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Injectable, High Density Collagen Gels for Annulus Fibrosus Repair: An *In Vitro* Rat Tail Model

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Abstract

A herniated intervertebral disc often causes back pain when disc tissue is displaced through a damaged annulus fibrosus. Currently the only methods available for annulus fibrosus repair involve mechanical closure of defect, which does little to address biological healing in the damaged tissue. Collagen hydrogels are injectable and have been used to repair annulus defects *in vivo*. In this study, high-density collagen hydrogels at 5, 10 and 15 mg/ml were used to repair defects made to intact rat caudal intervertebral discs *in vitro*. A group of gels at 15 mg/ml were also crosslinked with riboflavin at 0.03 mM, 0.07 mM or 0.10 mM. These crosslinked, high-density collagen gels maintained presence in the defect under loading and contributed positively to the mechanical response of damaged discs. Discs exhibited increases to 95% of undamaged effective equilibrium and instantaneous moduli as well as up to four fold decreases in effective hydraulic permeability from the damaged discs. These data suggest that high density collagen gels may be effective at restoring mechanical function of injured discs as well as potential vehicles for delivery of biological agents such as cells or growth factors that may aid in the repair of the annulus fibrosus.

Keywords

Intervertebral Disc; Riboflavin; Crosslinking; Biomaterial; Tissue Engineering

1. Introduction

A bulging or herniated intervertebral disc (IVD) can cause back pain, leg pain and neurological deficits when the nucleus pulposus (NP) is displaced through a damaged annulus fibrosus (AF). AF damage occurs in a variety of ways including natural weakening over time, trauma, or as an unintentional result of medical treatment (e.g. discography, discectomy). In the United States, an estimated \$90 billion per year is spent on assessment and treatment of lower back pain alone ¹. This damage also affects the motion segment

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mechanically, with loss of disc stiffness²⁻⁴ and biologically, in subsequent degenerative effects^{2,5-7}.

In most cases, partial discectomies alleviate the pain of a bulging or herniated disc; however the resulting annular defect is often left untreated. This increases the likeliness of recurrent disc herniations through the open defect. Clinical studies have shown that the rate of recurrent herniation after partial discectomy lies between 5%-20% with many of the patients requiring additional procedures. Furthermore, the rate of reherniation has been shown to correlate with the size of the original surgical defect⁸⁻¹⁰. In order to improve recovery after treatment, it is highly desirable to address the remaining AF defect after discectomy.

Annulus repair strategies have been devised to address many different defects in damaged AF tissue¹¹⁻¹⁷. These therapies aim to mechanically close lesions in the AF to prevent prolapse and perhaps slow or inhibit degeneration. The types of annulus closure treatments that are in development range from purely mechanical methods to tissue engineered strategies, all with varying degrees of success. Many mechanical strategies are already commercially available (i.e. suture, barrier, plug). These mechanical treatments block the AF defect while inhibiting short term reherniation. Studies have shown that disc compressive mechanical properties are affected when the annulus fibrosus is subject to full thickness defects, leading to diminished time dependant mechanical behavior and eventual degeneration¹⁸⁻²⁰. While these solutions address the load bearing requirements of the IVD, they do not encourage the long-term regeneration of tissue in the damaged area, which is intrinsically difficult to achieve due to the limited self-healing potential of the AF²¹.

Tissue engineered strategies are being developed with the goal of achieving biological healing, along with satisfying the requirements for mechanical support. Various scaffolds such as fibrin and silk have been used to develop laminates or adhesives to address the damaged AF. Furthermore, a host of studies have examined the effectiveness of different cell/scaffold combinations in creating AF-mimicking structures^{22,23}, while others have created tissue engineered total disc replacements with a construct that mimics the native AF²⁴⁻²⁸. These approaches are promising but their effectiveness in repairing AF defects *in situ* has not been reported. A major obstacle for many tissue engineering approaches is delivery of the material or device to an irregularly shaped defect. For clinical use, an injectable formulation is desirable due to the potential delivery by minimally invasive approaches.

Collagen hydrogels have been used for a variety of tissue engineering applications, enabling delivery of many cell types including chondrocytes and IVD cells^{29,30}. Collagen is injectable and highly biocompatible in the disc space as both an NP replacement material and AF repair material^{31,32}. Typically, collagen gels are weak and exhibit low stiffness at densities less than 5mg/ml. However, gel stiffness can be tuned by controlling concentration or crosslinking³³⁻³⁸.

A variety of crosslinking agents have been used with collagen, including formaldehyde, and glutaraldehyde. Although their effectiveness has been proven, these aldehydes are also cytotoxic, thus limiting their application in biological systems^{34,37}. To address these

limitations, groups have used sugars and flavins to induce crosslinking in collagen-based structures^{30,35}. Riboflavin is particularly attractive because it is used clinically to strengthen the collagen structure of the cornea in the treatment of keratoconus^{39,40}. Further, riboflavin is photo activated and as such crosslinking is tunable by exposure to UVA-wavelength light. Riboflavin crosslinking of collagen gel constructs was shown to increase their mechanical stiffness while maintaining high cell viability³⁰. These factors make riboflavin crosslinking a viable tool in the use of collagen hydrogels for tissue engineering.

We have successfully used high density, riboflavin-crosslinked collagen gels in an *in vivo* rat tail AF repair model. Crosslinked gels slowed or prevented the onset and progression of degeneration in rat caudal IVDs as evidenced by higher disc heights and NP hydration than untreated discs for up to 5 weeks after treatment³². Furthermore, discs treated with crosslinked collagen gels maintained healthy disc phenotype as seen in histological sections. Although these results were encouraging, the mechanical contribution of injectable repair with these gels is unknown. This study investigated the use of injectable, crosslinked high-density collagen gels to fill and mechanically repair focal defects in the annulus fibrosus of a rat caudal intervertebral disc. We report the effect of different defect sizes on effective disc mechanical properties, as well as the effects of increasing gel collagen density and riboflavin concentration on effective disc stiffness and hydraulic permeability.

2. Materials and Methods

Mechanical testing was performed on intact, cadaveric rat caudal motion segments to assess the extent to which injectable collagen gels restored performance to damaged intervertebral discs. In parallel studies, the effect of two different sized defects were examined, with each motion segment tested prior to damage, after the introduction of a defect to the AF, and after this defect was filled with a high density collagen gel. In separate studies the effect of crosslinking was assessed using a range of concentrations of the photocrosslinking agent riboflavin.

2.1 Collagen gel preparation

Collagen fibers were harvested from rat tail tendons as described previously^{36,41,42}. The resulting collagen mass was then weighed and digested in a 0.1% acetic acid at a concentration of 150 ml/g tendon for at least 48 hours. Digested collagen was then centrifuged at 9000 RPM for 90 minutes at 4°C and the supernatant collected and frozen at -80°C. After lyophilization for 48 hours, the dehydrated collagen was weighed and reconstituted in 0.1% acetic acid at the stock concentrations of 6, 12, and 20 mg/ml. Each collagen stock solution was stored at 4°C until use.

For high-density samples, final collagen gel solutions at 5, 10, and 15 mg/ml were made by mixing the acidic stock solutions with basic working solutions composed of 10x Dulbecco's Phosphate Buffered Saline (DPBS), 1N sodium hydroxide (NaOH) and 1x DPBS. Each working solution was dyed with trypan blue at a ratio of 10:1, respectively, to track the fate of the injected gel. Crosslinked collagen gels were mixed at the highest density (15mg/ml), with varying amounts of riboflavin. Each gel was prepared in the method above, however riboflavin was added to the 1X DPBS at concentrations of 0.25 mM, 0.50 mM, or 0.75 mM ,

resulting in gel concentrations of 0.03 mM, 0.07 mM, and 0.10 mM respectively. Upon delivery to the defect site, crosslinked gels were exposed to 468 nm blue light ($\sim 1400 \text{ kW/cm}^2$) for 40 seconds to initiate crosslinking.

2.2 Dissection and segment handling

Frozen tails from 34 7–8 week old Sprague-Dawley rats were thawed in room temperature DPBS. The skin was removed and the tissue was dissected from the most proximal three vertebrae. The most proximal complete motion segment (bone-disc-bone) was cut from the rest of the tail at the adjacent intervertebral disc. Remaining tail material was discarded and the dissected motion segments were kept in room temperature DPBS. After the first phase of mechanical testing, a defect was created at the midline of the intervertebral disc by either rotating a beveled 21 ga needle (small), or by removing a $\sim 1 \text{ mm}^2$ window of the AF using a #11 scalpel blade (large) (Figure 1). Each defect was limited to only the AF by using a depth stopper (small)⁴³ or using care to only remove the AF with the scalpel (large). The damaged motion segment was tested mechanically again before filling the defect with the desired final collagen gel solution. About 100 μL of the collagen gel was delivered to each defect using a 27 gauge precision tip needle (Nordson EFD, Robbinsville, NJ). Allowing 30 minutes for collagen gel polymerization after delivery, the motion segment was mechanically tested again. Upon completion of testing, the segments were either stored at -20°C for gross examination, or processed for histology.

2.3 Mechanical testing

Dissected motion segments were loaded into an Enduratec ELF 3200 test frame (Bose, Eden Prairie, MN) using custom grips (McMaster-Carr, Aurora, OH)²⁴. All manipulation of the motion segment was performed while mounted on the load frame so as to preserve the original undamaged height of the motion segment. Each motion segment was allowed to relax for 10 minutes to ensure all initial transient effects were gone before testing began. Stress-relaxation testing was performed on each motion segment in steps of 5% compressive strain to a total displacement of 20% initial disc height. Testing in this manner allowed us to observe compressive stiffness as well as the hydraulic permeability from relaxation. This loading scheme was performed on the undamaged motion segment and repeated for both damaged and treated phases.

2.4 Data Analysis and Statistics

Load data were recorded directly during mechanical testing at 1 Hz for 50 minutes. All data analysis was performed using Excel or MATLAB software. Load curves were generated for each test in order to qualitatively compare disc behavior before and after treatment with the collagen gels. Using a custom MATLAB script, a poroelastic model (Figure 2, Equ. 1) was fit to the raw load data to determine the effective equilibrium modulus ($A+B$), effective instantaneous modulus (B) and time constant of relaxation (τ) with time (t). Effective hydraulic permeability (k) was calculated using the equilibrium modulus (E), disc radius (r), and time constant from poroelastic model fit (Figure 2, Equ. 2)^{24,36,44}. One-way ANOVA with repeated measures including Tukey's HSD test for *post hoc* analysis was conducted to examine the general effect of injectable collagen gel treatment on all samples. Analysis of

the separate effects of collagen gel density or crosslink concentration was carried out using separate one-way ANOVA with Tukey's HSD test for *post hoc* pairwise analysis. All statistical analyses were conducted using JMP.

2.5 Histology

Whole IVDs were dissected from the frozen motion segments and fixed in 10% formalin for at least 48 hours before transfer to 70% ethanol. Fixed discs were embedded in paraffin wax and sectioned parallel to the transverse plane and affixed to glass slides. Histological slides were stained with Safranin-O with a Fast Green counterstain for proteoglycan content and general tissue architecture. Stained slides were viewed under bright-field microscopy for NP content and general tissue organization.

3. Results

The ability of high density, crosslinked collagen gels to repair defects in the AF of rat caudal IVDs was assessed both visually and quantitatively through effective disc mechanical properties. Photographs of transverse cross-sections of discs treated with 10 and 15 mg/ml gels in smaller defects (Figure 3a and 3b) and 15 mg/ml gel in the larger defect (Figure 3c) demonstrated the presence of dyed collagen gels in all cases, after the completion of the loading studies. In all cases collagen was present in the AF defect, while in the larger defect, some gel was also observed in the NP space. Histological analysis showed a clear defect in the AF created with both the 21 gauge needle and scalpel blade, severing all lamella and exposing the NP (Figure 4). Figure 4 shows a small defect repaired with a crosslinked collagen gel, with a gel patch adhered to the outer AF. Figure 4 also shows a large defect treated with a crosslinked collagen, however there is no evidence of the collagen gel after testing. These data indicate that injected high density collagen gels remained in place in AF defects either in whole or in part after IVDs had been compressed by up to 20%.

Based on temporal traces of the load, both defects had a profound effect on the compressive behavior of the motion segment. In particular, the instantaneous, peak stresses achieved directly after steps in compression were significantly diminished by the injury and were partially restored immediately after the injection of the collagen gel. Stress relaxation after steps in strain appeared to occur much more rapidly in damaged motion segments compared to either uninjured motion segments or those in which defects had been filled with collagen gels (Figure 5).

Using the temporal load data, the effective equilibrium and instantaneous moduli and effective hydraulic permeability were calculated for both defect types and for all collagen gel densities. The average effective equilibrium and instantaneous moduli of uninjured intervertebral discs were 170 ± 54 kPa and 272 ± 133 kPa, respectively, while the average effective hydraulic permeability was $2.07 \times 10^{-14} \pm 1.27 \times 10^{-14}$ m²/Pa•s. These values are similar to those reported previously for rat caudal intervertebral discs^{24,28,45}.

To specifically assess the effect of injury and treatment on the mechanical performance of the intervertebral discs, the mechanical properties after injury and treatment for each sample were normalized by the value of the uninjured disc. As such, all data displayed are unitless,

with 1 being the value of the uninjured sample. The efficacy of each collagen gel formulation at restoring the effective equilibrium and instantaneous moduli and effective hydraulic permeability were assessed separately, as well as pooled to assess the general effect of treatment.

Larger defects had profound effects on mechanical properties, resulting in 60% ($p<0.001$) decreases in effective equilibrium and instantaneous moduli, and a 400% ($p<0.05$) increase in hydraulic permeability, compared to smaller defects which produced 18% and 28% ($p<0.05$) decreases in moduli and a 114% ($p<0.01$) increase in permeability (Figure 4). Treating AF defects with collagen gel formulations increased the effective equilibrium modulus of samples with small defects to 85% of uninjured values. In contrast, filling large defects with collagen gels had little effect on the effective equilibrium modulus. Delivery of collagen gels nominally increased the effective instantaneous modulus by 6–8% of the undamaged values, but this change was not statistically different from the damaged case. The largest effect of delivering collagen gels to the defect was in restoring the hydraulic permeability of samples with small defects. Delivery of collagen gels decreased the effective hydraulic permeability from 215% of the undamaged value to 133% of the undamaged value ($p<0.05$ compared to damaged and $p>0.98$ compared to damaged). Treatment with collagen gels had a similar effect on the effective hydraulic permeability of intervertebral discs with large defects, which were 600% of control both before treatment and 400% after repair.

Collagen gel density showed no significant impact on AF repair in small defects. Collagen gels of 5 mg/ml increased effective equilibrium and instantaneous moduli slightly over the damaged value. All three tested gel densities decreased effective hydraulic permeability by 50% from damaged values. In samples with larger defects, both 5 and 10 mg/ml samples did not improve effective equilibrium or instantaneous modulus. However, samples treated with 15mg/ml gels exhibited dramatic improvement in effective equilibrium modulus, with an increase to 70% of undamaged from the damaged mean of 50%. Effective instantaneous moduli in these samples exhibited a greater increase of 30%, which was statistically significant ($p<0.05$). All three tested collagen gel densities decreased effective permeability in large defect samples. In small defects, riboflavin crosslinking of collagen did not statistically increase moduli; however the nominal increases in modulus were such that samples treated with crosslinked gels were not significantly different than uninjured samples. At the highest concentration of riboflavin, effective equilibrium modulus was 95% of undamaged. Crosslinked gels all decreased hydraulic permeability, with 0.10 mM group falling to within 5% of the undamaged mean. In large defect samples, none of the experimental groups exhibited increased effective equilibrium or instantaneous moduli significantly over damaged values. Non-crosslinked gels showed the most profound increase with effective equilibrium and instantaneous moduli rising to 75% and 70% of undamaged values, respectively. Although all three experimental groups decreased hydraulic permeability in large defect samples from 6-fold to 4-fold over undamaged, non-crosslinked controls exhibited the most profound decrease with a fall to 3-fold over undamaged discs.

4. Discussion

The goal of this study was to assess the effect of increased collagen density and riboflavin crosslinking on repair of AF defects with injectable collagen gels. This was accomplished through gross visual evaluation and comparison of mechanical properties with undamaged discs. We report several metrics that captured the mechanical performance including effective moduli and hydraulic permeability. It is shown here that injectable high density type I collagen gels had a positive effect on the mechanical behavior of injured IVDs once delivered to focal defects in the annulus fibrosus. Both large and small defects in the AF decreased effective disc moduli and increased effective permeability, which collectively represent the diminished ability of the disc to bear load. Furthermore, we report that crosslinking with riboflavin had limited positive effects, but did not diminish the effectiveness of high-density collagen gels for AF repair.

This study addressed the performance of an annular repair technique directly in an *ex vivo* whole motion segment. IVD defect models in rat tails are often used to study degeneration and to assess possible therapies^{2,46}. The decision to address two defect sizes, created differently, was motivated by the clinical need to address different damage types. Our beveled needle defect simulated standardized diagnostic procedures such as a discography, while our larger free-hand defect was meant to simulate a surgical intervention (e.g. annulotomy). Although defect variation adds to the variability in “damaged” data sets, it also enhances the rigorosity of our approach. An effective AF repair technique would be expected to comply with the irregularities of any AF defect. Our collagen hydrogel formulations were low viscosity upon delivery, allowing it to mold to the form of the defects.

Repaired IVDs were assessed based on effective equilibrium and instantaneous moduli for stiffness as well as effective hydraulic permeability for flow of water out of the disc. Effective equilibrium modulus and hydraulic permeability values were within the range reported previously for rat caudal IVDs^{26,45}. Diminished IVD performance from the smaller defect was not as profound as that seen with larger defect samples; however, in these samples collagen gels were more effective at repairing smaller defects. All three hydrogel densities improved IVD stiffness and hydraulic permeability in the small defects. IVDs with the larger defects also seemed to experience some benefit from treatment with collagen hydrogels, with the most profound increase in effective stiffness being delivered by 15 mg/ml collagen gels. Effective hydraulic permeability in large defect samples was improved by all collagen gel densities. The function of the IVD was highly dependent on the ability of the AF to keep the NP hydrated and enabling maintenance of large hydrostatic pressure in the disc space. Improvements in IVD stiffness were evident with repair using our collagen hydrogel; however the profound decreases in hydraulic permeability underscored the ability of high-density collagen gels to contain water within the IVD. Tested samples exhibited dramatic increases in permeability when damaged, and decreases in permeability of over 2 fold when repaired. When compared to slight changes in both effective equilibrium and instantaneous moduli, it seems that effective hydraulic permeability is better suited to evaluate disc mechanical function after AF repair in this model. The hydrostatic pressures and total stresses imposed on repaired samples during testing were as high as 83kPa and

150kPa respectively. These were about 20% of those seen in the human lumbar spine while standing⁴⁷ and over 80% of that seen by the human cervical spine under static loading or bending^{48,49}. While this result was encouraging, the pressures were only 4% of that seen in lumbar spine while lifting a 45lb weight⁴⁷, which supports our plan to continue testing these gels in a large animal model.

The improved mechanical responses in repaired IVDs discussed above strongly suggest that high-density collagen gels remained in AF defects. In order to help support the mechanical data, gross visual examination was conducted on repaired IVDs after mechanical testing. Gel formulations were dyed with trypan blue to allow tracking of the collagen throughout the testing process. After testing, motion segments were frozen and bisected transversely through the repaired disc (Figure 4). Blue gels were seen localized to the AF after testing in both the small and large defects. Some evidence of gel in the NP was seen, however it is unclear whether this happened during delivery, testing or bisection. The visual presence of gels after mechanical testing confirms that these high-density gel formulations were able to be delivered to the defect and maintained presence after compression and processing for visual analysis. Histology shows clear evidence of a patch in small defect samples, while there is evidence of material in large defects.

These studies were motivated in part by previous work demonstrating the efficacy of injected collagen gels for repair of AF defects *in vivo*³². While promising, these previous studies did not address the extent to which these gels restored mechanical function immediately after injury. In both *in vivo* and in the current study, we tested 15 mg/ml collagen gels containing varying amounts of the crosslinker riboflavin. Riboflavin crosslinked collagen gels were best in needle puncture defects in either study. Histology from both studies showed that collagen gel formulations work as a patch, with the majority of the injected gel localized to the outer AF. This patch type repair was successful in keeping the NP intact and in place. According to our study, we would move forward with higher concentrations of riboflavin. The highest concentration (0.1 mM) was observed to have the greatest impact on the damaged AF, however the other tested concentrations also had positive effects. As we move forward with larger animal models, we will use larger (0.07+ mM) concentrations of riboflavin and maintain a 15 mg/ml collagen gel unless a different formulation is needed for increased loading observed in larger animals.

Our previous *in vivo* study on AF repair in the rat model supports our observation that punctured discs treated with these crosslinked gel formulations retained healthy phenotype and NP hydration³², especially over the long term. Our *in vivo* samples treated with crosslinked collagen exhibited a brief dip in both disc height and NP hydration after surgery, but recovered and maintained higher properties by five weeks post-operation³². Therefore, the crosslinking of the gels may be more successful at promoting biological healing as opposed to a purely mechanical repair.

Until recently, the major focus for annular repair has been mechanical closure of the native AF with little attention to biological healing. Proposed criteria for effective AF repair⁹ include: filling the AF gap; mechanically augmenting injured disc function; maintaining or promoting cell survival and/or differentiation; integrating with surrounding tissues; and

exhibiting biocompatibility. Our study showed that collagen hydrogels can be injected into a gap in the AF and remain in the defect under load. Furthermore, once delivered the gels contributed to the mechanical function of the damaged IVDs. Stiffness of the discs, as assessed by effective modulus values, showed increases towards undamaged discs. Our collagen hydrogel formulations have proven to directly satisfy two of the criteria set forth by Bron et al. in an *ex vivo* AF defect model⁹.

Remaining factors considered in the literature and criteria above address the regenerative nature of AF repair treatments. An effective repair method must support cell survival, cannot be harmful to the surrounding native tissue and must have the potential for integration with surrounding tissue. Research groups have showed that collagen gels crosslinked with riboflavin are capable of supporting various cell types³⁰. Furthermore, our *in vivo* studies show that these gel formulations are not only harmless to surrounding tissue, but allow for cell migration into the repaired area with accompanied native tissue ingrowth³². Thus, according to the criteria above, our type I collagen hydrogel is an ideal candidate for AF repair.

While the rat caudal motion segment model is widely used in disc degeneration and repair studies, there were limitations to the model in this study that cannot be overlooked. The reported work only addressed the short term, mechanical contribution of both crosslinked and non-crosslinked high-density collagen gels. While it is known that the IVD experiences six degrees of motion *in vivo*⁵⁰, we only studied axial compression in this model. Compressive stress-relaxation allowed us to observe the dramatic changes in IVD effective permeability with puncture. Our studies have shown that once damaged, the hydraulic permeability increases dramatically (up to 6 fold with large defects) over undamaged values. Furthermore, other groups have seen changes in time-dependant compressive mechanical properties of IVDs with AF damage^{18,51}. Other testing modalities, such as torsion, do not allow us to study the effects on fluid flow out of the disc. As such, this model represented an important screening tool for assessing AF repair, but must be followed with assessment of efficacy in larger animal models, where loading better approximates that in humans.

The method of mechanical testing and analysis used in the current study reported effective material properties (i.e. moduli and hydraulic permeability) that assume material homogeneity. Although this did not allow us to address the mechanical integrity of the AF or NP directly, it did enable assessment of the effects of AF repair on function of the whole IVD as part of a motion segment. It may be beneficial to test the AF directly with the collagen hydrogel repair method once we expand the formulation to facilitate repair in larger IVDs, however the effective mechanical effects were suitable for establishing the feasibility of a crosslinked, high-density collagen hydrogel for AF repair. Such an approach could involve directly measuring local AF strains⁵² or analytic finite element models that account for differing properties between the AF and NP⁵³.

Another limitation was that AF defects in the reported work were made while maintaining the motion segment at constant height, preventing immediate collapse of the IVD upon puncture. Without immediate collapse, NP tissue was only displaced during testing instead of upon puncture, which we experienced during *in vivo* puncture. NP tissue is known to be

lost with puncture or aspiration, which has profound effects on mechanical behavior^{2,4,21}. Although keeping the motion segment in displacement control was a concern, histological sections show that we lost a considerable amount of NP, especially in large defects (Figure 3). Furthermore, loss of mechanical stiffness and corresponding increase in hydraulic permeability support our observation that we still do lose NP tissue.

This study demonstrated that crosslinked, high-density collagen gels mechanically enhanced the effective properties of damaged IVDs after injection into AF defects. Although the current study used gels alone, multiple studies have used type I collagen gels with various additions such as cells, growth factors, and other therapeutics to support tissue growth and development^{24,31,54}. As we move forward, we will also look to seed the collagen gels with ovine AF cells, as has been shown previously with tissue engineered total disc replacements, which exhibited enhanced integration with surrounding native AF tissue^{24,41}.

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Figure 1. Schematic of experimental design showing mounted, undamaged caudal motion segment, small and large defects as well as treated drawings.

Figure 2.

Representative temporal data set of an undamaged segment. Inlay shows custom MATLAB fit of poroelastic model to single step of stress relaxation data.

Figure 3.

Gross transverse cross-sections of treated IVDs after mechanical testing. High density, non-crosslinked collagen gels were dyed with trypan blue to track presence in the disc. Pictures show the presence of gel after loading small defect (A&B) as well as large defect (C) samples.

Figure 4. Safranin-O stained transverse sections of damaged and treated IVDs. Histology shows clear defects made with both 21ga. and scalpel blades. Arrows highlight defect sites. Collagen patch can be seen in treated small defect sample.

Figure 5.

Representative temporal data set of load during a series of stress relaxation tests. As shown here, each sample was tested prior to imposition of a defect (blue), after damage (red), and after the defect was filled with a collagen gel (green). The sample shown here was a small defect filled with a 15 mg/ml collagen gel.

Figure 6.

Normalized effective mechanical properties (pooled) of intervertebral discs after injury and after treatment with collagen gel formulations. The values of effective equilibrium modulus, effective instantaneous modulus, and effective hydraulic permeability were normalized to those of the undamaged disc (dashed line) on a sample by sample basis. % indicates that the noted conditions were significantly ($p < 0.05$) different from damaged. * indicates that the noted conditions were significantly ($p < 0.05$) different from undamaged condition. Error bars represent standard deviation $n = 34$.

Figure 7.

Normalized effective mechanical properties of intervertebral discs after injury and after treatment with high density collagen gels. % indicates that the noted conditions were significantly ($p < 0.05$) different from damaged. * indicates that noted conditions were significantly different from undamaged ($p < 0.05$). Error bars represent standard deviation. $n = 10 \pm 3$.

Figure 8.

Normalized effective mechanical properties of intervertebral discs after injury and after filling with riboflavin-crosslinked collagen gels. * indicates that noted conditions were significantly different from undamaged ($p < 0.05$). Error bars represent standard deviation. $n = 10 \pm 3$.