Design for a Cell Conditioning Platform with Nondamaging Cell Detachment Capability

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Purpose: Cells implanted following injury may remodel undesirably with improper mechanical stretching from the surrounding tissue.[1] Proper conditioning of such cells in vitro before implantation can lead to extracellular matrix (ECM) growth that more closely mimics the native tissue, and a scaffold is often used to promote ECM growth. However, because of adverse effects (eg, cytotoxicity, inflammation) from polymer degradation, implantation of the intact tissue without a scaffold is also highly desirable. Previous groups have created devices that mechanically stretch cells but require damaging treatment for removal from substrates.

We are creating a cell culture platform that combines mechanical conditioning in vitro and then allows nondamaging detachment of cells and ECM for therapeutic use. Whereas previous groups have demonstrated detachable cell capabilities on inelastic substrates,[2] our goal is to modify 6-well plates with elastic silicone surfaces, to incorporate the polymer poly(N-isopropylacrylamide) (PIPAAm). The thermally responsive nature of PIPAAm 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.

Methods: We applied our technology to a commercially available system FlexCell Tension Plus System (FlexCell International Corp., Hillsborough, NC) by modifying their pre-existing BioFlex Culture Plates. The BioFlex plates feature polydimethysiloxane (PDMS or silicone) membrane bottoms and can be used in conjunction with the Tension Plus System to control cell stretching parameters.

To modify the surfaces, PIPAAm was first co-polymerized with cross-linker allylamine (AA) (0.2% or 1% w/w AA:PIPAAm), and subsequently conjugated to cinnamoyl chloride (CC) (10:1 molar ratio CC:AA) to form UV-sensitive cross-linkable bonds. The silicone membranes were swollen in tert-butanol to allow co-polymer chains to entangle with the silicone to form an interpenetrating network. The solvent was then evaporated by air and vacuum dried before the cross-linkable bonds were activated by UV light. Phase transition temperature property changes of the resulting polymer were tested by measuring absorbance at 500 nm in temperatures ranging from 23°C to 42°C.

To determine interpenetration of PIPAAm with the silicone membrane, samples were tested for presence of PIPAAm before and after washing with room temperature water using Fourier transform infrared spectroscopy (FTIR) and contact angle.

Results: Phase transition temperature studies showed that both non-cross-linked and UV-activated cross-linked PIPAAm conjugated with allylamine has a slightly more hydrophobic shift in transition temperature that is negligibly different from PIPAAm alone.

FTIR showed that peaks characteristic to PIPAAm were present before washing (Figure 1); however, these peaks were not present following washing (data not shown).

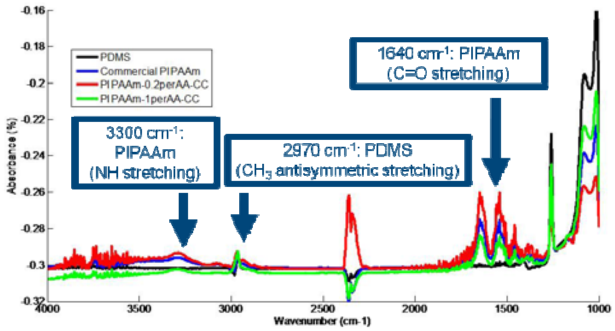

Figure 1. FTIR of PIPAAm on silicone before washing.

Polymer	Prewashed 21°C	Prewashed 37°C	Washed 21°C	Washed 37°C
Silicone[ab]	76.31 ± 0.18	69.34 ± 3.70	---	---
Commercial PIPAAm on silicone[a]	18.61 ± 0.18	48.32 ± 3.60	52.48 ± 18.94	66.58 ± 3.13
PIPAAm-0.2% AA on silicone[bc]	28.70 ± 9.37	50.00 ± 9.74	69.69 ± 1.13	62.28 ± 10.47

Table 1. Contact angle comparison of prewashed and washed silicone surfaces modified with PIPAAm.

Contact angle values are means of 4 measurements ± SD (Table 1). Single-factor analysis of variance (ANOVA) performed on data resulted in $P < 0.01$ comparisons (*ab*) between temperatures in prewashed samples and (*c*) between prewashed and washed samples at 21°C. Data analyses for surfaces modified with PIPAAm conjugated to 1% allylamine yielded similar results.

Conclusions: PIPAAm conjugated to allylamine retains its temperature responsive properties. Although PIPAAm is present before washing, it is not present following washing. This indicates that although PIPAAm can successfully initially entangle into the silicone, further studies are necessary to create a more robust network of PIPAAm that can remain entrapped in silicone even with repeated washing.

References:

1. Chiquet M. Biochem Cell Biol. 1996;74:737-44.
2. von Recum HA. J Biomed Mater Res. 1998;40:631-9.

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