A Degradable Poly(N-isopropyl acrylamide) Scaffold for Tissue Engineering Applications

Anna Galperin[1], Thomas J Long[1], Buddy D. Ratner[1]
[1]University of Washington Engineered Biomaterials (UWEB21), Seattle, WA 98195, USA

Statement of Purpose: Poly(N-isopropyl acrylamide) (polyNIPAM) hydrogels exhibit a volume phase transition temperature (VPTT) at 32-34 °C. Here, we report the preparation of thermoresponsive scaffolds based on a degradable polyNIPAM hydrogel for potential applications in tissue engineering. First, polyNIPAM hydrogels with degradable units within the polymer backbone and at the cross-linking sites were synthesized by Atom Transfer Radical Polymerization (ATRP) of NIPAM in the presence of di-chlorinated polycaprolactone-based macroinitiator (Cl-PCL-Cl) and polycaprolactone dimethacrylate (PCLDMA). Second, a sphere-templating technique[1] was applied to fabricate a scaffold based on the aforementioned hydrogel. Scaffolds with a pore diameter of 42±6 µm were successively loaded at 25 °C with smooth muscle cells (SMCs), which when cultured at 37 °C were locked within scaffolds of pore diameter 36±5 µm, a size optimal for vascularized, relatively non-fibrotic integration of a material into a tissue[1].

Methods: Degradable polyNIPAM hydrogels with theoretical Mw of 10, 20 and 40K were synthesized by ATRP of NIPAM in presence of Cl-PCL-Cl, PCLDMA, CuCl and tris[2-(dimethylamino)ethyl]amine (Me_2TREN) in DMSO at RT (Fig. 1). Mw of linear polymers and their degradation products were evaluated by GPC. The VPTT of the hydrogels was determined by DSC. Mechanical properties of the hydrogels as functions of Mw and temperature were measured by tensile tests at 25 and 37 °C. Swelling % of the hydrogels as a function of temperature was calculated by using the following equation: swelling (%) = $[(W_s-W_d)W_d]$x100, where W_d and W_s are dry and swollen weight, respectively. Degradability of hydrogels was explored by an accelerated hydrolysis in 0.007N NaOH at 25 and 37 °C. Cytotoxicity of the materials was measured by an MTT assay where NIH-3T3 cells were exposed to media eluted from the hydrogel and to media containing its degradation products at various concentrations. Cytocompatibility of the material was evaluated by measuring proliferation of NIH-3T3 cells cultured on the surface of the hydrogel by an Alamar Blue assay. PolyNIPAM-based scaffolds with pore diameters of 42±6 µm were then fabricated by using sphere-templating technique[1].The scaffolds' morphology was demonstrated by scanning electron microscopy (SEM). Pore diameters of the scaffolds were measured at 25 and 37 °C and shrinkage % was calculated[2]. The scaffold was loaded with A-10 SMCs at RT, which were cultured within the scaffold for 7 days at 37C. The scaffolds were then fixed, embedded in paraffin and stained with H&E.

Results: Mw of linear polymers and their degradation products, as well as polydispersity indexes support controlled polymerization. The VPTT of polyNIPAM hydrogels with theoretical Mw of 10, 20 and 40K are 31.7, 32.4 and 34.6 °C, respectively. Degradation rate, swelling and mechanical properties of hydrogels are greatly affected by temperature. MTT test results suggest that the hydrogels are non-toxic. Cytocompatibility of the material is supported by increase in alamar blue reduction % as a function of time. SEM images of the polyNIPAM-based scaffolds demonstrate a monodisperse, highly interconnected, porous structure. Histological images illustrate effective A-10 cells adhesion, distribution and infiltration after 7 days of culture (Fig. 2).

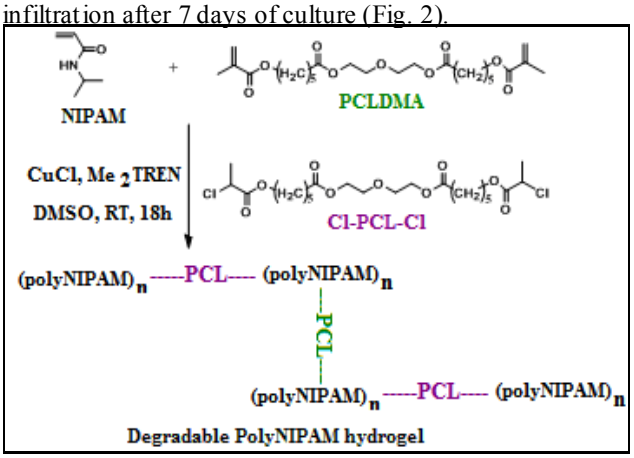

Figure 1. Synthesis of degradable polyNIPAM hydrogel.

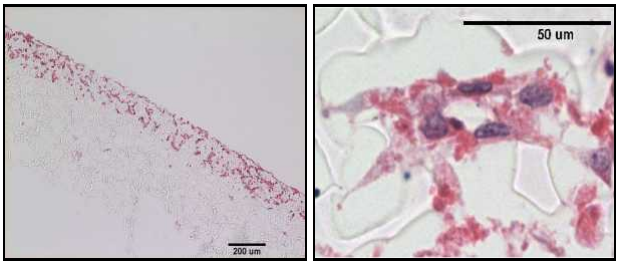

Figure 2. Histological images of polyNIPAM scaffold loaded with A-10 cells.

Conclusions: The concept for synthesizing degradable polyNIPAM hydrogels with defined Mw was developed. Thermoresponsive scaffolds with controlled porosity of 42±6 µm were then fabricated based on the developed hydrogel. The scaffolds were loaded with A-10 cells at RT to lock the cells within the scaffold with an optimal pore size of 36±5 µm at 37 °C. A-10 cells demonstrate sufficient adhesion, spreading and infiltration within the pores of the scaffold. The extension–contraction of the scaffold as a function of a temperature could mimic a radial force within a native artery and so the developed scaffold might serve as bioreactor for culturing SMCs by applying mechanical conditioning induced by cycling temperature between 37 and 25 °C. In a planned study we will explore the developed scaffold as a potential bioreactor for culturing SMCs by applying mechanical conditioning induced by alternating temperature.

References: 1. Marshal AJ. *et al.* ACS Polym Prepr. 2004;45:100-101. 2. Galperin A. *et al.* Biomacromoleculs 2010;11:2583-2592. **Funding:** UWEB21, Coulter Foundation, NIH R01HL64387.