Self-assembling "smart" hydrogels with bioadhesive properties for tissue engineering applications

Craig Wiltsey, Thomas Christiani, Jesse Williams, Jake Scaramazza, Cody Van Sciver, Katelynn Toomer, Joseph Sheehan, Amanda Branda, Cristina Iftode, Jennifer Kadlowec, Jennifer Vernengo
Rowan University, Glassboro, NJ

Statement of Purpose: For the repair of certain load-bearing tissues such as the intervertebral disc (IVD), success can be dependent on scaffold adhesion with the surrounding host tissue to prevent dislocation. In this work, we characterize a novel bioadhesive scaffold for IVD applications composed of poly(N-isopropylacrylamide) (PNIPAAm)-graft-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, it becomes hydrophobic, so the polymer and water separate, forming a compact gel. Therefore, aqueous solutions of the polymer can be implanted minimally invasively. In addition, the PNIPAAm-g-CS was blended with aldehyde-modified CS, allowing the hydrogel react with amines in the ECM upon contact with tissue, rendering it bioadhesive. However, aldehydes are known for limited biocompatibility. To overcome this, a "smart" self-assembling hydrogel system was designed by incorporating thermally sensitive liposomes. These lipid particles are thermally triggered to release the ECM-derived protein gelatin within the gel immediately after adhesion occurs. This end-caps the unreacted aldehyde groups that did not participate in bonding with tissue, potentially enhancing biocompatibility with encapsulated cells. In this study, the adhesive strength and cytotoxicity of the system were characterized.

Methods: Gelatin-loaded lipid vesicles with a melting point of 37°C were prepared (Messersmith PB. Chem. Mater. 1998;10: 117-124). Methacrylate-functionalized CS was prepared according to previously described methods **(**Bryant SJ. Macromolecules. 2004;37: 6726-6733). PNIPAAm-g-CS was synthesized via redox polymerization. CS aldehyde was prepared following established procedure (Reyes JMG. Invest Ophthalmol Vis Sci. 2005;46: 1247-1250). For all samples, a 5% (w/v) solution of PNIPAAm-g-CS was made in phosphate buffered saline (PBS) at room temperature. Aldehyde modified CS and liposomes were then combined with this solution at a concentration 3% (w/v) and 25 mg/mL, respectively. Properties of the adhesive in contact with porcine cartilage were tested in tension using the Shimpo E-Force stand. To determine cytotoxicity, HEK-293 cells were suspended in room temperature polymer solutions at $1x10^6$ cells/mL and were encapsulated within the polymer at 37°C for 5 days.

Results: Compared to PNIPAAm-g-CS alone, a significant increase ($p<0.05$) in the adhesive stress was seen with the incorporation of CS aldehyde into the hydrogels (Figure 1). Compared to PNIPAAm-g-CS + CS aldehyde, the scaffold containing liposomes exhibited a significant decrease in stress ($p<0.05$). Two control samples were also tested: 1) PNIPAAm-g-CS + CS aldehyde with un-encapsulated gelatin, 2) PNIPAAm-g-CS aldehyde with unloaded liposomes. Free gelatin slightly reduced adhesive stress to a level that was statistically similar to that seen with PNIPAAm-g-CS alone, supporting the rationale for encapsulation of gelatin. However, unloaded liposomes caused a statistically significant decrease in adhesive stress much greater in magnitude.

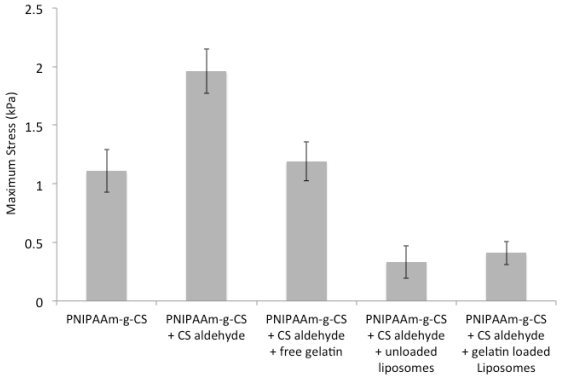

Figure 1. Adhesive strength of formulations tested in tension (error bars represent 95% confidence intervals)

Cytotoxicity results from a PicoGreen® assay are shown in Figure 2. As expected, the addition of CS aldehyde to PNIPAAm-g-CS caused a significant ($p<0.05$) decrease in DNA concentration, likely due to the cytotoxicity of aldehyde groups. However, the addition of free gelatin or gelatin-loaded liposomes, which allows for "end-capping" of the aldehyde groups within the network, resulted in increases in DNA concentration to levels statistically similar to that of PNIPAAm-g-CS alone.

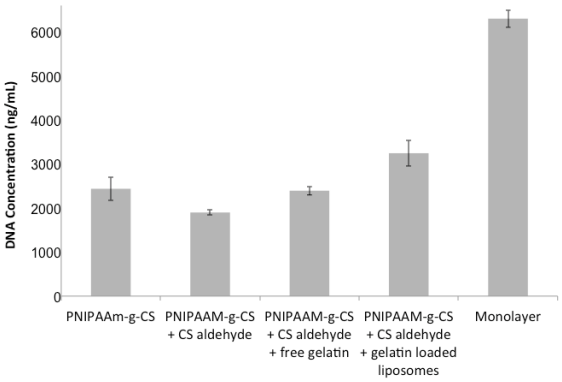

Figure 2. Cytotoxicity evaluated using PicoGreen assay at 5 days encapsulation *in vitro*.

Conclusions: These preliminary results demonstrate the potential of the self-assembling scaffold to function as a bioadhesive matrix permissive to encapsulated cell survival. However, the method of gelatin delivery needs to be optimized such that it does not interfere with adhesive strength. Alternative methods are currently being investigated.