423d Fabrication of Microarray Sensors Using a Temperature-Responsive Elastin Fusion Protein for Simultaneous Detection of Multiple Tumor Markers

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We present a new method to fabricate antibody microarray sensors using hydrophobic interactions between a temperature-responsive elastin fusion protein and a hydrophobic glass surface for simultaneous detection of multiple tumor markers. The elastin fusion protein is composed of an elastin-like polypeptide (ELP) domain, which undergoes a reversible hydrophobic-hydrophilic phase transition in aqueous solutions upon changing of the temperature, and an antibody-binding domain, protein L, which binds immunoglobulin G from several mammalian species with a high specific binding avidity for the Fc portion. The capture antibodies in a sandwich antibody microarray configuration are first conjugated to the elastin-Protein L fusion, and the conjugated complexes are separated from the unconjugated antibody by temperature-triggered precipitation and resolubilization. The purified complexes with the ELP domain triggered to its hydrophobic phase are directly spotted using a robotic DNA microarrayer onto glass slides with a hydrophobic surface modified by a self-assembled monolayer. By using this method, the capture antibodies are immobilized in a functionally active orientation directly onto the array surface through the hydrophobic interaction, which is advantageous compared with other antibody immobilization methods involving covalent coupling or interactions with a ligand on the surface. Based on this immobilization technique, we have fabricated microarray sensors that are able to quantitatively detect three tumor markers (carbohydrate antigen 19-9, carbohydrate antigen 125, and carcinoma embryonic antigen) involved in the diagnosis of liver cancer, choriocarcinoma and ovarian cancer, using a sandwich antibody microarray format. Calibration curves of the fluorescence intensity as a function of the concentration in a wide range for each antigen have been constructed. A microarray sensor with the capability of simultaneously detecting three tumor markers has also been demonstrated. We expect that the immobilization method presented here could be a simple and universal platform to directly print antibodies on hydrophobic surfaces via hydrophobic interactions for the fabrication of a variety of antibody microarray sensors.