A Comparative Analysis of Neurite Development in PC12 Cells Cultured on Nanopillars and Nanopores

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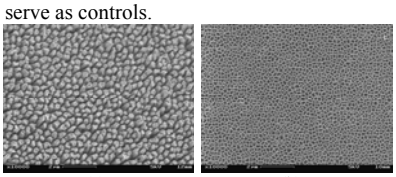
INTRODUCTION

To be able to control cell development for cell/tissue engineering applications, it is important to know the effect of the topography of basal membranes found *in vivo* and of substrates used *in vitro* on various cellular activities of attached cells. We investigated the role of substrate topography with nanoscale features on cellular activities such as differentiation and proliferation in PC12 cells.

MATERIALS AND METHODS

Four types of substrates were used. They are gold nanopillars with 229nm in diameter, 2123nm in height and 69nm in separations developed using electro-deposition (Fig-1a), gold coated porous anodized alumina (PAA, Whatman Inc.) with pores of 206nm in diameter and 41nm in separation (Fig-1b), along with gold coated coverslips and non-coated coverslips to serve as controls.



(a) (b)

Fig-1, SEM images show top-view of (a) nanopillars (b) nanopores.

Rat pheochromocytoma (PC12) cells were seeded at 5,000 cells/cm^2 on all substrate types in triplicates and maintained in RPMI-1640 medium (Sigma), supplemented with 10% horse serum (JRH Biosciences), 5% fetal calf serum (Atlanta Biological) and 2% v/v penicillin (Sigma). Cell culture was maintained at 37^{o}C in a humidified atmosphere of 5% CO_2/ 95% air. Nerve growth factor (Sigma) was added (100 ng/ml) to elicit neurite growth in all setups. Two sets of experiments were prepared: one for scanning electron microscope (SEM) analysis and the other for fluorescent microscopy (FM) analysis. Micro-tubule and actin were labeled for FM.

Cells in all setups were counted on day-4 using an areal count and fixed for SEM and FM. The neurite development was analyzed using a manual tracing method. Neurite length was measured from the leading tip (filopodia) to the base of the neurites. Number of neurites per neuron cell (neurite density) was counted. The percentage of the adhered viable cells (6 hours after cell seeding) out of the total plated cells was calculated as the plating efficiency. A paired wise *t*-test was done to compare the different groups and a *p*-value <0.05 was considered to be significantly different. A total of 20 cells were counted in all experimental groups.

RESULTS AND DISCUSSIONS

Neurite development was used to quantify differentiation and cell density was used to quantify proliferation. Table-1 lists the quantitative results of cellular activities in PC12 cells on different types of substrates. The data shows a similar trend in neurite length and neurite density between cells on different types of substrates in both analyses. Fig-2 shows that cells on nanopillars developed short and few neurite; cells on nanopores developed intermediate length and number of neurites; cells on smooth gold substrates and coverslips developed long and multiple neurites.

Table-1, Quantitative analysis of cellular activities in PC12 cells on different types of substrates

Type of Substrate	Plating Efficiency (%)	Cell Density x1,000 cells/cm^2	Neurite Length (microns)		Neurite Density (Neurites/Cell)	
			SEM	FM	SEM	FM
Nanopillars	83.4±2.2	22.6±0.6	14.5±1.6	11.9±0.8	2.3±0.1	3.0±0.1
Nanoporss	85.8±2.0	16.4±0.4	33.2±2.9	31.3±2.1	3.8±0.2	4.1±0.1
Gold Smooth	86.9±2.4	14.3±0.3	46.3±1.5	45.5±1.5	4.3±0.1	4.9±0.1
Coverslips	90.1±2.5	14.3±0.3	47.4±2.9	49.0±4.0	4.4±0.1	4.8±0.1

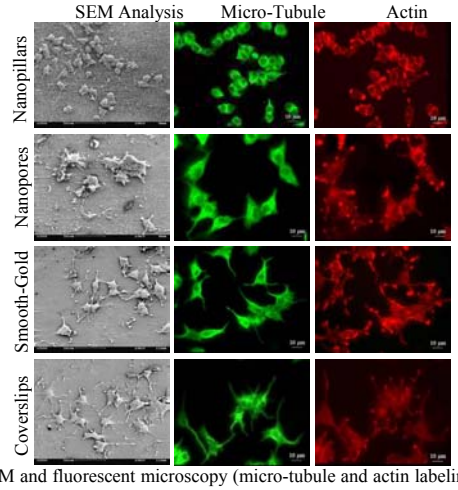


Fig-2, SEM and fluorescent microscopy (micro-tubule and actin labeling) images of PC12 cells on different types of substrates after 4 days of culture.

Fig-3 shows the plating efficiency (Fig-3a), cell density (Fig-3b), neurite length (Fig-3c) and neurite density (Fig-3d) of cells on different types of substrates. All substrates had a very high plating efficiency and furthermore there is no significant difference in the cell adhesion between the different types of substrates. Cells on nanopillars and nanopores had significantly shorter and fewer neurites and a higher cell density than cells on smooth gold substrates (@p<0.05) and coverslips (*p<0.05). Furthermore, cells on nanopores had significantly longer and multiple neurites and a lower cell density than cells on nanopillars (# p<0.05).

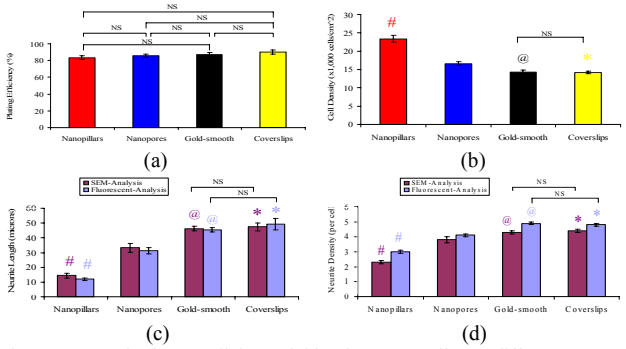


Fig-3, Bar graph shows cellular activities in PC12 cells on different types of substrates (a) mean plating efficiency (b) mean cell density (c) mean neurite length (d) and mean neurite density. Values reported are mean ± standard error; n = 3; #p < 0.05 (nanopillars compared to nanopores); @p < 0.05 (smooth gold substrates compared to nano-featured substrates); *p < 0.05 (coverslips compared to nano-featured substrates); NS = Not Significant.

These findings suggest that nanopillar substrates with smaller diameters and spacing restricted the growth of neuritis, leading to the inhibition of differentiation and enhancement of proliferation of PC12 cells cultured on nanopillar substrates.

CONCLUSION

Nanopillars elicited increased cell proliferation, and nanopores promoted intermediate cell proliferation and differentiation.

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