Prospective investigation evaluating the efficacy of the "Discseel Procedure" compared to the "Biologx" Procedure.

Investigator: Kevin Pauza MD

Abstract: This prospective investigation compares efficacy of the "Discseel Procedure" to the "Biologx Procedure". Both procedures utilize intra-discal fibrin, which is considered the most effective tissue bio-adhesive, and is also critical to all other tissue healing. However, the "Discseel Procedure" differs dramatically from the earlier "Biologx" procedure in that the Discseel Procedure embodies refinements constituting an inseparable series of procedures performed sequentially, which uniquely: (1) diagnose annulus fibrosus defects; and next (2) immediately repairs those defects by targeting them with fibrin bio-adhesive glue to treat discogenic symptoms. The Discseel Procedure incorporates refinements, which address weakness found in all other intra-discal treatments, hypothesizing that the Discseel Procedure is optimal over all other intra-discal procedures, including the Biologx procedure. This study determines if those refinements incorporated into the "Discseel Procedure" increase the procedure's safety and efficacy.

One refinement is the inclusion of diagnostic annulograms as a part of the overall procedure which improves the procedure's ability to diagnose abnormal disc annulus fibrosus tears by introducing radiopaque contrast diluted with antibiotics, to demonstrate disc tears while minimizing the likelihood of disc infection. Another Discseel Procedure methodology refinement theoretically optimizes efficacy by precisely targeting annular defects with fibrin bio-adhesive glue serving to constrain the disc's nucleus pulposus gel, instead of displacing it outwards through annular defects, as does all other treatment options.
Volume displacement is a fundamental law of physics, and all other spine disc treatments, with the exception of this Discseel Procedure, disregard this fundamental law of physics (fluid dynamics), and associated concerns caused by the iatrogenic displacement of nucleus pulposus outwards from a disc, because nucleus pulposus is perceived a

"noxious" foreign substance and must stay within the disc's center to optimize biochemical and biomechanical properties.
Therefore, this investigation compares the efficacy of the "Discseel Procedure" and the "Biologx procedure," to determine if those refinements increase efficacy and safety when treating discogenic axial spine pain.
Conclusions: Subjects who underwent the Discseel Procedure demonstrated statistically significant improvement in pain compared to those who underwent the Biologx procedure, with a mean VAS improvement of 76.1% vs 51.2% respectively, after one year ($p<0.001$). And at all time periods, there was a statistically significant improvement in pain reduction with the Discseel Procedure.
The Discseel Procedure subjects developed no infections and experienced no significant adverse events, whereas the Biologix Procedure cohort experienced infectious discitis and one subject was temporarily hospitalized for pain control.

Background context: Annulus fibrosis tears, no matter how miniscule, are the precursors for all disc pathology[66-67]. All spinal disc treatments, with the exception of the Discseel Procedure, fail to incorporate fundamental laws of physics in their attempt to repair abnormal spinal discs. Those laws define volume displacement, and all other treatments cause the displacement of nucleus pulposus gel.
The Discseel Procedure incorporates the fundamental laws of physics involving volume displacement[1], by sealing annular defects to stop displacement of nucleus pulposus outwards through annular defects. All other procedures including the "Biologx" procedure, disregard those laws of physics by increasing nucleus pulposus displacement through annular defects[11, 13]. Studies indicate that leakage of chemical mediators or inflammatory cytokines, which are produced in the painful disc, flow outwards through annular tears and lead to injury of adjacent spinal nerve roots[45-46], and constitute the primary pathophysiologic mechanism of low back and radiating leg pain in patients with or without disc herniation[2, 15]. Because there is little correlation between MRI or CT findings and symptom etiology[79-82], the Discseel Procedure utilizes diagnostic, dynamic annulograms to identify disc annular tears because of their high sensitivity and specificity in identifying annular tears. An annulus fibrosus tear, without herniation or any radiographic evidence of herniation or degeneration, (before the advent of diagnostic

annulograms), is sufficient to induce significant morphologic and functional changes of spinal nerve roots, causing weakness, pain, paraesthesias, and sensory deficit[14].

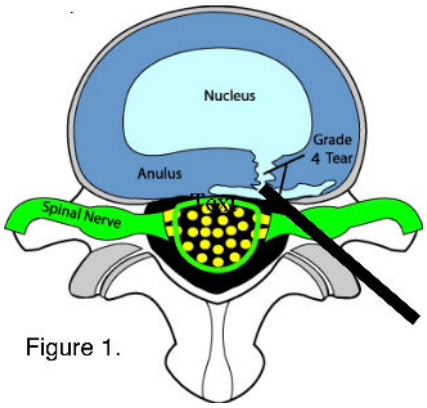


Figure 1.

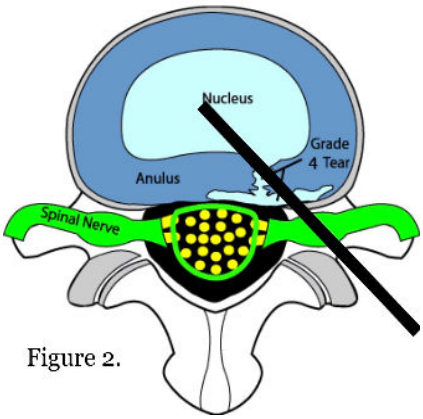


Figure 2.

(Figure 1) represents the "Discseel Procedure", with the introducer's tip positioned in the annulus fibrosus, to inject fibrin sealant, to contain the nucleus pulposus.
(Figure 2) represents the "Biolox Procedure", with the introducer's tip positioned in the nucleus pulposus, to inject fibrin tissue sealant, potentially displacing nucleus pulposus gel outwards through annular defects.

Discseel Procedure refinements:
(1) Diagnostic annulograms which inject radiopaque contrast diluted with antibiotic into the annulus fibosus to identify disc annular defects in the region of symptomology, and minimize the likelihood of infection by introducing intra-annular antibiotics[3].
(2) Subsequently, it immediately seals annular defects by introducing fibrin bio-adhesive glue into all annular defects identified by the annulogram, to seal those defects and to minimize displacement of nucleus pulposus outwards through those annular defects, and thus correct inflammation.
In comparison, all other spinal disc regenerative treatments are relegated to injecting their materials into a disc's center[4], because all other materials lack fibrin's bio-adhesive properties that allow fibrin to

adhere to annular defects. If other biologic materials were injected into the annulus fibrosus, they would immediately leak inwards or outwards[5]. Live animal *in vivo* investigations demonstrated that all radiolabeled stem cells leaked almost immediately from the discs they were injected. Those leaked stem cells were found within new, exuberant osteophyte and bone spur formations, large enough to be observed with gross macroscopic visualization. They concluded that those bone spurs were caused by the stem cells (mesenchymal precursor cells) that leaked[8,9,12]. To date, no research center conducted a similar investigation evaluating live humans, to determine if stem cells likewise leak in humans and instigate detrimental bone spur and osteophyte formation. In other intra-discal procedures, even the needle punctures are hypothesized to cause accelerated disc degeneration[48-50]. Fibrin seals those needle punctures, preventing iatrogenic disc degeneration.

Fundamental properties of physics defining volume displacement, were first described by Archimedes in 250 B.C., and remain crucial in describing the origins of disc symptomology, and should be incorporated into treatments meant to correct disc symptomology. More specifically, symptoms originate from heightened nocioceptor sensitivity caused by disc nucleus pulposus gel displaced outwards through annulus fibrosus tears, initiating the inflammatory cascade and autoimmune response[2,6]. The leaking nucleus pulposus is perceived as a "foreign substance" as it travels outwards through annulus fibrosus tears[10]. This nucleus pulposus leakage initiates the inflammatory cascade and the autoimmune response[7, 47]. The resultant inflammatory constituents heighten sensitivity of the nocioceptors residing within the disc's 22-25 annular rings, and the adjacent tissue, including the dura mater, pia mater, meninges, peri-radicular nocioceptors, and descending spinal nerves, causing low back and leg pain and other symptoms[7].

The "Discseel Procedure"[10] was developed to specifically target annulus fibrosus defects with fibrin bio-adhesive glue, to retain nucleus pulposus gel inside the disc, instead of allowing its further displacement outwards. Fibrin was chosen after comparing all other available bio-adhesives, which possessed varying, but lesser degrees of strength, and so their use was discounted[16-31]. Among the other tissue adhesive options, foremost was cyanoacrylate[32]. Although it possesses strong

adhesive bonds, those bonds lack biocompatibility, release formaldehyde as they degrade, and are mechanically stiff and brittle. In addition to cyanoacrylates, other tissue adhesives which offer relevant strength deserving consideration are the newer "marine adhesives" derived from molecules mimicking mussel adhesive proteins (MAPs)[33-36]. However, concerns associated with their cell toxicity and foreign body reactions outweighed their superior adhesive and cohesive properties, and therefore, fibrin remains the optimal tissue adhesive[38]. Even fibrin's degradation products are chemotactic agents, which heal tissue, and neither stem cells nor other constituents possess those unique healing attributes belonging to fibrin[37].

In nature, fibrin's precursors heal disc injuries, and because discs lack rich vascularity, fibrin constituents heal discs by diffusing through disc's Endplates, instead of by using their typical vascular route to heal injuries. The limited diffusion of anything into or out of spinal discs, including fibrin constituents into discs explains why natural disc healing occurs, but it occurs in a bridled fashion[37]. It's important to note that the human body deploys fibrin to heal discs, and does not deploy stem cells, which reflects that fibrin is a natural constituent whose sole purpose is to heal discs and other tissue[39-40].

Other biologics fail because none adhere to annulus fibrosus, and therefore would immediately leak, and if injected into the disc's center would cause nucleus pulposus displacement outwards, potentially worsening disc pathology[41-42].

Surgery also fails to maintain disc integrity because the annulus fibrosus tissue cannot be re-approximated with suture, ligature, staples, or other mechanical means, owing to its friable nature[43], and so all attempts at mechanical repair remain futile[44]. Mechanical anchors utilized in conjunction with fibrin, added no benefit, possibly because they interfered with fibrin's strong natural bonds. This explains why studies following discs which underwent prior surgical discectomy revealed accelerated disc degeneration and recurrent disc herniation[52-57]. Likewise, surgical fusions and disc arthrodesis also increase adjacent segment disc degeneration causing pain often without further treatment options. This is referred to as "the domino effect' and occurs with both minimally invasive and traditional fusions and disc replacements[58,71-78]. By comparison, targeting fibrin into the annulus fibrosus improves disc integrity.

A prospective, controlled human *in vivo* investigation demonstrated fibrin's ability to repair mechanically compromised discs and returned them to normal almost immediately. Additionally, several *in vivo* investigations, including a randomized, placebo controlled prospective investigation demonstrated fibrin's ability to return normal tensile and compressive properties to compromised discs, while also correcting the disc's chemical milieu from inflammatory to normal. These beneficial changes occurred because fibrin "transformed tissue", replacing damaged disc tissue with normal collagen Type I, Type II, analogous to fibrin healing skin tissue.

Purpose: To compare the abilities of the "Discseel Procedure" and the "Biologx procedure" to reduce axial spine pain, by utilizing a true VAS, and compare safety and adverse events.

Study design: Multicenter, prospective comparative cohort study, with the primary objective to assess reduction of axial spine pain, and the secondary objective to assess safety and adverse events.

Outcome Measures: Outcomes were measured utilizing 100mm Visual Analogue Scale (VAS), with VAS recorded during face-to-face encounters, and through VAS forms mailed to subjects, and recorded during face-to-face encounters. Adverse events were reported during face-to-face encounters and telephone interviews.

Patient Sample: At baseline, both cohorts were matched to gender, symptom intensity and symptom duration. The Biologx procedure cohort had a mean age of 43.9 yrs and Discseel Procedure cohort had a mean age of 53 yrs. Discseel Procedure subjects had mean number of 3.8 discs/subject treated and Biologx procedure subjects had 1.2discs/subject treated. Prior to enrollment, 3 Discseel Procedure subjects failed to experience pain relief following Regenexx intra-discal stem cell injections, and 21 subjects failed to experience relief with lumbar spine surgery performed more than one year prior to enrolment, (12 subjects underwent discectomy, 5 underwent discectomy with laminectomy and/or foramenotomy, and 4 underwent surgical fusion (1-3 levels). No subjects in the Biologx procedure cohort

underwent prior lumbar spine surgery. Cohort baseline differences are potentially biased against the Discseel Procedure cohort for experiencing relief, considering that a greater number of those subjects failed a greater number of prior invasive treatments, including surgery. In other words, the Discseel Procedure cohort was relatively "pre-destined' to fail when compared with the other cohort.

The baseline cohort differences seemingly biased against the Discseel Procedure cohort was intentional, and those subjects were typically considered "more difficult" to help because the investigator's tertiary spine center routinely treated patients from throughout the World who traveled to seek care who first failed many prior interventions elsewhere. In comparison, the Biologx procedure cohort intentionally demanded strict inclusion criteria in effort to select subjects less likely to be predisposed to fail. Even considering these baseline differences, the Discseel Procedure proved superior to reduce pain.

Subject Inclusion Criteria:

(1) Age 18–75 years (inclusive) and skeletally mature.
(2) Subject voluntarily signs the informed consent form.
(3) Physically and mentally able to comply with the protocol.
(4) Low back pain for at least 6 months.
(5) Pretreatment baseline LBP VAS of at least 40 mm.
(6) Referred leg pain, if present, is of non-compressive origin.
(7) Low back pain is greater than leg pain (if present.).
(8) LBP unresponsive to at least 6 weeks of non-operative treatment.
(9) Negative response to diagnostic medial branch block or facet joint injection.
(10) No sustained relief with epidural injection of corticosteroids.
(11) Relating to the Biologix procedure only, diagnosis of symptomatic lumbar internal disc disruption (IDD) requires discogenic pain at 1-2 contiguous levels through provocation discography.
(12) Relating to the Biologix Procedure only, disc provocation must demonstrate concordant pain (<50 psi above opening pressure) and demonstrate a fissure(s) in the outer one-third of the posterior or lateral annulus fibrosus.

Subject Exclusion Criteria:

(1) Cauda equina syndrome.
(2) Active malignancy or tumor.
(3) Infection.
(4) Previous lumbar spine surgery (for Biologx procedure cohort only).
(5) Previous lumbar disc invasive treatment procedure in the past 12 months (for Biologx procedure cohort only).
(6) Prior lumbar vertebral body fracture.
(7) Disc bulge or herniation at symptomatic level(s) >4 mm (for Biologx cohort only).
(8) Disc extrusion or sequestration.
(9) Clinical findings of lumbosacral motor or sensory radiculopathy (for Biologx procedure cohort only).
(10) Leg pain greater than low back pain.
(11) Lumbar intervertebral foramen stenosis at the affected level(s) resulting in significant spinal nerve root compression or impingement.
(12) Symptomatic central vertebral canal stenosis or absolute sagittal vertebral canal diameter <9 mm.
(13) Loss of disc space height at the symptomatic level(s) greater than one-third of an adjacent normal disc (or of the expected height in the case of an L5-S1 disc)(for Biologx procedure cohort only).
(14) Spondylolisthesis (≥grade 1) with or without spondylolysis at the symptomatic level(s)(for Biologx cohort only).
(15) Lumbar spondylitis or other undifferentiated spondyloarthropathy.
(16) Dynamic instability on lumbar flexion-extension radiographs.
(17) Positive response to diagnostic medial branch block or facet joint injections.
(18) Positive response to diagnostic sacroiliac joint injection for those patients with pain in the sacral region.
(19) Sustained relief obtained with epidural injection of corticosteroids.
(20) Symptomatic involvement of more than 2 lumbar disc levels (for Biologx procedure cohort only).

(21) Congenital or acquired coagulopathy or thrombocytopenia, or currently taking anticoagulant, antineoplastic, antiplatelet, or thrombocytopenia-inducing medications.
(22) History of unexplained, easy, or persistent bruising or bleeding.
(23) Aspirin-containing medication taken ≤7 days prior to the procedure.
(24) Significant systemic disease, including unstable angina, autoimmune disease, rheumatoid arthritis, and muscular dystrophy.
(25) Known or suspected hypersensitivity or allergy to drugs or components of the fibrin sealant, including aprotinin, used in the procedure.
(26) History of, or current psychiatric condition, substance or alcohol abuse that would potentially interfere with the subject's participation in the study.
(27) Ongoing or previous participation in another drug or device clinical study within the previous 2 months.
(28) Subject pregnant, nursing, or with plans to become pregnant within the planned length of follow-up.
(29) Body habitus precludes fluoroscopic visualization.
(30) Concomitant daily oral steroid usage.
(31) Presence of ferromagnetic implants that would disallow MRI of the symptomatic disc(s).

Study Methods: This study was conducted with Institutional Review Board approval at three centers in the United States. All applicable Federal regulations, including the FDA good clinical practice requirements, as well as other generally accepted standards of good clinical practice were followed at each center. Informed consent for participation in the study was obtained in accordance with FDA regulation 21 Code of Federal Regulations Part 50 and the Declaration of Helsinki. All study data were collated, processed, and audited by an independent clinical research organization. Serious adverse events unrelated to the procedure compared to adverse events related to the procedure were adjudicated by an independent Clinical Events Committee (CEC) composed of physicians knowledgeable in spine

interventions and surgery. CEC members were compensated hourly but otherwise had no financial relationship with the sponsoring company.

The Discseel Procedure utilizes annulograms to identify annular defects and IDD. Annulograms were performed following screening review of the lumbar MRI. After informed consent, under mild conscious sedation, with cardiopulmonary monitoring, the skin was aseptically prepared with clorhexidine gluconate 4% and alcohol. Percutaneous target disc access was achieved through a standard posterolateral approach utilizing local anesthesia on the spin puncture site only. Next, a styletted, curved-tip, 22-gauge trocar was advanced under real-time multi-planar fluoroscopic imaging into the posterior aspect of each disc's annulus fibrosus in every disc in the region of symptomology. Following multi-planar verification of trocar tip placement within the posterior annulus, approximately 0.5-1 .0 ml of radiopaque contrast diluted with antibiotic was slowly injected with live fluoroscopic visualization to identify contrast flow pattern through the annulus fibrosus, identifying potential annular defects. The 22G needles identifying annular defects remained in unchanged position within the annulus fibrosus defects.

Next, the precursors of fibrin, which include: prothrombin; fibrinogen; aprotinin; and calcium, merge together while traveling through the introducer needle towards the annulus. During this introduction into the annulus fibrin is formed. This occurs because fibrinogen is proteolytically cleaved by thrombin and converted into the active fibrin. Fibrin encircles the 22-25 annular rings, adhering to the damaged annular lamella. The fibrin monomers assemble into strong fibrin fibers, with bonds forming a three dimensional fibrin gel, stronger that innate annular tissue, before they have the opportunity to leak out of annular defects. If the preceding annulogram demonstrates a large annular defect with profuse leakage, the injection of fibrin is performed more slowly, thus keeping fibrin gel within the disc's defect. If the defects are smaller, the fibrin introduction is faster, so the fibrin can reach discrete, smaller tears. The fibrin's position of advancement is discerned by observing the radiopaque contrast it displaced in its wake. The operator observes defects occupied with radiopaque contrast, and then as fibrin fills these voids and defects, contrast is evacuated from the target site. This technique observing contrast also minimizes the likelihood of injecting fibrin intra-vascular. This process is engineered into a strong

adhesive system analogous to the resin and catalyst in a two-part epoxy kit. The aprotinin molecule included in the combination of constituents in a specific ratio, controls the timing of fibrin degredation products, which serve as chemotactic agents to construct normal disc tissue.

The Biologx procedure. After informed consent, the subject received intravenous antibiotics 60 minutes prior to skin puncture. Under mild conscious sedation, with cardiopulmonary monitoring, The skin was aseptically prepared with clorhexidine gluconate 4% and alcohol. Percutaneous target disc access was achieved through a standard posterolateral approach utilizing local anesthesia on the skin puncture site only, using a styletted, curved-tip, 18-gauge trocar, advanced under real-time multiplanar fluoroscopic imaging into the disc's center. Following multiplanar verification of trocar tip placement into the central third of the target disc nucleus. Fibrin sealant was slowly injected until continued injection resulted in one of three end points: sustained pressure above 100 psi; up to 4cc of fibrin sealant was injected, or the subject could not tolerate continuation.

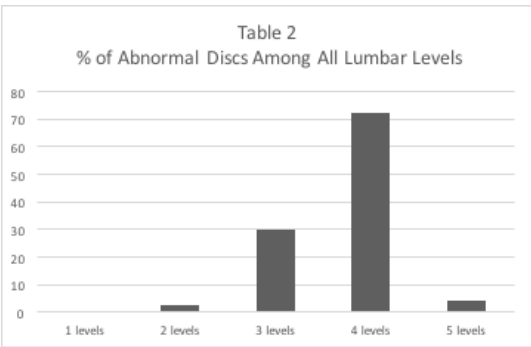
Immediately following either procedure, subjects lay supine for at least 30 minutes prior to discharge. A neurological examination was performed prior to discharge. A gradual return to full activity was encouraged with normal activities resumed as tolerated after three days. Strenuous activities, heavy lifting, and repetitive lumbar flexion and rotation were discouraged.

Follow-Up:

Clinical follow-up was performed at approximately 52 weeks post procedure, with additional interim safety and efficacy evaluations.

Results:

Discseel Procedure: 84 subjects underwent the Discseel Procedure and reported
low back and extremity pain for a mean duration of 8.2 years prior to undergoing the Discseel Procedure.
Subjects reported:
Baseline mean VAS of 7.4 and post procedure mean VAS of 2.0(-73.0 %) at 54.5 weeks. ($p < 0.001$).
94.0% of subjects reported $\geq$30% VAS improvement.
88.1% of subjects reported $\geq$50% VAS improvement.
60.0% of subjects reported $\geq$75% VAS improvement.
18 subjects reported increased lumbar and/or leg pain immediately following the procedure the resolved within one week. Pain was reported in their their typical region, or different region. No pain prevented the patient's discharge 30 minutes following their procedure.



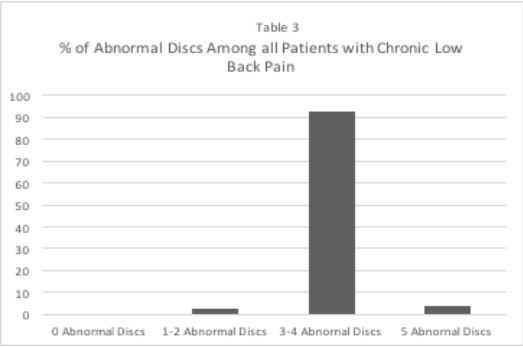


Table 2. Represents mean % levels of morphologically abnormal discs based on annulograms.
Table 3. Represents mean % of number of morphologically abnormal discs per subject, based on annulograms.

Biologx Procedure: 13 subjects underwent the Biologx procedure and reported
low back and extremity pain for a mean duration of 6.0 years prior to undergoing the Biologx procedure.
Subjects reported:
Baseline mean VAS of 7.2 and post procedure mean VAS of 3.5(-51.2 %) at 52.0 weeks.($p < 0.001$).
61.5% (8/13) reported $\geq$30% VAS improvement.
53.8% (7/13) reported $\geq$50% VAS improvement.
38.5% (5/13) reported $\geq$75% VAS improvement.

Conclusions: Subjects who underwent the Discseel Procedure demonstrated statistically significant improvement in pain compared to those who underwent the Biologx procedure, with a mean VAS improvement of 76.1% vs 51.2% respectively, after one year ($p<0.001$). Likewise, there was statistically significant pain reduction of the Discseel Procedure cohort compared to the other cohort when considering all percentages of pain relief, and at all times of outcome procurement. 88.1% of the subjects who underwent the Discseel Procedure and 53.1% of the subjects who underwent the Biologx treatment reported $\geq$ 50% VAS improvement one year following treatment ($p<0.001$).
No Discseel Procedure subjects developed infection or experienced any significant adverse events with the exception of <40% of subjects experiencing transient post-procedure increased pain which resolved. Less than 50% of subjects experienced transient increased pain post procedure which resolved, and none underwent spine-surgery post procedure. In comparison, one Biologx treatment subject developed infectious discitis at the treated disc, and one subject was briefly hospitalized for increased pain.

Discussion: The Biologx procedure methodology mimics all other commonly performed intra-discal regenerative medicine treatments, in that they all possess an increased likelihood of displacing nucleus pulposus outwards, initiating the inflammatory cascade. Some current "stem cell investigations" are focusing on constructing three-dimensional matrices or hydrogels, all of which would displace nucleus pulposus outwards, an action opposite of that which is desirable.

Although baseline pre-treatment data was biased against this Discseel Procedure cohort, these subjects experienced statistically significant pain reduction and minimal adverse events when compared to the Biologx procedure cohort.

Subject's increased pain reduction may be attributable to one or a combination of the following methodology refinements:

(1) The superior diagnostic ability of the annulogram to identify annular tears.

(2) Painful nocioceptors only occupy the annulus fibrosus, and so therefore the annulus fibrosus is the only logical target, and not the inner nucleus pulposus, which is targeted in every other regenerative medicine treatment.

(3) Displacement of the annulus fibrosus causes inflammation, which causes symptoms. So therefore, containing the "noxious" nucleus pulposus gel by making a mechanical barrier with fibrin sealant, to seal the annulus fibrosus and reduce inflammation is both logical and necessary. No other regenerative medicine treatment accomplishes this necessity.

References

1. Razin, N., Voituriez, R., Elgeti, J., & Gov, N. S. (2017). Generalized Archimedes' principle in active fluids. *Physical Review E*, *96*(3).

2. Peng, B., Wu, W., Li, Z., Guo, J., & Wang, X. (2007). Chemical radiculitis. *Pain*, *127*(1–2), 11–16.

3. Klessig, H. T., Showsh, S. A., & Sekorski, A. (2003). The use of intradiscal antibiotics for discography: An in vitro study of gentamicin, cefazolin, and clindamycin. *Spine*, *28*(15), 1735–1738.

4. Huang, S., Tam, V., M.C. Cheung, K., Long, D., Lv, M., Wang, T., & Zhou, G. (2011). Stem Cell-Based Approaches for Intervertebral Disc Regeneration. *Current Stem Cell Research & Therapy*, *6*(4), 317–326.

5. Pirvu, T., Blanquer, S. B. G., Benneker, L. M., Grijpma, D. W., Richards, R. G., Alini, M., … Li, Z. (2015). A combined biomaterial and cellular approach for annulus fibrosus rupture repair. *Biomaterials*, *42*, 11–19.

6. Caner, D. (2002). Wandering, Begging Monks. *University of California Press*, 1–227.

7. Burke, J. G., Watson, R. W. G., McCormack, D., Dowling, F. E., Walsh, M. G., & Fitzpatrick, J. M. (2002). Intervertebral discs which cause low back pain secrete high levels of proinflammatory mediators. *J Bone Joint Surg Br*, *84*(2), 196–201.

8. Vadalà, G., Sowa, G., Hubert, M., Gilbertson, L. G., Denaro, V., & Kang, J. D. (2012). Mesenchymal stem cells injection in degenerated intervertebral disc: Cell leakage may induce osteophyte formation. *Journal of Tissue Engineering and Regenerative Medicine*, *6*(5), 348–355.

9. Yang Zeng, Chun Chen, Wei Liu, Qinyouen Fu, Zhihua Han, Yaqian Li, Siyu Feng, Xiaokang Li, Chunxiao Qi, Jianhong Wu, Deli Wang, Christopher Corbett, Barbara P. Chan, Dike Ruan and Yanan Du, Injectable microcryogels reinforced alginate encapsulation of mesenchymal stromal cells for leak-proof delivery and alleviation of canine disc degeneration, *Biomaterials*, 10.1016/j.biomaterials. 2015.04.029, **59**, (53-65), (2015).

10. Pauza K, Wright C, and Fairbourn A, Treatment of annular disc tears and "leaky disc syndrome" with fibrin sealant, *Techniques in Regional Anesthesia and Pain Management*, 19, 1-2, (45), (2015).

11. Jandial, R., Aryan, H. E., Park, J., Taylor, W. T., & Snyder, E. Y. (2008). Stem cell–mediated regeneration of the intervertebral disc: cellular and molecular challenges. *Neurosurgical Focus*, *24*(3–4), E21.

12. Li, Y. Y., Diao, H. J., Chik, T. K., Chow, C. T., An, X. M., Leung, V., … Chan, B. P. (2014). Delivering Mesenchymal Stem Cells in Collagen. Microsphere Carriers to Rabbit Degenerative Disc: Reduced Risk of Osteophyte Formation. *Tissue Engineering Part A*, *20*(9–10), 1379–1391.

13. Yin, W., Pauza, K., Olan, W. J., Doerzbacher, J. F., & Thorne, K. J. (2014). Intradiscal injection of fibrin sealant for the treatment of symptomatic lumbar internal disc disruption: Results of a prospective multicenter pilot study with 24-month follow-up. *Pain Medicine (United States)*, *15*(1), 16–31.

14. Kayama, S., Konno, S., Olmarker, K., Yabuki, S., & Kikuchi, S. (1996). Incision of the anulus fibrosus induces nerve root morphologic, vascular, and functional changes: An experimental study. *Spine*, *21*(22), 2539–2543.

15. Levi, D., Horn, S., Tyszko, S., Levin, J., Hecht-Leavitt, C., & Walko, E. (2016). Intradiscal platelet-rich plasma injection for chronic discogenic low back pain: Preliminary results from a prospective trial. *Pain Medicine (United States)*, *17*(6), 1010–1022.

16. Pirvu, T., Blanquer, S. B. G., Benneker, L. M., Grijpma, D. W., Richards, R. G., Alini, M., … Li, Z. (2015). A combined biomaterial and cellular approach for annulus fibrosus rupture repair. *Biomaterials*, *42*, 11–19.

17. Guterl, C. C., See, E. Y., Blanquer, S. B. G., Pandit, A., Ferguson, S. J., Benneker, L. M., … Grad, S. (2012). Challenges and strategies in the repair of ruptured annulus fibrosus. *European Cells and Materials*, *25*, 1–21.

18. Epstein, N. (2017). Tisseel's impact on hemostasis for 2–3 and 4–6-level lumbar laminectomies. *Surgical Neurology International*, *8*(1), 299.

19. Boyce, S. T., Holder, I. A., Supp, A. P., Warden, G. D., & Greenhalgh, D. G. (1994). Delivery and activity of antimicrobial drugs released from human fibrin sealant. *Journal of Burn Care and Rehabilitation*, *15*(3), 251–255.

20. Khyati, D. (2012). *Characterization of Rheological Properties and Degradation of Genipin Crosslinked Fibrin Hydrogel For Annulus Repair*. *City University of New York (CUNY)*.

21. Long, R. G., Bürki, A., Zysset, P., Eglin, D., Grijpma, D. W., Blanquer, S. B. G., … Iatridis, J. C. (2016). Mechanical restoration and failure analyses of a hydrogel and scaffold composite strategy for annulus fibrosus repair. *Acta Biomaterialia*, *30*, 116–125.

22. Buser, Z., Kuelling, F., Liu, J., Liebenberg, E., Thorne, K. J., Coughlin, D., & Lotz, J. C. (2011). Biological and biomechanical effects of fibrin injection into porcine intervertebral discs. *Spine*, *36*(18).

23. Buser, Z., Kuelling, F., Liu, J., Liebenberg, E., Thorne, K. J., Coughlin, D., & Lotz, J. C. (2011).
(2011). Biological and biomechanical effects of fibrin injection into porcine intervertebral discs. *Spine*, *36*(18), E1201–E1209.

24. T.K. Chik, X.Y. Ma, T.H. Choy, Y.Y. Li, H.J. Diao, W.K. Teng, S.J. Han, K.M.C. Cheung and B.P. Chan, Photochemically crosslinked collagen annulus plug: A potential solution solving the leakage problem of cell-based therapies for disc degeneration, *Acta Biomaterialia*, 9,9, (8128), (2013).

25. Fibrin promotes proliferation and matrix production of intervertebral disc cells cultured in three-dimensional poly(lactic-co-glycolic acid) scaffold. *Journal of Biomaterials Science, Polymer Edition*, *19*(9), 1219–1237.

26. Schek, R. M., Michalek, A. J., & Iatridis, J. C. (2011). Genipin-crosslinked fibrin hydrogels as a potential adhesive to augment intervertebral disc annulus repair. *European Cells and Materials*, *21*, 373–383

27. Hoyland, J., & Freemont, T. (2007). Intervertebral disc tissue engineering. In *Tissue Engineering Using Ceramics and Polymers* (pp. 357–378). Elsevier Ltd.

28. Yuk Yin Li, Hua Jia Diao, Tze Kit Chik, Cin Ting Chow, Xiao Meng An, Victor Leung, Kenneth Man Chi Cheung and Barbara Pui Chan, Delivering Mesenchymal Stem Cells in Collagen Microsphere Carriers to Rabbit Degenerative Disc: Reduced Risk of Osteophyte

Formation, *Tissue Engineering Part A*, 10.1089/ten.tea.2013.0498, 20, 9-10, (1379-1391), (2014).

29. Colombini, A., Lopa, S., Ceriani, C., Lovati, A. B., Croiset, S. J., Di Giancamillo, A.,Moretti, M. (2015). *In Vitro* Characterization and *In Vivo*Behavior of Human Nucleus Pulposus and Annulus Fibrosus Cells in Clinical-Grade Fibrin and Collagen-Enriched Fibrin Gels. *Tissue Engineering Part A*, *21*(3–4), 793–802.

30. Sha'Ban, M., Yoon, S. J., Ko, Y. K., Ha, H. J., Kim, S. H., So, J. W., … Khang, G. (2008). Fibrin promotes proliferation and matrix production of intervertebral disc cells cultured in three-dimensional poly(lactic-co-glycolic acid) scaffold. *Journal of Biomaterials Science, Polymer Edition*, *19*(9), 1219–1237.

31. Lauto, A., Mawad, D., & Foster, L. J. R. (2008, April). Adhesive biomaterials for tissue reconstruction. *Journal of Chemical Technology and Biotechnology*.

32. Sagar, P., Prasad, K., Lalitha, R. M., & Ranganath, K. (2015). Cyanoacrylate for Intraoral Wound Closure: A Possibility? *International Journal of Biomaterials*, *2015*.

33. Kaushik, N. K., Kaushik, N., Pardeshi, S., Sharma, J. G., Lee, S. H., & Choi, E. H. (2015, November 1). Biomedical and clinical importance of mussel-inspired polymers and materials. *Marine Drugs*. MDPI AG.

34. Ninan, L., Monahan, J., Stroshine, R. L., Wilker, J. J., & Shi, R. (2003). Adhesive strength of marine mussel extracts on porcine skin. *Biomaterials*, *24*(22), 4091–4099.

35. Wei, W., Tan, Y., Martinez Rodriguez, N. R., Yu, J., Israelachvili, J. N., & Waite, J. H. (2014). A mussel-derived one component adhesive coacervate. *Acta Biomaterialia*. Elsevier BV.

36. Matos-Pérez, C. R., White, J. D., & Wilker, J. J. (2012). Polymer composition and substrate influences on the adhesive bonding of a biomimetic, cross-linking polymer. *Journal of the American Chemical Society*, *134*(22), 9498–9505.

37. Rodriguez, A. G., Slichter, C. K., Acosta, F. L., Rodriguez-Soto, A. E., Burghardt, A. J., Majumdar, S., & Lotz, J. C. (2011). Human disc nucleus properties and vertebral endplate permeability. *Spine*, *36*(7), 512–520.

38. Likhitpanichkul, M., Kim, Y., Torre, O. M., See, E., Kazezian, Z., Pandit, A., … Iatridis, J. C. (2015). Fibrin-genipin annulus fibrosus sealant as a delivery system for anti-TNFα drug. *Spine Journal*, *15*(9), 2045–2054.

39. Scalcione, C., Ortiz-Vaquerizas, D., Said, D. G., & Dua, H. S. (2018). Fibrin glue as agent for sealing corneal and conjunctival wound leaks. *Eye (Basingstoke)*, *32*(2), 463–466.

40. J., Z., N., Z., T., Y., Y., Z., M., N., M.V., H., & J.H.-C., W. (2016). Kartogenin regenerates wounded tendon-bone junction. *Journal of Orthopaedic Research*, *34*.

41. Marta Tibiletti, Nevenka Kregar Velikonja, Jill P. G. Urban and Jeremy C. T. Fairbank, Disc cell therapies: critical issues, *European Spine Journal*, 10.1007/s00586-014-3177-2, 23, S3, (375-384), (2014).

42. Sandra Reitmaier, Ludwika Kreja, Katharina Gruchenberg, Britta Kanter, Joana Silva-Correia, Joaquim Miguel Oliveira, Rui Luís Reis, Valeria Perugini, Matteo Santin, Anita Ignatius and Hans-Joachim Wilke, In vivo biofunctional evaluation of hydrogels for disc regeneration, *European Spine Journal*, 10.1007/s00586-013-2998-8, 23, 1, (19-26), (2013).

43. Linderman, S. W., Kormpakis, I., Gelberman, R. H., Birman, V., Wegst, U. G. K., Genin, G. M., & Thomopoulos, S. (2015). Shear lag sutures: Improved suture repair through the use of adhesives. *Acta Biomaterialia*, *23*, 229–239.

44. Lauto, A., Mawad, D., & Foster, L. J. R. (2008, April). Adhesive biomaterials for tissue reconstruction. *Journal of Chemical Technology and Biotechnology*.

45. Ulrich, J. A., Liebenberg, E. C., Thuillier, D. U., & Lotz, J. C. (2007). ISSLS prize winner: Repeated disc injury causes persistent inflammation. *Spine*, *32*(25), 2812–2819.

46. Alkhatib, B. G., Rosenzweig, D., Krock, E., Gawri, R., Beckman, L., Steffen, T., … Haglund, L. (2014). Acute Mechanical Injury to the Human Intervertebral Disc Initiates Events Implicated in Disc Degeneration. *Global Spine Journal*, *4*(1_suppl), s-0034-1376578-s-0034-1376578.

47. Alkhatib, B. G., Rosenzweig, D., Krock, E., Gawri, R., Beckman, L., Steffen, T., … Haglund, L. (2014). Acute Mechanical Injury to the Human Intervertebral Disc Initiates Events Implicated in Disc Degeneration. *Global Spine Journal*, *4*(1_suppl), s-0034-1376578-s-0034-1376578.

48. Wang, J. L., Tsai, Y. C., & Wang, Y. H. (2007). The leakage pathway and effect of needle gauge on degree of disc injury post anular puncture: A comparative study using aged human and adolescent porcine discs. *Spine*, *32*(17), 1809–1815.

49. Hsieh, A. H., Hwang, D., Ryan, D. A., Freeman, A. K., & Kim, H. (2009). Degenerative anular changes induced by puncture are associated with insufficiency of disc biomechanical function. *Spine*, *34*(10), 998–1005.

50. Carragee, E. J., Don, A. S., & Hurwitz, E. L. (2010, June 15). Erratum: ISSLS prize winner: Does discography cause accelerated progression of degeneration changes in the lumbar disc: A ten-year matched cohort study (Spine (2009) 34 (2338-2345). *Spine*.

51. M.E., Y., A., K., N.D., Y., F., B., & G., A. (2017). Factors that influence recurrent lumbar disc herniation. *Hong Kong Medical Journal*, *23*(3)

52. Rajasekaran, S., Bajaj, N., Tubaki, V., Kanna, R. M., & Shetty, A. P. (2013). ISSLS prize winner: The anatomy of failure in lumbar disc herniation: An in vivo, multimodal, prospective study of 181 subjects. *Spine*, *38*(17), 1491–1500.

53. Watters, W. C., & McGirt, M. J. (2009, March). An evidence-based review of the literature on the consequences of conservative versus aggressive discectomy for the treatment of primary disc herniation with radiculopathy. *Spine Journal*.

54. McGirt, M. J., Eustacchio, S., Varga, P., Vilendecic, M., Trummer, M., Gorensek, M., Carragee, E. J. (2009). A prospective cohort study of close interval computed tomography and magnetic resonance imaging after primary lumbar discectomy: Factors associated with recurrent disc herniation and disc height loss. *Spine*, *34*(19), 2044–2051.

55. Trummer, M., Eustacchio, S., Barth, M., Klassen, P. D., & Stein, S. (2013). Protecting facet joints post-lumbar discectomy: Barricaid annular closure device reduces risk of facet degeneration. *Clinical Neurology and Neurosurgery*, *115*(8), 1440–1445.

56. Kuelling, F. A., Foley, K. T., Liu, J. J., Liebenberg, E., Sin, A. H., Matsukawa, A., & Lotz, J. C. (2014). The anabolic effect of plasma-mediated ablation on the intervertebral disc: Stimulation of proteoglycan and interleukin-8 production. *Spine Journal*, *14*(10), 2479–2487

57. M., K., T., N., A., M., & H., N. (2016). Prevalence and impact on outcome of reoperation following minimally invasive decompression alone for degenerative lumbar disease. *European Spine Journal*, *25*, S303–S304

58. Schroeder, J., Dettori, J., Brodt, E., & Kaplan, L. (2013). Disc degeneration after disc herniation: are we accelerating the process? *Evidence-Based Spine-Care Journal*, *3*(04), 33–40.

59. Hilibrand, A. S., & Robbins, M. (2004). Adjacent segment degeneration and adjacent segment disease: The consequences of spinal fusion? *Spine Journal*, *4*(6 SUPPL.).

60. Helgeson, M. D., Bevevino, A. J., & Hilibrand, A. S. (2013, March). Update on the evidence for adjacent segment degeneration and disease. *Spine Journal*.

61. Kim, J. Y., Ryu, D. S., Paik, H. K., Ahn, S. S., Kang, M. S., Kim, K. H., … Kuh, S. U. (2016). Paraspinal muscle, facet joint, and disc problems: risk factors for adjacent segment degeneration after lumbar fusion. *Spine Journal*, *16*(7), 867–875.

62. Maldonado, C. V., Paz, R. D. R., & Martin, C. B. (2011). Adjacent-level degeneration after cervical disc arthroplasty versus fusion. *European Spine Journal*, *20*, S403–S407.

63. Nakashima, H., Kawakami, N., Tsuji, T., Ohara, T., Suzuki, Y., Saito, T., Imagama, S. (2015). Adjacent Segment Disease after Posterior Lumbar Interbody Fusion: Based on Cases with a Minimum of 10 Years of Follow-up. *Spine*, *40*(14), E831–E841.

64. Ishihara, H., Kanamori, M., Kawaguchi, Y., Nakamura, H., & Kimura, T. (2004). Adjacent segment disease after anterior cervical interbody fusion. *Spine Journal*, *4*(6), 624–628.

65. Lee, C. K. (1988). Accelerated degeneration of the segment adjacent to a lumbar fusion. *Spine*, *13*(3), 375–377.

66. Sharma, A., Pilgram, T., & Wippold, F. J. (2009). Association between annular tears and disk degeneration: A longitudinal study. *American Journal of Neuroradiology*, *30*(3), 500–506.

67. Munter, F. M., Wasserman, B. A., Wu, H. M., & Yousem, D. M. (2002). Serial MR imaging of annular tears in lumbar intervertebral disks. *American Journal of Neuroradiology*, *23*(7), 1105–1109.

68. Farshad-Amacker, N. A., Hughes, A. P., Aichmair, A., Herzog, R. J., & Farshad, M. (2014). Is an annular tear a predictor for accelerated disc degeneration? *European Spine Journal : Official Publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society*, *23*(9), 1825–1829.

69. Osti, O., Vernon-Roberts, B., Moore, R., & Fraser, R. (1992). Annular tears and disc degeneration in the lumbar spine. A post-mortem study of 135 discs. *The Journal of Bone and Joint Surgery. British Volume*, *74-B*(5), 678–682.

70. Sharma, A., Parsons, M. S., & Pilgram, T. K. (2009). Temporal association of annular tears and nuclear degeneration: Lessons from the pediatric population. *American Journal of Neuroradiology*, *30*(8), 1541–1545.

71. Ghiselli, G., Wang, J. C., Bhatia, N. N., Hsu, W. K., & Dawson, E. G. (2004). Adjacent segment degeneration in the lumbar spine. *Journal of Bone and Joint Surgery - Series A*, *86*(7), 1497–1503.

72. C.-H., J., N.-S., C., H.-D., L., & S.-Y., L. (2014). Adjacent disc degeneration and facet joint arthritic change after lumbar fusion: Which is more affected? *European Spine Journal*, *23*, S470.

73. Kim, J. Y., Ryu, D. S., Paik, H. K., Ahn, S. S., Kang, M. S., Kim, K. H., … Kuh, S. U. (2016). Paraspinal muscle, facet joint, and disc problems: risk factors for adjacent segment degeneration after lumbar fusion. *Spine Journal*, *16*(7), 867–875.

74. Park, P., Garton, H. J., Gala, V. C., Hoff, J. T., & McGillicuddy, J. E. (2004, September 1). Adjacent segment disease after lumbar or lumbosacral fusion: Review of the literature. *Spine*.

75. Harrop, J. S., Youssef, J. A., Maltenfort, M., Vorwald, P., Jabbour, P., Bono, C. M., Hilibrand, A. S. (2008). Lumbar adjacent segment degeneration and disease after arthrodesis and total disc arthroplasty. *Spine*, *33*(15), 1701–1707.

76. McAfee, P. C. (2016). Adjacent Segment Degeneration Versus Disease After Lumbar Spine Fusion for Degenerative Pathology A Systematic Review With Meta-Analysis of the Literature. *Clinical Spine Surgery*, *29*(1), 21–29.

77. Kumar, A., Beastall, J., Hughes, J., Karadimas, E. J., Nicol, M., Smith, F., & Wardlaw, D. (2008). Disc Changes in the Bridged and Adjacent Segments After Dynesys Dynamic Stabilization System After Two Years. *Spine*, *33*(26), 2909–2914.

78. M., K., T., N., A., M., & H., N. (2016). Prevalence and impact on outcome of reoperation following minimally invasive decompression

alone for degenerative lumbar disease. *European Spine Journal*, *25*, S303–S304.

79. Okubo, T., Watanabe, A., Yamada, H., Inou, S., Ono, R., Ozawa, T.,Wada, Y. (2010). Comparison of axial T2 mapping with CT discography in assessment of lumbar intervertebral disk degeneration. *Neuroradiology Journal*, *23*, 428–429.

80. B., P., S., H., W., W., C., Z., & Y., Y. (2006). The pathogenesis and clinical significance of a high-intensity zone (HIZ) of lumbar intervertebral disc on MR imaging in the patient with discogenic low back pain. *European Spine Journal*, *15*(5), 583–587.

81. Ernst, C. W., Stadnik, T. W., Peeters, E., Breucq, C., & Osteaux, M. J. C. (2005). Prevalence of annular tears and disc herniations on MR images of the cervical spine in symptom free volunteers. *European Journal of Radiology*, *55*(3), 409–414.

82. Stadnik, T. W., Lee, R. R., Coen, H. L., Neirynck, E. C., Buisseret, T. S., & Osteaux, M. J. (1998). Annular tears and disk herniation: prevalence and contrast enhancement on MR images in the absence of low back pain or sciatica. *Radiology*, *206*(1), 49–55.