Microfabrication and Patterning of Protein Arrays

Ben Whatley [1], Brooke Damon[2], Xuejun Wen [1,2,3]
[1]Clemson - MUSC Bioengineering Program, Department of Bioengineering, Clemson University, Clemson, SC, 29634
[2]Department of Cell Biology & Anatomy, Medical University of South Carolina, Charleston, SC, 29425
[3]Department of Orthopaedic Surgery, Medical University of South Carolina, Charleston, SC, 29425

Statement of Purpose: Surface patterning of proteins onto different materials using a variety of proteins is an attractive method to study individual cellular and protein functions. Protein patterning enables the a variety of interactions to be studied including: protein-protein, protein-cell, protein-antibody, protein-DNA, protein-lipid, protein-drug, and protein-receptor.[1-3] A novel method developed in our lab uses high-precision robotics to fabricate proteins in a spatially controlled manner and permits the use of different biomolecules to selectively pattern a variety of surfaces. This method preserves protein functional domains which are important to allow their various interactions to be studied. Studying these protein interactions allows each protein to be characterized leading to a better understanding of the complexity of cellular signaling. A patterned protein microarray may allow cell behavior, specifically attachment and differentiation, to be controlled. Different protein molecules may be used to selectively attach cells or to selectively differentiate progenitor cells towards a desired phenotype. This ability to bind specific proteins within a cell membrane in combination with different biomaterials proves the abilities of this technique to enhance materials and their surface interactions. Protein micropatterning also has a broad spectrum of novel biological applications including the study of cell shapes, cell migration, and cell division as cells can be attached to certain patterns and studied to determine how they interact with the biomolecules patterned on the different substrates. Microcontact printing to a resolution of around 100 μm is common. However, we have the ability to decrease the size of patterned spots and lines, thereby increasing the resolution to make the applications more useful. The extrusion tip of our device was modified to control the size of protein spots and lines, while permitting the use of multiple solutions simultaneously. This technique is beneficial as it controls the spatial patterning of a variety of proteins.

Methods: To prove that the resolution of the device created is better than current robotic microarrayers, different fluorescently labeled protein solutions were deposited onto glass slides. The proteins used for this project include laminin, fibronectin, and albumin, which have shown to have different bioactivity in cell behaviors. The device incorporates a freezing stage which can control the temperature, to around 4-8 °C, which is necessary for protein preservation. The cold temperature also increases the solution viscosity and helps decrease the surface tension between the liquid spots and the substrate surface. Ring structures are prevented by adding surfactant (triton X-100) which displaces molecules at the air-liquid interface.[4] Multiple surface chemistries of glass slides were tested, and octadecyltrichlorosilane (OTS) achieved the best repulsive surface. OTS is hydrophobic and aids in protein attachment to ensure the creation of high resolution/quality microarrays. OTS binds proteins randomly through amine groups and preserves protein structure. Confocal microscopy was used to determine spatial orientation and size of the spots.

Results: Using this robotic microarray fabrication technique, it is possible to create spatially ordered protein patterns at high resolution. Small amounts of protein solutions on the microliter scale can be used, and spots/lines of different size can be created, making this method attractive. Spots and lines can be created down to a thickness of several to tens of microns (Fig. 1). Therefore, this technique is more accurate than conventional microarray technologies. The device can create surfaces with multiple different protein spatial patterns, in alignment, while also providing reproducibility. This technique does not use chemical reactions that may alter protein structure. The microfabrication device used to create patterns has the ability to work with nearly any protein.

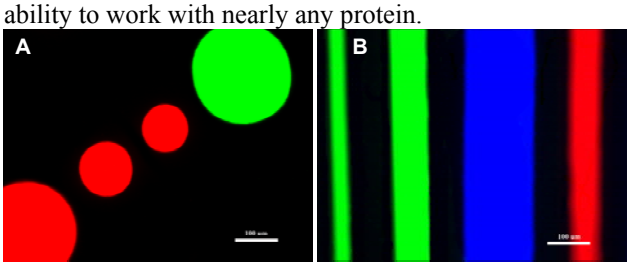

Fig. 1: A) Protein spots of different sized printed in alignment, B) Protein lines printed in alignment with different spacing and different sizes (scale bar= 100 um, laminin-red, fibronectin-green, albumin-blue).

Conclusions: This fabrication method permits the study of protein spatial distributions and offers different and versatile surface modification techniques. Multiple protein solutions can be used simultaneously. The technique is user friendly, allowing multidisciplinary researchers to effectively study protein interactions or selectively modify different material surfaces with bioactive molecules. The microarray apparatus provides a low cost fabrication method that can be translated to a clean room setting and provide an alternative to high cost robotic arrayers currently available. This technique is simple to use, can be implemented in any basic laboratory, and is a proof of concept method that will permit the downstream creation of biomolecular arrays in the future.

References:

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