Development of thermoresponsive non-woven 3D scaffold for smart cell culture

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INTRODUCTION

Conventional cell culture of adherent cells, based on the use of tissue culture polystyrene trays is an inefficient method to culture cells. The method employs the use of 2D surfaces and enzymatic treatment to release propagated cells. It is well-known that cells grown on 2D surfaces display vast differences to physiological tissue, also enzymatic cell release damages and disaggregates the cells' extracellular matrix (ECM), which contains crucial surface proteins. The CSIR developed a 3D scaffold which is based on a polypropylene (PP), non-woven fabric (NWF), grafted with poly (*N*-isopropylacrylamide) (PNIPAAm) for use in non-invasive 3D cell culture [1]. PNIPAAm is a thermo-responsive polymer with a lower critical solution temperature of 32°C. At temperatures above 32°C, PNIPAAm is hydrophobic and below 32°C, the polymer becomes hydrophilic (**Figure 1**). This phenomenon is used to culture the cells at 37°C, which resembles the normal body temperature, and to release the cells at 20°C as the cells being cultured only attach to hydrophobic surfaces[2-4].

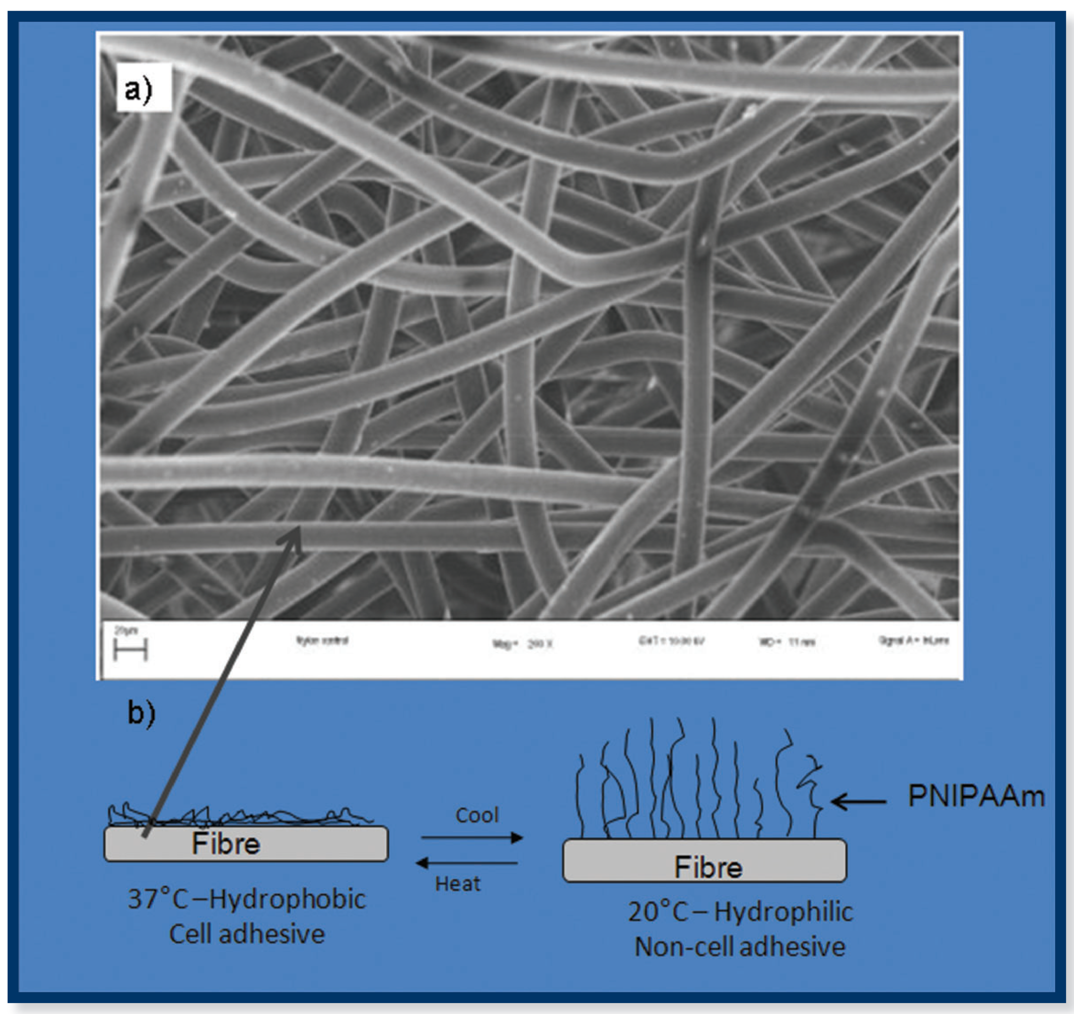


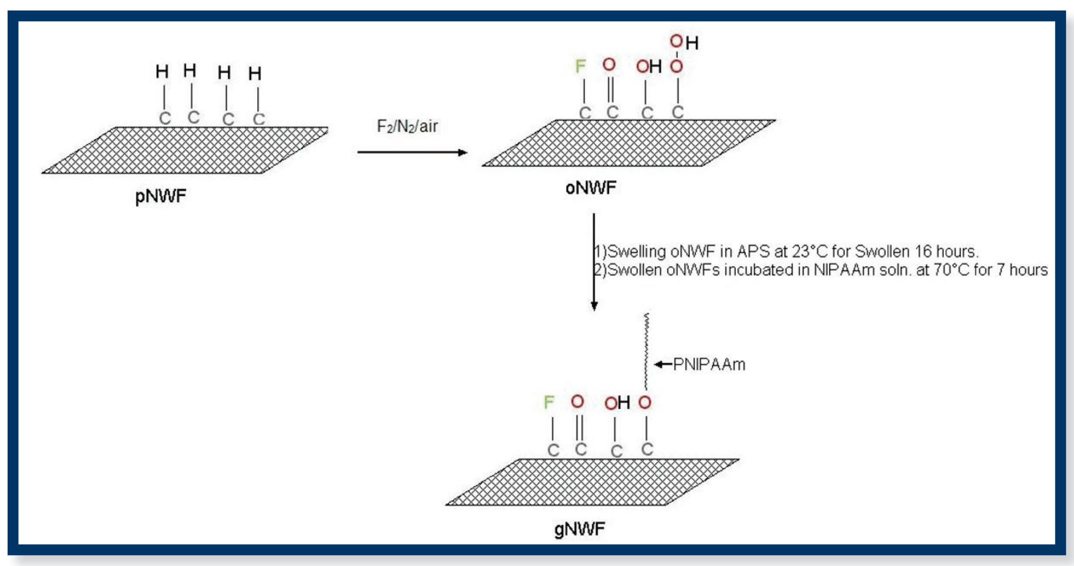
Figure 1: The image of the transition of PNIPAAm at temperatures above and below its LCST of 32°C

AIM

The aim of this study was to develop PP-g-PNIPAAm (gNWF) non-woven 3D scaffolds using an oxyfluorination-assisted graft polymerisation (OAGP) method. A further objective was to quantify the graft yield and to characterise the physical and chemical properties of the PP pure NWF (pNWF); oxyfluorinated NWF (oNWF), and the gNWF scaffolds. The gNWF was developed for non-invasive 3D cell culture.

EXPERIMENTAL

The OAGP method involved a two step process. Firstly the pNWF scaffold was functionalised by oxyfluorination at Pelchem SOC Ltd. The oNWF was then grafted in an aqueous NIPAAm solution using ammonium persulphate (APS) to form gNWF as shown in Scheme 1. The graft yield was quantified using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) based on the peak area ratios of the C=O peak at 1650 cm^{-1}: PP methyl peak at 1455 cm^{-1}. Graft thickness was calculated from the graft yield in $\mu g/cm^2$. The graft yield was optimised by varying some of the polymerisation parameters. Scanning Electron Microscopy (SEM) was used to investigate the surface morphology of the NWF scaffolds. 2-2-Diphenyl-1-picrylhydrazyl (DPPH), a stable free radical, was used to quantify the amount of peroxy free radicals on the oNWF.



Scheme 1: A schematic representation showing the possible mechanism for oxyfluorination assisted grafting of PNIPAAm onto PP

X-Ray Diffraction (XRD) was used to investigate the crystalline structures of the pNWF, oNWF and gNWF scaffolds.

RESULTS

Graft polymerisation was confirmed by both ATR-FTIR and SEM (**Figures 2–3**). The parameters that resulted in the highest graft yield of 26.20±8.44 $\mu g/cm^2$ and a graft thickness of 238.17±76.69 nm were: Soaking oNWF scaffolds for an hour in 10 wt% APS, grafted for seven hours at 50°C and using 10 wt% NIPAAm monomer concentration.

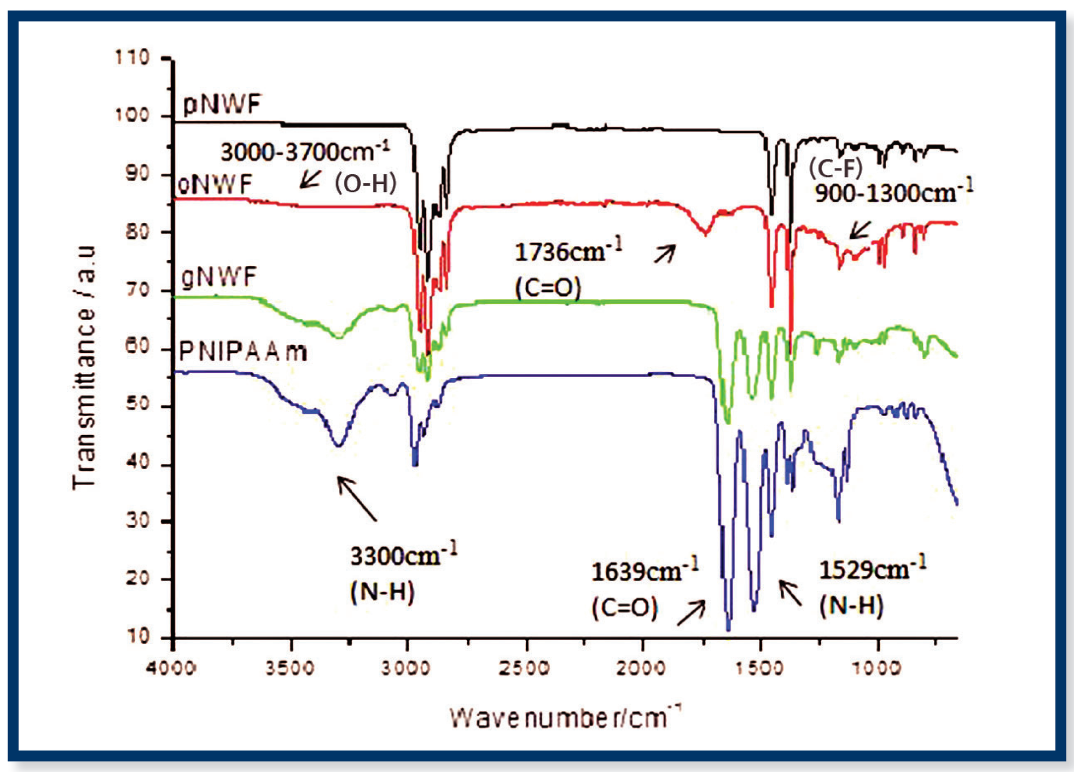


Figure 2: Typical ATR-FTIR spectra of pNWF, oNWF, PP-g-PNIPAAm (gNWF) and PNIPAAm homopolymer

It is well-known that oxyfluorination produces peroxy free radicals on polymer surfaces. After oxyfluorination, new bands were observed on the oNWF at 3 700–3 000 cm^{-1}, 1 736 cm^{-1} and 900 cm^{-1}–1 300 cm^{-1} which are characteristic bands of the O-H; C=O and C-F stretching vibrations respectively. After graft polymerisation, new bands appeared on the gNWF, at 3 300 cm^{-1}; 1 639 cm^{-1}, and 1 529 cm^{-1}, which are the characteristic bands of the broad amide II (N-H) stretch, amide I (C=O) stretching vibration and amide II deformation respectively. The gNWF spectra resembles that of PNIPAAm; therefore confirming that grafting has taken place.

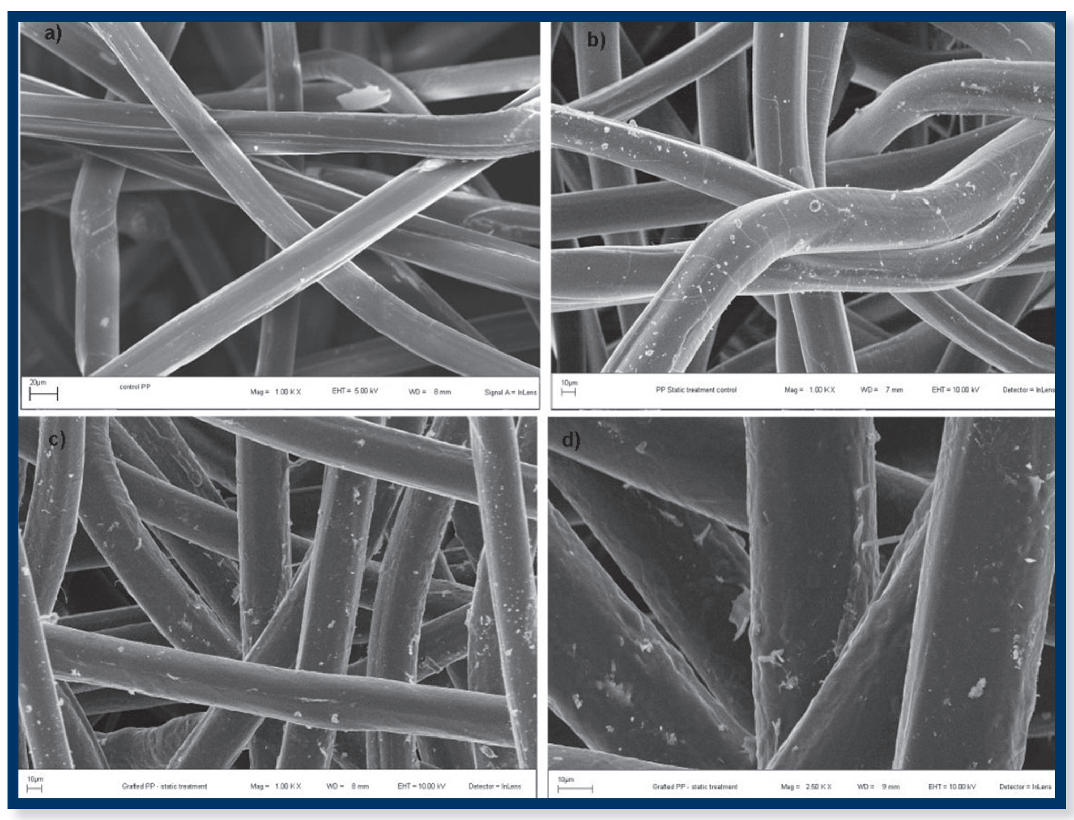


Figure 3: SEM images of a) pNWF; b) oNWF; and c-d) gNWF

A change in surface roughness between the pNWF, oNWF, and the gNWF was observed but the structure of the NWF was retained (**Figure 3**). The solution containing the oNWF changed colour due to the relatively high number of peroxy free radicals present on the oNWF than those on the pNWF (**Figure 4**).

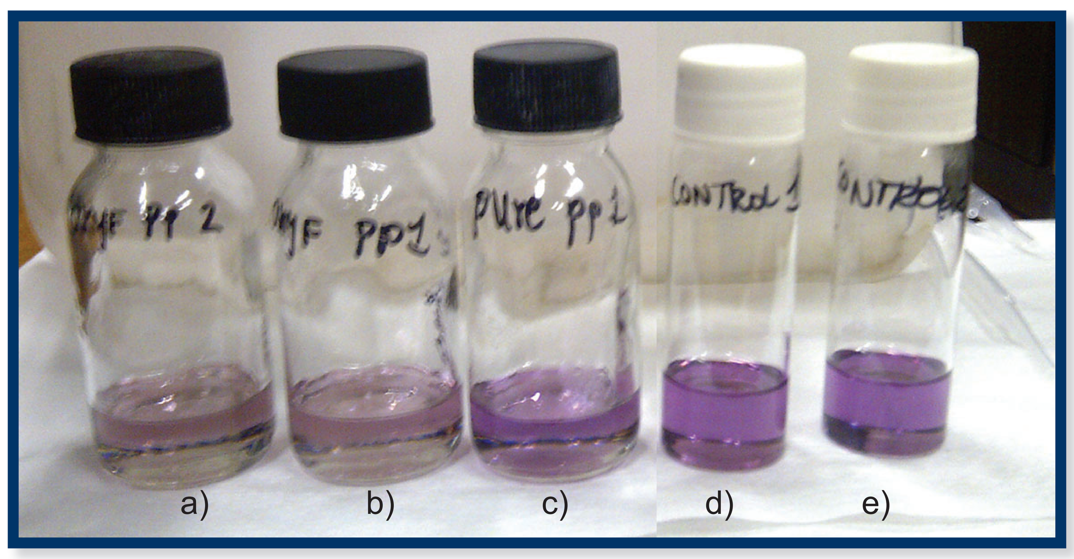
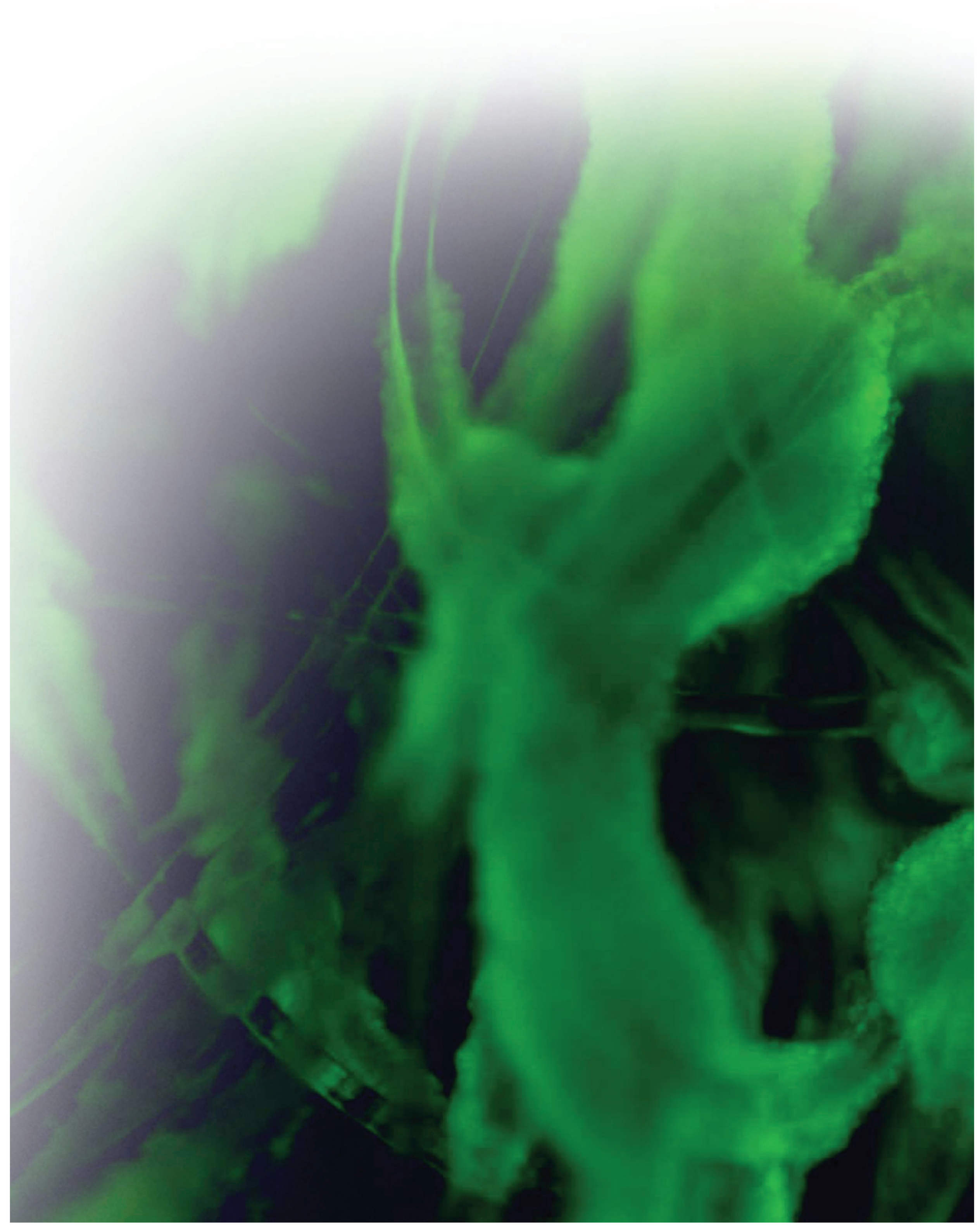


Figure 4: Colour observation after reaction with DPPH at 70°C for seven hours for (a-b) oxyfluorinated PP NWF; (c) pure PP NWF; and (d-e) DPPH blank

Non-woven 3D thermoresponsive scaffold for non-invasive cell culture



The amount of peroxy free radicals on oNWF scaffolds and pNWF scaffolds were 4.344×10^{-9} mol/cm^2 and 4.488×10^{-10} mol/cm^2 respectively.

The oNWF, pNWF and gNWF crystallised in the α- monoclinic form showing a characteristic diffractogram of isotactic polypropylene. The PP-g-PNIPAAm scaffolds have recently been tested in cell culture using hepatocytes cells, and temperture-induced cell release has been demonstrated[1].

CONCLUSION

Using the OAGP method, PNIPAAm was successfully grafted onto PP NWF. Oxyfluorination increases the graft yield by increasing the number of active peroxy sites on the polypropylene NWFs scaffolds.

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