Two-Dimensional Micropatterns of Self-Assembled Poly(N-isopropylacrylamide) Microgels for Adhesion, Alignment, and Temperature-induced Detachment of NIH 3T3 Fibroblast Cells

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Statement of Purpose: A range of tools have been employed to pattern cells on substrates at a variety of length scales. Cell patterning allows control over cell morphology, alignment, and even cell functions via interaction with underlying biochemical and/or topographical cues. However, most of the current patterning approaches, such as microcontact printing, inkjet printing, and laser bioprinting, require costly, clean-room generated lithography-based "templates"[1] . Here we report a template-free two-dimensional micropatterning of poly(*N*-isopropylacrylamide) (PNIPAM) microgels that self-assemble on optically-transparent polystyrene substrates via a simple dip coating technique[2]. This method allows flexible generation of PNIPAM micropatterns of varying dimensions, which in turn can be employed for cell patterning. Also, the thermo-responsiveness of the PNIPAM-coated surface can be utilized for enzyme-free cell detachment, which is very desirable for applications such as "cell-sheet engineering" with sheets of predetermined sizes and shapes.

Methods: PNIPAM microgels were synthesized using free-radical polymerization and dispersed in deionized water to a final concentration of 0.02 wt%. Polystyrene substrates were immersed into the PNIPAM microgel dispersion maintained at 40 ˚C. Upon upward withdrawal of the polystyrene substrate at an alternating speed of 90 µm/min and 50.9 mm/min and a variety of time scales, a variety of PNIPAM patterns resulted. Coated substrates were seeded with NIH3T3 fibroblasts at 12,000 cells/cm^2 and incubated at 37 °C. After 24 hr and 72 hr, cell adhesion on micropatterned substrates was observed and analyzed for the uniformity and sizes of patterns. For cell detachment studies, the cell-seeded substrates were washed with tissue culture medium maintained at 25 °C.

Results:

Polystyrene substrates were successfully patterned with PNIPAM microgels to form "stripe/spacing" patterns simply by controlling the substrate withdrawal speed (Figure 1a-c). "Stripes" were composed of single layers of densely-packed PNIPAM microgels, while the "spacings" had sparsely distributed microgels. NIH3T3s cells cultured on the substrates for 24 hrs adhered only on PNIPAM micropatterns (Figure 1d-f). For example, for 50/100 PNIPAM micropatterns, the dimension of patterned cells was found to be 86.5 ± 26.8 µm stripes of cells and 109.3 ± 18.4 µm spacings (absent of cells). The detachment of fibroblast cells from micropatterned PNIPAM substrates was then tested. After 24 hr of incubation, patterned cells were washed with tissue culture media at 25 ˚C. Nearly 100% of cells detached from the substrates within a few minutes of washing (Figure 2a,b). 72 hr after seeding, cells proliferated to cover the entire substrate, forming confluent cell layers (Figure 2c). This cell sheet rolled up from the edges and detached when temperature was lowered to 25 °C (Figure 2d).

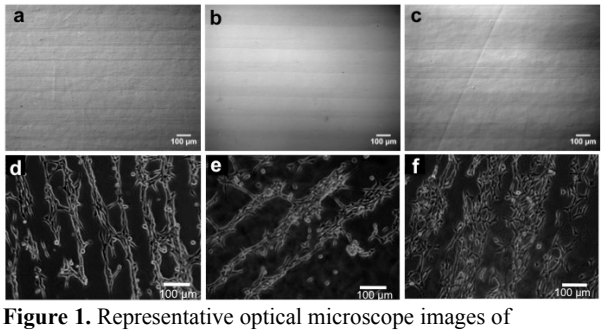

Figure 1. Representative optical microscope images of polystyrene substrate patterned with PNIPAM microgels (a) 50 µm stripe/50 µm spacing (b) 50 µm stripe/100 µm spacing and (c) 100 µm stripe/100 µm spacing. (d,e,f) Patterned NIH 3T3 fibroblasts on substrates a,b, and c respectively.

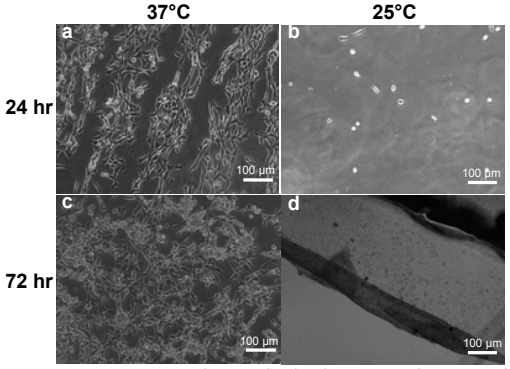

Figure 2. Representative optical microscope images of NIH3T3s cultured on the polystyrene substrates coated with 100 µm stripe/100 µm spacing PNIPAM micropatterns at 37 °C for (a) 24 hr and (c) 72 hr, and after cell detachment upon lowering the temperature to 25 °C (b, d).

Conclusions: We have demonstrated that 2-dimensional PNIPAM micropatterns can be controllably created on polystyrene substrates via a simple and low cost approach. When fibroblasts were seeded on the PNIPAM-patterned substrates, they adhered and formed patterns that replicate the original PNIPAM patterns achieved with dip coating. With time, adhered fibroblasts proliferated on the PNIPAM-coated substrates to form confluent cell layers. Cell sheets were released upon reduction in temperature to 25 °C. This approach can be used to pattern cell substrates for tissue engineering/cell-sheet engineering applications or fundamental cell-material interactions studies.

References:

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