

## Carbohydrate and Single Cell-based Platform

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It is often desirable to sequester cells in specific locations on the surface and to integrate sensing elements next to the cells. In the present study, surfaces were fabricated so as to position cytokine sensing domains inside non-fouling poly(ethylene glycol) (PEG) hydrogel microwells. Our aim was to increase sensitivity of micropatterned cytokine immunoassays through covalent attachment of biorecognition molecules. To achieve this, glass substrates were functionalized with a binary mixture of acrylate- and thiol-terminated methoxysilanes. During subsequent hydrogel photopatterning step acrylate moieties served to anchor hydrogel microwells to glass substrates. Importantly, glass attachment sites within the microwells contained thiol groups that could be activated with a hetero-bifunctional cross-linker for covalent immobilization of proteins. This result highlighted the advantages of covalent attachment of avidin inside the microwells. The rational design described here will be leveraged in the future for rapid detection of multiple cytokines secreted by individual immune cells.