Development of a novel microwell-patterned PNIPAM-based hydrogel for temporary disruption of cell membrane

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Introduction: Current research in drug delivery and immunoengineering has faced the barrier of the selectivity of diffusion across the cellular membrane. These effects can be detected in experiments involving cellular uptake of compounds, such as growth factors and/or drugs. Previous studies have shown that inducing mechanical pressure on the cellular membrane increases its permeability by altering osmotic pressure and disrupting membrane fluidity, as described by the fluid-mosaic model. This increase in permeability allows for decrease in diffusion time and enables diffusion of larger molecules that were previously unable to diffuse. A tunable platform for inducing uniform or isotropic mechanical stress on the cellular membrane would optimize diffusion rates.[1,2] Poly(*N*-isopropylacrylamide) (PNIPAM) based hydrogels have been extensively researched for biomedical applications.[3] These hydrogels are of elevated interest, because of their temperature-responsive properties at temperatures within the range of physiological (37°C) and room (25°C) temperatures. The polymer, PNIPAM, has a reversible lower critical solution temperature (LCST) of 32°C allowing it to exhibit an increase in volume when cooled from 37°C to 25°C.[3,4] During this process, microwells imprinted on the surface of the hydrogels decrease in volume. The decrease in microwell volume will induce isotropic mechanical stress on the cellular membrane of cells seeded in these microwells.[2,5]

Materials and Methods: Poly(*N*-isopropylacrylamide) (PNIPAM) hydrogels were synthesized via free radical solution polymerization from the monomers *N*-isopropylacrylamide (NIPAM) and *N*,*N*'-methylenebisacrylamide (BIS). Potassium persulfate (KPS) and Tetramethylethylenediamine (TEMED) were used as the radical initiator and radical generator accelerator, respectively.[4] Due to the dependence of the reaction on the lack of external oxidative species, the solutions were first purged with nitrogen and reacted under argon gas. To remove unreacted compounds, the hydrogel underwent a thermocycling process depending on resulting hydrogel thickness. To quantify the degree of swelling, hydrogels were incubated with DI water at 37°C for 24 hrs and weighed. The hydrogels were then moved to 25°C DI water and weighed periodically. An MTS assay was performed on cells seeded on the surface of the hydrogels that have undergone 3, 6, and 9 repetitions of the thermocycling process and compared to cells that were 2D cultured; this determined the overall cytotoxicity of the PNIPAM hydrogel and identified the relationship between thermocycling and cytotoxicity. The MTS Assay was performed on 3 cell lines: HDF (human dermal fibroblast), MDA-MB-231 (breast cancer), and THP-1 (monocyte).

Results and Discussion: We observed a significant microwell volume reduction based on hydrogel swelling kinetics with a temperature change from 37°C to 25°C. This volume reduction provides the mechanism for inducing isotropic stress on cellular membrane. The MTS Assay showed that there was no statistically significant difference between the cytotoxic effect of the 2D culture and hydrogels that have undergone the thermocycle process more than three repetitions at a thickness of 3 mm.

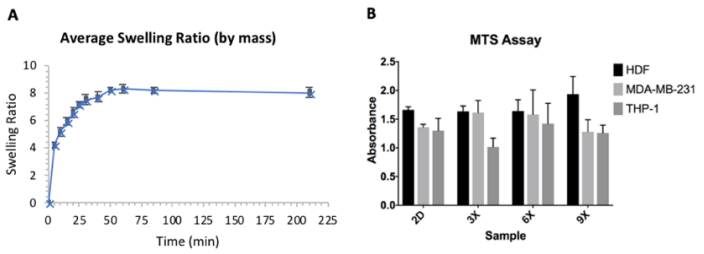

Figure 1. Quantification of PNIPAM swelling properties and cell-compatibility were assessed by A) periodic mass ratio measurements and B) MTS Assay, respectively. MTS Assay was performed on three hydrogel samples of varying thermocycle purification and three different cell lines: HDF, MDA-MB-231, and THP-1.

Conclusions: We demonstrated that PNIPAM hydrogels exhibit significant swelling properties between room (25°C) and physiological (37°C) temperatures. Furthermore, the hydrogels do not exhibit apparent cytotoxicity given adequate thermocycle purification. Further experiments need to be performed to determine the effects of microwell-volume reduction on cells. The insight gained from this project provides a rational basis for utilizing PNIPAM-based hydrogels in drug-delivery and stem cell engineering systems in both research and clinical settings.

Keywords: PNIPAM hydrogel; microwells; membrane disruption; drug delivery; MDA-MB-231

Acknowledgements
This work was supported in part by the South Carolina Honors College (SCHC) Science Undergraduate Research Fellowships (SURF) Program and the University of South Carolina Magellan Scholar Program.

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