Fibrin-Genipin Annulus Fibrosus Sealant as a Delivery System for Anti-TNFα Drug

Morakot Likhitpanichkul1,2, Yesul Kim1, Eugene See3, Olivia M. Torre1, Zepur Kazezian2,3, Abhay Pandit2,3, Andrew C. Hecht, MD1, James C. Iatridis, PhD1.
1Icahn School of Medicine at Mount Sinai, New York, NY, USA, 2Collaborative Research Partner Annulus Fibrosus Rupture Program of AO Foundation, Davos, Switzerland, 3Network of Excellence for Functional Biomaterials, National University of Ireland, Galway, Ireland.


Introduction: Regenerative repair of intervertebral discs (IVDs) with annulus fibrosus (AF) defects is a significant clinical challenge due to the large spinal loads that must be applied immediately after repair. Inflammation is involved in IVD injuries and often progresses into a chronic state where a prolonged pro-inflammatory environment results in cell catabolism and inhibits tissue regeneration [1]. TNFα is a key initiator that increases downstream production of pro-inflammatory cytokines (IL-1β, IL-6, IL-8) and propagates matrix degrading enzymes and pain mediators [2,3]. TNFα and hyperphysiological level of cell stretch, as may occur from IVD injuries and herniation, can have synergistic detrimental effects on the production of IL-1β, IL-6, and IL-8 by human AF cell in vitro [4], possibly due to increased cell mechanosensitivity with TNFα. We propose that the restoration of physiological strain environment by the structural repair of AF, in concert with a sustained inhibition of TNFα is an attractive therapeutic strategy to repair herniated IVDs. Fibrin-genipin (FibGen) adhesive hydrogel was an effective sealant for AF defects because of its biocompatibility with human AF cells, its ability to partially restore IVD biomechanical behaviors and its extended degradation rate [5]. FibGen sealant as a carrier for sustained delivery of an anti-TNFα drug may represent a promising treatment strategy for a painful ruptured IVD that may promote tissue regeneration in the long-term. Collagen type I hollow spheres (CHS), which are an extracellular matrix-based reservoir system that allow for a simple and non-damaging drug loading process may be suitable for drugs such as anti-TNFα antibodies [6], and may provide an improved drug release profile when incorporated within FibGen. This study aimed to functionalize FibGen for controlled and sustained release of an anti-TNFα drug, infliximab, either by direct mixing of the drug or by embedding the gel with drug-loaded CHS.

Methods: Five drug delivery systems for infliximab were prepared (n≥4): 1) CHS alone (1μm, 400μg) loaded with 320 μg drug (CHS_320 μg); 2) FibGen alone (250 μl) directly mixed with 320 μg (FibGen_320 μg), 2.5 mg (FibGen_2.5 mg), or 7.5 mg (FibGen_7.5 mg) drug; 3) FibGen +CHS loaded with 320 μg drug (FibGen+CHS_320 μg); 4) FibGen with 2.5 mg drug+ CHS with 320 μg drug (FibGen_2.5 mg + CHS_320 μg); and 5) Fibrin alone (250 μl) directly mixed with 394 μg infliximab. FibGen gel contained 6 mg/ml genipin, 200 mg/ml fibrinogen and 26 U/ml thrombin and was prepared as previously described [7], with or without drug/CHS. Infliximab doses and amount of CHS were selected from pilot studies maximizing drug release in that system. Transmission Electron Microscopy (TEM) compared microstructures of each system. To obtain drug release kinetics, 300 μl of DMEM 5 U/ml plasmin (a broad spectrum serine proteinase capable of degrading fibrin) was added to each sample. All systems were incubated in 37°C on a shaker, and media was collected and freshly replaced every 3-4 days for 20 days. Drug released to the media was measured with an infliximab specific ELISA [8]. To test bioactivity of the released drug,
collected media was applied on TNFα-treated (10 ng/ml) human AF cells isolated from surgical tissue and autopsy (N=4, grade 2-4, 105 cells/ml, 37°C, 5% O2, 5% CO2). Pro-inflammatory cytokines (TNFα, IL1-β, IL-6, IL-8) in AF cell supernatant were measured using a multiplex ELISA and averaged.

**Results:** CHS was successfully embedded into FibGen without altering the spherical hollow structure based on TEM images (not shown). To assess drug loading efficiency, 320 µg infliximab was initially loaded into CHS and measured residual drug release (1.0±1.3 µg) indicated high loading efficiency (99.7±0.4 %). Cumulative infliximab concentration in Fibrin alone showed rapid drug release from day 1 (283.3±73.9µg/mL) to day 6 (937.2±32.3µg/mL) but degraded within 6 days. Cumulative infliximab concentration over 20 days showed increased drug release from CHS_320 µg, steady release at low levels from FibGen_320 µg, and impeded drug release in FibGen+CHS_320 µg (Fig. 1). Loading FibGen with increased amount of infliximab (2.5 mg and 7.5 mg) increased cumulative drug release, however addition of infliximab-loaded CHS into FibGen_7.5 mg did not further increase drug release compared to FibGen_7.5 mg alone (Fig. 2A). Cumulative drug release from FibGen_320 µg in the presence of 1.5U plasmin was increased over 20 days compared to the non-plasmin treated system (Fig. 2B) and resulted in approximately 8% cumulative drug release over the 20-day period. The bioactivity of infliximab following release from CHS_320 µg and FibGen_7.5 mg was confirmed by the averaged reduction of all 4 pro-inflammatory cytokines; TNFα, IL1-β, IL-6, IL-8, produced by human AF cells, which were significantly different compared to the TNFα treated control case (Fig. 3).

**Discussion:** FibGen with direct drug incorporation was effective for sustained delivery of infliximab, and the dose of released drug can be optimized by adjusting the amount of initially incorporated drug. Rapid and high drug release was measured in Fibrin alone but the Fibrin completely degraded at day 6 and therefore was not suitable as a drug carrier for sustained release. Although CHS alone was highly effective at delivering sustained high doses of infliximab at early time points, a carrier would be required for retention within the IVD space in vivo. Addition of drug-loaded CHS into FibGen showed no benefit to cumulative drug release over time, which could be due to changes in the drug release mechanism from CHS during the gel cross-linking process, or entrapment of the released drug from CHS within the FibGen gel. Infliximab was proven to be bioactive on human AF cells following release, which further confirmed the suitability of FibGen as a carrier for infliximab. Because higher drug concentration was achieved when FibGen was degraded by plasmin treatment, we expect that the drug release in vivo will be increased as the gel continues to slowly degrade overtime. Therefore, we believe that FibGen with direct mixing of infliximab is an attractive delivery system, as the gel can partially restore IVD biomechanical behavior while allowing for local and sustained release of infliximab to inhibit the catabolic effects of TNFα and to promote regeneration. These results warrant future investigations and assessment in organ culture or in vivo models for FibGen as an effective carrier for sustained delivery of infliximab.

**Significance:** FibGen as an effective AF sealant that can provide sustained delivery of an anti-TNFα drug may serve as a therapeutic strategy for painful IVD with ruptured AF that can inhibit progressive IVD degeneration and promote regeneration.
Figure 3 Averaged reduction of IL-1β, IL-6, IL-8 and TNFα confirmed that infliximab remains bioactive following release (**p<0.001)
Figure 2  
A Increased cumulative release with higher drug incorporation into FibGen  
B Higher release of infliximab when FibGen’s degradation was induced with plasmin
Figure 1: Cumulative infliximab release showed no benefit of CHS addition.