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Effect of Intra Discal High-Frequency Ultrasound Application in Degenerative Disc Disease: A Seamless Sequential Translational *Ex Vivo* and *In Vivo* Study

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Abstract

Background: Degenerative Disc Disease (DDD) is a highly prevalent condition, with over 400 million people estimated to experience symptomatic DDD annually as of 2018 [1]. Its prevalence increases significantly with age [2]. Despite its widespread occurrence, definitive treatment options for DDD remain limited to surgical interventions. These include procedures such as discectomy with fusion and other invasive techniques [3]. However, many elderly patients are not suitable candidates for surgery due to multiple comorbidities. Therefore, the importance of searching for a novel, effective, minimally invasive treatment is paramount. **Study Design:** A seamless sequential *ex vivo* and *in vivo* translational animal study has been conducted to evaluate the dose-finding matrix and the efficacy of intra-discal high-frequency ultrasound (HF-US) application in treating mechanically and surgically-induced hyper-acute disc bulging in a porcine model, for *ex vivo* and *in vivo*, respectively. **Materials and Methods:** Mechanical disc prolapse was induced through rhythmic spinal flexion and axial compression, and surgical prolapse was generated by annulotomy using 14 G Veress needle puncture on the spinal functional unit (SFU) (lumbar vertebra-disc-lumbar vertebra) from L1 - L6. Disc bulging observed by direct visualization in L3/L4, L4/L5 and L5/L6 (>25% disc protrusion from baseline), and L4/L5, L5/L6 demonstrated with indocyanine green intra-discal injection observed with special laparoscopic camera marking with >25% disc protrusion from baseline and reduced in IVD disc height, for *ex vivo* and *in vivo* subjects, respectively. Intra-discal HF-US applied in multiple frequencies,

power/acoustic intensity, exposure duration, and duty cycle has been conducted during the *ex vivo* study to determine the dose matrix. Once the optimal treatment measurement was obtained during *ex vivo*, a seamless, sequential *in vivo* test conducted using the same parameters with HF-US. The healthy spines have been used as control, treated with sham energy source. **Result and conclusion:** Results demonstrated that treatment with high-frequency ultrasound significantly reduced disc bulging and restored the intervertebral disc height in the intervention group compared to the control group.

Keywords

Degenerative Disc Disease, Disc Bulging, High-Frequency Ultrasound, Translational Research for Disc Bulging

1. Introduction

Degenerative disc disease, along with associated disc bulging and prolapse, represents a common age-related condition with a broad spectrum of clinical manifestations. While many individuals remain asymptomatic, others may experience significant pain, disability, and neurological impairment. Understanding the pathophysiology and tailoring interventions to individual risk profiles remain key to improving outcomes—particularly as less invasive therapies (e.g., high-frequency ultrasound) are being explored as alternatives to surgery in high-risk populations.

Degenerative Disc Disease (DDD) is highly prevalent and increases with age. Estimated that over 400 million people globally suffer from symptomatic DDD annually [1]. More than 90% of individuals over the age of 50 show radiographic signs of disc degeneration [2] [4]. Obesity, smoking, genetics, and sedentary lifestyle are significant risk factors [5].

Disc bulging and prolapsed discs are common manifestations of DDD and can be found in up to 60% - 80% of asymptomatic adults on MRI [6]. Lumbar disc herniation is the most common cause of sciatica and affects about 1% - 2% of the population annually [7].

DDD is not truly a "disease", but rather a collection of age-related changes and mechanical wear-and-tear in the intervertebral discs. Dehydration of the nucleus pulposus (loss of proteoglycans and water content) is a common finding [8]. The annular fissures and weakening of the annulus fibrosus lead to loss of disc height, resulting in disc prolapse [8]. Inflammatory mediator release (e.g., IL-1β, TNF-α), contributing to pain and degradation, including vertebral endplate changes (Modic changes) [9]. Other changes included reduced disc nutrition and impaired healing.

Disc bulging has been defined as a symmetrical extension of the disc margin beyond the vertebral body, usually affecting more than 25% of the disc circumference [10]. Disc prolapse/herniation occurs when nucleus pulposus material breaches the annulus fibrosus, often due to annular tears or fissures [11]. There are various types of disc bulging, including protrusion, extrusion, and sequestra-

tion. The bulging of the disc often compresses adjacent neural structures (e.g., spinal nerves, cauda equina, transverse nerve), leading to numerous clinical outcomes [12].

Although the vast majority of patients may be asymptomatic, once symptoms appear, there is a wide range of symptoms with various degrees of severity, ranging from mild low back pain (mechanical or inflammatory in nature) to radiculopathy (e.g., sciatica) due to nerve root compression, neurogenic claudication (especially in spinal stenosis), numbness, tingling, or weakness in limbs to severe conditions that sometimes require serious emergency intervention such as cauda equina syndrome or conus medullaris involvement [13].

Not all DDD leads to disc herniation, but disc herniation is often part of the degenerative cascade. Spinal instability, facet joint arthritis, and stenosis may develop as a result of prolonged degeneration [14]. In many cases, symptoms may improve over time with conservative management, though recurrence is common [15].

Conservative treatments (physical therapy, NSAIDs, epidural injections) are first-line for most. Surgical intervention (laminectomy with discectomy, fusion, or disc replacement) is considered for refractory cases or those with neurological deficits. Nevertheless, long-term outcomes are highly variable: some patients recover fully while others develop chronic pain or disability [4]. Elderly patients or those with comorbidities often have limited treatment options due to surgical risk.

High-frequency ultrasound (HFUS) is a novel way to treat intervertebral disc bulging. When it is applied directly inside the intervertebral disc (intra-discal application) it represents a promising minimally invasive treatment approach for prolapsed (herniated) discs. The proposed mechanisms of action are reduction of disc size. HFUS delivers targeted energy that precisely cuts and ablates displaced disc material, reducing the volume of the herniated nucleus pulposus responsible for nerve compression [16]. It has been postulated that HFUS will also act as a nucleus pulposus modulator by breaking down and coagulating the inner gel-like nucleus pulposus; HFUS decreases intradiscal pressure, relieving mechanical stress on the annulus fibrosus and surrounding neural structures [16]. Another hypothesis depicts that the annulus fibrosus can be repaired during intra-discal HFUS application while simultaneously sealing the annular defect created by the herniation or prior injury through thermal coagulation, promoting closure of the annulus fibrosus and preventing re-herniation [17]. The ultrasound energy acts as a precise cutting tool that minimises collateral damage, while the coagulation effect seals the wound, enhancing tissue healing and structural integrity. The dual actions of disc size reduction and wound sealing demonstrated by HFUS may become a minimally invasive treatment option for DDD with a prolapsed disc.

2. Study Design

2.1. Objective

Develop and validate a minimally-invasive intra-disc HFUS procedure to reduce

nucleus pulposus volume/pressure and diminish radicular compression from contained disc herniation while preserving annulus fibrosus integrity and adjacent neural/vertebral tissues.

2.2. Rationale/Background (Brief)

Focused ultrasound has been shown to be feasible for heating/ablating nucleus pulposus and is being explored as an alternative to thermal intra-discal techniques and percutaneous discectomy [16]. The translational pathway requires *ex vivo* characterization (dose-response, thermal spread, biomechanical effect) and then seamless validation in an appropriate large-animal *in vivo* model applying the optimal characteristics of HFUS [18]. Reviews of animal models and trans-spinal HFUS delivery guide model selection and endpoints [2].

2.3. Overall Study Design (Phased)

Phase A—*Ex vivo*/bench; dose-finding matrix, establish safe/efficacious ultrasound parameters and delivery technique; measure thermal distribution, tissue volume reduction, and biomechanical changes; acoustic dosimetry: measure delivered acoustic intensity, duty cycle, focal-spot size in water/phantom. Once the optimal efficacy and safety characteristics of HFUS have been determined during *ex vivo* experiments, the authors applied the full characteristics of HFUS in Phase B sequentially in a large animal *in vivo* model with combined surgical and mechanical induced disc prolapse.

Phase B—Large animal *in vivo* study of intra-discal HF-US application for acute disc bulging to demonstrate safety and efficacy.

3. Materials and Methods

3.1. Phase A: *Ex Vivo*

3.1.1. Materials

The materials applied in this study were supplied by World Laparoscopic Hospital (WLH), New Delhi, India. The high-frequency ultrasound equipment applied in both *ex vivo* and *in vivo* studies was the Harmonic™ system manufactured by Ethicon™, J&J Medtech, provided by WLH. Indocyanine green (ICG) used in this study was the Aurogreen brand, produced by Aurolab and supplied by WLH. The special modified telescope camera utilized to capture intervertebral spine ICG imaging was the Invislabs™ system, also provided by WLH. Measurement of intervertebral disc (IVD) dimensions was carried out using sophisticated image-processing software ImageJ™.

3.1.2. Specimens

Ex vivo study was conducted by dissecting spinal functional units (SFU) (lumbar motion segments: vertebra–disc–vertebra) from a 7-month-old male porcine, weighing 18 kg, half an hour postmortem. The SFU was kept moist by soaking in normal saline (0.9% NaCl) at temperature approx. 37 ˚C (room temperature) and

immediately applied for surgical and mechanical induced disc prolapse. The authors noted that discs can desiccate quickly, so this pre-emptive measure was applied. Due to its similarity to the human spine, porcine models have been used widely in orthopaedic and spine research [5].

3.2. Method (Phase A)

3.2.1. Primary Measurements

Volume/weight change: The nucleus region was weighed before/after to quantify tissue removal or denaturation; ICG injection (0.1 ml into each disc) allowed visualisation of disc substance; measurement of volume/weight change was performed using digital image-measurement software.

3.2.2. Combined Surgical and Mechanical Induced Hyper-Acute Disc Bulging

1) Surgical-Induced Disc Prolapse

Potting the ends — Embed L1 and L6 vertebral bodies in PMMA (polymethyl methacrylate) blocks to allow secure fixation in a testing machine.

Positioning — Mount the spine vertically in the testing machine; align the segment to neutral lordosis to mimic physiological posture.

Pre-conditioning — Apply a low axial preload (100 N) for 10 - 15 minutes to equilibrate disc hydration and annular tension.

2) Surgical Induction

The posterior or posterolateral exposure of target discs (L1/L2, L2/L3, L3/L4, L4/L5, L5/L6) was performed; soft tissue retraction allowed annulus visualisation. An annulotomy defect (3 mm wide × 3 mm deep) was created at the posterolateral annulus fibrosus using a 14 G Veress needle and scalpel size 1. This standardized defect determines likelihood and extent of herniation. Nucleus pressurisation/extrusion was achieved using manual pressure or syringe introduced via the anterior endplate or nucleus puncture. "End-point" was confirmed by visible HNP (herniated nucleus pulposus) extrusion, annular rupture, or sudden drop in force under load.

To enhance effect, mechanical-induced disc bulging followed surgical induction: vertebra–disc–vertebra motion segments were potted in a test rig/bioreactor and subjected to axial compression, flexion/extension, bending, shear, and cyclic fatigue until annulus failure and nucleus material extrusion. These models reproduce clinically relevant herniation morphologies and are widely used to test repair techniques and implants [18].

3.2.3. Dose-Finding Matrix of HFUS

Tested a matrix of frequency × intensity × exposure time × duty cycle: frequency from 0.5, 1.0, 1.5, up to 3.0 MHz; power/acoustic intensity (low/medium/high); exposure times of 10 s, 30 s, 60 s, 120 s; duty cycles (e.g., pulsed every 3 s). Endpoint: ≥25% NP volume reduction without outer annulus fibrosus (AF) temperatures exceeding safe thresholds (avoid > 37˚C at outer AF/adjacent neural structures). Thermometry data collected accordingly.

3.3. Phase B: *In Vivo* Studies

Purpose: demonstrate safety, device delivery workflow, and hyper-acute efficacy in an animal model using data validated from *ex vivo* study. Hyper-acute disc bulging was defined as observable disc bulging ≥25% of baseline soon after surgical + mechanical induction. Spinal segments L1 - L6 were included. When a disc is mechanically or surgically over-pressurized, incised or loaded beyond its viscoelastic limit, it undergoes a pathophysiologic cascade mimicking early human acute disc herniation: abrupt NP hydrostatic pressure shift, migration through weakened annular fibres (typically posterolateral annulus), focal annular delamination and NP extrusion/bulging; accompanied by acute inflammatory milieu (IL-1β, TNF-α, IL-6), matrix metalloproteinase activation, rapid osmotic pressure increase, endplate microfractures, disrupted nutrient diffusion, cell death and matrix breakdown, lamellar annular distortion, nociceptive nerve ingrowth—all within hours [9]. The "hyper-acute disc bulging" model simulates the window between structural disruption and full herniation, offering a translationally relevant platform to test early interventions.

Materials: same as Phase A.

Study Subject: 10-month old male porcine, weighing 21.2 kg.

Safety/Ethical: Approval from ethics committee of World Laparoscopy Hospital, India; followed ARRIVE guidelines [12].

Method: Using the characteristic model from Phase A, HFUS device (Harmonic™) with frequency 1.5 MHz, medium power, 30 s duration, pulsed exposure every 3 s was applied intra-discus.

Study arms: Treatment vs sham.

Endpoints:

Primary: Safety — no injury to AF beyond planned zone, no nerve-root thermal injury, no vertebral endplate necrosis, no infection. Biological efficacy: reduction in herniation size/NP volume > 25% as visualised by telescope camera (Invislab™) detecting ICG (Aurogreen™). Histologic evidence of controlled NP coagulation/necrosis not obtained in this study—future work planned.

Secondary: Biomechanical preservation (disc height/stiffness), absence of adverse systemic effects; pain/behavioural surrogate improvements not obtained in this study.

Induction of mechanical + surgical disc prolapse: To visualise discs, a 5 mm laparoscopic camera (Invislabs™) was inserted into the dorsal portion of the porcine; after small dorsal incision, lumbar levels L1 - L6 identified; ICG intra-discal injection permitted direct visualisation and digital size measurement of nucleus pulposus and disc via ImageJ™ software; the process of induction followed same surgical + mechanical protocols as Phase A.

4. Result

Pre-treatment result depicted in **Table 1** and elaborated with **Figure 1**.

Table 1. Phase A: Pre-treatment (before induction) IVD dimensions.

	Height (mm)	Width (mm)	Depth (mm)
L1/L2	5.12	37.56	25.77
L2/L3	5.36	37.39	25.96
L3/L4	5.40	37.98	25.08
L4/L5	5.59	37.86	25.99
L5/L6	5.58	36.55	25.30

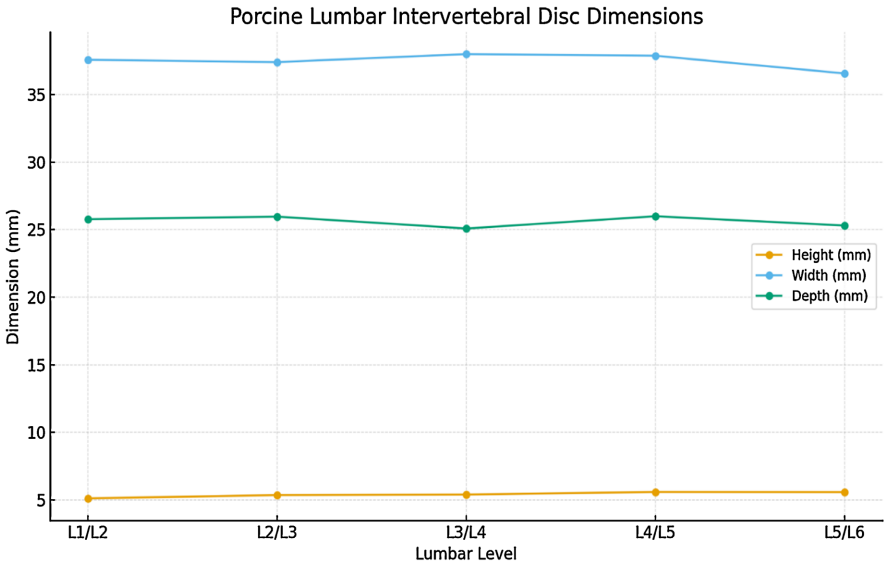

Figure 1. The dimension (height, width and depth) of the inter vertebra lumbar spine discs (IVD) at the base line.

After induction endpoints revealed in **Table 2** and further elaborated in **Figure 2**.

Table 2. Intra-discal HFUS.

Height (mm)	HNP sign(s)/stop signs
L1/L2	5.12 — No sign of HNP
L2/L3	5.16 — No sign of HNP
L3/L4	3.09 — visible HNP extrusion/protrusion
L4/L5	3.16 — visible HNP extrusion + annular rupture
L5/L6	3.08 — visible HNP extrusion/protrusion

Intra-discal HFUS was implemented on L3/L4, L4/L5, L5/L6 with endpoint > 25% shrink of disc bulging measured by ImageJ™; sham treatment applied to L1/L2 and L2/L3.

The upper segments (L1/L2 and L2/L3) show minimal change, while the lower segments (L3/L4 to L5/L6) demonstrate marked height loss—around 40% - 45%—corresponding with visible HNP extrusion and annular rupture.

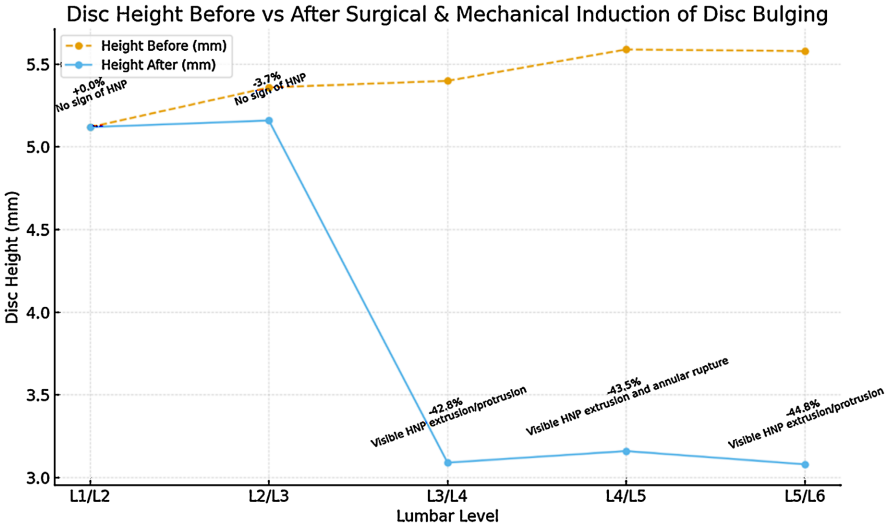

Figure 2. The comparison chart showing disc height before and after surgical + mechanical induction of disc bulging at each lumbar level.

This created a clear biomechanical picture: herniation coincides with disc height collapse.

After the application of intra-discal HFUS treatment, there were significant changes in the affected lumbar spines as described in **Table 3** and further elaborated in **Figure 3**.

Table 3. Post intra-discal HFUS treatment.

Height (mm)	Disc Bulging Reduction
L1/L2	5.02 — No reduction
L2/L3	5.16 — No reduction
L3/L4	4.14 — reduction of disc bulging > 25%
L4/L5	5.16 — reduction of disc bulging > 25%
L5/L6	5.08 — reduction of disc bulging > 25%

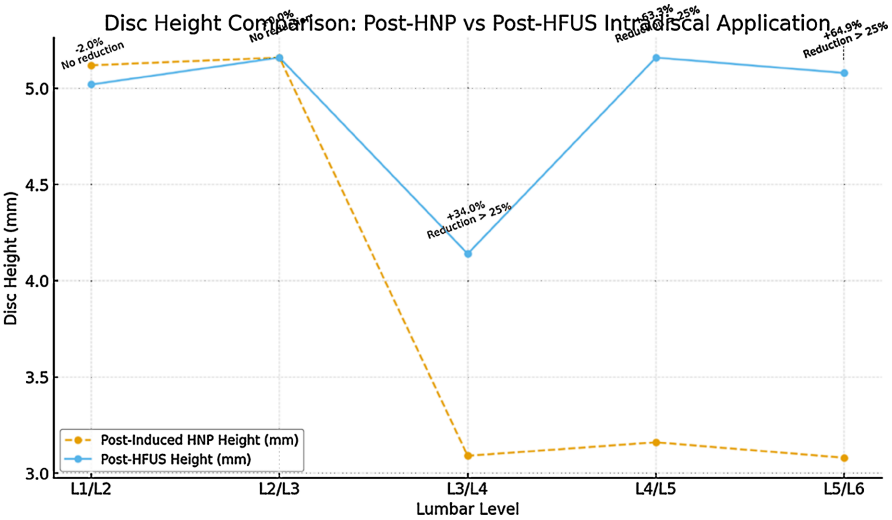

Figure 3. Disc Height Comparison: Post-HNP vs Post-HFUS Intradiscal Application.

Figure 3 visually shows: lower lumbar levels (L3/L4-L5/L6) show height recovery > 25% reduction in bulging, while upper levels (L1/L2, L2/L3) show no change. Dose matrix summary: frequency 1.5 MHz, medium power, 30 s duration, pulsed every 3 s.

By applying dose matrices obtained from phase A, in Phase B the pretreatment, post disc-prolapse-induction, and post intra-discal HFUS treatment, were summarized in Tables 4-6, respectively and further comprehensive results had been displayed in Figure 4.

Table 4. Phase B: Pre-treatment IVD dimensions.

	Height (mm)	Width (mm)	Depth (mm)
L1/L2	5.92	38.49	27.77
L2/L3	5.99	38.39	27.96
L3/L4	6.03	38.98	28.08
L4/L5	6.96	38.86	25.99
L5/L6	6.58	37.55	25.30

Table 5. Post intra-discal HFUS treatment.

Height (mm)	Disc Bulging Reduction
L1/L2	5.36 — No sign of HNP
L2/L3	5.57 — No sign of HNP
L3/L4	6.0 — No sign of HNP
L4/L5	4.16 — visible HNP extrusion + annular rupture/tear
L5/L6	4.08 — visible HNP extrusion/protrusion + annular rupture/tear

Intra-discal HFUS implemented on L4/L5, L5/L6 with endpoint > 25% shrink. Sham applied to L1/L2, L2/L3, L3/L4.

Table 6. Post HFUS.

Height (mm)	Disc Bulging Reduction
L1/L2	5.44 — No reduction
L2/L3	5.46 — No reduction
L3/L4	5.97 — No reduction
L4/L5	6.02 — reduction of disc bulging > 25%
L5/L6	5.98 — reduction of disc bulging > 25%

A clear visualization of IVD height progression across three key stages:

- Pre-treatment (baseline anatomy)
- Post-induction (hyper-acute disc bulging with HNP at L4/L5, L5/L6)
- Post-HFUS (marked > 25% height recovery and bulge reduction at treated seg-

ments)

4.1. Results—Summary

Control group (L1 - L3): pre-treatment mean 5.64 mm, post-treatment mean 5.62 mm (Table 7).

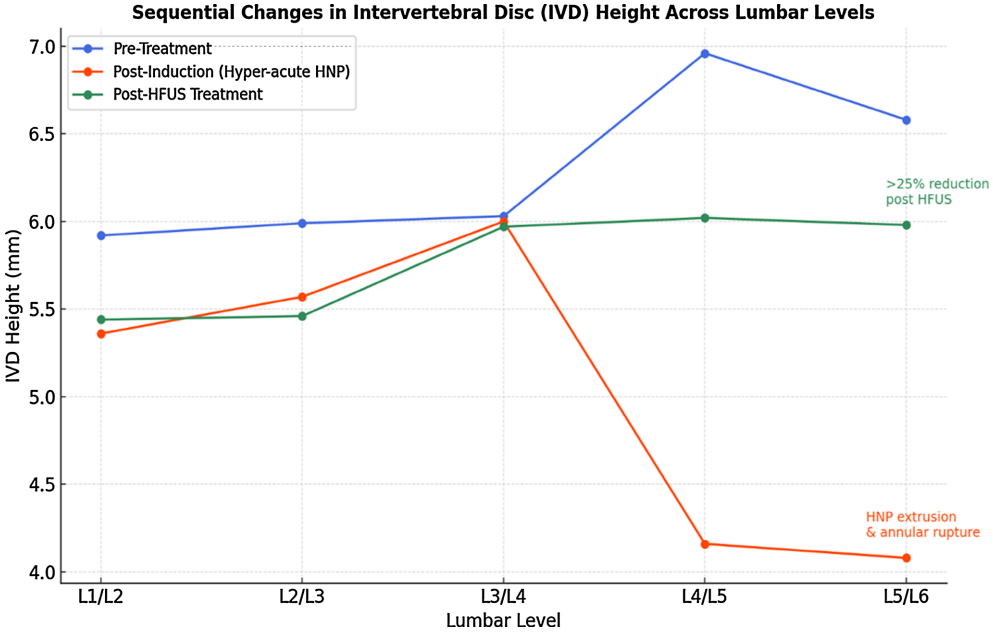

Figure 4. Sequential changes in intervertebral disc height across lumbar levels during hyper-acute induction and post-HFUS treatment.

Table 7. Phase B: Pre-treatment IVD dimensions.

Pre-treatment height (mm)	Post-treatment height (mm)	HNP sign/ Bulging	Disc Bulging Reduction
L1/L2	5.36 → 5.44	No HNP	No reduction
L2/L3	5.57 → 5.46	No HNP	No reduction
L3/L4	6.00 → 5.97	No HNP	No reduction
L4/L5	4.16 → 6.02	Visible extrusion	>25% reduction
L5/L6	4.08 → 5.98	Visible extrusion	>25% reduction

Treatment group (L4 - L5): pre-treatment mean 4.12 mm, post-treatment mean 6.00 mm.

SD (Treatment Post): 0.058 mm; SD (Control Post): 0.040 mm.

SEM (Treatment): 0.041 mm; SEM (Control): 0.023 mm.

Paired t-test: $p \approx 0.03$.

4.2. Data Interpretation and Analysis

Mean IVD height – treatment group: before 4.12 mm, after 6.00 mm; control group: before 5.64 mm, after 5.62 mm.

Standard deviation (SD) of change: control group 0.0954 mm; treatment group 0.0283 mm.

Standard error (SE) of change: control group 0.0551 mm; treatment group 0.0200 mm.

Paired t-test (within group): control group $t = -0.36$, $p = 0.751$ → no significant difference; treatment group $t = 94.00$, $p = 0.0068$ → statistically significant increase in disc height after HFUS.

Independent t-test (between groups): $t = -32.43$, $p = 0.00026$ → highly significant difference between groups.

The effect size was substantial: average increase of 1.88 mm in treatment vs 0.02 mm in controls. No spontaneous IVD height increase in control group, indicating observed difference associated with HFUS treatment rather than measurement variability.

5. Discussion

Herniated nucleus pulposus (HNP) and disc bulging arise from a cascade of pathophysiological changes in the intervertebral disc. Degeneration leads to reduced proteoglycan content and hydration of the nucleus pulposus (NP), annular fissuring and altered load transfer. Increased intradiscal pressure under physiological loading can force NP material outward, resulting in focal protrusion or extrusion. This mechanical deformation compresses neural structures and alters segmental biomechanics [8]. Over time, annular defects may enlarge and persistent inflammation perpetuates nociceptive sensitization, contributing to radicular pain [9].

Current treatment options for lumbar disc herniation reflect this pathophysiology. Conservative therapy—physical rehabilitation, analgesics, and anti-inflammatory agents—can provide symptom relief but does not directly modify disc geometry. Interventional techniques such as epidural steroid injections reduce inflammation but have only transient benefits. More invasive modalities, including microdiscectomy and endoscopic discectomy, aim to remove herniated NP but carry procedural risks and may accelerate degenerative changes by altering load distribution [3]. Energy-based minimally invasive options such as radiofrequency and nucleoplasty attempt to reduce NP volume, but their effects are often modest and operator-dependent [17].

The present study demonstrates a clear, immediate increase in intervertebral disc height following targeted intra-discal high-frequency ultrasound (HFUS) exposure in an *ex vivo* porcine lumbar spine model. Because the control and treatment groups were handled and measured identically, with HFUS being the sole variable, the observed effect can be plausibly attributed to the intervention itself. The absence of measurable change in the control group helps exclude confounders such as passive fluid redistribution or spontaneous disc expansion. Moreover, the temporal sequence—intervention preceding structural change—strengthens the causal inference between HFUS application and increased disc height.

In this context, HFUS offers an intriguing dual-function mechanism. First, ul-

trasonic energy at appropriate frequencies can modulate NP material, inducing local heating and microstructural changes that reduce the volume of herniated nucleus tissue. This volumetric reduction may relieve annular stress and decrease disc bulging. Second, the thermal effect of HFUS may facilitate coagulation and sealing of annular fissures, similar to hemostatic ultrasound effects in other tissues. By combining decompression with annular stabilization, HFUS could theoretically address both mechanical and structural components of disc protrusion—a feature not shared by most current modalities [16].

The *ex vivo* design provides a high degree of experimental control, allowing clear isolation of treatment effects. However, several limitations must be acknowledged. The sample size was small (n = 2 for treatment, n = 3 for control), which restricts statistical power and generalisability. The study is translational and proof-of-concept in nature; it does not assess long-term durability, histological changes, or functional outcomes. The absence of *in vivo* factors such as vascular perfusion, systemic inflammatory response and mechanical loading limits direct extrapolation to clinical scenarios.

The results indicate a clear cause–effect relationship between HFUS application and increased disc height. Because the control and treatment groups were subjected to identical experimental conditions aside from HFUS exposure, the significant increase in IVD height observed exclusively in the treatment group is most plausibly attributed to the intervention itself. The absence of measurable change in the control group helps rule out confounding factors such as passive fluid redistribution, spontaneous disc expansion or measurement error. The temporal relationship—with HFUS applied before the increase—strengthens the causal inference.

Mechanistically, this rapid increase in height may reflect HFUS-induced biomechanical changes such as enhanced nucleus pulposus hydration, reduced annular compression or modulation of disc pressure dynamics. These findings are consistent with the hypothesis that targeted ultrasonic energy can reverse early disc bulging by promoting acute fluid inflow or tissue decompression.

Although the sample size was limited, the large effect size and low variability in treatment response provide compelling preliminary evidence for a direct treatment effect. Future studies with larger cohorts and histological confirmation are warranted to further substantiate this causal link.

This study demonstrates a clear treatment-associated increase in IVD height following intra-discal HFUS exposure in an *ex vivo* porcine lumbar spine model. Because both groups were subjected to identical handling and measurement protocols, and the intervention was the only variable, the observed effect can be plausibly attributed to HFUS application. The lack of measurable change in the control group further supports this interpretation.

While the sample size was limited, the magnitude of the effect and the low variability yielded a statistically robust result. These findings provide preliminary evidence that HFUS may acutely modify disc geometry, supporting its potential as a

therapeutic tool for early disc herniation or bulging. Larger studies with histological and biomechanical correlation are warranted to further validate these results and to explore the durability and clinical relevance of the observed changes.

These limitations highlight the need for subsequent investigations: a larger preclinical cohort is essential to confirm reproducibility and explore dose–response characteristics; integration of imaging (MRI or ultrasound elastography) and histological analysis will help define mechanisms at tissue-level including NP shrinkage and annular remodelling; progression toward first-in-human feasibility studies will be required to assess safety, targeting precision and real-world clinical benefit.

This study provides preliminary evidence that HFUS can acutely modify disc geometry through targeted modulation of the nucleus pulposus and possible annular sealing effects. These dual mechanisms, if confirmed *in vivo*, could represent a novel minimally invasive strategy for early-stage disc bulging and contained herniation. Although preliminary, the findings lay the groundwork for future translational research aimed at bridging experimental feasibility with clinical application.

6. Conclusion

In summary, intra-discal HFUS application produced a significant and reproducible increase in IVD height in the treatment group compared with controls, supporting a direct biomechanical effect of the intervention. The controlled experimental design, absence of spontaneous disc height change in controls and temporal sequence of intervention and response collectively strengthen the causal inference. These findings suggest that HFUS may represent a promising modality for acute modulation of disc bulging and height restoration. Further investigation with larger sample sizes and *in vivo* models is warranted to assess long-term efficacy, safety and clinical translatability.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix A

Standard Operating Procedure (SOP)

Creation of Acute Lumbar Disc Prolapse in a Porcine Model (Mechanical & Surgical Compression)

1. Purpose and Scope

This SOP provides a reproducible and ethically sound surgical protocol to induce acute lumbar disc prolapse in pigs using two techniques: mechanical compression and surgical annulotomy. This model mimics human lumbar disc prolapse and is used for this preclinical testing of intra-discal therapies such as high-frequency ultrasound.

2. Animal Model

Species	Porcine (Sus scrofa domesticus)
Weight	15-20 kg
Target Disc Level	L3/L4, L4/L5 and L5/L6
Group Size	Pilot: 2 animals; Study: ex-vivo and in-vivo

3. Equipment and Materials

Surgical instruments (scalpel, retractors, rongeur), Special fluorecense telescope, syringe pump with pressure gauge, needles (14G) (Veress needle, 120 mm), electrocautery unit, high-frequency ultrasound equipment, sutures, sterile saline, antibiotics, analgesics, sedative and anesthesia agents.

4. Anesthesia and Perioperative Care

Premedication	Ketamine 10 mg/kg IM + Xylazine 2 mg/kg IM
Induction	Propofol 4 mg/kg IV
Maintenance	Propofol 1 - 2 mg/kg IV
Analgesia	Buprenorphine 0.01 mg/kg IM + Meloxicam 0.2 mg/kg SC
Antibiotics	Cefazolin 25 mg/kg IV

5. Mechanical Compression Procedure

1) Prone positioning, skin prep, and incision over the target level.

2) Needle insertion into disc under fluoroscopy (special telescope that can capture ICG images)

3) Apply 150–250 psi pressure via syringe pump for 10–30 seconds.

4) Confirm extrusion fluoroscopically.

5) Closure of fascia and skin.

6. Surgical Annulotomy Procedure:

1) Midline incision and paraspinal muscle dissection.

2) Partial hemilaminectomy or facetectomy for exposure.

3) 3 - 5 mm annulotomy and manual extrusion of nucleus pulposus.

4) Irrigation and layered closure.

a) Postoperative Care

Analgesia	Buprenorphine 0.01 mg/kg IM q8–12 h + NSAIDs for 48 h
Antibiotics	Cefazolin 25 mg/kg IV q12 h × 3 days
Monitoring	Daily neuro and wound check
Mobilization	Free cage movement after 24 h

b) Ethical Considerations

All procedures have been approved by World Laparoscopy Hospital, New Delhi, India, institutional ethics board. Adhere to ARRIVE guidelines. We ensure adequate perioperative analgesia and humane endpoints.

Appendix B

Photograph

Figure S1. Biomechanical-induced IVD prolapse used in the *ex vivo* experiment.

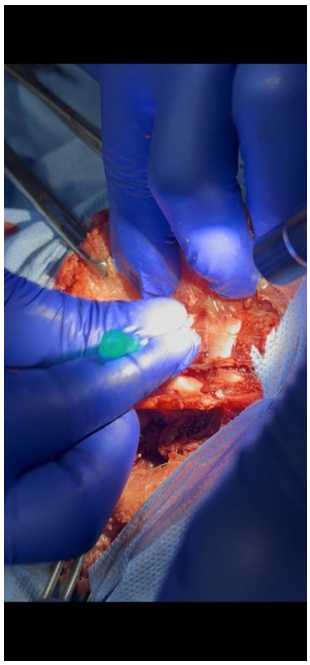

Figure S2. Surgically-induced intervertebrae disc prolapse.

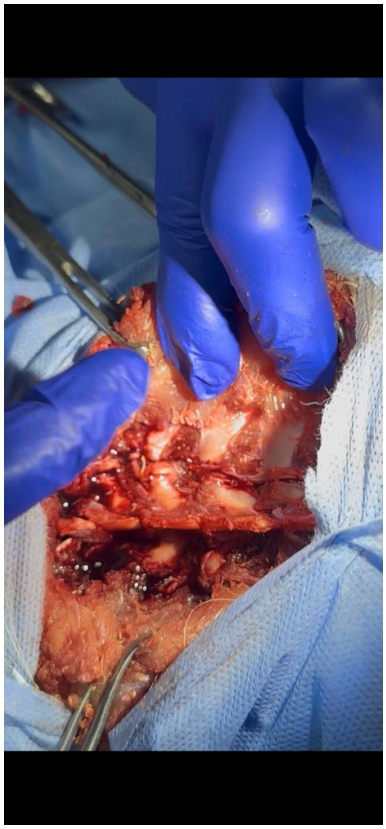

Figure S3. Notable Disc Prolapse L4/L5 and L5/L6 after Combined Mechanical and Surgical-Induced Herniated Disc.