1. Three dimensional neuronal cell culture on polymer scaffolds more accurately modal in vivo voltage gated calcium channel functionality
2. Polymer scaffold: Substrate for three dimensional cell-based assays

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Statement of Purpose: Many whole-cell-based assays in use today rely on flat, two-dimensional (2-D) glass or plastic substrates that may not produce results characteristic of in vivo conditions [1]. Polymer scaffolds have been widely used in tissue engineering and regenerative medicine studies. However, their applications as substrata for three dimensional (3-D) cell-cultures for assay purposes has not been explored. The purpose of this study was to investigate how well neuronal cells cultured on 3-D polymer scaffolds model in vivo functionality with respect to voltage gated calcium channels (VGCC) activity.

Methods: Poly-l-latic-acid (PLLA) and polystyrene scaffolds were fabricated by the salt leaching/gas forming method. Scanning election microscopy and fluorescence microscopy were used to examine the cell morphology and spatial distribution. VGCC functionality was reflected by calcium influx in response to high K^+ (50 mM) depolarization. The intracellular calcium concentration was recorded continuously in time by the membrane permeable dye Calcium Green-1 AM coupled with a confocal laser scanning microscopy. The magnitude of the response from each cell was expressed as a peak fractional increase over basal fluorescence intensity $(F-Fo)/Fo$, where F is the peak fluorescence intensity and Fo is the basal fluorescence intensity.

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

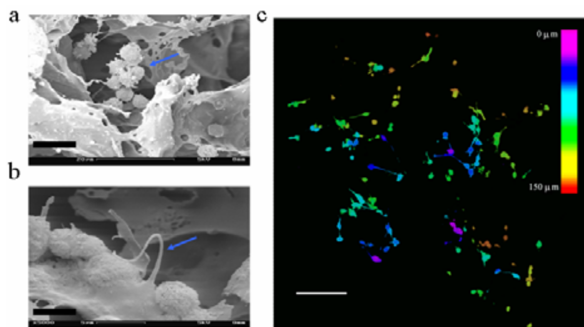

Fig. 1: PLLA scaffolds seeded with mouse SCG cells. (a) SEM image of a SCG cell cluster (indicated by arrow) inside a pore on day 2 after plating. (d): SEM image showing a neurite (indicated by arrow) from one cell to another on day 7 after plating (c): Confocal depth projection micrograph of a 20:1 polymer scaffold with 60-100 µm pores, seeded with SCG cells. Bars represent 10 µm in (a), 5 µm in (b), and 100 µm in (c).

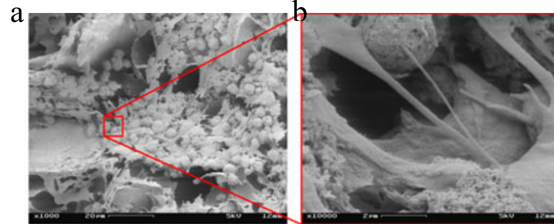

Fig. 2: Polystyrene scaffolds seeded with human neural progenitor cells. Cells developed well-defined neurites (b). Bars represent 20 µm in a and 2 µm in c.

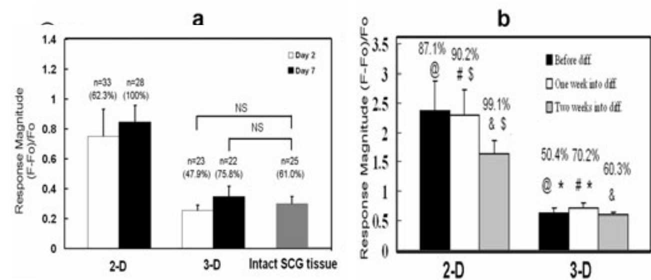

Fig. 3: Cellular VGCC functionality from mouse SCG cells (a) and human neural progenitor cells (b). "NS" indicates that the means of the two samples are not significantly different with at level of $p>0.8$. @, # , and $ indicate the two means in are significantly different at a level of $p<0.01$. $ and * shows the means are significantly different at a level of $p<0.05$. "n" is the number of responsive cells. The percentage of responsive cells from the total cell pool is indicated in parenthesis. Error bars are the 95% confidence intervals.

Conclusions: Both porous PLLA and polystyrene scaffolds created proper three dimensionalities for neuronal cells to attach and differentiate. The mouse SCG cells cultured on 3-D PLLA scaffolds more closely mimicked the cells in intact SCG tissue than those cultured on 2-D substrates. Similarly, the human neural progenitors cultured on polystyrene scaffolds had lower VGCC response magnitude than the cells on 2-D substrates. These results provide the first evidence in support of the hypothesis that some cellular responses under traditional 2-D environment may be exaggerated [2].This study brings attention to the potential importance of introducing three dimensional cell-based assays in drug discovery programs.

References:
1. Abbott, A. Nature. 2003; 424:870-872.
2. Cukierman, E. Science. 2001; 294:1708-383.