PNIPAAm-Grafted-Collagen as an Injectable, *In Situ* Gelling Delivery Scaffold for Retinal Cell Therapy

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Statement of Purpose: Permanent vision loss associated with diseases affecting the retina affects over 10 million people worldwide[1]. Recent advances in cell based therapies have demonstrated the potential to repopulate diseased retinal tissue with viable, functioning cells which may lead to restoration of site[2]. However, current cell delivery techniques suffer significant drawbacks. Bolus cell injections result in poor delivery efficiency, whereas, degradable cell scaffolds require invasive surgical techniques for implantation. Therefore, we hypothesize that the development of an injectable, *in situ* scaffold forming cell delivery vehicle, which combines the non-invasive delivery of bolus injections with the high delivery efficiency of scaffold transplantations will improve retinal pigmented epithelial (RPE) cell transplantation, thereby improving outcomes in retinal cell therapy. Three novel biomaterial scaffolds have been synthesized consisting of collagen, a naturally occurring cell matrix protein and thermoresponsive poly(N-isopropylacrylamide) (PNIPAAm). All scaffolds undergo a phase transition, from liquid to gel, below physiological temperature allowing the delivery of a liquid suspension of cells that forms a scaffold upon delivery into the body.
Methods: PNIPAAm was grafted onto a collagen backbone through EDC/NHS chemistry (PCol) and via UV photocrosslinking (UV PCol). A blend of fibronectin functionalized PNIPAAm and collagen was also created (PColFn). LCST properties of the different materials were characterized by DSC, turbidity testing and by assessing gelling kinetics. The molecular weight of grafted PNIPAAm chains was determined via GPC. Retinal pigmented epithelial (RPE) cell compatibility was tested with the biomaterial scaffolds in 2D and 3D cultures. ESEM was used to obtain high resolution images of the internal pore structure of the scaffolds.
Results: The LCST of the different scaffolds were all found to be below physiological temperature, thus allowing the delivery of a liquid suspension of cells that gels *in situ* to form a scaffold. Turbidity testing confirmed DSC findings and gave insight into phase transition behaviour, Figure 1.

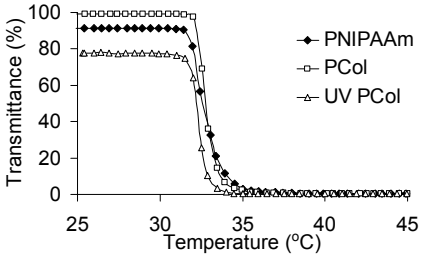

Figure 1: Phase transition analysis of the different PNIPAAm-collagen based scaffolds.

All three scaffolds demonstrated rapid gelation, essential for localization of treatment. Gelling kinetics were assessed by analyzing the time to reach cloud point at various temperatures, Figure 2.

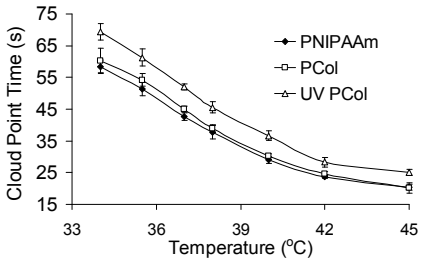

Figure 2: Gelling times of amine terminated PNIPAAm, PCol and UV PCol at varying temperatures.

All scaffolds were found to have highly porous internal microstructures which varied greatly depending on chemistry of synthesis.

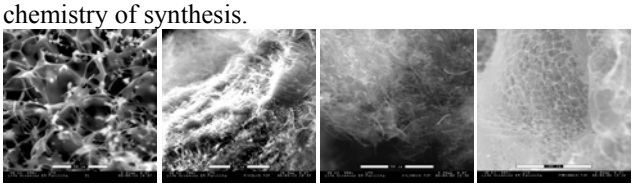

Figure 3: High resolution ESEM images of PNIPAAm, PCol, UV PCol and PColFn (left to right).

RPE cells demonstrated excellent viability when cultured in the presence of and within PCol and UV PCol scaffolds. Viabilities were all greater than 90% and there was no statistical difference between the means.

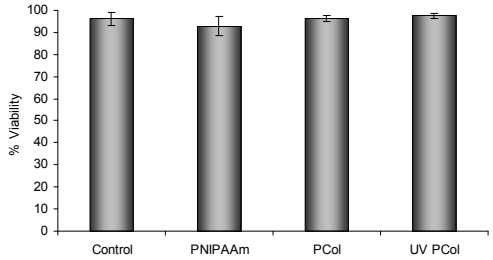

Figure 4: Viability of RPE cells cultured in the presence of amine terminated PNIPAAm, PCol and UV PCol for 96 hours.

Conclusions: Novel biomaterials consisting of PNIPAAm and collagen were designed to deliver RPE cells into the sub-retinal space via minimally invasive techniques. It is envisioned that these scaffolds will ease the transition from implantation to integration of transplanted RPE cells, thereby improving outcomes in retinal cell therapy. Although designed specifically for sub-retinal cell delivery, these generic scaffolds may ultimately be used as cell and drug delivery vehicles for a vast array of different applications.

References:

1. World Health Organization: http://www.who.int/en/. Accessed 11, 03, 2009.
2. MacLaren RE, et al., Nature, 2006: 444, 203-207.