INTRODUCTION

In this application note the Nano eNabler™ system is used to print proteins onto various surface types including hydrogel, nitrocellulose, and chemically treated surfaces such as silanes and self-assembling monolayers (SAMs). This demonstrates the utility of these surfaces as printing substrates for a variety of potential applications, some of which may require a flat silane or SAM surface while others may benefit from the increased loading capacity and activity retention of a 3D matrix. Observation of the printed surfaces provides information on the efficiency of protein transfer and consistency in ultramicron spot formation.

DATA SUMMARY

The surfaces used in this study included ultrathin nitrocellulose coated PATH™ Protein Microarray slides (GenTel BioSurfaces, Inc.), FAST™ proprietary nitrocellulose based slides (Schleicher & Schuell), ArrayIt Microarray Technology SuperAldehyde 2 (TeleChem International, Inc.), polyacrylamide based HydroGel slides (PerkinElmer) and Sindex™ (BioForce Nanosciences) silicon chips functionalized with ProLinker (Proteogen) providing an amine reactive SAM. Rat anti-mouse IL-6 (R&D Systems) was diluted in Spotting Buffer (BioForce Nanosciences) at 0.5 mg/ml. A surface patterning tool (SPT™ print cartridge, catalog # SPT-S-C30) was inserted into the Nano eNabler™ system and front loaded with the antibody solution. The SPT™ print cartridge was then used to create spots on each of the surfaces using varied contact times. Resulting spot diameters ranged from 3-10 µm. The spots were visible via bright field illumination through the high magnification video system in the Nano eNabler™ system. Relative humidity was maintained at 55% throughout the deposition process.

The printed arrays were processed as follows. The amine reactive Sindex™ chips and HydroGel slides were incubated at 70% humidity. The other materials were placed at ambient humidity and room temperature overnight. All surfaces were blocked with ViriBlock (BioForce Nanosciences) and incubated with murine IL-6 protein (R&D Systems) at 10 ng/ml followed by wash and incubation with biotinylated anti-mouse IL-6 (R&D Systems). The slides and chip were then washed and treated with Streptavidin 594 (Molecular Probes). Following a wash with PBST, the arrays were imaged with a Nikon TE-2000 fluorescence microscope and a cooled CCD camera (Figure 1).

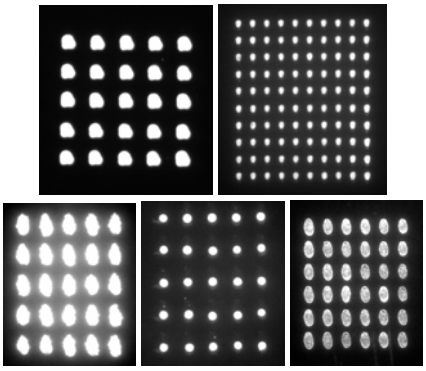

Figure 1. Fluorescence images of ultramicro arrays. Top panel left to right shows amine reactive ProLinker (D=5 µm/spot) and amine reactive SuperAldehyde (D=6 µm/spot) surfaces. Bottom panel left to right shows the nitrocellulose based FAST™ slide (D=10 µm/spot), HydroGel (D=3 µm/spot) and nitrocellulose PATH™ (D=8 µm/spot) surfaces. The pitch of the arrays varied from 10-20 microns and the images are not all scaled the same.

CONCLUSIONS

The Nano eNabler™ system was used to print proteins on an assortment of surfaces with varying roughness and contact angles. Writing proteins on the smooth, amine reactive SAM and silane surfaces was straightforward. Protein droplets dispensed on the nitrocellulose FAST™ slides disappeared as they were rapidly absorbed by the hydrophilic matrix, which resulted in larger spots and higher background. In contrast, printing on PATH™ nitrocellulose slides was straightforward, but the similarly hydrophilic nature also resulted in larger spots. HydroGel surfaces produced some of the smallest spots and best morphology, however they require pretreatment and must be used immediately. In all cases we demonstrated detectable and relatively uniform protein capture on all five surfaces. This opens a large number of possibilities for effective use of the Nano eNabler™ system as both a research and manufacturing tool for use with either smooth or rough surfaces.

REFERENCES

[1] Xu, J., Lynch, M., Huff, J., Mosher, C., Vengasandra, S., Ding, G., and E. Henderson. Microfabricated quill-type Surface Patterning Tools for the creation of biological micro/nano arrays. *Biomedical Microdevices* **6 (2)**: 117-123, 2004.