Three Dimensional Polymer Scaffolds for High Throughput Cell-Based Assay Systems

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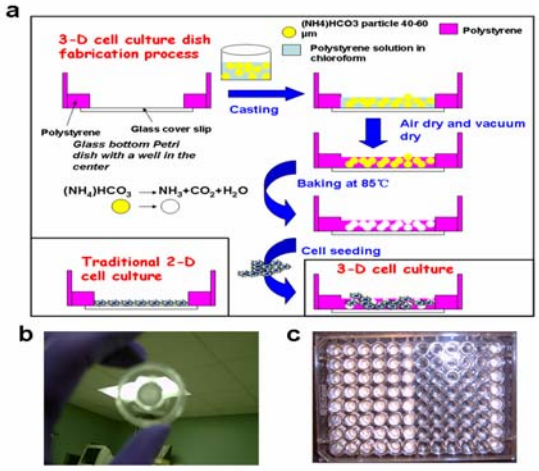
Statement of Purpose: Many whole cell-based assays in use today rely on flat, two-dimensional (2D) glass or plastic substrates that may not produce results characteristic of in vivo conditions. This lack of predictability has been partially attributed to the fact that such systems commonly employed 2D cellular assays, which do not mimic the response of cells in the three-dimensional (3D) milieu present in a tissue, or in vivo Ideal cell-based screening systems call for research efforts to create simple, robust and effective 3D cell-based platforms so that cellular responses will be more representative of those under in vivo conditions.

Methods: In this study, a three dimensional (3D) cell-based assay platform was established by integrating 3D synthetic polymer scaffolds with standard cell culture dishes and multi-well plates. This technology can be used to feasibly modify any traditional 2D cell-based assay vessels for 3D cell-based assay with currently used high throughput screening (HTS) systems. Human neural stem cells were seeded onto the scaffolds. We conducted 2D and 3D cellular activity comparison in scopes of proliferation, cell morphology, cell-matrix adhesion, gene expression and Voltage gated calcium channel (VGCC) functionality.

Results/Discussion:
The results are summarized in Figure 1, 2, and 3.



Fabrication and characterization of 3D cell culture vessels. (a): Schematic of the fabrication process. (b): Outlook of a 3D cell culture dish with a 3D polystyrene scaffold in the center. (c): Outlook of a 3D cell culture 96-well plates with 3D polystyrene scaffolds in the left half of the columns.

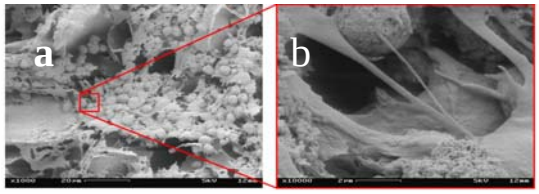


Fig. 2: Polystyrene scaffolds seeded with human neural progenitor cells. Cells developed well-defined neurites (b). Bars represent 20 μm in a and 2 μm in c.

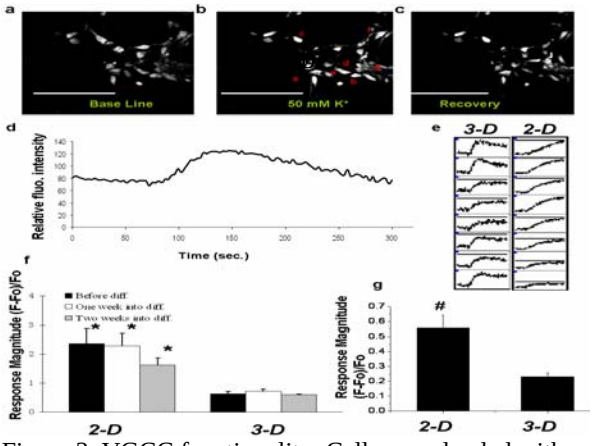


Figure 3. VGCC functionality. Cells were loaded with calcium indicator Calcium Green-AM and Fluo-4. The cells were exposed to 50 mM high K^+ depolarization for calcium imaging. (a)–(c): Confocal micrographs of cells on a 3D scaffolds within a Petri dish showing changes in $[Ca^{2+}]$ levels following addition of high K^+ buffer. (d) Plot of relative fluorescent intensity versus recording time for a cell labeled "d" in (b). The increase in fluorescence intensity is proportional to the increase in intracellular $[Ca^{2+}]$ concentration. (e): Typical $[Ca^{2+}]$ time courses of cells from a column in both 2D and 3D culture 96-well plates. (f): High K^+ buffer stimulated VGCC response magnitudes from NS cells cultured on 2D surface and in 3D vessels. (g): High K^+ buffer stimulated VGCC response magnitudes from NS cells cultured in 2D and 3D 96-well plates. * and # indicate that the 2D and 3D response magnitude means compared were significantly different at $p<0.00001$ and $p<0.01$, respectively. Error bars are standard deviations.

Conclusions: By integrating 3D polymer scaffolds with standard cell culture vessels, we created a ready-to-use, robust and highly compatible 3D cell-based assay platform for HTS cell-based drug discovery programs.