A MICROFLUIDIC PLATFORM OPTIMIZING BEAD-BASED ELISA FOR THE DETECTION OF CELL SECRETION

B. Lincoln¹, J.Garcia Cordero¹, C.R. Poulsen¹, and L.P. Lee¹,²
¹Biomedical Diagnostics Institute, Dublin City University, Dublin, IRELAND,
²Biomolecular Nanotechnology Center, Berkeley Sensor & Actuator Center,
Department of Bioengineering, University of California, Berkeley, USA

ABSTRACT
With the goal of enabling the dynamic measurement of cell secretion within a microfluidic cell culturing platform, we have developed chambers that efficiently deliver the surrounding media to a bead-based ELISA detection site while maintaining optimal culture conditions. Platforms for the optimization of this system are introduced, including a size-varied chamber array that can test for the number of cells required for a detectable signal.

KEYWORDS: ELISA, Secretion, Culture Platform, Systems Biology

INTRODUCTION
An on-chip bead-based ELISA system has the potential for quantitative, dynamic local measurement of proteins secreted from living cells with higher sensitivity than standard “snapshot” ELISA techniques (Figure 1). Sandwich ELISA utilizing beads as a means of increasing surface-to-volume ratio has been combined with microfluidics using a few different bead trapping methods; including via a dam [1], and a sieve [2]. While these researchers have cited a reduced reaction time as benefits of a microfluidic ELISA system, we additionally propose a design capable of culturing cells on-chip and local to a detection zone that is separated from bulk flow.

Figure 1. Standard sandwich ELISA (left column) relies on slow diffusion and requires several washing steps, while bead-based sandwich ELISA in a microfluidic channel (right column) has the advantage of a steady flow delivering the antigen and labelled antibody to the site optimized for a high density of capture antibody.
**DESIGN/FABRICATION**

For each specific application of a culture/detection system, the optimal microfluidic conditions will depend on a number of factors, including the minimum antigen concentration that can be detected, the number of cells needed to secrete a detectable amount, and the interplay between the binding dynamics of the ELISA system and the flow characteristics in the vicinity of the beads. Some of these properties are best explored by using a dam configuration similar to [1] (Figure 2) to calibrate the beads themselves by flowing controlled concentrations of antigen and labelled antibody and observing the fluorescent signal over time. However, a more sophisticated design is needed to test the secretion behaviour of various types of growing cells under culture-friendly conditions. Such devices were constructed using multilayer negative photoresist as a mold for the final PDMS chamber, which was bonded to a floor of glass (Figure 3). The multilayer design, which enables control over features both vertically and horizontally, allowed for the creation of cell-trapping regions with low flow relative to bulk channel flow with each chamber containing its own detection site, or “nose”. This enabled a steady supply of nutrients without placing the cells under significant shear while at the same time ensuring that the contained medium will pass slowly and continuously through the packed capture antibody coated beads.

A flow system with size varied chambers was designed in order to compare cell count with the local detected signal (Figure 4). The examples shown here vary in size either radially or through elongation in order to control the number of cultured cells associated with a single detection site over a range from ~1 to ~10,000 cells.

**CHARACTERIZATION**

Beads were successfully loaded into the “noses” in both geometries (Figure 4). The flow has been modelled (Figure 5) in order to quantify the relative velocities for both an empty “nose” (top) and a “nose” filled with beads (bottom). The flow was...
experimentally confirmed to pass through the bead location, even in the largest chambers (Figure 6).

**Figure 4.** Two examples of microfluidic platforms with size varied culture chambers, with the top being varied radially, and the bottom being varied through elongation. The center SEM images show the successful construction of these shapes, while the right DIC images show the successful loading of beads into the chamber “noses”.

**Figure 5.** Simulation to show the flow conditions in and around the chamber containing a “nose”. The flow rate around the chamber is much higher than inside the chamber, while a majority of the slow moving fluid inside the chamber will eventually pass through the nose.

**Figure 6.** Physical characterization of the flow properties, in this case using the largest chamber from the radially size-varied chamber platform.

**CONCLUSIONS**

In summary, we developed an integrated microfluidic bead-based ELISA system for the detection of cellular secretions. The cancer biological characterizations are in progress. We will perform new systems biology experiments capable of measuring secreted cell proteins over time and with high sensitivity.

**REFERENCES**
