

CELL CULTURE PLATFORM WITH MECHANICAL CONDITIONING AND NONDAMAGING CELLULAR DETACHMENT

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Introduction Cells implanted following injury may remodel undesirably with improper mechanical stimulation from surrounding tissue. Proper cell conditioning *in vitro* before implantation can lead to extracellular matrix (ECM) growth that more closely mimics native tissue, and scaffolds are often used to promote ECM growth. However, because of adverse effects (eg, cytotoxicity, inflammation) from polymer degradation, implanting intact tissue without a scaffold is also highly desirable. Previous groups have created devices that stretch cells but require damaging treatment for removal, or conversely, devices with detachable cell capabilities on inelastic substrates.

Methods We have created a cell culture platform that combines mechanical conditioning *in vitro* and then allows nondamaging detachment of cells and ECM for therapeutic use. Poly(N-isopropylacrylamide) (P(NIPAAm)) is a thermally responsive polymer that, when attached to culture surfaces, allows cell attachment at 37°C, and spontaneous detachment at room temperature, without using damaging enzymatic treatments. We have modified commercially available silicone membranes with amine-conjugated surfaces (available from FlexCell), to incorporate P(NIPAAm) to create an elastic substrate that can also change surface properties with temperature change. NIPAAm was first copolymerized with 10% w/w acrylic acid (AAc). P(NIPAAm-co-AAc) composition and phase transition temperature were verified as suitable for cell culture, and then conjugated with the amine-treated silicone surface through N,N'-dicyclohexylcarbodiimide chemistry.

Results P(NIPAAm-co-AAc) composition was verified using x-ray photoelectron spectroscopy (XPS) and acid titration. P(NIPAAm-co-AAc) addition to the amine-treated silicone surface was verified by XPS to have a higher nitrogen content than amine-treated silicone alone, and infrared spectroscopy (FTIR) revealed the presence of the P(NIPAAm) peak even after washing the modified surface. Temperature change properties were verified through contact angle analysis. Cells attached to the modified surfaces at 37°C and showed a rounded morphology, indicating cell detachment, at 25°C. Following mechanical stretching on the FlexCell Tension Plus System, cells still spontaneously detached from our modified silicone surfaces after a temperature change to 25°C.

Conclusion We have created a cell culture platform that allows nondamaging cell detachment following mechanical conditioning of cells as a first step to engineering *in vitro* replacement tissue requiring specific mechanical environments.

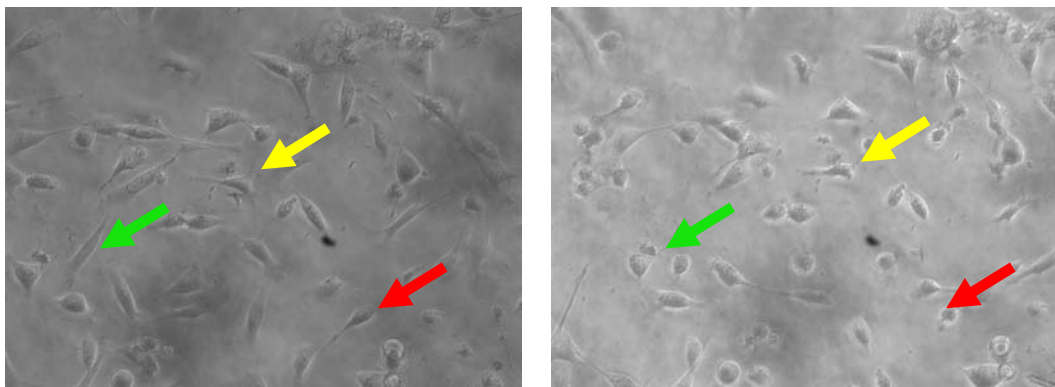


Figure. Cells are attached to the silicone membrane surface modified with P(NIPAAm-co-AAc) at 37°C (left) and releasing focal adhesions to exhibit a detached, rounded morphology at 25°C (right).

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