Cell Culture Platform with Mechanical Conditioning and Non-damaging Cellular Detachment

Elaine L. Lee, BS, and Horst von Recum, PhD
Case Western Reserve University

Statement of Purpose: Cells implanted following injury may remodel undesirably with improper mechanical stimulation from surrounding tissue.[1] Proper conditioning of tissue engineered constructs before implantation can lead to suitable tissue architectures, along with an extracellular matrix (ECM) environment that more closely mimics native tissue. Additionally, cell implantation without bulky polymeric scaffolding is often desirable. Previous researchers have created devices capable of applying mechanical forces to cells (e.g. stretch), but cellular removal from these devices, such as by trypsin, often results in irreversible damage.[2] Conversely, devices are available that can detach intact cells, but these are inelastic, non-stretchable substrates.[3] We have created a cell culture platform that allows for mechanical conditioning and then subsequent non-damaging detachment of those cells.

Methods: We have modified silicone culture surfaces, to incorporate thermally responsive polymers of N-isopropylacrylamide (NIPAAm) to create an elastic substrate that can also change surface properties with temperature change. The thermally responsive nature of P(NIPAAm) allows for cell attachment at 37°C, and spontaneous detachment at room temperature, allowing for cell removal from a tissue culture surface without using damaging enzymatic treatments. A copolymer of NIPAAm and 10% w/w acrylic acid (AAc) was conjugated to a commercially available amine-bonded silicone surface (BioFlex Culture Plate-Amino, Flexcell International, Hillsborough, NC). The silicone membrane was first swollen using tert-butanol, and then the copolymer was conjugated overnight at room temperature using carbodiimide chemistry. Excess reagents were removed in three cycles by first swelling the membranes in excess tert-butanol at 30°C for 20 min and then rinsing in excess deionized water. FTIR, XPS, and contact angle were used to verify the surface modification.

To determine single cell detachment capability both before and after mechanical stretching, 3T3 cells (100,000/well) were allowed to detach as the culture media was brought to room temperature over the course of 1 hr. Commercially available P(NIPAAm) electron beam-grafted to inelastic polystyrene (UpCell, Thermo Scientific Nunc, Waltham, MA) were used as a positive control for cell detachment. Cells were conditioned using the Flexcell FX-4000 Tension System at 5% elongation strain, 1 Hz for 24 hrs. Following stretching, cells were stained with phalloidin to elucidate filament alignment.

Results: Cells were able to attach to the resulting surfaces at 37°C and showed detachment by rounded morphology at 25°C (Figure 1). Following mechanical stretching, cells were still able to spontaneously detach from these modified silicone surfaces with temperature change. In addition, cells also showed increased alignment.

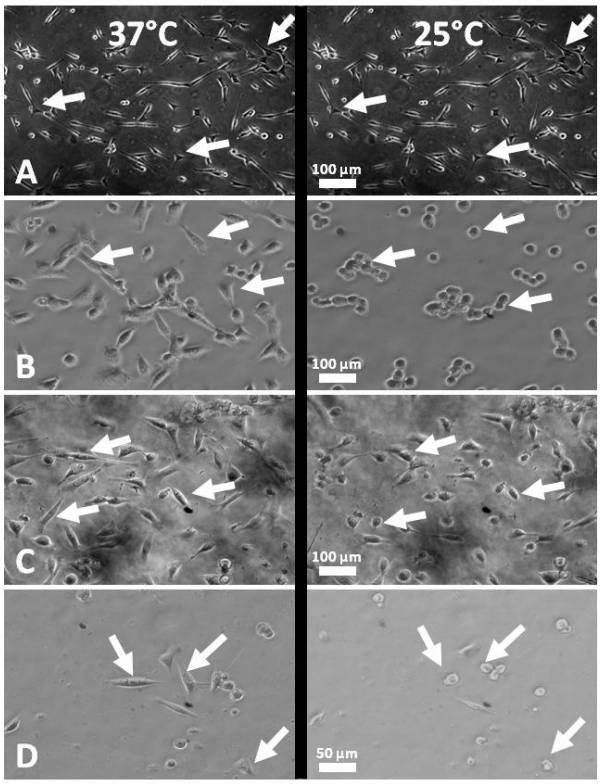

Figure 1. Cells grown on (A) negative control unmodified silicone, (B) positive control P(NIPAAm) electron-beam grafted onto polystyrene, and (C) silicone modified with P(NIPAAm-co-AAc) before stretching, and (D) following mechanical stretching were compared at 37°C at time 0 min (Left) and again at 25°C after 1 hr (Right).

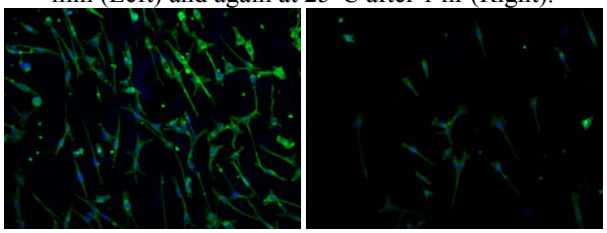

Figure 2. Cells were aligned following mechcanical stretching for both unmodified silicone (Left) and silicone modified with P(NIPAAm-co-AAc).

Conclusions: We were able to modify silicone surfaces with P(NIPAAm) copolymers to allow cell detachment with a change in temperature. Following mechanical conditioning, the modified membranes were able to retain the ability to detach cells. We are currently investigating detachment of cellular sheets.

References:

1. Chiquet M. Biochem Cell Biol. 1996;74:737-44.
2. Eastwood M.Biochim Biophys Acta.1994;1201:186-92.
3. von Recum HA. J Biomed Mater Res. 1998;40:631-9.

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