Thermal Cycling to Eliminate Water Loss upon Gelation of Injectable Hydrogels for Nucleus Pulposus Replacement

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Introduction. Currently, the major surgical treatments for lower back pain are spinal fusion and total disc arthroplasty [1]. Because these treatments involve highly invasive surgeries, a promising alternative is the replacement of the nucleus pulposus alone. There are several pre-formed polymeric implants currently under investigation as synthetic nucleus pulposus substitutes. However, the development of an injectable hydrogel nucleus replacement would have important clinical consequences because it could be injected non-invasively using a needle, minimizing soft tissue trauma. *In situ* gel formation can be achieved using a thermo-responsive polymer, such as poly(N-isopropylacrylamide) (PNIPAAm). Aqueous solutions of PNIPAAm have a lower critical solution temperature (LCST), typically around 32 ºC. Below the LCST, PNIPAAm chains hydrate, forming a free flowing solution in water. Above the transition temperature, the polymer becomes hydrophobic, causing the water and polymer to separate. The PNIPAAm chains collapse, forming a compact gel.

Previous work has shown that polymerizing the NIPAAm monomer in the presence of poly(ethylene) glycol (4600 g/mol) dimethacrylate (PEGDM) produces a PNIPAAm-PEG branched copolymer with suitable equilibrium swelling and mechanical properties for nucleus pulposus replacement [2]. However, the phase transition of PNIPAAm is marked by an initial de-swelling until equilibrium is reached. The accompanying volume loss could prevent intimate contact with the inner annulus, which is necessary for the restoration of biomechanical function [3]. This study focuses on the optimizing the copolymer formulation to minimize implant water loss following implantation into the intradiscal environment.

Materials and Methods. The synthesis of PNIPAAm-PEG (4600 g/mol) branched copolymers has been described previously [2]. The polymers were ground into a fine powder and combined with water to form 15wt% copolymer solutions at room temperature. The solutions were then injected into dialysis tubing (Spectrum Spectra/Por Biotech cellulose ester, 500 Dalton MWCO) and sealed with dialysis clips. The bags were immersed in 37ºC aqueous solutions of poly(ethylene glycol (20,000 g/mol) with an osmotic pressure of 0.4 MPa. Preliminary studies indicate that this osmotic pressure will decrease the time to equilibration of hydrogel water content. After 48 hours immersion, the gels were subsequently removed from the dialysis bags, and cooled to room temperature, forming uniform copolymer solutions due to the reversibility of the phase transition of PNIPAAm. The water content was determined according to Equation 1. The same solutions were re-injected into dialysis bags and immersed in the same 37ºC osmotic environment for an additional 48 hours. This thermal cycle was repeated five times. Solution volume retention after each cycle was also measured using the heptane density determination system [3].

$$Water\ content = 1 - \frac{m_{dry}(t)}{m_{wet,37^oC}(t)} \qquad \textbf{Equation 1}$$

Results and Discussion. Our previous studies have shown that 15wt% PNIPAAm-PEG (4600 g/mol) solutions will shed approximately 30% of the solution water content above the LCST. To ensure the implant remains space filling, it is necessary to eliminate this water loss. This can be achieved by starting with more a concentrated polymer solution at room temperature. However, solutions higher than 25wt% polymer cannot be prepared uniformly by combining the dry powder and water.

For this reason, thermal cycling studies were performed, the results shown in Figure 1. The water content and volume retention of the solutions before and after the initial immersion period is indicated as thermal cycles 0 and 1. As expected, there was significant water loss during this period ($p<0.05$). Additionally, the solutions only retained 25% of their initial volume. The water content of the solutions after the second thermal cycle is shown in the figure as thermal cycle 2. There were no significant changes in water content or gel volume ($p>0.05$) compared to cycle 1. For the subsequent cycles, there were no more appreciable changes in water content and the volume of the solutions remained constant.

These results indicate that the implant can be made space filling by equilibrating dilute polymer solutions in a 37 ºC environment. Upon cooling to room temperature, the polymers will form uniform, concentrated solutions which should show minimal water loss when injected into the disc.

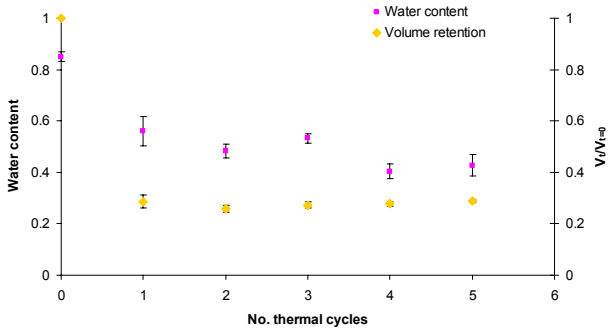

Figure 1. Effect of pre-conditioning the polymer solution by equilibrating in a 37 ºC osmotic environment, followed by cooling to room temperature.

References.

[1] Herring, S.A., *Artificial discs and future of spine surgery*, in *Bone and Joint*. 2005. p. 53-54.
[2] Vernengo, J., G.W. Fussell, N. Smith, A.M. Lowman. "Evaluation of Novel Injectable Hydrogels for Nucleus Pulposus Replacement". Society for Biomaterials Annual Meeting, Pittsburgh, PA, April 27, 2006.
[3] Joshi, A. B. *Mechanical Behavior of the Human Lumbar Intervertebral Disc with Polymeric Hydrogel Nucleus Implant: An Experimental and Finite Element Study*. PhD Thesis, February 2004
[4] Peppas, N.A., B.D. Barr-Howell, in: N.A. Peppas (Ed.). Hydrogels in Medicine and Pharmacy, Vol. I, Fundamentals, CRC Press, Boca Raton, Florida, 1987.