Shear Strength of Thermogelling Scaffold for Intervertebral Disc (IVD) Tissue Engineering

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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) 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 this work, we characterize the shear behavior of the adhesive. This testing mode was chosen because it is akin to the likely mode of failure in an intervertebral disc if the hydrogel were to be extruded through the annulus due to insufficient adhesion.

Methods: PNIPAAm-g-CS was synthesized via redox polymerization (Wiltsey C. J Mater. Sci. Mat. Med. 2013; 837-847). Alginate microparticles were produced by crosslinking with calcium chloride in a water in oil emulsion based on previously described methods (Paques JP. Food Hydro. 2013; 31: 428-434). 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 for shear behavior with the fixture we designed and fabricated, as shown in Figure 1. A test method was developed based on ASTM standard F 2255-05. Two tissue samples of porcine cartilage were cut to dimensions 5mm x 10mm, affixed to plexiglass slides with cyanacrylate adhesive and warmed to 37°C in a saline bath. Hydrogel (60 μL) was applied to the samples by pipette and allowed to solidify for 5 minutes. The cartilage samples were displaced relative to one another using a FGS-200PV E-Force Test Stand at a rate of 5 mm/min until the hydrogel failed in shear, while displacement and force were recorded. For the XTT viability studies, adipose stem cells (ASCs) suspended in polymer solutions were plated as 5 X 10^4 cells/cm^2 and grown in DMEM, low glucose, with 10 ng/mL EGF for 7 days.

Results: Eight tests were run in order to demonstrate the efficacy of this method of testing for shear strength and shear modulus. These tests verified that the equipment was capable of generating consistent results using the developed testing method. The average shear strength was 2.32 kPa, with a standard deviation of 1.25 kPa.

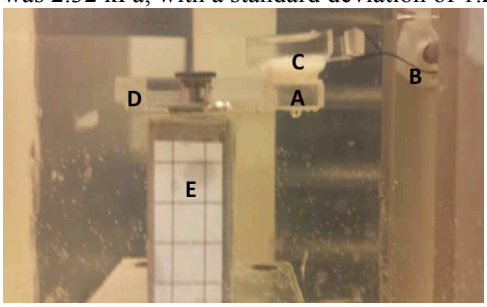

Figure 1. Testing apparatus for shear. A: Cartilage samples affixed to slides, with hydrogel in between. B: String to transfer vertical test force to horizontal force on apparatus. C: Top slide, free to move with force gauge. D: Bottom slide, fixed to baseplate. E: 5mm reference grid for optical distance measurements.

Results of the XTT assay (Figure 2) indicate that, at 7 days encapsulation in PNIPAAm-g-CS, with or without alginate particles, ASC viability was not significantly different than the monolayer, indicating good viability within the scaffold.

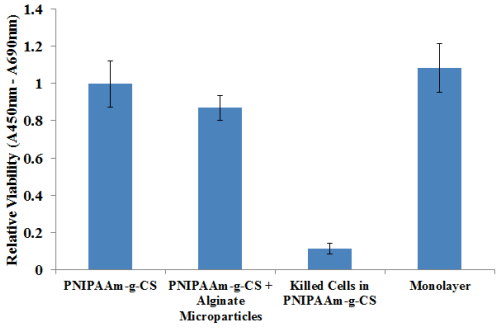

Figure 2. XTT assay performed by seeding ASCs within PNIPAAm-g-CS, with and without alginate microparticles. Relative viability in polymer is compared with killed cell control (70% methanol) and a cell monolayer.

Conclusions: In our current studies, we are characterizing the shear modulus of the PNIPAAm-g-CS, with and without alginate microparticles, in contact with porcine annulus fibrosus tissue. A fibrin-based sealant (Tisseel®) will be tested in parallel, since it is the current standard for biocompatible adhesives. Our aim is to show significantly higher adhesive strength compared to fibrin glue, which would indicate greater potential for using the adhesive for tissue engineering applications.

Acknowledgements: Research reported in this publication was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases and the National Institute of Biomedical Imaging and Bioengineering of the National Institutes of Health under Award Number 1R15 AR 063920-01. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.