

PC12 Cell Attachment, Proliferation, and Differentiation on Polymer Spherulites

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Statement of Purpose: Rat pheochromocytoma (PC12) cell attachment, proliferation, and neurite extension were regulated on spherulites-bearing polymer surfaces, specifically, banded and non-banded spherulites of poly(ϵ -caprolactone) (PCL) and poly(3-hydroxybutyrate) (PHB) prepared through isothermal crystallization.

Methods: PCL used here had M_n and M_w of 77350 and 142660 g mol⁻¹, respectively.^{1,2} PHB was purchased from Sigma-Aldrich and used as received. PCL was dissolved in CH₂Cl₂ at 0.1 g mL⁻¹ and spin-coated onto a round glass coverslip (15 mm, diameter) at 1000 rpm for 30 s at room temperature. After the PCL films on coverslips were dried completely, they were melted at 80 °C for 10 min and then transferred to a hot stage with a preset crystallization temperature. PHB films were prepared by drop-casting 60 μ L PHB solution in CHCl₃ (0.1 g mL⁻¹) directly on round glass coverslips. After being completely dried, PHB films were melted at 190 °C for 10 min and then quickly transferred to a hot stage with a preset crystallization temperature. Flat films of PCL and PHB as the control groups were prepared by compressing their melts between two glass slides and cooled down to room temperature. Prior to cell studies, the polymer films were immersed in 70% alcohol solution for 30 min for cleaning, rinsed in 70% alcohol solution again for 10 min twice for sterilization, and completely dried in vacuum. Rat PC12 cells were cultured on these polymer films according to our previous report.³

Results: PC12 cells had a lower density on hot-compressed PCL than on the spherulites and the highest value was found on the banded spherulites at all time points. The opposite trend was found for PHB as PC12 cells preferred to grow on the hot-compressed spherulites while the slowest proliferation occurred on the banded spherulites. This finding agreed with our previous report that MC3T3 cell proliferation was enhanced on rougher spherulites of PCL because the higher surface roughness resulted in higher protein adsorption.^{1,2} Although PCL is a tough semi-crystalline polymer with a T_m of 60 °C, its T_g is as low as \sim -60 °C and thus the chain flexibility and mobility are higher at the cell culture condition than those of PHB with a much higher T_g of \sim 0 °C and T_m of 175 °C. Fluorescent cell images were taken with the banded spherulitic background (Fig. 1A). PC12 cells did not show alignment on the banded spherulites, either PCL or PHB. PC12 cell nuclei did not show evident alignment along any specific direction on the banded spherulites but they indeed preferred to stay in the valleys. The percentages of cell nuclei located in the valleys of the banded spherulites were \sim 65% and \sim 70% for PCL and PHB, respectively. PC12 cell differentiation or neurite extension was demonstrated in Fig. 1B after the cells were cultured in the presence of NGF for 7 days on the PCL and PHB substrates. NGF-induced neurite growth was evident on all the samples. The neurites on the hot-compressed

samples were few and short. PC12 cell neurite growth was significantly promoted on the spherulites, in particular, on the banded ones. PC12 cell neurites on the banded spherulites were dendrites and preferentially followed the ridge or groove direction. The number of neurites per cell, the percentage of cells bearing neurites, and the average length per cell were significantly higher on the spherulites than on the hot-compressed samples, and the best results were on the banded spherulites.

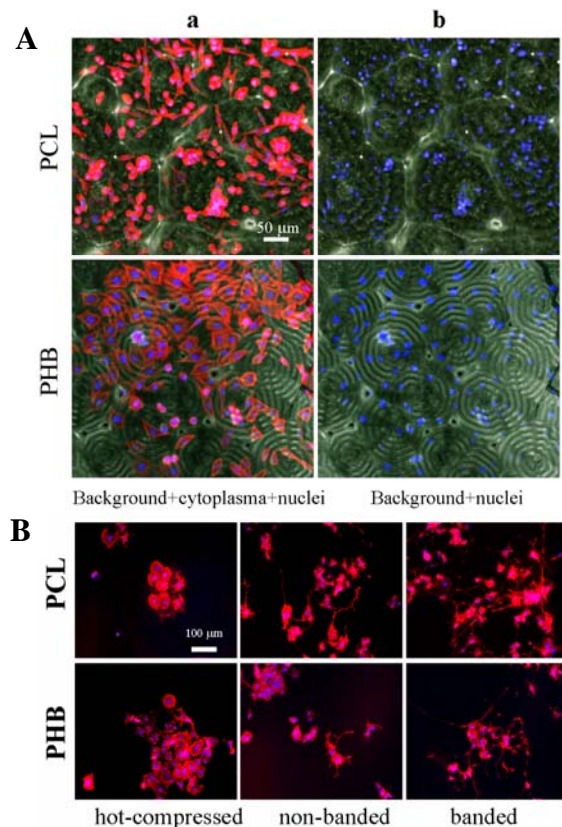


Figure 1. (A) PC12 cells (a) stained with RP (red) and DAPI (blue) and their nuclei (b) stained with DAPI (blue) at day 1 on the banded spherulites of PCL and PHB. (B) PC12 cell neurites on the hot-compressed samples, non-banded spherulites, and banded spherulites.

Conclusions: PC12 cell attachment and proliferation were higher on the spherulites of PCL, especially on the banded spherulites, than on the hot-compressed sample. In contrast, PHB spherulites inhibited PC12 cell attachment and proliferation. The spherulites of both PCL and PHB promoted NGF-induced neurite outgrowth of PC12 cells and the banded spherulites showed evident neurite guidance along the groove direction.

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References: 1. Wang K. *Langmuir* **2012**, 28(9), 4382-95. 2. Wang K. *Macromol. Chem. Phys.* **2012**, 213(12), 1239-50. 3. Cai L. *Langmuir* **2012**, 28(34), 12557-12568.