

## Microparticle-Mediated Adhesion of a Thermogelling Scaffold for Intervertebral Disc (IVD) Tissue Engineering

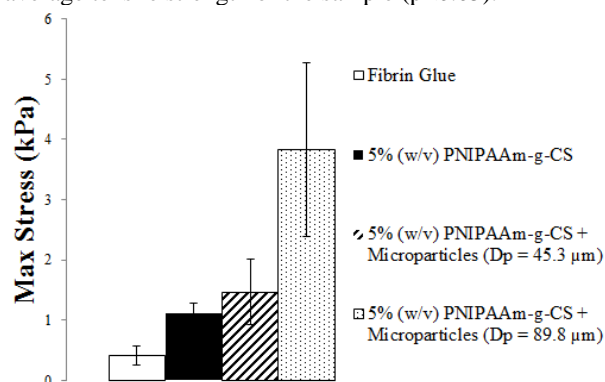
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**Statement of Purpose:** In this work, we characterize a novel bioadhesive scaffold for IVD applications composed of poly(N-isopropylacrylamide) (PNIPAAm) grafted with chondroitin sulfate (CS) (PNIPAAm-g-CS). Below its lower critical solution temperature (LCST) at 32°C, PNIPAAm forms a miscible solution with water. Above the LCST, the polymer becomes hydrophobic, expels water from its matrix, and forms a compact gel. Therefore, aqueous solutions of the polymer can be implanted through a needle. The PNIPAAm-g-CS was blended with alginate microparticles to mediate bioadhesion. At body temperature, PNIPAAm-g-CS precipitation localizes the microparticles, enhancing bioadhesion to tissue through ionic and/or hydrogen bonding interactions. In our preliminary investigations, we determine how size of the microparticles influences bioadhesive strength of the hydrogel-microparticle composite and qualitatively assess encapsulated cell viability.

**Methods:** PNIPAAm-g-CS was synthesized via redox polymerization (Wiltsey C. *J Mater. Sci. Mat. Med.* 2013; 837-847). Alginate microparticles with a range of diameters were produced by varying the calcium chloride concentration and stir speed in a water in oil emulsion based on previously described methods (Paques JP. *Food Hydro.* 2013; 31: 428-434). The average diameter of the alginate microparticles was measured by randomly selecting 50 spheres and measuring their dimensions under a light microscope. For all samples, a 5% (w/v) solution of PNIPAAm-g-CS was made in phosphate buffered saline (PBS) at room temperature. Alginate microparticles were suspended in the solution at a concentration of 50 mg/mL. Adhesive properties in contact with porcine cartilage were tested in tension using a Shimpo E-Force stand. The adhesive tensile stress was taken as the highest stress recorded during the experiment. A fibrin-based sealant (Tisseel®) was tested in parallel, since it is the current standard for biocompatible adhesives. Qualitative cell viability was determined by performing a Live/Dead® assay. HEK-293 cells were suspended in polymer solutions at 10<sup>6</sup> cells/mL at 37°C for 6 days. Cells were treated with 6 μM ethidium homodimer-1 and 2 μM calcein AM for 40 minutes at room temperature, then separated from the polymer and examined under fluorescence microscopy.

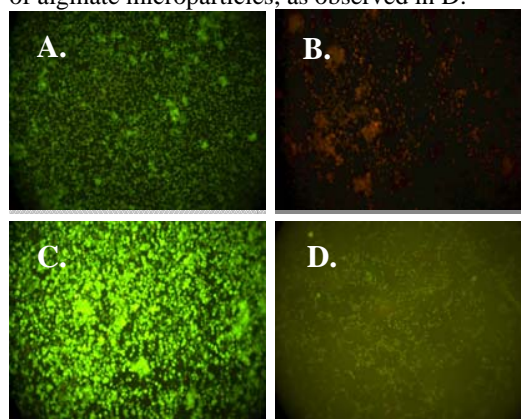
**Results:** Likely due to the inability of fibrin to perform within an aqueous environment, the PNIPAAm-g-CS-based adhesives exhibited tensile strengths two to eight-fold higher than Tisseel. (Figure 1). The 5% PNIPAAm-g-CS containing 50 mg/mL of 45.3-micron diameter particles exhibited similar strength to 5% PNIPAAm-g-CS alone (p<0.5). However, keeping the alginate microparticle concentration constant but increasing the

particle diameter to 89.8 microns more than doubled the average tensile strength of the sample (p<0.05).



**Figure 1. Adhesive strength of formulations tested in tension (error bars represent 95% confidence intervals).**

Cell viability results from a Live/Dead® assay are shown in Figure 2. The control samples for live and killed cells can be seen in A and B, respectively. Cells exhibited good viability within 5% PNIPAAm-g-CS as seen in C. Importantly, cell viability was still intact with the addition of alginate microparticles, as observed in D.



**Figure 2. A Live/Dead assay was performed by seeding 10<sup>6</sup> cells/mL within: A) Monolayer, B) Killed Control (with 70% methanol), C) PNIPAAm-g-CS, and D) PNIPAAm-g-CS with alginate microparticles (89.8μm) for 6 days of incubation at 37°C.**

**Conclusions:** These results indicate potential for using the thermogelling microparticle-hydrogel composite for tissue engineering applications where adhesion or integration with the surrounding host tissue is necessary to prevent dislocation, such as in the IVD. Currently, we are optimizing assays for quantification of cell viability over long-term encapsulation in the scaffolds.

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