Scaffolds with Controlled Porosity Based on Fully Degradable Poly(N-isopropyl acrylamide) Hydrogel

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Statement of Purpose: Poly(N-isopropyl acrylamide) (polyNIPAM) hydrogels exhibit a volume phase transition temperature (VPTT) at 32-34 °C. Herein we demonstrate the preparation of thermosensitive, monodisperse pore size scaffolds, based on a fully degradable polyNIPAM hydrogel for applications in tissue engineering. The pore size of the scaffold can be engineered to have a large pore size at room temperature (RT) that at 37 °C would be reduced to smaller size. Pores of approximately 40 µm have been found to be optimal for vascularized, relatively non-fibrotic integration of a material into a tissue [1, 2]. The polyNIPAM-based scaffold with large pore size may efficiently be loaded with cells at RT and then at 37°C cells would be locked within the scaffold with an optimal pore size of 40 µm. Thus the cell loaded scaffold may regenerate a tissue through increased angiogenesis and decreased fibrosis, and, after providing mechanical and biochemical support, to degrade and be eliminated from the body.

Methods: Fully degradable polyNIPAM hydrogel was synthesized by copolymerization of NIPAM with 2-methylene-1,3-dioxepen (MDO) (40% mol/mol) and polycaprolactone dimethacrylate (PCLDMA) (2% mol/mol) contributing ester linkages within the polyNIPAM backbone [3] and the crosslinking site, respectively (Fig. 1). The VPTT of the hydrogel was determined by DSC and its degradability was explored by an accelerated hydrolysis in 0.007N NaOH at 25 and 37 °C. PolyNIPAM-based scaffolds with pore diameters of 36±2, 55±5, 90±8 and 204±26 µm were then fabricated by using sphere-templating technique [2]. The scaffolds' morphology was demonstrated by scanning electron microscopy (SEM). Pore diameter of the scaffolds was measured at 25°C (D_{25}) and 37 °C (D_{37}) and shrinkage percent was calculated using the following equation: shrinkage (%) = $[(D_{25}-D_{37})D_{25}]$x100. NIH-3T3 cells were exposed to media eluted from the scaffolds and to media containing their degradation products at concentrations of 5, 10 and 15 mg/ml for 48h and cytotoxicity of the materials was measured by an MTT assay. To demonstrate cell adhesion, a scaffold with a pore size of 55±5 µm was used as a surface for culturing NIH-3T3 cells for 2 and 5 days. At each point in time, the scaffold was washed with H_2O, fixed in formalin solution, frozen in liquid nitrogen, lyophilized and observed under SEM.

Results: The VPTT of the fully degradable polyNIPAM hydrogel is 30.2 °C and its degradation rate at 37 °C is slower comparing to 25 °C. SEM images of the polyNIPAM-based scaffolds demonstrate a monodisperse, highly interconnected, porous structure. The shrinkage study indicates that at 37°C the pore diameter of the scaffolds decreases from 36±2, 55±5, 90±8 and 204±26 µm to 29±1, 40±3, 67±4 and 138±19 µm, respectively (on average by 26%) (see Fig. 2). MTT test results suggest that the scaffolds and the degradation products are not toxic. SEM images illustrate effective cell adhesion and spreading after 2 days of culturing. Formation of a cell sheet on the surface of the scaffold as well as cell infiltration within the pores was observed after 5 days of culturing.

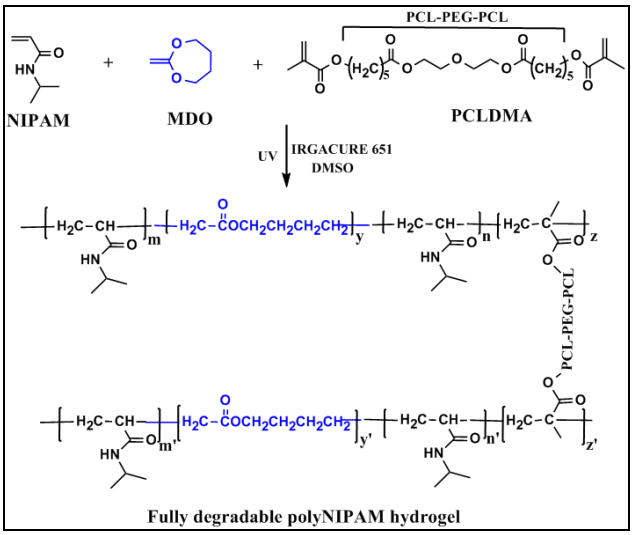

Figure 1. Synthesis of fully degradable polyNIPAM hydrogel.

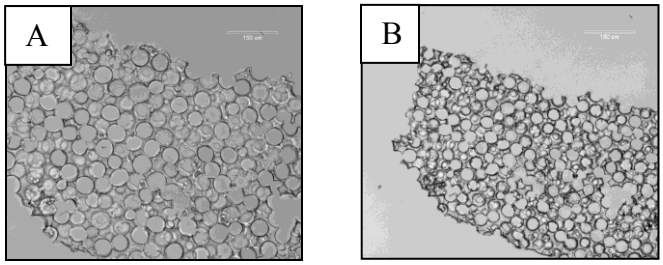

Figure 2. Light microscope images of polyNIPAM scaffold sections with pore diameter of 55±5 µm at 25 (A) and 40±3 µm at 37 °C (B). Scale bar is 150 µm.

Conclusions: The model for preparation of thermosensitive scaffolds with two important criteria, full degradability and controlled porosity, was developed. It was found that a scaffold with the pore size of 55±5 µm at RT gives an optimal pore diameter of 40±3 µm at 37 °C. This scaffold could be loaded with the cells at RT and then implanted to achieve tissue regeneration with increased angiogenesis and decreased fibrosis. Neither scaffolds nor their degradation products at different concentrations show cytotoxicity. NIH-3T3 cells demonstrate sufficient adhesion and spreading on the surface, as well as infiltration within the pores of the developed scaffold. The scaffolds are based on synthetic polymers and therefore have tunable physicochemical properties that could be adjusted to optimize performance for tissue engineering.

References: 1. Ratner BD. Polym Int. 2007;56:1183-1185. 2. Marshal AJ. *et al.* ACS Polym Prepr. 2004;45:100-101. 3. Sun L. *et al*. Macromol Biosci. 2003;3:725-728.