ABSTRACT

With the increasing demand for higher throughput systems for biological applications, new systems need to be validated for use in the life sciences. Here we validate high throughput “tubeless microfluidics” by characterizing robustness and consistency as well as comparing the platform using a well-established assay against traditional biological methods. We show consistent results between microfluidic methods and immunocytochemistry, western blots, and IR scanned in-cell westerns. In addition, we demonstrate a high throughput dose response for TGF-β-induced epithelial to mesenchymal transition in microfluidic channels on an automated liquid handler.

KEYWORDS: High-throughput, automated, tubeless, microfluidic, growth factor

INTRODUCTION

Many microfluidic systems require numerous connections to outside equipment which presents a barrier to wide spread use and throughput - particularly outside engineering labs. Our group has previously shown the potential advantages of “tubeless” microfluidic systems [1]. We and others have also performed analysis using plate readers as compared to lower throughput microscopy [2, 3].

Here we expand upon these technologies by integrating “tubeless” microfluidics, automated liquid handlers and automated analysis via an IR scanner to realize an automated solution for high throughput cell-based assays. We characterized robustness and repeatability in the system and performed a dose response for TGF-β-induced epithelial to mesenchymal transition (EMT) [4]. Decreases in E-Cadherin (E-cad) expression were used as the readout. We further validated this system by comparing IR scanned In-Cell Westerns (ICW) in microfluidic channels with ICW from 96 well plates, immunocytochemistry (ICC), and traditional Western blots. Results were consistent between all tested methods.

EXPERIMENTAL

MCAs of 192 channels (Figure 1) were fabricated in poly(dimethylsiloxane) (PDMS) using standard techniques. Fluid was manipulated via surface tension droplet based “passive pumping” [1]. Liquid handling (Figure 1) was performed via a single pipette robot (223 Sample Changer, Gilson Inc.). During the entirety of an experiment each channel was addressed for 35 individual operations (dispenses and aspirations) i.e: channel filling, cell seeding, replacing media, washing, fixing, permeabilizing and staining (blocking buffer, primary and secondary antibodies and nuclear stains).
Normal murine mammary gland epithelial cells (NMuMG, ATCC) were seeded into channels and 96 well plates at equal surface densities (400 cells/mm²). After 24 and 48 hours media was replaced with either media containing 100 pM TGF-β or control media containing no TGF-β, at 72 hours cells were fixed and stained. An automated dose response to TGF-β was performed on an ALH by replacing the media with 8 different concentrations in the range of (0 and 100 pM). For all n=5.

RESULTS AND DISCUSSION

Liquid handling was characterized for robustness and cell seeding consistency and was found to be 99.97% accurate over 28,000 operations. Seeding consistency was analyzed because many biological experiments are density depended. Cells were seeded to be 35% confluent after 24 hours (consistent with flask culture) and automated image analysis over a plate revealed a confluence of 34.32% with a standard deviation of 4.97%. These results indicate that this method is both robust and accurate in performing biological experiments.

Results were consistent between all methods for EMT induction in NMuMGs as shown in Figure 2 (visually via ICC, Western blots, ICWs for microchannels and ICWs for 96 well plates). Fold reductions of E-cad are shown in Table 1 for quantitative methods. Consistent results were also found for increases in N-Cadherin in the presence of 100 pM TGF-β. The automated dose response to TGF-β revealed consistent fold reductions and are summarized in Figure 3.

Figure 2: From left to right: Immunocytochemistry showing a decrease in E-cad (green) after TGF-β exposure (nuclei-blue). Western blot for E-cad (green) and actin (red). ICW(E-cad-green, nuclei-red, overlay-yellow) from MCA (top) and 96-well plate (bottom). ICW graph shows a decrease in E-cad with TGF-β and no significant difference between macro and micro scale cultures.
Table 1: Fold reductions of E-cadherin in response to 100 pM TGF-β. Western blot results show specific reduction when selecting just the band and expected reduction when selecting the entire lane (ICW technique used for micro and macro does not filter the result based on molecular weight).

<table>
<thead>
<tr>
<th>Method</th>
<th>Western Blot</th>
<th>In Cell Western</th>
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<tbody>
<tr>
<td></td>
<td>Specific</td>
<td>Expected</td>
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<td>Fold reduction</td>
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<td>1.60</td>
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Figure 3: Automated dose response of E-cad to TGF-β in an MCA via ICW. Fold reductions compared to 0pM in E-cad are noted above each column.

CONCLUSIONS
The repeatability and accuracy of this method compares to traditional culture methods and provides a new tool to perform unique biological experiments in high throughput with reduced sample sizes, decreased assay time and the ability to integrate more complex geometries and microfluidic functionality.

ACKNOWLEDGEMENTS
The authors would like to thank the entire MMB lab. D. J. Beebe has an ownership interest in Bellbrooks Labs, LLC which has licensed technology presented in this manuscript. Funding by the NIH K25-CA104162 and NIH R21-CA122672.

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