

## Chondrocytes in Monolayer Culture on a Thermoresponsive Polymer vs. Conventional TCPS

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**INTRODUCTION:** Expansion of cells is an essential step in tissue engineering to provide sufficient numbers from a tissue biopsy. In monolayer culture, trypsin is commonly used to detach cells. To remove the potential risk of cross infection<sup>1</sup>, alternatives to animal-derived products are being investigated. Culture of cells on thermoresponsive polymer grafted surfaces offers one such alternative method of cell detachment<sup>2</sup>. When cooled below their lower critical solution temperature (LCST), approximately 32°C, these hydrogels become swollen due to hydration and cause cell detachment. The aim of the study was to compare the detachment of bovine articular chondrocytes (BAC) from tissue culture polystyrene (TCPS) surfaces using trypsin/EDTA (0.5 g/L trypsin/0.2 g/L EDTA), with the detachment from a thermoresponsive polymer surface.

**METHODS:** A thermoresponsive hydrogel system (poly(NIPAM)-g-GMMA-co-EGDMA) was synthesised<sup>3</sup>. Chondrocytes were isolated from bovine metacarpophalangeal joints, expanded, and seeded onto the different surfaces (as described previously<sup>4</sup>). When confluent, the cells were stained with CellTracker™ Green CMFDA (Molecular Probes, C2925). Live imaging was acquired to follow cell detachment, using a fluorescence microscope (Zeiss Axiovert 200M). Cell viability by Alamar blue assay was also studied as a result of the different culturing conditions.

**RESULTS:** Cultures with similar morphologies were obtained on both surfaces looking at the micrographs.



Fig. 1: Micrographs showing BAC (passage 2) after 7 days of culturing on (a) poly(NIPAM)-g-GMMA-co-EGDMA and (b)TCPS

Assessment of cell viability with Alamar blue indicated that cells were equally viable on both surfaces.

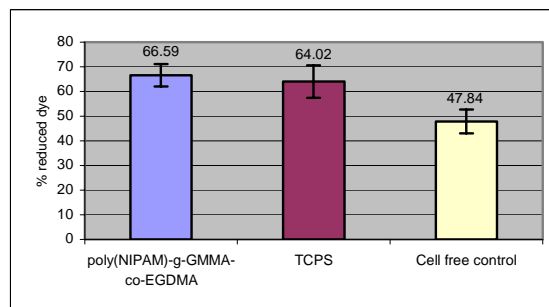


Fig. 2: Cellular activity of BAC on poly(NIPAM)-g-GMMA-co-EGDMA and TCPS respectively was determined by Alamar blue assay after five days of culture as percentage reduction of the stain.

**DISCUSSION & CONCLUSIONS:** The results indicated that poly(NIPAM)-g-GMMA-co-EGDMA grafted surfaces offered a promising alternative for the expansion of chondrocytes. Our interim conclusion was that the mechanism may be more complex than previously reported, encompassing changes in both hydrophilicity and dimension. Work is in progress to study the arrangement of the cytoskeleton by following the actin filaments on the different surfaces.

**REFERENCES:** <sup>1</sup>Jochems, CEA, et al, *Altern Lab Anim*, 2002. **30**(2): p. 219-227. <sup>2</sup>Okano, T, et al., *Biomaterials*, 1995. **16**(4): p. 297-303. <sup>3</sup>PhD Thesis of Jon Collett, 2005. <sup>4</sup>Hollander, A.P. and Hatton, P.V., (2004). *Biopolymer Methods in Tissue Engineering*. Totowa, New Jersey, Human Press Inc.

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