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# Thermoresponsive 3D scaffolds for non-invasive cell culture

by

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**Thermoresponsive 3D scaffolds for non-invasive cell culture**

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# ABSTRACT

Conventionally, adherent cells are cultured *in vitro* using flat 2D cell culture trays. However the 2D cell culture method is tedious, unreliable and does not replicate the complexity of the 3D dynamic environment of native tissue. Nowadays 3D scaffolds can be used to culture cells. However a number of challenges still exist, including the need for destructive enzymes to release confluent cells. Poly(*N*-isopropylacrylamide) (PNIPAAm), a temperature responsive polymer, has revolutionised the cell culture fraternity by providing a non-invasive means of harvesting adherent cells, whereby confluent cells can be spontaneously released by simply cooling the cell culture medium and without requiring enzymes. While PNIPAAm monolayer cell culturing is a promising tool for engineering cell sheets, the current technology is largely limited to the use of flat 2D substrates, which lacks structural and organisational cues for cells.

The aim of this project was to develop a 3D PNIPAAm scaffold which could be used efficiently for non-invasive 3D culture of adherent cells. This project was divided into three phases: Phase 1 (preliminary phase) involved development and characterisation of cross-linked PNIPAAm hydrogels; Phase 2 involved development and characterisation of PNIPAAm grafted 3D non-woven scaffolds, while Phase 3 focused on showing proof of concept for non-invasive temperature-induced cell culture from the 3D PNIPAAm grafted scaffolds.

In Phase 1, PNIPAAm was cross-linked with N,N'-methylene-bis-acrylamide (MBA) using solution free-radical polymerisation to form P(PNIPAAm-*co*-MBA) hydrogels. A

broad cross-link density (i.e. 1.1 - 9.1 Mol% MBA) was investigated, and the effect of using mixed solvents as the co-polymerisation medium. The P(PNIPAAm-*co*-MBA) gels proved unsuitable as a robust cell culture matrix, due to poor porosity, slow swelling/deswelling and poor mechanical properties.

Subsequently, in Phase 2, polypropylene (PP), polyethylene terephthalate (PET), and nylon fibers were processed into highly porous non-woven fabric (NWF) scaffolds using a needle-punching technology. The NWF scaffolds were grafted with PNIPAAm using oxyfluorination-assisted graft polymerisation (OAGP). The OAGP method involved a 2 step process whereby the NWF was first fluorinated (direct fluorination or oxyfluorination) to introduce new functional groups on the fibre surface. The functionalised NWF scaffolds were then graft-polymerised with NIPAAm in an aqueous medium using ammonium persulphate as the initiator.

Following oxyfluorination, new functional groups were detected on the surface of the NWF scaffolds, which included C-OH; C=O; $CH_2$-CHF, and CHF-CHF. PP and nylon were both easily modified by oxyfluorination, while PET displayed very little changes to its surface groups. Improved wetting and swelling in water was observed for the oxyfluorinated polymers compared to pure NWF scaffolds. PP NWF showed the highest graft yield followed by nylon and then PET. PNIPAAm graft yield on the PP NWF was ~24 ± 6 µg/cm$^2$ on grafted pre-oxyfluorinated NWF when APS was used; which was found to be significantly higher compared to when pre-oxyfluorinated NWF was used without initiator (9 ± 6 µg/cm$^2$, p= 1.7x10$^{-}$7); or when grafting was on pure PP with APS (2 ± 0.3 µg/cm$^2$, p = 8.4x10$^{-12}$). This corresponded to an average PNIPAAm layer thickness of ~220 ± 54 nm; 92 ± 60 nm; and 19 ± 3 nm respectively. Scanning electron microscopy (SEM) revealed a rough surface morphology and confinement of the PNIPAAm graft layer to the surface of the fibers when oxyfluorinated NWF scaffolds were used, however when pure NWF scaffolds were used during grafting, homopolymerisation was observed as a loosely bound layer on the NWF surface. The OAGP method did not affect the crystalline phase of bulk PP as was determined by X-ray diffraction (XRD), however, twin-melting thermal peaks were detected from DSC for the oxyfluorinated PP and PP-*g*-PNIPAAm NWF which possibly indicated crystal defects. Contact angle studies and microcalorimetric DSC showed that the PP-*g*-PNIPAAm NWF scaffolds exhibited thermoresponsive behaviour. Using the 2,2-Diphenyl-1-1-picrylhydrazyl (DPPH) radical method and electron-spin resonance (ESR), peroxides, as well as trapped long-lived peroxy

radicals were identified on the surface of the oxyfluorinated PP NWF, which are believed to be instrumental in initiating graft polymerisation from the NWF. A free radical mechanism which is diffusion controlled was proposed for the OAGP method with initiation via peroxy radicals ($RO^{\bullet}$), as well as $SO_4^{\bullet-}$ and $OH^{\bullet}$ radicals, whereby the latter result from decomposition of APS.

In Phase 3 of this study, proof-of-concept is demonstrated for use of the PNIPAAm grafted NWF scaffolds in non-invasive culture of hepatocytes. Studies demonstrated that hepatocyte cells attached onto the 3D PNIPAAm scaffolds and remained viable in culture over long periods. The cells were released spontaneously and non-destructively as 3D multi-cellular constructs by simply cooling the cell culture medium from 37 ℃ to 20 ℃, without requiring destr uctive enzymes. The PP-*g*-PNIPAAm NWF scaffolds performed the best in 3D cell culture. Additionally the CSIR is developing a thermoresponsive 3D (T3D) cell culturing device, whereby the 3D thermoresponsive NWF scaffolds are used in the bioreactor for cell culture. Temperature-induced cell release was also verified from the 3D thermoresponsive scaffolds in the bioreactor. This technology could lead to significant advances in improving the reliability of the *in vitro* cell culture model.

*Key-words:* Poly-*N*-Isopropylacrylamide; graft polymerisation; 3D scaffolds; non-wovens; hydrogels; cell culture

# OUTPUTS EMANATING FROM STUDY

## Papers published

1. **Avashnee S. Chetty**, Viktoria Vargha, Arjun Maity, F. Sean Moolman, Claire Rossouw, Rajesh Anandjiwala, Lydia Boguslavsky, Dalu Mancama, Walter W. Focke, Development of thermoresponsive PP-*g*-PNIPAAm non-woven 3D scaffold for smart cell culture using oxyfluorination-assisted graft polymerisation, Colloids and Surfaces A: Physicochemical and Engineering Aspects, Colloids and Surfaces A: Physicochem. Eng. Aspects, 2013; 419: 37– 45.
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3. **Avashnee S. Chetty**, János Kovács, , Viktória Vargha, Ágnes Mészáros, Jenő Fekete, Attila Domján, A versatile characterisation of poly(*N*-isopropylacrylamide-*co*-N,N’-methylene-bis-acrylamide) hydrogels for composition, mechanical strength, and rheology, EXPRESS Polymer Letters, 2012; 7(1): 95–105
4. Viktoria Vargha, **Avashnee S. Chetty,** Zsolt Sulyok, Judith Mihály, Zsófia Keresztes, Andraś Tóth, Istvań Sajó, László Korecz, Rajesh Anandjiwala, Lydia Boguslavsky, Functionalisation of polypropylene non-woven nonwoven fabrics (NWF's) by oxyfluorination as a 1st step for graft polymerisation, Journal of Thermal Analytical Calorimetry, 2012; 109:1019-1032.

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1. **Avashnee S. Chetty**, Claire Rossouw, Luvo Ntsangani, Sean Moolman, Kobus van Wyk, Dalu Mancama, Rajesh Anandjiwala, Lydia Boguslavsky, Viktoria Vargha, Smart 3D cell culture using a thermoresponsive PP non-woven scaffold”, 11th ICFPAM conference- International Conference on Frontiers of Polymers and Advanced Materials: Symposium 8: Biomaterials Africa 2011, Pretoria, South Africa, May 2011, Paper 8A133C, pg 279.
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# DEFINITIONS AND ABBREVIATIONS

| | |
|---|---|
| 2D | Two dimensional |
| 3D | Three dimensional |
| APS | Ammonium persulphate |
| ATR-FTIR | Attenuated total reflectance Fourier transform infrared |
| CSIR | Council for Scientific and Industrial Research |
| DPPH | 2,2-Diphenyl-1-1-picrylhydrazyl |
| DMEM | Dulbecco's Modified Eagle Medium |
| DSC | Differential scanning calorimetry |
| ECM | Extracellular matrix |
| ESR | Electron spin resonance |
| FCS | Foetal calf serum |
| FDA | Fluorescein diacetate |
| LCST | Lower critical solution temperature |
| LVE | Linear viscoelastic |
| MBA | N,N'-methylenebisacrylamide |
| NIPAAm | *N*-isopropylacrylamide |
| NWF | Non-woven fabric |
| OAGP | Oxyfluorination-assisted graft polymerisation |
| PBS | Phosphate buffered saline |
| PE | Polyethylene |
| PET | Polyethyleneterephthalate |
| PNIPAAm | Poly(*N*-isopropylacrylamide) |
| PP | Polypropylene |
| TCPS | Tissue-culture polystyrene |
| TEMED | N,N,N'-N'-tetramethylenediamine |
| R | Cross-link density (nMol NIPAAm/nMol MBA) |
| SEM | Scanning electron microscopy |
| THF | Tetrahydrofuran |
| UV-VIS | Ultraviolet-visible |
| XRD | X-ray diffraction |
| XPS | X-ray photoelectron spectroscopy |