Effect of Cyclic Uniaxial Strains on Neurite Development in PC12 Cells Cultured on Deformable Micro-Textured Substrates

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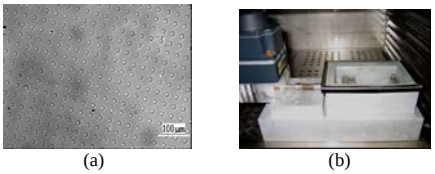
INTRODUCTION

We studied the neurite outgrowth in PC12 cells by exposing the cells to substrates with topographical features subjected to various mechanical strains and analyzing the cytoskeletal actins and microtubules in extending neurites. This study provides some insight into the synergistic effect of the micro-textured substrates, strain amplitudes and strain rates on the neurite outgrowth in PC12 cells.

MATERIALS AND METHODS

Polydimethylsiloxane (PDMS; Dow Chemical) deformable substrates (smooth and textured) with dimensions of 2.5×1.0×0.01 (inch) were developed. The textured substrates have circular shaped micro-islands of equal heights arranged in a square array pattern (Fig.1a). For applying mechanical strains to the substrates, a cell stretching device was built (Fig.1b). Prior to all experiments, the PDMS substrates and the cell stretching device were sterilized by autoclave. The sterilized substrates were then chemically treated with 0.05% poly-L-lysine (70-150 kD.) for four hours followed by two 10-minute washes in deionized water to promote cell adhesion by ionic interactions.



(a) (b)

Fig-1, Micro-patterned PDMS substrates (a) and the stretching device (b).

PC12 cells were seeded at 5,000 cells/cm^2 on all substrates 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) in an incubator. Nerve growth factor (Sigma) was added (100 ng/ml) to elicit neurite growth. Triplicate cultures in each of the static (non-stretched) and dynamic (cyclically stretched) groups with either smooth or micro-textured substrates were set up. In the dynamic conditions, cells were subjected to strains at 4%, 8% or 16% under three different strain rates of 0.1Hz, 0.5Hz and 1.0Hz.

Fluorescent analysis of neurite development was performed using fluorescent microscopy (Zeiss Axioskop 40, Carl Zeiss Inc.). The stained microtubules and actins were observed using fluorescein and rhodamine respectively. Statistical analysis was performed using a 3-factorial design (4×4×2) with three fixed effects: strain level (static, 4%, 8% and 16%), strain rate (static, 0.1Hz, 0.5Hz and 1.0Hz) and topography (smooth and micro-textured). Comparisons were made between cells on smooth and micro-textured substrates at each strain condition to determine the combined effect of applied strain and topography and the combined effect of strain level and strain rate on neurite development. Tukey tests were performed to compare the different groups with a p-value <0.05 being considered as statistically different.

RESULTS AND DISCUSSIONS

In general cells had shorter and fewer neurites on smooth substrates than on textured substrates in the static condition. In the static and 4% at 0.1Hz conditions, cells on textured substrates had greater neurite development and lower cell density than cells on smooth substrates. In the 4% at 0.5Hz and 1.0Hz, the 8% and the 16% at 0.1Hz, 0.5Hz and 1.0Hz conditions, cells showed similar neurite development and cell density on both types of substrates. These results suggest that under the static or small strain conditions, texture had a significant effect on neurite outgrowth.

At the strain level of 4%, a higher strain rate enhanced the neurite development, while at the strain levels of 8% or 16% a lower strain rate induced a similar degree of enhanced neurite development. These facts suggest that there is a synergistic relationship between the strain rate and the strain level: at a higher strain level, increasing the strain rate will decrease the neurite outgrowth, while at a lower strain level, increasing the strain rate will increase the neurite outgrowth. Fig. 2 shows some representative images of stained actins and microtubules of cells on smooth (S) and textured (T) substrates subjected to 4% and 16% at 0.1Hz, 0.5Hz and 1.0Hz.

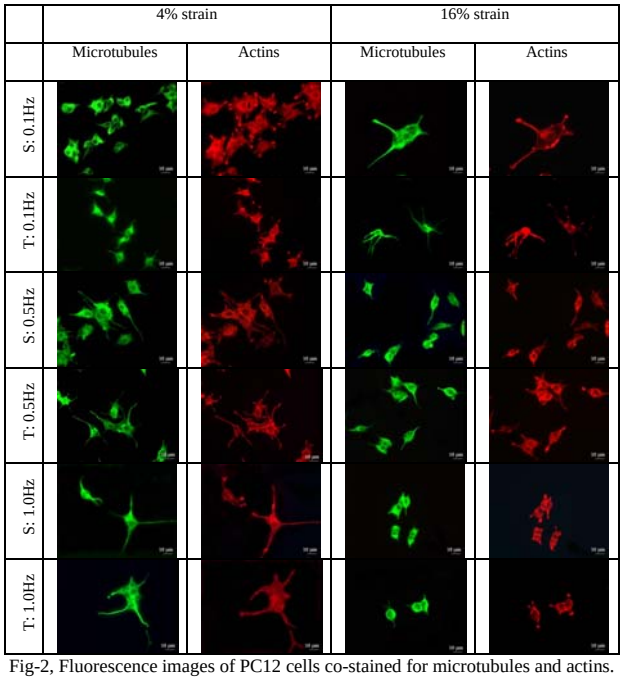


Fig-2, Fluorescence images of PC12 cells co-stained for microtubules and actins.

Cells in the 4% at 0.1Hz and the static conditions showed no major difference in neurite length, neurite density and cell density on both types of substrates. That is to say that the low strain condition of 4% at 0.1Hz did not have any stimulatory effect. But the strain condition of 16% at 1.0Hz had some detrimental effect on neurite outgrowth. This is attributed to the fact that such a high mechanical condition may be responsible for causing the rupture of the actin network leading to neurite retraction. The conditions of 4% at 1Hz, 8% at 0.1Hz and 16% at 0.1Hz, however, are beneficial for stimulating neurite outgrowth. These conditions may be responsible for initiating actin polymerization and facilitating the advancement of the microtubules leading to neurite outgrowth.

CONCLUSION

Micro-textured substrates enhanced neurite development only in the static and 4% at 0.1Hz conditions. Strain level and strain rate have an interrelated effect on neurite development in cells on smooth and micro-textured substrates. On both types of substrates, the conditions of a lower strain level at a higher strain rate (e.g., 4% at 1.0Hz) and a higher strain level at a lower strain rate (e.g., 16% at 0.1Hz) have some stimulatory effects on neurite outgrowth. But the condition of a higher strain level at a higher strain rate (e.g., 16% at 1.0Hz) has adverse effect on neurite development, and the condition of a lower strain level at a lower strain rate (e.g., 4% at 0.1Hz) does not have any stimulatory effect on neurite development.

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