Effects of microstructured topography on the differentiation of H945RB.3 human neural progenitor cells

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Introduction: We have been interested in developing a whole cell-based assay format using three-dimensional topographical scaffolds (Wu ZZ et al., Biosens Bioelectron. 2006, 22: 685-693; Wu ZZ et al., Colloids and Surfaces B: Biointerfaces. 2006; 52: 14-21). With neuroblastoma cells, we found cells on topographical scaffolds were less spread, had fewer and shorter neurite extensions and developed higher resting membrane potential and lower voltage-gated calcium channel (VGCC) activity in comparison to cells on flat surfaces. We speculated that cell spreading and morphology was an important factor affecting neuronal cell differentiation. In the present study, we fabricated substrates with microstructured topography formed by packed polystyrene microbead array. H945RB.3 neural progenitor cells were cultured on flat surfaces and microbead array. Neuronal differentiation was analyzed in terms of cell morphology and VGCC function in an attempt to understand the importance of cell morphology in VGCC function development. The small size of the microbeads (1.98±0.20 μm) as compared to cell dimension ascertained that the effects of microstructured topography were not obscured by those of physical three dimensionality when larger beads were used.

Materials and Methods: Polystyrene was dissolved in toluene at 2.5% and spun onto 25 mm coverslips to achieve a coating thickness of around 0.1 μm. Polystyrene microbeads (1.98±0.20 μm, Bangs Laboratories, Inc.) were arrayed on the flat polystyrene surfaces with a simple tilting angle method. Before cell culture the whole set was etched with oxygen plasma for 2 minutes, sterilized and coated with 40 μg/ml polyornithine and 5 μg/ml murine basement membrane laminin (Sigma). H945RB.3 neural progenitor cells were provided by the Rhodes Animal Science Center, The University of Georgia. They were cultured on both flat polystyrene surfaces and microbead arrays with neural basal medium supplemented with 2 mM L-glutamine, 20 ng/ml basic fibroblast growth factor (bFGF), 1 μg/ml leukemia inhibition factor (LIF) and 1% B-27 supplements. Cell differentiation was initiated by starvation of bFGF. On days 0 (before differentiation) and 7 into differentiation, cells were loaded with 5 μM Calcium Green-1 AM in neurobasal medium and the increase in Calcium Green-1 fluorescent intensities in response to 50 mM KCl stimulation was evaluated with a laser scanning confocal microscope system (PCM-2000, Nikon). Fluorescent intensities were expressed as average gray level readings within created regions of interests (ROIs), which range from 0 to 255 artificial gray level units with 0 and 255 indicating highest darkness and brightness, respectively.

Results and Discussion: Cells on both flat surfaces and microbead arrays were well spread (Figs 1 *a* and *b*) before differentiation. They had a projection area of 262±108 μm^2 (n=249) and 264±131 μm^2 (n=263), respectively (P>0.05). Upon differentiation, cells became smaller in size. On day 14 into differentiation, cells on flat surfaces were less spread while cells on the bead arrays remained highly spread (Figs 1 *c* and *d*). They had a projection area of 120±48 μm^2 (n=134) and 135±73 μm^2 (n=210), respectively (P<0.05). On days 0 and 7 into differentiation, cells on flat surfaces had magnitudes of increase in Calcium Green-1 fluorescence intensity of 0.86±0.46 (n=54) and 0.90±0.43 (n=60) when stimulated with 50 mM KCl, while cells on microbead arrays had magnitudes of 0.88±0.70 (n=73) and 1.07±0.57 (n=280), respectively. This suggests that, upon differentiation, cells on microbead arrays had significantly higher VGCC responsiveness than cells on flat surfaces (P<0.01).

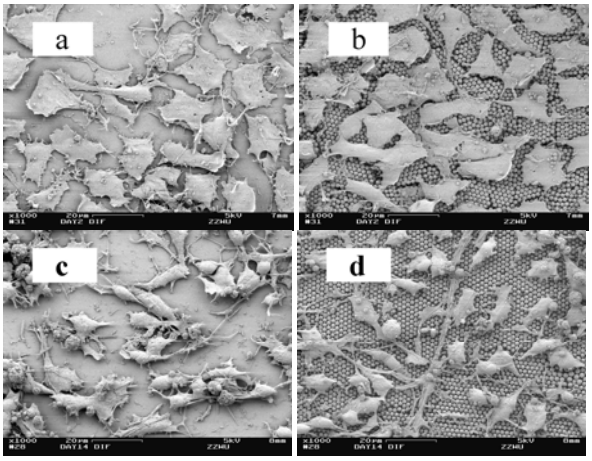

FIG. 1. SEM images showing H945RB.3 cells growing on polystyrene flat surfaces (a, c) and microbead array (b, d) before differentiation (a, b) and on day 14 into differentiation (c, d). The microbeads had a nominal mean diameter of 1.98 μm. Bar=20 μm.

Conclusions: Microbead arrayed substrates enhanced H945RB.3 neural progenitor cell adhesion, spreading and VGCC responsiveness upon differentiation. This echoed our speculation that cell spreading and morphology play an important role in directing neuronal differentiation.

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