

RESEARCH ARTICLE

Pathological Degeneration of Disc and Bone Is Associated With Chronic Low Back Pain in a Rat Model With Intradiscal Monosodium Iodoacetate

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ABSTRACT

This study aimed to determine whether degeneration of intervertebral discs and subchondral bone correlates with the development of persistent low back pain (LBP) in a rat model with intradiscal monosodium iodoacetate (MIA), focusing on structural alterations, neuronal sensitization, and pain-related behavior. Male Sprague Dawley rats received MIA injections (2 mg in 2 μ l) into L4/5 and L5/6 lumbar discs to induce degeneration. LBP-related behaviors were assessed over 42 days via dynamic weight-bearing and hindpaw withdrawal thresholds. Neuronal sensitization was evaluated via electrophysiological recordings of mechanosensitive afferent nerves (MSAN) and calcium imaging of labeled dorsal root ganglia (DRG) neurons innervating discs. Structural alterations in subchondral bone and disc were analyzed using in vivo/ex vivo μ CT imaging, along with immunohistochemistry for TRPV1 and CGRP expression. Intradiscal MIA induced long-term LBP behaviors, characterized by forelimb weight-bearing shifts and reduced hindpaw withdrawal thresholds. MSAN exhibited hyperexcitability and lowered firing thresholds to intradiscal pressure, while DRG neurons showed enhanced TRPV1- and TRPA1-mediated calcium influx. μ CT imaging revealed decreased disc volume and deteriorated trabecular bone quality. Histology confirmed disc and subchondral bone degeneration, with upregulated TRPV1 and CGRP expression in subchondral bone, indicating nociceptive fiber ingrowth. Linear discriminant analysis identified weight-bearing asymmetry (HindFore AUC), trabecular thickness, connectivity, and trabecular pattern factor as strong predictors of LBP development. Pathological degeneration of disc and bone is associated with chronic LBP through structural remodeling and peripheral neuronal sensitization. These findings suggest that therapeutic strategies targeting both tissues can alleviate chronic pain in degenerative spine conditions.

Yun-Ho Cho, Cham Choi, Minji Kwon, and Jinju Kwon contributed equally to the present work.

1 | Introduction

Low back pain (LBP) is a highly prevalent musculoskeletal disorder affecting individuals across all age groups, leading to reduced quality of life and persistent economic burden (Knezevic et al. 2021; Hartvigsen et al. 2018). Among its many causes, lumbar intervertebral disc degeneration is recognized as a major contributor to LBP (Risbud and Shapiro 2014). Structural deformation of disc, such as fissures, bulging, and herniation, can lead to mechanical compression of adjacent spinal nerves or the spinal cord, eliciting pain signals (Endean et al. 2011). Furthermore, biochemical alterations within degenerated disc can activate the infiltration of immune cells and upregulate subsequent release of pro-inflammatory cytokines (e.g., IL-1 β , TNF- α , IL-6) and neurotrophic factors (e.g., NGF, BDNF) (Lyu et al. 2021). Inflammation and NGF-dependent neuronal sprouting within degenerated discs have been observed in patients with LBP symptoms (Freemont et al. 2002; Pedersen et al. 2015).

However, LBP pathogenesis cannot be attributed to intervertebral disc degeneration alone. Instead, LBP represents a multi-tissue condition involving pathological interplay among the disc, endplate, and subchondral bone (Ashinsky et al. 2020; Maruyama et al. 2024; Zehra et al. 2022). Subchondral bone and vertebral endplates are essential for regulating nutrient and ion exchange, providing mechanical integrity and structural support for the intervertebral disc. Prolonged and repetitive mechanical stress can produce microfractures in the subchondral bone, initiating a cascade of spine tissue degeneration. Excessive or dysregulated Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) signaling promotes osteoclast activity and accelerates pathological bone modeling, such as sclerosis, thickening of the subchondral bone plate, and abnormal sensory innervation (Zhu et al. 2019; Aso et al. 2016). In parallel, heightened RANKL signaling often correlates with an upregulation of inflammatory cytokines and growth factors (e.g., IL-1 β , TNF- α , VEGF) that diffuse across the vertebral endplates (Ono et al. 2020). It is possible, therefore, that this subchondral bone degeneration shifts mechanical stress onto nucleus pulposus (NP) and annulus fibrosus (AF) regions of disc, accelerating fissuring or herniation processes. These concurrent alterations weaken the structural and biochemical integrity of lumbar spine, creating a vicious cycle of degenerative bone and disc. For example, advanced micro-computed tomography (μ CT) imaging reported that trabecular bone quality deteriorates and subchondral endplate became increasingly sclerotic, together with disc degeneration progresses (Choi et al. 2018; Rutges et al. 2011; Teboul-Coré et al. 2018). Multiple clinical studies revealed that patients with severe degeneration or symptoms exhibit elevated levels of pain-related neurotrophic factors, angiogenesis, and aberrant neuronal sprouting in disc and bone tissue (Freemont et al. 2002; LA Binch et al. 2014; Krock et al. 2014; Purmessur et al. 2008). These findings imply that extended degeneration across subchondral bone and disc play a critical role in developing LBP, reinforcing the transition from acute to persistent pain states.

To better understand the interplay between disc degeneration, bone remodeling, and chronic pain, some in vivo animal models have been suggested. One model is applied with needle puncture to induce annulus fibrosus injury or endplate microfractures. Although this model progressively causes disc and

bone degeneration, it has shown relatively heterogenous phenotype and limits its ability to replicate consistent pain conditions reliably (Huang et al. 2024; Shi et al. 2018). Another model involves the intradiscal injection of monosodium iodoacetate (MIA), a metabolic inhibitor that disrupts glyceraldehyde-3-phosphate dehydrogenase and induces chondrocyte apoptosis, thereby accelerating disc and subchondral bone degeneration. Experimental studies have demonstrated that a sufficient dosage of MIA results in comparable structural remodeling of the disc and subchondral bone, consistently triggering pain-related behavior patterns over a short-term induction period (Suh et al. 2022; Michael et al. 1997).

Despite advancements in LBP research, the precise pathological alterations between disc and subchondral bone and their impact on LBP processing remains insufficiently understood. The present study aims to explore whether concurrent structural degeneration after intradiscal MIA correlates with neuronal sensitization and the development of LBP. Our previous study demonstrated that afferent nerve fibers innervating disc become hypersensitive to intradiscal pressure after lumbar disc puncture and NP aspiration, producing LBP-related behavioral changes (Park et al. 2019). In the current study, we used an in vivo rat model via an intradiscal MIA injection to examine tissue structure and pain processes in more depth. Under multimodal evaluation of LBP-related behavior, in vivo electrophysiology, calcium imaging, in vivo and *ex vivo* μ CT imaging, and immunohistochemistry, we intend to clarify tissue structure underlying chronic LBP.

2 | Methods

2.1 | Animals

Male Sprague–Dawley rats (Orient Bio, Seongnam, Republic of Korea; 10 weeks old, 350–400 g) were housed in a temperature- and light-controlled room (22°C–25°C, 12-h light/dark cycle). Food and water were provided ad libitum.

2.1.1 | Ethics Statement

All animal procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) at Korea University and were approved by the IACUC (approval number: KOREA-2021-0012-C2). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2 | Animal Model With Intradiscal Injection of Monosodium Iodoacetate

Rats (total $n = 81$, Table 1) were anesthetized with a mixture of alfaxalone (10 mg/kg) and medetomidine (0.5 mg/kg), and a midline ventral longitudinal incision was made. For the chemical injury model with NP presence, monosodium iodoacetate (MIA; 1 mg/ μ l, total 2 μ l; dissolved in 0.9% saline) was individually injected into L4/5 and L5/6 discs, with a Hamilton syringe (26s-gauge). Muscles and skin margins were sutured with 6-0 and 5-0 silk. Saline-injected control (SAL) group underwent intradiscal injection of 0.9% saline (2 μ l). Sham-surgery group underwent the same surgical procedures. If the body

TABLE 1 | Experimental design, animal allocation, and outcome measures.

Group (No. of animal)	Nerves or DRG (Drugs [No. of Neurons])	Experiments	Pre	Post-surgery days							Figures
			−1	7	14	21	28	35	42	44	
Sham, SAL, and MIA (<i>N</i> = 14/group)		Behavior (Weight bearing & withdrawal threshold)									Figure 1A–F
└ Sham, SAL, and MIA └ (<i>N</i> = 7/group) └ Sham, SAL, and MIA └ (<i>N</i> = 7/group)		Ex Vivo μ CT imaging (Bone)									Figure 4A–L
		In Vivo μ CT imaging (Disc)									Figure 3A–C
		Histology & Immunofluorescence (Bone & Disc)									Figure 5A–F
		Linear discriminant analysis									Figure 6
Sham, SAL, and MIA (<i>N</i> = 8/group)	One Nerve per Animal	In Vivo Electrophysiology (Intradiscal pressure)									Figure 2A–C
Unlabeled (<i>N</i> = 5/group)	Capsaicin (<i>N</i> = 118), AITC (<i>N</i> = 143)	Calcium imaging									Figure 2D–G
DiI*+SAL (<i>N</i> = 5/group)	Capsaicin (<i>N</i> = 61), AITC (<i>N</i> = 58)										
DiI*+MIA (<i>N</i> = 5/group)	Capsaicin (<i>N</i> = 46), AITC (<i>N</i> = 30)										
Total <i>N</i> = 81			DiI* was performed 7 days before surgery								

weight of rat was reduced drastically (more than 20% compared to before surgery), they were excluded.

2.3 | Experimental Design

Our experimental design is included in Table 1.

2.4 | Randomization and Blinding in Experiments

We randomly assigned rats to Sham/SAL/MIA groups. Upon the group assignment, outcome assessment of all the experiments was performed under a blinded condition.

2.5 | In Vivo and Ex Vivo Micro-Computed Tomography (μ CT)

For scan and reconstruction, the animals were scanned before and 14–28–42 days after the surgery. The field of view (FOV) included both surgery sites: L4/5 and L5/6 disc. SkyScan1176 μ CT (Bruker, Kontich, Belgium) was used. The image pixel size was 36 μ m for *in-vivo* (18 μ m for *ex-vivo*), the filter option was copper 0.1 mm, the applied voltage was 90 kV. The scanned projection image datasets were reconstructed in NRecon (Bruker). The dynamic range was from 0 to 0.02 for *in-vivo* (0 to 0.025 for *ex-vivo*), the ring artifact correction was 9, the beam hardening correction was 30%. The reconstructed cross-section datasets were reoriented and analyzed in Dataviewer and CTAn (Bruker).

For *in-vivo* data analysis, the reoriented cross-sectional datasets were analyzed using CTAn (Bruker). The volume of interest (VOI) encompassed the intervertebral space (IVS) of L4/5 and L5/6. These VOIs exhibit two key characteristics: first, they include the cartilaginous endplates; second, they do not precisely delineate the disc boundaries but rather interpolate them. Consequently, these regions are designed as intervertebral space (IVS) VOIs rather than intervertebral disc (IVD) VOIs, as illustrated in Supporting Figure 1A.

For *ex-vivo* data analysis, VOI for the trabecular bone of L5 spine (trab VOI) were delineated as Supporting Figure 1B. Ten parameters were measured in the VOI (Chappard et al. 2008; Bouxsein et al. 2010). Bone mineral density (BMD) describes the calcium density in the VOI, including the volume of bone and bone marrow. The BMD phantom set of 8 mm diameter was also prepared for BMD measurement. Tissue volume (TV) describes the total trabecular volume. Bone volume (BV) represents the trabecular bone volume. Percent bone volume (BV/TV) indicates the proportion of the bone relative to the trabecular volume. Trabecular thickness (Tb. Th) describes the mean thickness value of all the trabecular structures. Trabecular number (Tb.N) describes the number of trabecular bone strands per unit length. Trabecular pattern factor (Tb. Pf) reflects the concavity and convexity of trabecular structure. A negative Tb. Pf value indicates a predominantly concave structure, whereas a positive value suggests a more convex structure. In trabecular bone analysis, a lower Tb. Pf value signifies better trabecular connectivity, while a higher value suggests more isolated trabeculae (Hahn et al. 1992). Trabecular separation (Tb. Sp) represents the mean thickness of all void spaces with the trabecular structure.

Connectivity (Conn) measures the degree of structural connectivity, indicating the maximum number of branches that can be disrupted before the structure splits into two separate parts (Odgaard and Gundersen 1993). Connectivity density (Conn. Dn) represents the number of trabeculae per unit volume (Odgaard and Gundersen 1993), calculated as connectivity divided by TV.

2.6 | Histology

Harvested disc and subchondral bone were fixed with 10% neutral buffered formalin for 7 days and then decalcified in RDO gold (Apex Engineering Products Corporation, IL, USA) for 1 day. The material was processed for paraffin embedding, and sagittal sections of 5 μ m thickness were then obtained using a microtome. After deparaffinization in xylene and a graded series of alcohols, hematoxylin and eosin (H&E) and safranin O staining were performed to stain the sections. Histopathological score was applied to grade the degeneration (Lai et al. 2021).

2.7 | Immunofluorescence

After paraffin embedding, tissues were coronally sectioned at a thickness of 8 μ m. The sectioned tissue samples were first permeabilized and treated with avidin and biotin blocking solutions, followed by overnight incubation at 4°C with primary antibodies: anti-TRPV1(1:1000, PA1-29421, Invitrogen, Carlsbad, CA, USA), anti-CGRP(1:4000, Abcam, Boston, MA, USA), and DAPI(0.3 μ l/10 ml, Abcam, Boston, MA, USA). Secondary antibodies were applied for 1.5 h at room temperature (anti-rabbit IgG H&L (Alexa Fluor® 488), 1:2000, Abcam; anti-Mouse IgG H&L (Alexa Fluor® 568), 1:2000, Abcam). Fluorescent images were captured using a Leica DFC 450 C fluorescence camera (Leica Microsystems GmbH). The relative expression area (%) was quantified using ImageJ software (NIH) by applying a threshold within a defined region of interest (ROI; 100X). For each tissue section, ROIs were selected in both rostral and caudal regions of the L5 subchondral bone. The average of these two measurements was used as the expression value for each section, with a total of 8 tissue sections analyzed per subject.

2.8 | In Vivo Single Nerve Recording

After the animal was anesthetized with urethane (250 mg/kg; Sigma-Aldrich, St Louis, MO, USA) the left jugular vein, carotid artery, and trachea were cannulated to inject drugs and monitor blood pressure (80–120 mmHg)/the end-tidal CO₂ (3.0–4.0%). A midline ventral longitudinal incision was made, and the abdominal viscera were gently retracted and covered with gauze soaked in warm saline to maintain moisture and temperature. Autonomic afferent nerve fibers, located in paravertebral sympathetic fibres, were identified and carefully dissected free from connective tissue. A single fiber was isolated by teasing and placed over a platinum bipolar electrode to record neuronal activity. The firing frequency (maximal spikes/sec) of mechanosensitive afferents (MSA) was recorded and compiled into a post-stimulus time histogram (PSTH) with a bin width of 1 s. The MSA threshold was defined as at least two firings over a 2-second stimulation period. Mechanical stimulation was applied by generating intradiscal pressures at

lumbar 5/6. Intradiscal pressure (100, 500, 1000, 1500, 2000, and 2500 mmHg, applied at 70-second intervals) was produced by injecting saline through a 23-gauge needle connected via tubing to a pressure transducer (BLPR2, WPI, Sarasota, FL, USA) and a pressure monitor (BP-1, WPI, Sarasota, FL, USA), which were calibrated prior to the experiment.

2.9 | DiI-Labeling From Lumbar Intervertebral Discs

Rats were anesthetized with a mixture of alfaxalone (10 mg/kg) and medetomidine (0.5 mg/kg), and a midline ventral longitudinal incision was made in the supine position. The L4/5 and L5/6 disc were exposed under a microscope, intradiscal administration of 1,1'-diiododecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI, 2 μ l) were cautiously performed.

2.10 | Intracellular Ca^{2+} Imaging

After being loaded with Fura-2 AM (1 μ l/1 ml, Invitrogen, CA, USA; 30 min), DRG neurons were monitored by sequential dual excitation (340 nm and 380 nm; every 5 s) and DiI (Invitrogen)-labeling were also identified (540/25 nm of excitation, 605/55 nm of emission). DRG neurons were exposed to baseline period (70-sec), 1 μ M capsaicin (Sigma-Aldrich, St. Louis, United States; 20-sec) or 100 μ M AITC (Sigma-Aldrich; 20-sec), interval period (105-sec), and 50 mM potassium chloride (KCl; 20-sec). Only small-sized DRG neurons (diameter of less than 20 μ m) were analyzed within 10 h after processes of dissociation and stabilization. Detailed description is attached in the [Supporting Information](#).

2.11 | Dynamic Weight Bearing and Hindpaw Withdrawal Threshold

For dynamic weight bearing, the weight load difference between the averaged hind and fore limbs (Hind-Fore), averaged right and left limbs (Left-Right), and averaged crossing limbs (Cross) of rats were analyzed (Park et al. 2019). To measure the mechanical threshold for hindpaw withdrawal, a series of von Frey filaments (0.41–15.10 g, Stoelting, Wood Dale, IL, USA) were applied. The 50% withdrawal threshold was determined using the up-down procedure. Detailed description is attached in the [Supporting Information](#).

2.12 | Statistical Analysis

Data were expressed as means with 95% confidence interval (CI) and were analyzed using SPSS software version 26 (IBM corp., Armonk, NY, USA). Parametric or non-parametric statistics analysis was applied based on the pass of normal distribution testing. Dynamic weight bearing parameters (across time points and animal groups) and withdrawal thresholds in left and right hindpaw (between time points and animal groups) were analyzed using a two-way repeated measures ANOVA. *In vivo* μ CT imaging of intervertebral space (IVS) were analyzed using a mixed model with a post hoc Tukey test. Firing rates and threshold of MSAN, peak normalized ratio of calcium imaging, *ex-vivo* μ CT imaging parameters, and histopathology scoring were analyzed using a Kruskal-Wallis test with a post

hoc Bonferroni correction. Linear discriminant analysis with stepwise selection (LDA-S) was applied into a total of 12 variables, including 10 *ex-vivo* μ CT parameters and 2 behavioral variables-HindFore(pre) and HindFore(AUC). Differences between experimental conditions were considered statistically significant when $p < 0.05$.

3 | Results

3.1 | The Intradiscal Administration of Monosodium Iodoacetate (MIA) Leads to Low Back Pain-Related Behavior

We evaluated whether the administration of MIA into two-level (L4/5 and L5/6) lumbar discs (Figure 1A) can induce pain-related behaviors, such as dynamic weight bearing and hindpaw withdrawal threshold, over a 42-day period after MIA injection surgery (Figure 1B–F). We observed that normal or sham-operated rats normally walked, with greater weight load on the hindlimbs than the forelimbs (Hind-Fore; 10%–12%). However, this walking pattern was significantly altered following MIA administration, resulting in a shift toward forelimbs-centered weight distribution (Hind-Fore; 2–4%; groups, $F_{2,39} = 56.796$, $p < 0.001$; days, $F_{6,234} = 6.165$, $p < 0.001$; groups $\times$ days, $F_{12,234} = 9.447$, $p < 0.001$). Post hoc analysis revealed a significant decrease in Hind-Fore in MIA group compared to sham and SAL groups between days 7 and 42 post-injection (Figure 1B; all $p < 0.01$), while no significant changes were observed in Left-Right and Cross (Figure 1C,D). In addition, MIA group also elicited significant changes of withdrawal threshold in both left (groups, $F_{2,39} = 251.867$, $p < 0.001$; days, $F_{6,234} = 60.917$, $p < 0.001$; groups $\times$ days, $F_{12,234} = 45.565$, $p < 0.001$) and right hindpaw (groups, $F_{2,39} = 295.364$, $p < 0.001$; days, $F_{6,234} = 43.017$, $p < 0.001$; groups $\times$ days, $F_{12,234} = 34.334$, $p < 0.001$). Post hoc analysis revealed a significant decrease of MIA group compared to sham and SAL group from 7 to 42 days post-injection (Figure 1E,F; all $p < 0.01$). Our current findings indicate the intradiscal application of MIA can contribute to the development of long-term low back pain.

3.2 | Degeneration in Lumbar Disc and Bone Induces Hyperexcitability of Mechanosensitive Afferent Nerve Fibers and Increases in TRPV1- or TRPA1-Mediated Calcium Influx of DRG Neurons

We examined the mechano-sensitivity of afferent nerve fiber innervating lumbar disc tissue in response to intradiscal pressure 28 days post-MIA surgery (Figure 2). Figure 2A showed representative electrophysiological data of the responsiveness of mechanosensitive single afferent nerve (MSAN) among groups. We found that MIA model expressed the hyperexcitability and reduced firing threshold of MSAN (Kruskal Wallis test; all $p < 0.05$). Post hoc analysis (Bonferroni correction) indicated that MIA group elicited not only a significant increase in the peak firing number of MSAN regarding 1500 to 2500 mmHg (vs. sham, $p = 0.015$, 0.008, and 0.005; vs. SAL, $p = 0.014$, 0.029, and 0.036; Figure 2B), but also a significant decrease in firing threshold of it compared to sham and SAL groups ($p = 0.021$ and 0.032; Figure 2C).

To confirm the peripheral sensitization of afferent neurons retrogradely labeled from degenerated spine, we examined

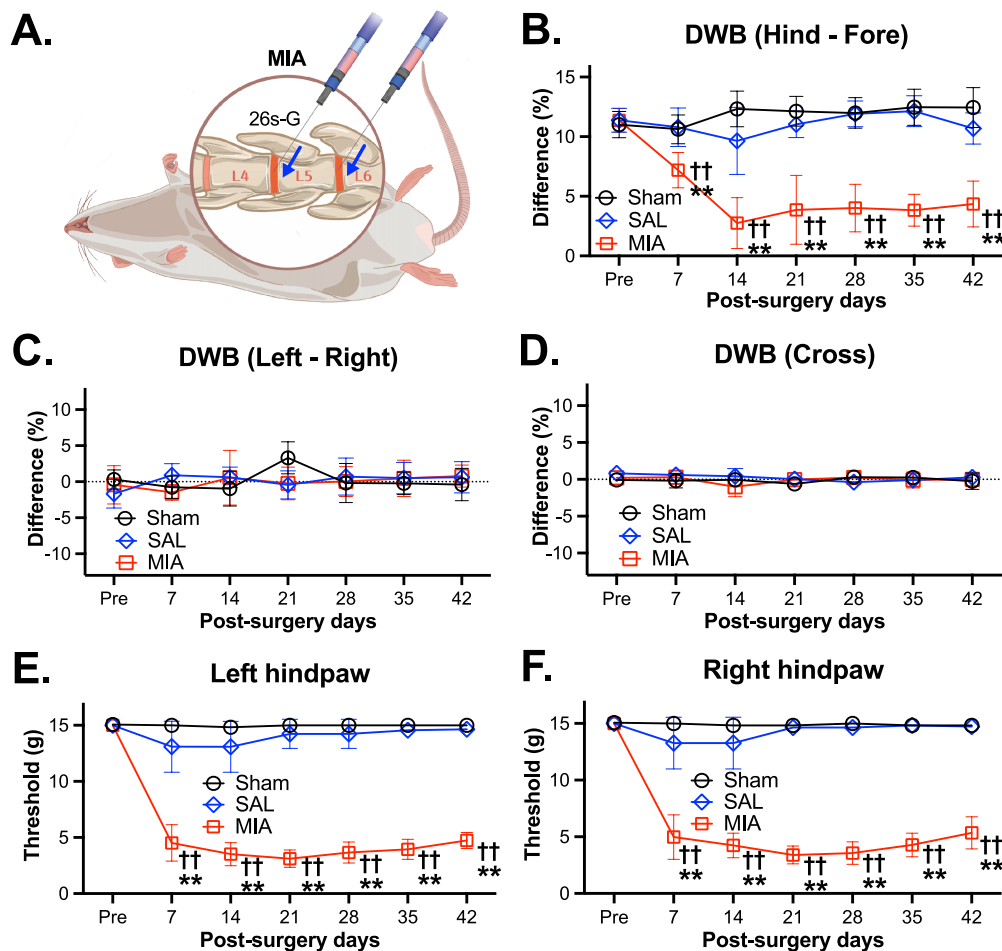


FIGURE 1 | Behavioral assessment of dynamic weight bearing and mechanical hyperalgesia following intradiscal MIA injection. (A) Schematic illustration of MIA injection at L4/5 and L5/6 lumbar discs in rats. (B) MIA group ($n=14$) showed remarkable forelimbs-dependent walking of dynamic weight bearing (DWB) compared to Sham ($n=14$) and SAL groups ($n=14$) over 42 days. (C and D) However, there was no significant difference of Left-Right and Cross among all groups. (E and F) MIA groups significantly decreased in the withdrawal threshold (g) of left and right hindpaws, measured by von Frey filaments, over 42 days. Data represent mean $\pm$ 95% CI. ** symbolizes $p < 0.01$ versus sham, and †† symbolizes $p < 0.01$ versus SAL (Repeated measure ANOVA with post hoc Tukey test).

calcium influx in primary neurons of dorsal root ganglia (DRG) 28 days post-MIA surgery (or 35 days post-DiI injection). Figure 2D and F showed the normalized ratio (340/380) of capsaicin (TRPV1 agonist) or allyl isothiocyanate (AITC; TRPA1 agonist)-evoked calcium influx in DiI labeled-DRG neurons. We found that both MIA groups induced an enhanced pattern of calcium influx following capsaicin or AITC application in labeled DRG neurons (Kruskal Wallis test; all $p < 0.05$). Post hoc analysis (Bonferroni correction) indicated that DiI-MIA groups expressed significant increases in the peak normalized ratio of 1 μ M capsaicin- ($p = 0.001$ and 0.008) or 100 μ M AITC- ($p = 0.042$ and 0.018) evoked calcium influx compared to unlabeled and DiI-SAL group (Figure 2E-G).

3.3 | Low Back Pain Correlates With Distinct Pathological Alterations of Lumbar Disc and Bone, as Well as Painful Nerve Ingrowth

To determine whether these pain-related behaviors can be associated with degenerated structure of disc and bone, we utilized multi-modal analysis including μ CT imaging, histology,

immunohistochemistry, and linear discriminant analysis (LDA). Using *in vivo* μ CT imaging, we assessed intervertebral disc volume (IVD.V; L4/5 and L5/6) from pre-surgery to 42 days post-surgery, with testing performed at 14-day intervals (Figure 3). Figure 3A showed representative 3D images of intervertebral discs. Notably, the MIA groups displayed morphometric changes of lumbar discs ($F_{2, 30.5} = 7.54$, $p = 0.002$; Mixed model). In detail, MIA group showed a significant decrease in IVD.V compared to sham and SAL groups (all $p < 0.01$). However, no significant difference was observed across days ($F_{2, 21.6} = 1.87$, $p = 0.178$), disc levels ($F_{1, 21.1} = 0.02$, $p = 0.896$), or their interactions including group $\times$ days ($F_{4, 23.5} = 0.36$, $p = 0.832$), group $\times$ disc levels ($F_{2, 21.1} = 1.19$, $p = 0.323$), days $\times$ disc levels ($F_{2, 27.9} = 2.64$, $p = 0.089$), and group $\times$ days $\times$ disc levels ($F_{4, 31.6} = 0.95$, $p = 0.449$; Figure 3B,C).

Using *ex vivo* μ CT imaging, we assessed 3D morphological parameters of lumbar vertebrae 5 (L5) 44 days post-MIA injection (Figure 4). Representative images of bone mineral density (BMD) and trabecular thickness (Tb. Th) were shown in Figure 4A,B. The MIA group expressed inferior quality of trabecular bone compared to controls (Kruskal Wallis test;

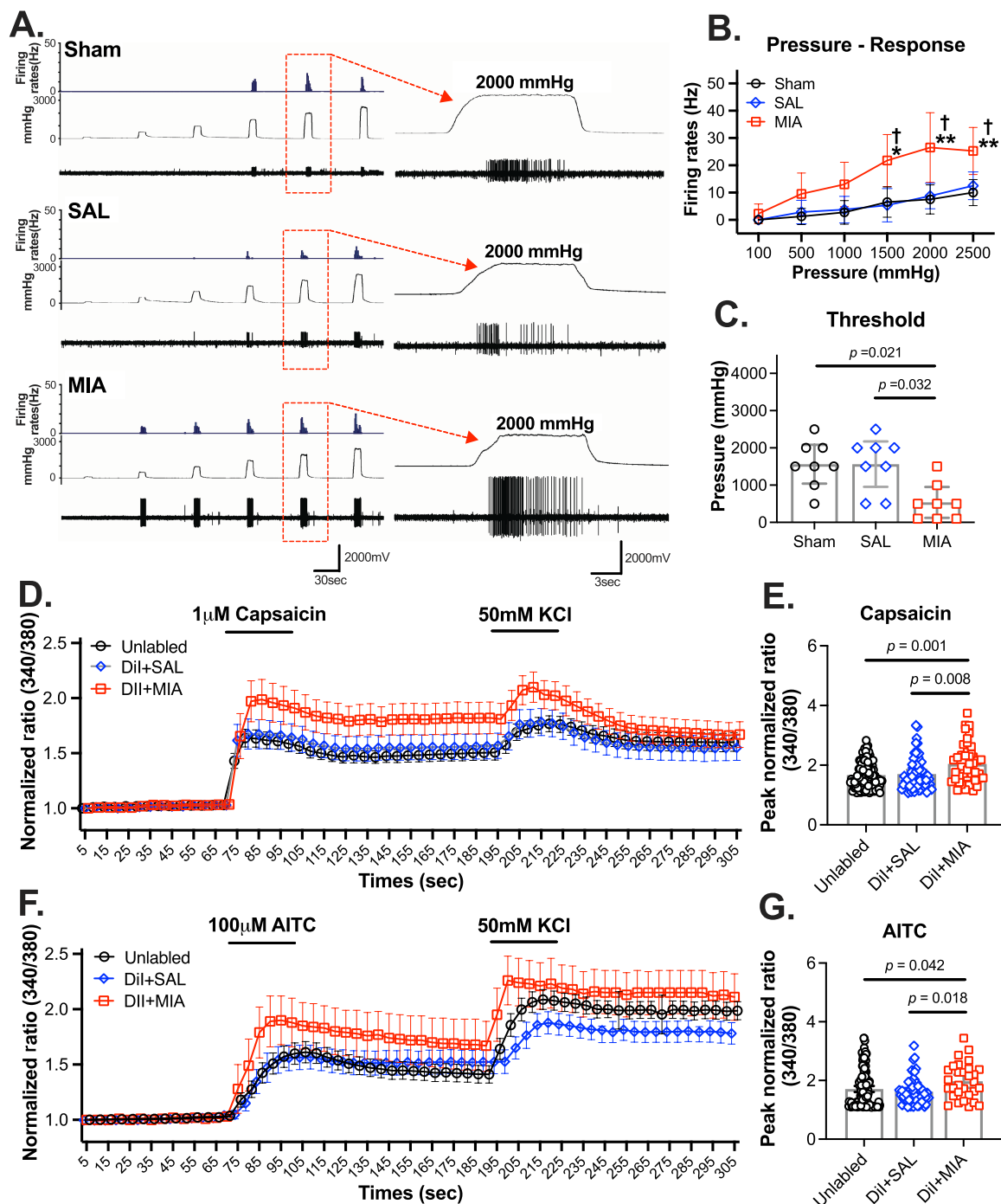


FIGURE 2 | Neuronal hyperexcitability and calcium influx after intradiscal MIA injection. (A) Representative firing rates traces of mechanosensitive single afferent nerve (MSAN) in response to intradiscal pressure in sham, SAL, and MIA groups. (B and C) Peak firing frequencies (spikes/sec) of MSAN in MIA groups (nerve/animal; $n = 8/8$) were significantly enhanced in ranges of 1500 to 250 mmHg compared to sham ($n = 8/8$) and SAL groups ($n = 8/8$). In addition, intradiscal pressure thresholds of MIA group significantly reduced. Data represent mean + 95% CI. *** symbolize $p < 0.05$ and 0.01 versus sham, and † symbolize $p < 0.05$ versus SAL (Kruskal–Wallis with post hoc Bonferroni correction). (D and F) Normalized ratio traces (340/380 nm; Fura-2AM) of calcium influx in unlabeled or DiI-labeled DRG neurons, stimulated with 1 μ M capsaicin or 100 μ M AITC and 50 mM KCl. (E and G) DiI-MIA group expressed significant increases in capsaicin- or AITC-evoked calcium influx compared to unlabeled and DiI-SAL group. DRG neurons with less than 20 μ m diameter were analyzed. Data represent mean + 95% CI, and p values were determined using Kruskal–Wallis with post hoc Bonferroni correction. Unlabeled (neuron/animal for capsaicin or AITC; $n = 118$ or 143/5), DiI-SAL ($n = 61$ or 58/5), and DiI-MIA ($n = 46$ or 30/5).

$p < 0.05$). In detail, despite borderline significance in trabecular thickness (Tb. Th; $p = 0.057$, Figure 4G), MIA group exhibited a significant increase in Tb. Sp ($p = 0.0002$), along with a significant decrease in Tb.N ($p = 0.00003$), Conn. Dn ($p = 0.0001$), and Conn. Dn ($p = 0.0007$), compared to sham

group (Bonferroni correction; Figure 4I–L). In addition, MIA group showed a significant increase in Tb. Th ($p = 0.011$) and a significant decrease in Tb.N ($p = 0.007$) and Conn. Dn ($p = 0.020$) compared to SAL group (Bonferroni correction; Figure 4G, I and L).

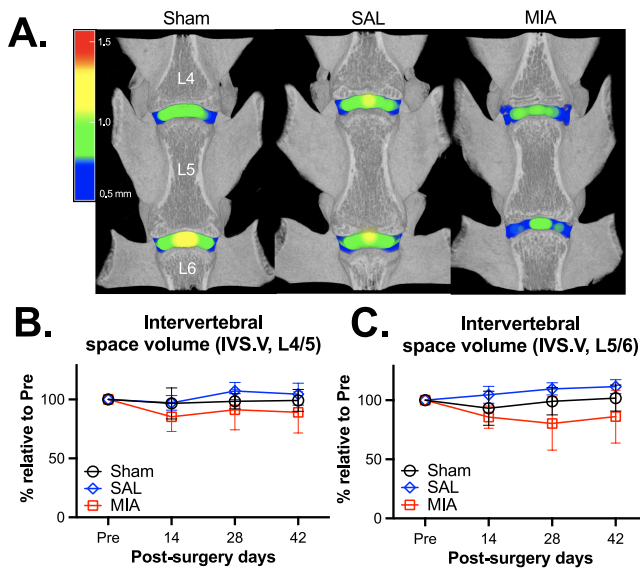


FIGURE 3 | In vivo μ CT analysis of lumbar intervertebral disc spaces in rats with MIA-induced low back pain. (A) Representative in vivo μ CT images of lumbar intervertebral spaces (L4/5 and L5/6) in Sham, SAL, and MIA groups. The blue, green, yellow, and red color bar indicate 0.50–0.75, 0.75–1.00, 1.00–1.25, and >1.25 mm, respectively. (B and C) Time-course quantification shows progressive reductions of L4/5 and L5/6 intervertebral space volume (IVS.V, percentage relative to pre) in MIA group ($n = 7$) 14 to 42 post-surgery days compared to Sham ($n = 7$) and SAL groups ($n = 7$). Data represent mean $\pm$ 95% CI (Mixed model with post hoc Tukey test).

Degenerative disc and bone were evaluated in histopathology scoring system 46 days post-MIA injection (Figure 5A–C). Figure 5A showed the representative images of hematoxylin/eosin (H&E) and safranin O staining. MIA group showed shrunk NP, deformity or fissure within AF lamella, partial irregularity of endplate and subchondral bone. In detail, MIA group significantly increased histopathological scores of L4/5 and L5/6 disc, endplate, and L4–6 subchondral bone compared to sham or SAL group (Kruskal–Wallis test and post hoc; $p < 0.05$ or 0.01 , Figure 5B,C). In addition, TRPV1- and CGRP-positive area of subchondral bone (L5) were evaluated 46 days post-MIA surgery (Figure 5D–F). Figure 5D showed the representative images of TRPV1 and CGRP. MIA group showed ingrowth of nociceptive fiber. In detail, MIA group exhibited significant increases of TRPV1- ($p = 0.012$) and CGRP- ($p = 0.021$) positive areas compared to sham group, as well as TRPV1- ($p = 0.007$) positive area compared to SAL group (Kruskal Wallis test and post hoc; Figure 5E,F).

To identify key features distinguishing among the groups, we applied linear discriminant analysis with stepwise selection (LDA-S) using a total of 12 variables (10 variables: *ex vivo* uCT parameters, 2 variables: HindFore(pre) and HindFore(Area under curve [AUC])). The stepwise method selected 4 variables (HindFore(AUC), Tb. Th, Conn, and Tb. Pf) as the most significant predictors. The LDA model identified two discriminant functions, which explained 90.14% and 9.86% of the total variance, respectively. Both functions were statistically significant ($p < 0.0001$ for dimension 1; $p = 0.0002$ for dimension 2). The canonical correlations for the functions were 0.929 (dimension 1) and 0.640 (dimension 2), indicating strong group separation.

The overall classification accuracy was 88.10% (95% CI: 74.37–96.02) for raw data and 83.33% (95% CI: 68.64–93.03) for leave-one-out cross-validation (LOOCV). The kappa statistic was 0.8214 (raw data) and 0.7500 (LOOCV), demonstrating substantial agreement between predicted and actual group assignments. A scatter plot of two discriminant functions revealed clear group clustering, supported by an LDA biplot illustrating the contributions of the selected variables (Figure 6).

4 | Discussion

This current study provides strong evidence that intradiscal injection of monosodium iodoacetate (MIA) induces persistent low back pain (LBP)-related behaviors alongside pathological remodeling in both intervertebral discs and subchondral bone. These changes correlate with neuronal hyperexcitability and enhanced TRPV1/TRPA1-mediated calcium influx in peripheral nerves and DRG neurons. TRPV1/CGRP upregulation shows the presence of painful nerve ingrowth within degenerated spine. These findings reflect that chronic LBP is driven by structural and functional alterations across the disc and subchondral bone tissues.

It remains controversial whether or how intervertebral disc, normally aneuronal and avascular tissue, can transmit nociceptive information as a ‘painful disc’. However, human and animal studies have consistently demonstrated that nerve ingrowth and inflammation within lumbar disc degeneration play a critical role in LBP (Risbud and Shapiro 2014; Lyu et al. 2021; Freemont et al. 2002). Rodent models using mechanical puncture or chemical injury into lumbar disc were useful for elucidating pain-related processes, though certain methodological considerations should be addressed. For instance, annulus fibrosus-isolated disc degeneration increases inflammation and apoptosis, but expresses a relatively moderate nociceptive phenotype (Huang et al. 2024). Disc puncture with endplate microfracture or NP removal induces mechanical or thermal hyperalgesia of hindpaw and impaired rearing behavior, accompanied by nerve ingrowth (NGF) and upregulated expression of IL-1 β , TNF- α , CGRP, and substance P (Shi et al. 2018; Park et al. 2019; Wang et al. 2023). Chemical injury using intradiscal MIA also reflects key features of LBP. Intradiscal application of MIA (L4–5 and L5–6; 4 mg/5 μ l) impaired dynamic weight bearing and rearing of rats at 14 to 28 days, with decreased disc height and subchondral bone deformation (Suh et al. 2022). Lower MIA dosages (L4–5 and L5–6; 0.5 or 1 mg/5 μ l) enhanced morphological irregularity and CGRP-positive innervation in rat endplate, reducing rearing distance and frequency in the open field over 6 to 11 weeks (Maruyama et al. 2024). In light of these findings, we optimized MIA dosage and volume (2 mg in 2 μ l) to reliably induce disc/subchondral bone degeneration without volume leakage. Critically, we established a multimodal methodology with pain-related behavior, single or labeled neuronal response, and tissue analysis using μ CT and IHC to elucidate the relationship between tissue alteration and LBP processing (Park and Choi 2025). Indeed, we observed long-lasting nociceptive behaviors, such as forelimb weight-bearing shifts and decreased hindlimb mechanical thresholds. Concurrently, in vivo electrophysiology revealed hyperexcitability of single afferent fibers to intradiscal pressure, while calcium imaging showed

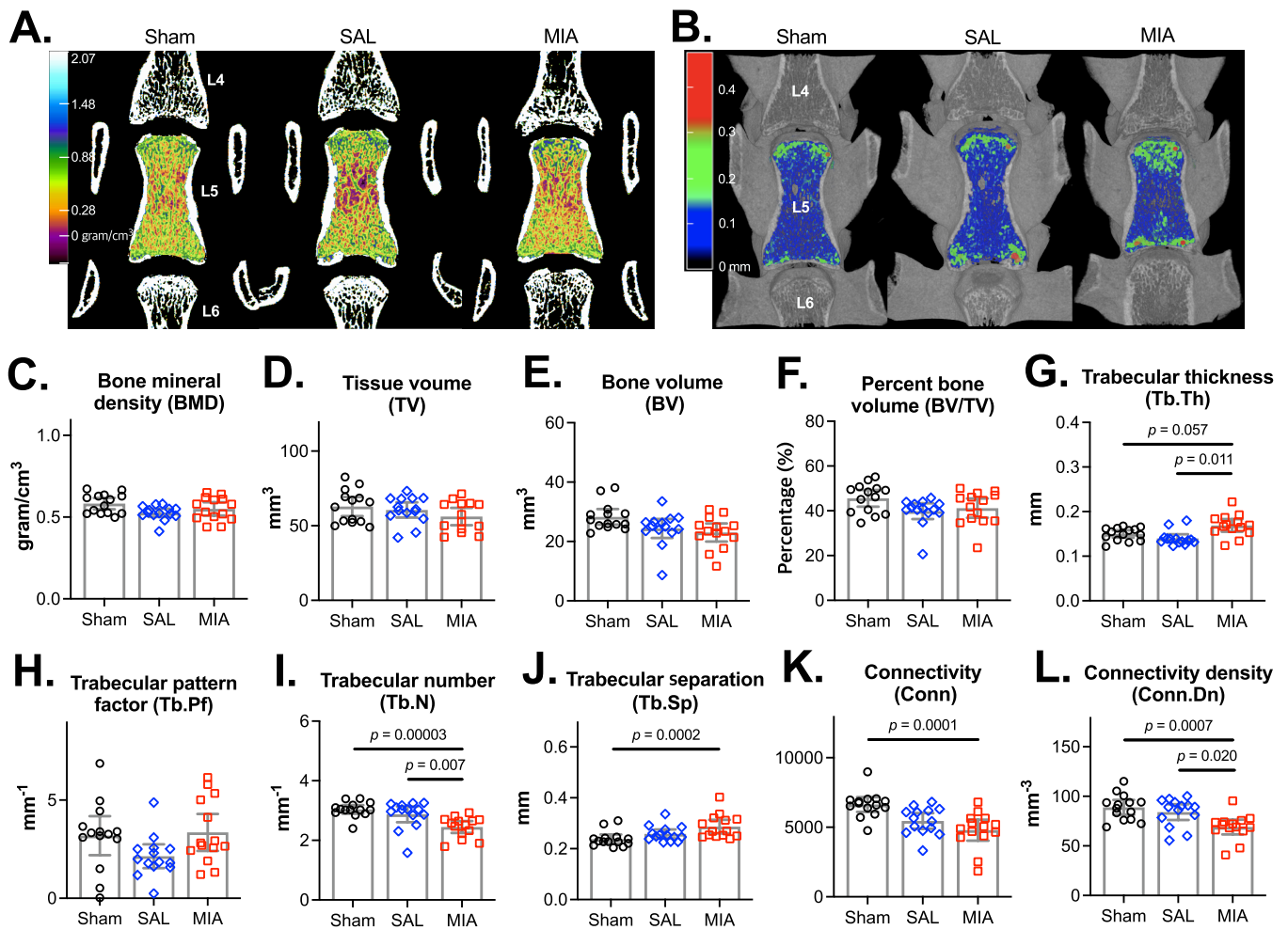


FIGURE 4 | Ex vivo μ CT analysis of subchondral bone in rats with MIA-induced low back pain. (A–B) Representative ex vivo μ CT images of L5 subchondral bone in Sham, SAL, and MIA groups 44 days post-surgery, showing bone mineral density (BMD) and trabecular thickness (Tb. Th) distribution. The purple, orange, green and blue color bar for BMD indicate 0, 0.3, 0.9, and 1.5 g/cm³, respectively. The blue, green, and red color bar for Tb. Th indicate 0–0.15, 0.15–0.30, and > 0.30 mm. (C–L) MIA group ($n = 14$) expressed inferior bone quality by showing significant increases of Tb. Th and trabecular separation (Tb. Sp) and significant decreases of trabecular number (Tb.N), connectivity (Conn) and connectivity density (Conn. Dn) compared to Sham ($n = 14$) or SAL group ($n = 14$). Data represent mean + 95% CI, and p values were determined using Kruskal–Wallis with post hoc Bonferroni correction.

enhanced TRPV1/TRPA1-mediated responses in labeled DRG neurons. Importantly, μ CT and histological evidence confirmed disc and bone tissue degeneration strongly associated with LBP.

It is increasingly apparent that combined structure degeneration in both subchondral bone and disc, rather than the disc alone, can represent a critical factor of LBP pathogenesis. In current study, intradiscal MIA model illustrates how pathological processes impair subchondral bone–disc metabolic exchange, which weakens the lumbar disc against mechanical stress and ultimately render it a source of pain. Our histopathological evaluation revealed NP/AF deformation and endplate–subchondral bone irregularities. Furthermore, 3D μ CT analysis revealed reduced disc volume (IVS.V) and deteriorated trabecular bone quality, reflected by increased Tb. Th and Tb. Sp and decreased Tb.N, Conn, and Conn. Dn. From a statistical perspective, LDA identified Tb. Th, Conn, and Tb. Pf parameters, in combination with HindFore (AUC) behavior, as strong indicators for LBP. These results are consistent with previous studies showing severe endplate and disc degeneration in MIA models in a dose- and time-dependent

manner (Maruyama et al. 2024; Elmounedi et al. 2023), along with elevated iNOS levels in the endplate (Suh et al. 2022). Notably, a recent study using μ CT reported significant increases in BV/TV, BMD, and Tb. Th of rat vertebral body 6 to 12 weeks after the intradiscal MIA injection (Maruyama et al. 2024), paralleling nociceptive behavior.

Comparisons with other MIA-induced joint models deepen LBP understanding, given that disc/endplate tissues share cellular and functional similarities with joint/cartilage. For example, MIA-induced facet joint of rat leads to cartilage breakdown, proteoglycan loss, and structural alteration of subchondral bone observed using μ CT and IHC, correlating with pressure-evoked hyperalgesia (Kim et al. 2011). Likewise, MIA-induced knee joint degeneration of rats involves synovial inflammation, osteophyte formation, and subchondral bone loss (reduced BV/TV and lowered Tb. Th), which closely corresponds to mechanical hypersensitivity and abnormal weight load (Tan et al. 2022; Allen et al. 2017; Kwon et al. 2023; Li et al. 2021). However, a distinct discrepancy presents in μ CT outcomes between disc models (increased BV/TV and Tb. Th) and knee joint models (decreased

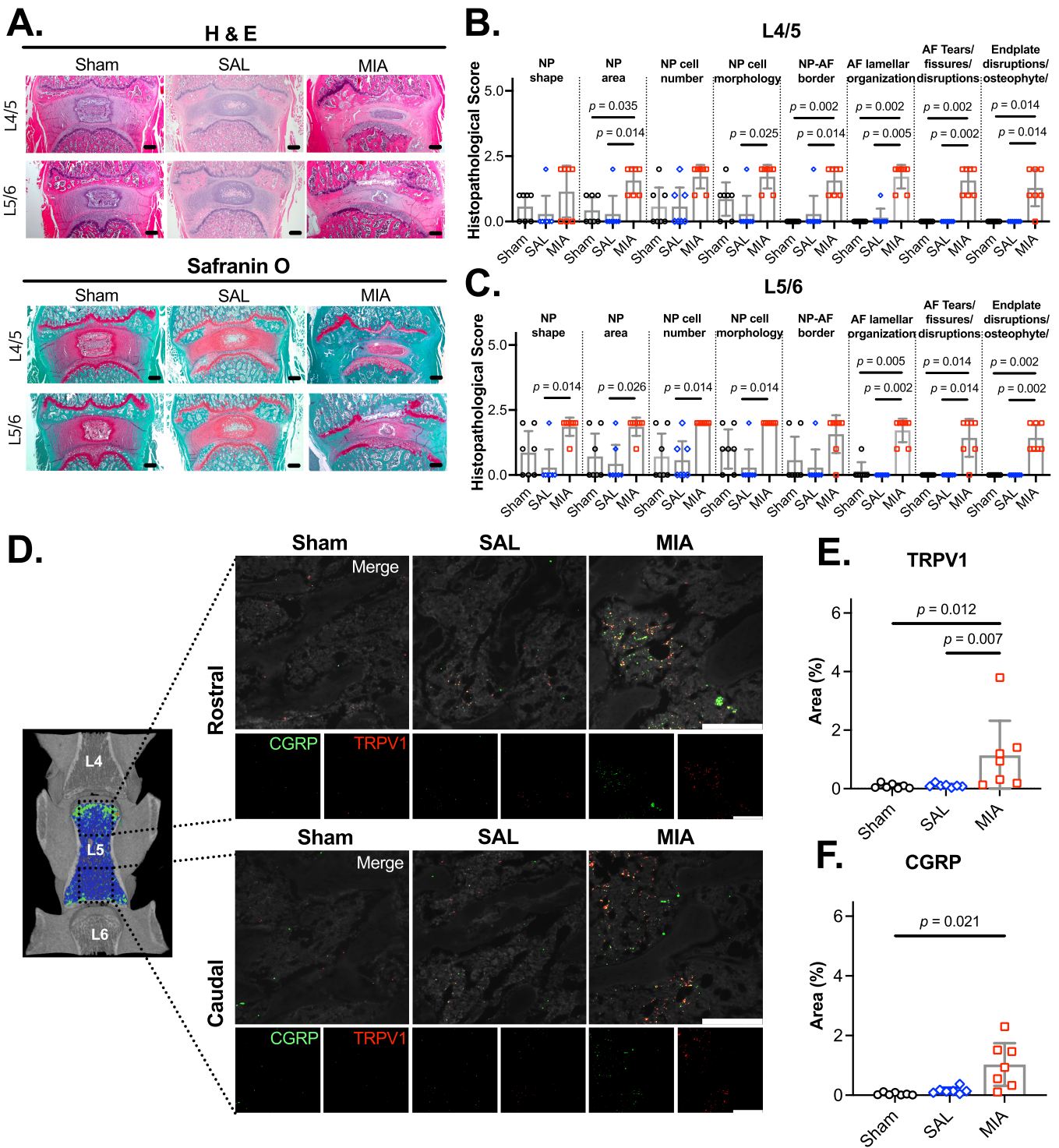


FIGURE 5 | Histopathological and immunohistochemical analysis of degenerative changes in rats with MIA-induced low back pain. (A) Representative histological images of discs (L4/5 and L5/6) and subchondral bone (L5) 46 days post-surgery. Upper images are H&E staining, and lower images are safranin O staining. Scale bar is 100 μ m. (B and C) Histopathological score of MIA group ($n = 7$) showed shrunk nucleus pulposus (NP), deformity or fissure within annulus fibrosus (AF) lamella, and partial irregularity of endplate and subchondral bone compared to Sham ($n = 7$) and SAL group ($n = 7$). (D) Representative images of TRPV1- and CGRP-positive areas (%) in L5 subchondral bone (rostral and caudal parts) in Sham, SAL, and MIA groups. (E and F) MIA group ($n = 7$) expressed significant increases of TRPV1 and CGRP in subchondral bone compared to Sham ($n = 7$) or SAL group ($n = 7$). Data represent mean + 95% CI, and p values were determined using Kruskal–Wallis with post hoc Bonferroni correction.

BV/TV and Tb. Th) following local MIA administration. One possible explanation lies in the anatomical divergence between the lumbar spines and knee joints: the lumbar disc's extracellular matrix structure limits fluid exchange and localizes inflammatory

processes, whereas the synovial fluid and capsule of knee joint extensively diffuse inflammatory cytokine. Supporting this, erosion at the center of the endplates was observed 6 weeks after intradiscal MIA injections (Maruyama et al. 2024). Consequently,

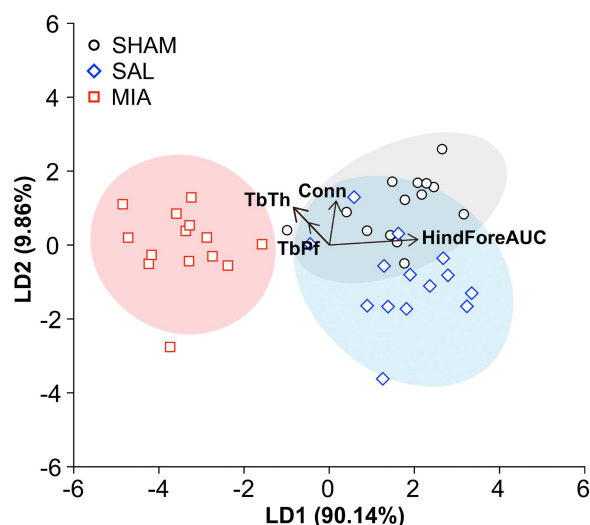


FIGURE 6 | Linear discriminant analysis of low back pain features. Scatter plot of the two discriminant functions from linear discriminant analysis (LDA) with stepwise selection, distinguishing Sham ($n = 14$), SAL ($n = 14$), and MIA ($n = 14$) groups based on 12 variables (10 ex vivo μ CT parameters, HindFore(pre), and HindFore(AUC)). The stepwise selection identified HindFore(AUC), Tb. Th, Conn, and Tb. Pf as the key variables contributing to the group separation. The LDA identifies two functions, with the first discriminant function (LD1) explaining 90.14% of the variance and the second (LD2) explaining 9.86%.

the structural difference leads to distinct mechanical force distributions and localized versus diffuse inflammatory responses, creating different patterns of subchondral bone remodeling.

LBP progression is ultimately determined by the neuronal sensitization from inflammation and aberrant mechanical distribution in degenerated spines. TRP channels, nonselective cation transmembrane channel, play physiological roles in detecting changes in temperature, pH, and osmolarity across multiple tissue types. Of particular note, TRPV1 and TRPA1 channels are enriched in small-diameter DRG neurons and upregulated under inflammatory conditions within degenerated spine tissues, thereby contributing to chronic pain (Gao et al. 2024; Masuoka et al. 2017). The neuropeptide CGRP, predominantly synthesized in DRG, is secreted at peripheral and central terminals in Ca^{2+} -dependent pathways. Damage within degenerated subchondral bone and disc upregulate CGRP, activating vasodilation, immune cells infiltration, and pain signaling (Kim and Granstein 2021). In turn, CGRP influence bone modeling by regulating osteoclast differentiation and inhibiting bone resorption in Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL)/osteoprotegerin (OPG) signaling pathways (He et al. 2016; Wang et al. 2010). It was reported that both TRPV1 and TRPA1 channels can evoke CGRP exocytosis through elevated intracellular calcium levels (Hoffmann et al. 2013; Pozsgai et al. 2012). Our current study supports these observations: increased TRPV1- and CGRP-positive areas in the subchondral bone indicate ingrowth of nociceptive fibers exacerbating LBP. Calcium imaging demonstrated co-activation of TRPV1 and TRPA1 in labeled DRG neurons innervating degenerated bone and disc, supporting that both channels participate in peripheral sensitization. Although a few immunohistology or molecular studies reported TRPV1

without TRPA1 in DRG neurons innervating intervertebral disc (Shi et al. 2018; Aoki et al. 2005; Leimer et al. 2019; Ohtori et al. 2003), our MIA injection likely establishes a more intensive inflammatory microenvironment in the disc–bone interface, thereby activating both TRPV1- and TRPA1-expressing nociceptors (Zhang et al. 2021). In support of this, mice osteoarthritis (OA) models with destabilization of the medial meniscus (DMM), featuring cartilage erosion, subchondral bone sclerosis, and osteophyte, upregulates CGRP, TRPV1, TRPV4, and TRPA1 genes in lumbar DRG at 16 weeks of OA (Zaki et al. 2021).

Despite no direct evidence, TRPV1/CGRP upregulation in subchondral bone could be related to Modic changes of MRI in LBP patients. Multiple human and animal studies have reported that endplate/subchondral bone microfracture or trauma increases vascular density and proinflammatory environment, with CGRP- or TRPV1-positive nerve innervation (Zhu et al. 2019; Hu et al. 2021; Brown et al. 1997; Morisako et al. 2022; Liu et al. 2021; Ma et al. 2024). As Modic type 1 or 2 changes strongly reflect fatty or non-fatty inflammatory process of endplate and bone degeneration, these signals implicate pathological nerve ingrowth which lead to very high specificity and positive predictive value of LBP (Weishaupt et al. 2001; Thompson et al. 2009). For example, patients with Modic changes exhibit more protein gene product (PGP) 9.5-positive nerve fibers and TNF immunoreactivity than those with normal endplates (Ohtori et al. 2006; Fields et al. 2014). Similarly, degenerated endplate of rats showed increased CGRP-immunoreactivity and low T1- and T2-weighted MRI 8 to 12 weeks post-lumbar endplate lesion (Morisako et al. 2022). Mouse endplate expressed not only CGRP- and TRPV1-positive nerve ending, but also high percentage of porosity in μ CT following lumbar spine instability (Liu et al. 2021). Importantly, a few clinical therapeutics targeting TRP channels and CGRP receptors in chronic LBP patients were reported. Compared to placebo control, oral administration of TRPA1 antagonist, LY3526318 (250 mg daily; 8 weeks), tend to improve numerical rating scale (NRS) pain intensity in chronic low back pain (CLBP) population despite no significant difference (Mellado Lagarde et al. 2024). In a patient population with comorbid degenerative spinal disease and migraine, CGRP inhibitors in patients significantly decreased back/neck pain and improved activities of daily living (ADLs) in 25% of the patients (Canseco et al. 2023).

In conclusion, this study provides a comprehensive, multimodal characterization of chronic LBP driven by concurrent disc and bone pathology. It was strongly shown that the pathological co-degeneration of discs and subchondral bone contributes to the development of chronic low back pain in a rat model with intradiscal MIA. Specially, single nerve recordings and DRG calcium imaging confirmed that peripheral neurons innervating this degenerated spine exhibited hyperexcitability and enhanced TRPV1/TRPA1-mediated calcium influx. Histological and μ CT analyses further revealed structural deterioration in disc and bone tissues, along with increased expression of nociceptive markers (TRPV1 and CGRP). Furthermore, linear discriminant analysis identified trabecular thickness, connectivity, trabecular pattern factor, and pain behavior (Hind-For AUC) as key predictors of LBP development. However, the current experimental design does not include a ‘disc-only degeneration’ group and thus cannot isolate the independent contribution of subchondral bone from that of disc degeneration. Therefore, while our findings

strongly support that combined degeneration of disc and subchondral bone is associated with chronic LBP, it cannot be completely established that subchondral bone involvement *per se* exacerbates pain beyond disc pathology alone. Our findings highlight the need for therapeutic strategies that target the disc-bone unit as a whole. Further research is required to clarify the detailed mechanistic relationship between spine degeneration and nociceptive processing, particularly through targeted modulation of TRPV1/TRPA1 pathways.

Author Contributions

Yun-Ho Cho: writing – original draft, methodology, investigation. **Cham Choi:** writing – original draft, methodology, investigation, validation. **Minji Kwon:** writing – review and editing, methodology, investigation, visualization. **Jinju Kwon:** methodology, investigation, visualization. **Hogwang Ok:** investigation. **Dawon Ryu:** investigation. **Kyung-Sook Yang:** formal analysis. **Junesun Kim:** writing – review and editing, supervision. **Eui Ho Park:** conceptualization, methodology, writing – original draft, writing – review and editing, visualization, funding acquisition.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting Figure 1: The volume of interest (VOI) for lumbar disc and trabecular bone. (A) Coronal and sagittal view of VOI at L4/5 and L5/6 lumbar discs in rats. (B) Coronal and sagittal view of VOI at L5 trabecular bone in rats.