Life science at the sharp end: recent developments in tip-based nanolithography in sensing, cell biology and diagnostics

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The depostion of biomaterials at the micro and nanoscale holds huge potential for medical and life sciences. Dip Pen Nanolithography® (DPN®) is an established method of nanofabrication in which materials are deposited onto a surface via a sharp tip.[1] Recent advances in DPN technology have resulted in the ability to directly print biologically relevant materials, including DNA, antibodies and smaller proteins, onto a variety of surfaces under ambient conditions thus maintaining biological activity. We will present data that demonstrates the generation of features for ELISA-type systems and biosensors (electronic, optical and mechanical).

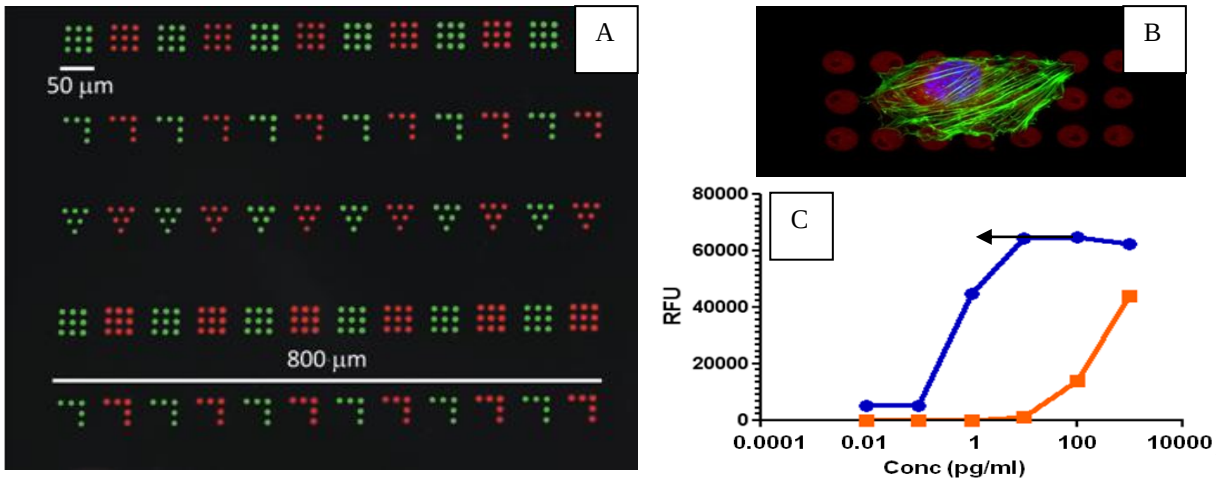
Direct deposition of biomaterials onto existing chips or microstructures is significant in the development of biosensors and diagnostics.[2] In recent years several different micro mechanical machining systems (MEMS)-based sensing elements have found applications in biomolecular and chemical detection. An important advantage of MEMS sensors is that they can be easily multiplexed in a high density fashion to simultaneously detect multiple analytes from very small volumes. One of the factors limiting a wider application of these sensors is the fact that chemical or biological functionalization of these elements has been challenge. We will present data demonstrating the feasibility of placing multiple proteins on microscale structures. Examples presented will include placement of proteins on microcantilever and microelectrodes.

Recent research has focused on precisely controlling the microenvironment of cells. Tip-based direct protein printing is a relatively new technique in this field. The flexibility of the methods presented here enable the construction of complex patterns for cell culture studies and the ability to address cells at an individual level. We will report novel and fundamental demonstrating the placement of multiple proteins with subcellular resolution. We will also present the effect of these patterns on cell polarization.[3] Co-culture studies have been useful for mimicking the in vivo environment and studying effects on stem or progenitor cell function. However, there are many experimental variables that cannot be properly controlled and may lead to confounding results. Herein we demonstrate a technique that allows spatial control of multiple cell types at single cell levels on a substrate. This single cell co-culture concept is demonstrated by utilizing the binding dynamics with fibronectin and laminin of 3T3 fibroblasts and C2C12 myoblasts. We further demonstrate the delivery of biology-effecting agents, including toxins, to a fraction of cells on a surface and determine the effects.

References

[1] R. D. Piner, J. Zhu, F. Xu, S. Hong, C.A. Mirkin, Science 283 (1999) 661.
[2] R. J Stokes, J. A. Dougan, D. Graham Chem. Commun. (2008) 5734.
[3] D.K. Hoover, E. W. L. Chan, M. N. Yousaf. J. Am. Chem. Soc. 130 (2008) 3280.

Figures



Figures: A: Arrays of Fibronectin and Laminin depositions enabling cell co-culture. B: Flexible patterning at sub-cellular scales. C: Example fluorescence response curves from IL-1β (example from library) in an ELISA type assay showing the increase in sensitivity that is obtained from smaller features fabricated by the DPN process.