SU-8 microstructure for three-dimension cell-based biosensing

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Statement of Purpose: In the design of cell-based biosensors for drug discovery, three-dimension (3-D) cell culture systems have been proposed to replace the traditional cell culture systems in which cells are cultured on flat surfaces (2-D).[1] Microfabrication is an ideal way to produce a miniaturized, inexpensive platform for "cell-culture-on-a-chip". In this work, SU-8 photoresist which can form structures with high aspect ratios and can cover a wide range of thicknesses from <1μm to >200μms, was chosen to microfabricate 3-D structures.[2,3] The purpose of this study was to engineer a SU-8 microstructure for 3-D cell-based biosensing.

Methods:

SU-8 processing

SU-8 (2025, MicroChem, Newton, MA, USA) was spun onto the 25-mm coverslips. SU-8 was then exposed in soft contact mode with a Karl Suss MJB 3 HP Mask Aligner. Patterns were developed with SU-8 developer (MicroChem, Newton, MA) and then briefly immersed in isopropyl alcohol (Fisher Chemicals, Fairlawn, NJ) before drying with nitrogen.

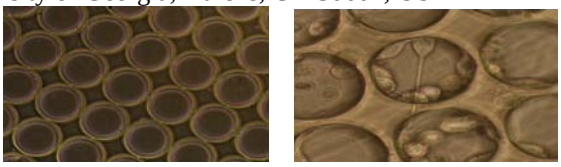
Cell culture and cell seeding

Before plating SH-SY5Y cells, SU-8 flat substrates and microwell patterns were coated with poly-lysine. Approximately 5×10^5 cells were plated on each patterned substrate or flat substrate in growth medium. On the second day after plating, the medium was changed from growth medium to differentiation medium. Differentiation medium was changed daily.

Evaluation of VGCC function

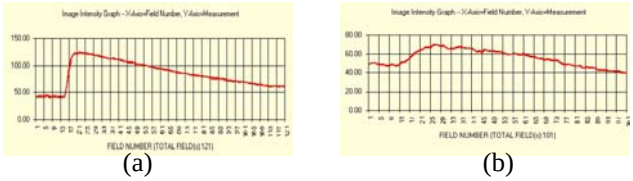
VGCC functionality was evaluated with the dynamics of calcium influx in response to high K^+ (50 mM) depolarization. The membrane permeable fluorescent dye, Calcium Green-1, acetoxymethyl ester (AM) (Molecular Probes, Eugene, OR), was used to visualize the calcium influx dynamics. Calcium Green-1 was excited with 488 nm argon laser and the emission was captured through a 515 nm long-pass filter.

Results/Discussion: SH-SY5Y human neuroblastoma cells were successfully integrated into the microwells fabricated from SU-8 photoresist (Figure 1). VGCC function of SH-SY5Y cells on 2-D substrates and in 3-D microstructure was evaluated (Figure 2). In response to 50 mM high K^+ depolarization, cells in 3-D structure were less responsive in terms of increase in intracellular Ca^{2+} in comparison to cells on 2-D substrates (Figure 3).



(a) (b)

Fig.1. Confocal images of SU-8 pattern with a diameter of 100 μm (a) and pattern with SH-SY5Y cells on day 8 into differentiation (b)



(a) (b)

Fig. 2. Typical time course of changes in relative intracellular Calcium Green-1 fluorescence intensities. On 2-D substrates, cells responded to high K^+ depolarization with HBS containing 50 mM K^+ on day 2 (a). Within 3-D microwells, cells responded on day 2 (b).

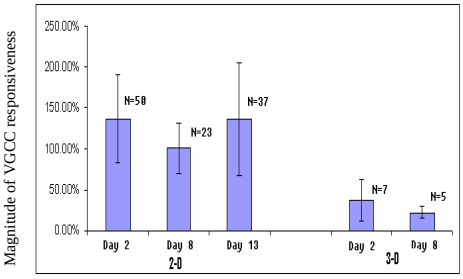


Fig. 3. The magnitude of VGCC responsiveness of SH-SY5Y cells on 2-D substrates and in 3-D patterns. There is significant difference between 2-D and 3-D ($P<0.05$).

In comparison to previous studies,[4] which also focused on the VGCC function of SH-SY5Y cells, the magnitude of the response of cells on SU-8 flat surface was larger. For example, at day 2, the magnitude of VGCC response of cells on SU-8 flat surface was about 200% higher than that on polystyrene surface.

Conclusions: This result supports the speculation that 2-D cell functions may represent an exaggeration of those in vivo. Also, this result demonstrates that the SU-8 may promote SH-SY5Y cell differentiation with respect to the VGCC function. This work will provide a 3-D platform for the development of cell-based biosensor.

Reference:

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