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Multiplexed Sub-Cellular Scale Microarrays from direct DNA Nanolithography

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The multiplexed, high-throughput fabrication of microarrays is of vital importance for many applications in life sciences, including drug screening, medical diagnostics and cell biology. In single cell investigations, features smaller than 10 µm are needed for functional manipulation of sub-cellular structures. Several top-down methodologies like electron beam lithography and microcontact printing can be employed for indirect surface patterning at this scale, however those approaches often require clean rooms and multiplexing of several different biomolecules on the same surface is limited [1]. To overcome these obstacles, we combined Dip-pen nanolithography (DPN) and DNA-directed immobilization (DDI) of proteins to fabricate cell-compatible functionalized glass surfaces [2]. We optimized ink formulation for ssDNA printing and the produced arrays were then functionalized with epidermal growth factor (EGF) taking advantage of covalent ssDNA-streptavidin conjugates as adaptor molecules. The surface-immobilized EGF was used for recruiting EGFR in the plasma membrane of MCF7 cells. Via this bottom-up structuring approach, we were able to analyse multiple protein-protein interactions simultaneously in individual living cells [3].
To improve the efficiency of multiplexed surface patterning, we developed a prototype of a robust custom plotter based on 2D polymer-pen lithography (2D-PPL) [4]. This device enables rapid fabrication of microarrays at ambient conditions in a multiplexed direct-writing mode. The printing process was carried out by polymeric pyramidal pens onto which multiple (up to 36) ssDNA solutions can be loaded through a microfluidic inkwell device. Subsequent to optimization of ink viscosity and surface tension by glycerol and tween-20, DNA arrays were plotted and used for DDI of EGF-bearing ssDNA-streptavidin conjugates. The resulting microarrays covered areas of about 0.5 cm^2, and were capable of recruiting and activating EGF receptors in sub-cellular regions within human MCF7 cells [4].

References

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