595f Fabrication of Antibody Microarray Sensors Using Thermally Responsive Elastin-Protein a Fusion for Detection of Microbial Pathogens

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An antibody microarray sensor platform has been developed by taking advantage of a smart biopolymer with thermally tunable hydrophobic properties that can be used to immobilize antibodies onto a target hydrophobic region on an array surface through hydrophobic interactions. The biopolymer composed of a elastin-like polypeptide (ELP), a temperature-responsive biopolymer that undergoes a reversible hydrophobic-hydrophilic phase transition upon changing of temperature, fused to Staphylococcal protein A (SpA), a cell wall component of Staphylococcus aureus that binds Fc portion of immunoglobulin G with high affinity, is used to enable the non-covalent immobilization of antibodies in a functionally active orientation directly onto an array surface by hydrophobic interactions between the ELP and the hydrophobic surface. This platform creates a basis for the fabrication of self-assembled antibody sensor arrays to incorporate sandwich immunoassays for quantitative detection of microbial pathogens.

Based on this platform, two different approaches have been demonstrated to immobilize antibodies in a spatially patterned manner. In the first approach, the ELP-SpA fusion is selectively immobilized on a previously defined hydrophobic pattern created on a hydrophilic background by spotting a hydrophobic ink onto glass slides using a robotic DNA microarrayer. The antibody is then loaded to the glass slide and immobilized by binding to the SpA. In the second approach, the antibody is first conjugated with the ELP-SpA fusion to form an ELP-SpA-antibody complex, and the complex is directly spotted using the microarrayer onto glass slides with a hydrophobic surface modified with a self-assembled monolayer. For both immobilization approaches, the feasibility to capture and detect a model pathogen, E. coli O157:H7, over a wide range of concentrations is demonstrated.