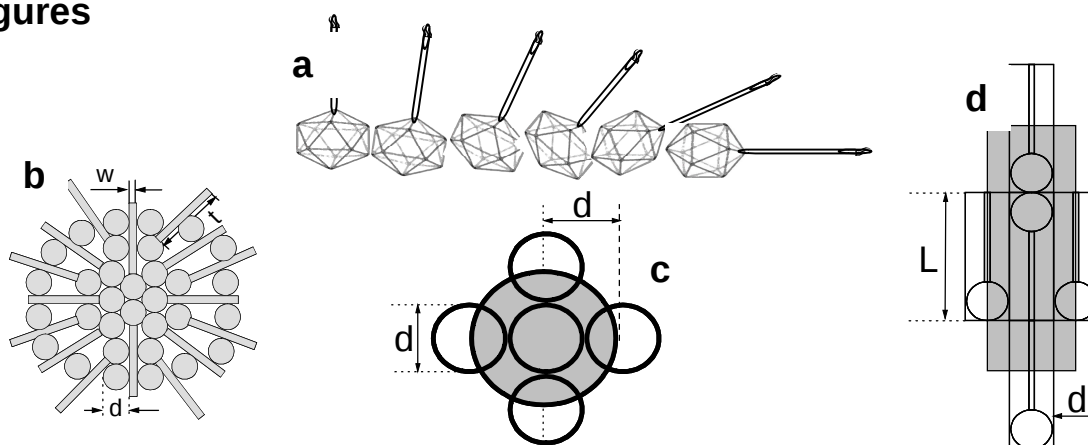


Fig. 1. Packing models of phage on the water/air interface. **(a)** One dimensional model of phage assuming that phages can change orientation from horizontal to oblique, and to vertical as surface pressure change from small to high (from right to left). **(b)** A sample of phage surface arrangement at horizontal positions. Density of space-filling is ~ 0.6 . **(c)** Two dimensional arrangement of phages illustrating the excluded area of phages in the vertical orientation. The phage's heads are approximated by discs with diameter of d . The central phage, occupies an area $A=\pi d^2/4$. **(d)** The sketch for excluded area of phages in horizontal orientation. The central phage occupies an area $A=Ld$, where L is a length of a whole phage.

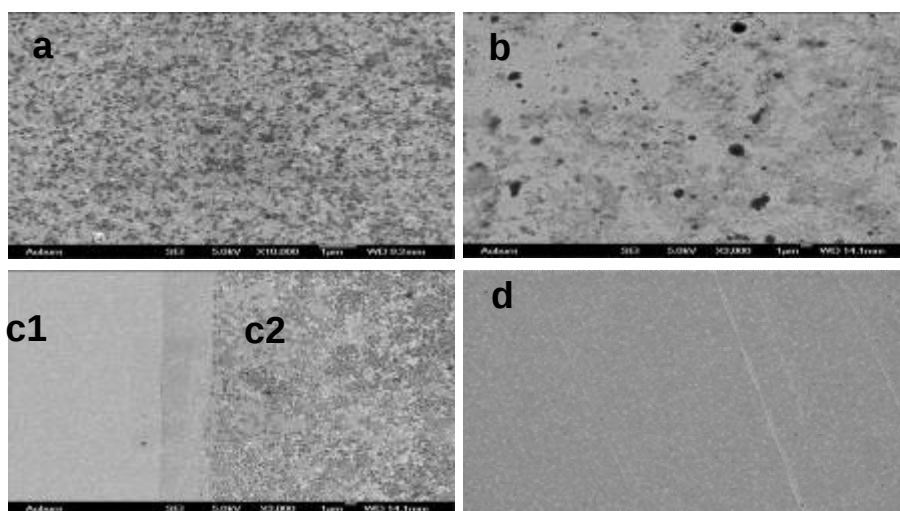


Fig. 2. SEM micrographs of (a) lytic phage on substrate, (b) phospholipid on substrate, (c1) substrate devoid of monolayer (c2) stearic acid, and (d) gold-coated silica substrate devoid of LB monolayer.

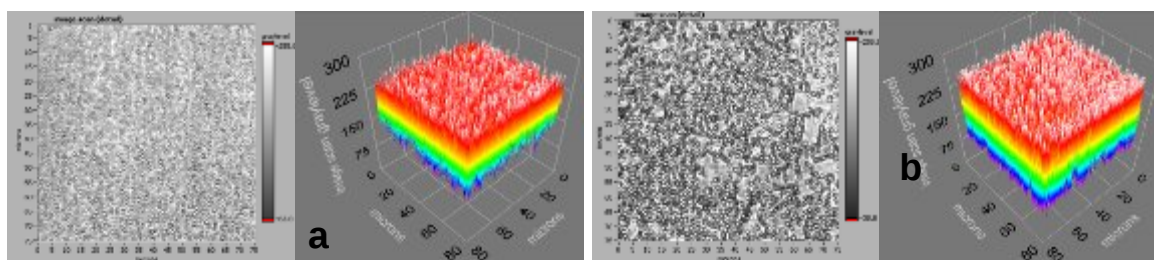


Fig. 3. Representative CCD camera image and 3D intensity profile of MRSA at concentrations of (a) 10^8 CFU/ml, and (b) 10^9 CFU/ml, attached to phage immobilized on glass substrate.