360d Total Internal Reflectance Microscopy on a Microfabricated High-Throughput Glass Chip: Application to Cholesterol-Modulated Antibody Binding to Supported Lipid Membranes

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A high-throughput microfabricated all-glass mcirochip, Lipid Biochip, was created and used to measure fluorescently-tagged antibody binding to dinitrophenol (DNP) haptens in planar supported phospholipid/cholesterol lipid bilayers as a function of cholesterol-to-lipid molar ratio (XCHOL). Multiple parallel microchannels etched in the Lipid Biochip allowed simultaneous measurement of antibody binding to hapten-containing and hapten-free lipid bilayers, for a range of aqueous antibody concentrations. Specific and non-specific antibody binding to the supported lipid bilayers was determined from the internally calibrated intensity of the surface fluorescence using total internal reflectance fluorescence (TIRF) microscopy. The TIRF intensity data of the specific antibody binding were fitted to the Langmuir isotherm and Hill equation models to determine the apparent dissociation constant Kd, the maximum fluorescence parameter F and binding cooperativity n. As XCHOL increased from 0 to 0.50, Kd exhibited a minimum of ~ 4 μM and n reached a maximum of ~ 2.2 at XCHOL ~0.20. However, F appeared to be insensitive to the cholesterol content. The non-specific binding fraction (NS), defined as the ratio of the TIRF intensity for hapten-free bilayers to that with hapten, showed a minimum of ~ 0.08 also at XCHOL ~0.20. The results indicate that cholesterol regulates the specific binding affinity and cooperativity, as well as suppresses non-specific binding of aqueous antibody to planar supported lipid bilayer surface at an optimal cholesterol content of XCHOL ~0.20. Interestingly, for XCHOL ~0.40, NS reached a maximum of ~0.57, suggesting significant packing defects in the lipid bilayer surface, possibly as a result of lipid domains formation as predicted by the lipid Superlattice model. We conclude that cholesterol plays a significant role in regulating both specific and non-specific antibody/antigen binding events on lipid bilayer surface, and that our Lipid Biochip represents a new and useful high resolution microfluidic device for measuring lipid/protein surface binding activities in a parallel and high-throughput fashion.