Additional Table 1. Animal models used to study disc degeneration

Model type	Species	Manipulation	Reference
Disc disruption			
Spontaneous	Mouse	Aging	200, 206
		Ercc1 mutation	252
		Cmd aggrecan knockout	236, 237
		Inherited kyphoscoliosis	238
		Collagen II mutation	239
		Collagen IX mutation	235
		Myostatin knockout	240
		Defect at *ank* locus, ankylosing spondylitis	241
		twy mouse—IVD calcification and ankylosis	242
		SPARC null	29, 30, 255
		HLA B27 transgenic, spondylolisthesis	243
	Rat	Aging	244
	Sand rat	Chondrodystrophy, aging, breed	245-247
	Dog	Spondylosis; aging	228, 248
	Chinese hamster	Aging	249
	Baboon	Aging	200
Mechanical alteration	Mouse	Lumbar spine instability mouse model with/without ovariectomy	31, 277
	Mouse, rat	Static compression	32, 290, 292, 783
	Rabbit	Shear stress	282
	Rabbit	Compression injury, lumbar spine and caudal disc compression	264, 265
	Rat	Tail suspension	272
		Shear stress	281
		Amputation of upper limbs and tail	266
	Mouse	Amputation of upper limbs	271
	Rabbit	Resection of the cervical supraspinous and interspinous ligaments and detachment of the posterior paravertebral muscles from the cervical vertebrae; the removal of facet joints	267, 268
	Dog	Static compression	293
	Pig	Resection of facet joint, interspinous and anterior ligament injury	269
	Rabbit	Facetectomy/capsulotomy torsional lumbar injury	270
Disc herniation	Cavine	A partial laminectomy of the caudal part of the 6th lumbar vertebrae; puncture of dorsolateral portion of the annulus fibrosus	33, 372
	Rat	NP obtained from tail amputation and placed on nerve root	373
	Rabbit	Bilateral facet joint resection at L7–S1 and rotational manipulation	294
		External annular wound (2 mm)	295, 296
	Rat	Flexion, lateral bending and rotational forces	297
Disc lesions	Rabbit	Multiple 5 mm stab incisions using 16, 18 or 21G needles	298, 337, 725
		NP removal	299, 300
		3–5 mm outer anterolateral annular incision (rim-lesion)	110, 301-303, 435
	Ovine	Circumferential annular tear (delamellation)	304
		A lateral retroperitoneal drill bit injury	790
		Anular lesion by surgical incision through the left anterolateral AF	305
	Pig	Combined lesions in AF (1.2 cm), NP (1.5 cm), facet joint and capsule	306
	Rat	5 mm stab by 18–30G needles	307
	Dog	4 mm posterior annulotomy	308
Local chemical stimulation	Rat	Chondroitinase ABC	212
	Rabbit		213
	Sheep		380-382
	Macaque	Chymopapain	375

Additional Table 1. Continued

Model type	Species	Manipulation	Reference
	Rabbit	Chymopapain	214, 374
	Rhesus monkeys	Pingyangmycin	210
		Bleomycin	211
	Dog	Fibronectin fragments	406
	Rabbit	Fibronectin fragments	405
		Chymopapain, krill proteases	215-217
	Rat	Complete Freund's adjuvant	218, 398-400
	Rat	IL-1β	409
	Rat	AGE	397
Systematic reagents stimulation	Mouse	Immunized with aggrecan and/or versican, develops spondylitis	413
		Dietary AGE	393
		Diabetic	389
Fusion	Rabbit	Lumbar arthrodesis	419
	Sheep	Lumbar arthrodesis	420
	Rat	Lumbar arthrodesis	421
	Rabbit	Controlled dynamic distraction	422
Pinealectomy models of scoliosis	Chicken	Pinealectomy	220
	Rat	Pinealectomy + bipedal	221
Appendix			
Loss of nutrient supply	Mouse, rat	Endplate perforation	426
	Pig	Disc allograft transplantation	424
		Endplate perforation and cryoinjury	425, 427
	Goat	Ethanol injection to bone marrow vertebrae body	428
		Cement injection to the adjecant vertebrae body	429
	Rat	Nd: YAG laser on the CEP of the degenerated IVD	222
Nerves and vessels ingrowth	Pig	Annulus fibrosus puncture and poly(lactic-co-glycolic acid)/ fibrin gel sealing	336
	Mouse	Disc puncture and nucleus pulposus removal	436
	Sheep	Annulus fibrosus puncture	435
Nerve associated degeneration	Rabbit	Surgical narrowing of intervertebral neural foramen, vibrational stimulation of dorsal root ganglia	223
Others			
Hyperactivity	Dog	Long distance running training	224-226

Note: AF: annulus fibrosus; AGE: glycation end products; ank: ankylosis; CEP: cartilage endplate; Ercc1: Excision repair cross-complementing 1; HLA: human leukocyte antigen; IL-1β: interleukin-1β; IVD: intervertebral disc; Nd: YAG: neodymiumyttrium-aluminum-garnet; NP: nucleus pulposus; SPARC: secreted protein acidic and rich in cysteine; twy: tiptoe walking-Yoshimura.

Additional Table 2. Needle gauge and corresponding size

Gauge number	Needle nominal O.D. (mm)	Needle nominal I.D. (mm)	Needle wall thickness (mm)
10G	3.404	2.693	0.356
11G	3.048	2.388	0.33
12G	2.769	2.159	0.305
13G	2.413	1.804	0.305
14G	2.109	1.6	0.254
15G	1.829	1.372	0.229
16G	1.651	1.194	0.229
17G	1.473	1.067	0.203
18G	1.27	0.838	0.216
19G	1.067	0.686	0.191
20G	0.908	0.603	0.152
21G	0.819	0.514	0.152
22G	0.718	0.413	0.152
23G	0.642	0.337	0.152
24G	0.566	0.311	0.127
25G	0.515	0.26	0.127
26G	0.464	0.26	0.102
27G	0.413	0.21	0.102
28G	0.362	0.184	0.089
29G	0.337	0.184	0.076
30G	0.312	0.159	0.076
31G	0.261	0.133	0.064
32G	0.235	0.108	0.064
33G	0.21	0.108	0.051
34G	0.159	0.051	0.051

Note: I.D.: inner diameter; O.D.: outer diameter.

Additional Table 3. Parameters for needle puncture-induced intervertebral disc degeneration models

Animal	Needle size	Needle diameter/disc height (%)	Approach	Depth	Puncture position	Segements	Additional	Degenerated time point/ longest recorded time	Mechanical	Biochemical	Height (longest recorded time)	Histologic and gross	Radiograph and MRI	Neuropathic pain	Reference
Rat	18G	128%	Open/ percutaneous puncture	Needle bevel completely inserted	Tail	C3/4	–	1/4 months	–	–	–	Yes, degenerated, NP herniation (more severe in open puncture)	Yes, progressed (more severe in open puncture)		367
	20G	95%	Percutaneous puncture	5 mm (through the annulus fibrosus); 10 mm (full penetration)	Tail	C6/7–C9/10	–	2–4/4–8 weeks	–	Decreased GAG (by ~11% for 5 mm, by ~16% for 10 mm)	Decreased (by ~10% for 5 mm; by ~20% for 10 mm)	Yes, degenerated, NP herniation (more severe in full penetration)	Yes, progressed (more severe in full penetration)	Yes	312-314
	20G	95%	Percutaneous puncture	Through the annulus fibrosus	Tail	C6/7–C8/9	–	1–4/4–24 weeks	–	Decreased water, GAG and type I collagen expression	Decreased (by 25–75%)	Yes, degenerated	Yes, progressed	–	532-534, 550
	21G	85%	Open puncture	3 mm (through the annulus fibrosus)	Posterial approach	L4/5	–	4/8 weeks	–	Altered collagens expression	–	–	Yes, progressed	–	349
	21G	85%	Open puncture	3 mm (through the annulus fibrosus)	Posterior/ anterior approach	L4/5	–	2/6 weeks	–	–	–	Yes, degenerated	Yes, progressed	Yes (more significant for posterior puncture)	370
	21G	85%	Open puncture	3 mm (through the annulus fibrosus)	Tail	C4/5, C8/9	–	2 weeks	–	–	–	Yes, degenerated, NP herniation	Yes, progressed	Yes	322
	21G	85%	Open puncture	5 mm (through the annulus fibrosus)	Tail	C5/6, C7/8	–	4 weeks	–	Altered collagens expression	–	Yes, degenerated	Yes, progressed	–	323
	21G	85%	Percutaneous puncture	Through the annulus fibrosus	Tail	C4/5–C8/9	–	1–2/14–42 days	–	–	–	Yes, degenerated	Yes, progressed	–	360-366
	23G	64%	Open puncture	Through the annulus fibrosus	Lateral approach	L5/6	Repetitive puncture for five times	1/2 weeks	–	–	–	–	–	Yes, increased neurons staining	324-326
	27G	51%	Open puncture	Through the annulus fibrosus	Dorsal approach	L4/5, L5/6	–	2/8 weeks	–	Altered collagens, SOX9, aggrecan expression		Yes, degenerated, NP herniation	Yes, progressed	–	327
	31G	26%	Percutaneous puncture	1.5 mm (through the annulus fibrosus)	Tail	C6/7	–	4 weeks	–	Altered collagens, aggrecan, MMP13, Adamts4 expression		–	–	–	328
	18G/22G	128%/74%	Percutaneous puncture	Through the annulus fibrosus	Tail	C6/7, C8/9	–	2/4 weeks	–	–	–	Yes, degenerated	–	–	329
	18G/22G/26G	128%/74%/20%	Percutaneous puncture	2 mm (through the annulus fibrosus)	Tail	C6/7, C8/9	–	1/4 weeks	Altered creep behavior (for 18G)		–	Yes, degenerated (more severe for 18G)	Yes, progressed	–	321
	18/20/22G	128%/95%/74%	Percutaneous puncture	5 mm (through the annulus fibrosus)	Tail	C6/7, C8/9	–	2/8 weeks	–	Increased proteoglycan (for 18G, 20G)	Decreased (for 18G)	Yes, degenerated, NP herniation (more severe in 18G)	Yes, progressed (more severe in 18G)		359

Additional Table 3. Continued

Animal	Needle size	Needle diameter/disc height (%)	Approach	Depth	Puncture position	Segements	Additional	Degenerated time point/ longest recorded time	Mechanical	Biochemical	Height (longest recorded time)	Histologic and gross	Radiograph and MRI	Neuropathic pain	Reference
	18/21/23/ 25/27/29G	128%/85%/64%/ 53%/51%/36%	Percutaneous puncture	5 cmm (through the annulus fibrosus)	Tail	NA	–	2/8–12 weeks	–	Altered collagens, SOX9, aggrecan expression	Decreased (by ~10% for 29/27/25G, by ~30% for 23/21G, by ~35% for 18G)	Yes, degenerated (more severe in 18G)	Yes, progressed (more severe in 18G)		319, 320
	16G/18G/26G	170%/128%/50%	Percutaneous puncture	Full penetration	Tail	C8/9	–	2/4 weeks	–	Altered Collagens expression		Yes, degenerated, NP herniation (more severe in 16G, 18G)	Yes, progressed (more severe in 16G, 18G)		763
Mice	26G	100%	Percutaneous puncture	2/3 of the disc thickness	Tail	C3/4–C6/7	–	4–8/4–32 weeks	–	Altered Collagens, MMPs, Adam8, Cxcl-1 expression	–	Yes, degenerated, NP herniation	–	–	330, 331
	27G	90%	Open puncture	Through the annulus fibrosus	Anterior approach	L3/4, L4/5	–	1/7 days	–	–	–	Yes, degenerated, NP herniation	–	–	332
	30G	63%	Percutaneous puncture	Needle bevel completely inserted	Tail	NA	–	14 weeks	–	–	Decreased (by ~25%)	Yes, degenerated	–	–	333
	31G	55%	Open puncture	1 mm (through the annulus fibrosus)	Tail	C9/10	–	1/12 weeks	–	Altered collagens, GAG, aggrecan expression	Decreased (by ~30%)	Yes, degenerated	–	–	334, 335
	26G/29G	100%/65%	Percutaneous puncture	1.75 mm or 90% of the dorsoventral width	Dorsal approach	C6/7–C8/9	–	8 weeks	Decreased compressive stiffness, torsional stiffness, torque range, nc compressive ROM, increased creep displacement (for 26G)	Decreased GAG (by ~30% for 26G)	Decreased (by ~30% for 26G)	Yes, degenerated, NP herniation	–	–	353
	27G/29G/31G	90%/70%/55%	Percutaneous puncture	Through the annulus fibrosus/full penetration	Tail	C7/8, C9/10	–	4 weeks	–	–	NS	Yes, degenerated, NP herniation (more severe in full penetration)	Yes, progressed (more severe in full penetration)		317
	27/30/33G	90%/63%/40%	Open puncture	NA	Ventral approach	L4/5–L6/S1	–	1/8 weeks	–	Decreased GAG expression (for 27G and 30G)		Yes, degenerated (more severe for 27G and 30G)		–	318
	33G/35G	50%/42%	Open puncture	Through the annulus fibrosus	Ventral/ central/dorsal approach	L4/5	–	1/12 weeks	–	–	–	Yes, degenerated (more severe in central/dorsal approach)	Yes, progressed (more severe in central/dorsal approach)		371
Rabbit	16G	66%	Percutaneous puncture	Through the annulus fibrosus	Lateral approach	L2/3–L4/5	NP removal with negative pressure	6/12 weeks	–	Decreased collagen X expression	Decreased (by ~25%)	Yes, degenerated	–	–	369
	16G	66%	Open puncture	5 mm (through the annulus fibrosus)	Posterolateral approach	L3/4, L5/6	–	4/12 weeks	–	–	Decreased (by ~45%)	Yes, degenerated, NP herniation	Yes, progressed	–	368

Additional Table 3. Continued

Animal	Needle size	Needle diameter/disc height (%)	Approach	Depth	Puncture position	Segements	Additional	Degenerated time point/ longest recorded time	Mechanical	Biochemical	Height (longest recorded time)	Histologic and gross	Radiograph and MRI	Neuropathic pain	Reference
	16G	66%	Open puncture	5 mm (through the annulus fibrosus)	Anterolateral approach	L2/3–L6/7	NP removal with negative pressure	2–8/12–24 weeks	Decreased ROM, increased creep displacement	Altered GAG, collagens, aggrecan, MMP3, SOX9 expression	Decreased (by ~25%)	Yes, degenerated, NP herniation	Yes, progressed	–	336-340
	18G	50%	Open puncture	5 mm (through the annulus fibrosus)	Anterior/ lateral approach	L2/3–L6/7	NP removal with negative pressure	1–4/4–14 weeks	–	Decreased GAG, proteoglycan (by ~30%)	Decreased (by ~30%)	Yes, degenerated	Yes, progressed	–	341
	18G	50%	Percutaneous puncture	Through the annulus fibrosus	Lateral approach	L5/6	NP removal with negative pressure	1–4/4–12 weeks	–	Decreased GAG, collagens expression	Decreased (by ~50%)	Yes, degenerated, NP herniation	Yes, progressed	–	342-344
	18G	50%	Open puncture	1 mm (superfical annulus defect); 5 mm (through the annulus fibrosus)	Anterior approach	L2/3–L4/5	–	2/12–24 weeks	–	–	Decreased (NS for 1 mm puncture, by ~25% for 5 mm puncture)	Yes, degenerated, NP herniation (for 5 mm puncture)	Yes, progressed (for 5 mm puncture)		309-311
	19G	44%	Percutaneous puncture	5 mm (through the annulus fibrosus)	Posterolateral approach	L2/3–L4/5	NP removal with negative pressure	9/20 weeks	–	–	Decreased (by ~40%)	Yes, degenerated	Yes, progressed	–	345, 346
	21G	27%	Open puncture	5 mm (through the annulus fibrosus)	Anterior approach	L3/4–L5/6	NP removal with negative pressure	4/12–28 weeks	–	Decreased proteoglycan expression	-	Yes, degenerated	–	–	347, 348
	16/18/21G	66%/50%/27%	Open puncture	5 mm (through the annulus fibrosus)	Anterior approach	L2/3–L5/6	–	4/8 weeks	–	–	Decreased (by ~30% for 16G, by ~10% for 18G/21G)	Yes, degenerated, NP herniation (for 16G/18G)	Yes, progressed (for 16G/18G)		725
Pig	3.2 mm diameter trephine	62%	Open puncture	NA	Anterolateral approach	NA	–	8/39 weeks	–	–	Decreased (by ~15%)	Yes, degenerated	–	–	357
	16G	30%	Open puncture	Through the annulus fibrosus	Anterolateral approach	L2/5	NP removal with negative pressure	3/12–24 weeks	–	Altered Collagens, MMPs, aggrecan, TIMPs expression		Yes, degenerated	Yes, progressed	–	354-356
	20G	17%	Open puncture	Through the annulus fibrosus	NA	L2/3, L4/5	NP removal with negative pressure	12/24 weeks	–	–	–	Yes, degenerated	Yes, progressed	–	315
Rhesus monkeys	15G/20G	41%/20%	Percutaneous puncture	Through the annulus fibrosus	Anterolateral approach	L1/2–L5/6	–	4/12 weeks	–	–	–	Yes, degenerated (more severe in 15G)	Yes, progressed (more severe in 15G)		209
Ovine	3.2–4.5 mm drill	94–100%	Open puncture	9–15 mm (through the annulus fibrosus)	Lateral approach	L1/2–L5/6	–	16 weeks	–	–	–	Yes, degenerated	Yes, progressed	–	602, 603
CD-Canine	NA	30–50%	Open puncture	Through the annulus fibrosus	Dorsal approach	L1/2, L3/4, L5/6	–	14 weeks	–	Altered aggrecan, collagens expression	–	Yes, degenerated	Yes, progressed	–	604

Note: Adam8: a disintegrin and metalloproteinase domain-containing protein 8; Adamts4: a disintegrin and metalloproteinase with thrombospondin motifs-4; Cxcl-1: C-X-C motif chemokine ligand-1 28863006; GAG: glycosaminoglycan; MMP: matrix metalloproteinase; NA: not announced; NP: nucleus puposus; NS: not significant; ROM: range of motion; SOX9: SRY-related high mobility group box 9; TIMP: tissue inhibitors of metalloproteinases.

Additional Table 4. Stimuli-evoked hypersensitivity measurement in rodent model

Subtype	Method	Protocol	Reference
Mechanical	Tactile responses	Rats are placed in individual plexiglass boxes on a stainless-steel mesh floor and are allowed to adjust for at least 20 minutes.	653, 656, 657
		A series of calibrated von Frey filaments (range 4–28 g) is applied perpendicularly to the plantar surface of a hindpaw with sufficient force to bend the filament for 6 seconds.	
		Brisk withdrawal or paw flinching is considered as a positive response.	
		Once a positive response is seen, the previous filament is applied.	
		If positive, the lower filament is determined to be the 50% paw-withdrawal threshold.	
		If negative, the next ascending filament is applied.	
		If that next filament provokes a positive response, the original filament is considered to be the 50% withdrawal threshold.	
		If the next ascending filament is negative, further ascending filaments are applied until a response is provoked.	
		Cautions: Avoid obscure foot pads and surgical incisions, and ensure that the position of the pain measurement is fixed in the central area of the foot; repeat the test four to five times at 5-min intervals on each animal.	
	Mechanical algesia	A von Frey anesthesiometer and rigid von Frey filaments are used to quantifying the withdrawal threshold of the hindpaw in response to mechanical stimulation.	653, 658
		Rats are placed in individual plexiglass boxes on a stainless-steel mesh floor and are allowed to acclimate for at least 20 minutes.	
		A 0.5-mm diameter polypropylene rigid tip is used to apply a force to the plantar surface of the hindpaw.	
		The force causing the withdrawal response is recorded by the anesthesiometer.	
		The anesthesiometer is calibrated before each recording.	
		The test is repeated four to five times at 5-minute intervals on each animal, and the mean value is calculated.	
	Mechanical hyperalgesia/pressure hyperalgesia	The vocalization threshold based on the force of an applied force gauge is measured by pressing the 0.5-cm^2 device tip directly on the dorsal skin over the punctured disks (L4/5).	659, 660
		The force was slowly increased 100 g/s until an audible vocalization is heard.	
		A cut off force of 1000 g is used to prevent tissue trauma.	
		The tests should be carried out in duplicate, and the mean value is taken as the nociceptive threshold.	
		Caution: Postoperative testing should be delayed until one week after surgery to allow the abdominal tissue to heal.	
Thermal	Hot algesia (plate)	Rats were placed within a plexiglass chamber on a transparent glass surface and allowed to acclimate for at least 20 minutes.	653, 654, 661
		A thermal stimulation meter is used with the temperature set to 50°C and the stimulating time set to 30 seconds.	
		Brisk withdrawal or paw flinching is considered as a positive response.	
		The duration from stimulation to positive responses is recorded and noted as paw withdrawal latency.	
		Individual measurements were repeated four to five times. The intermittent period for repetitive measurements of each rat is 15 minutes.	
		The mean value was calculated as the thermal threshold.	
		Cautions: The tests should be restricted to a certain period in a day, like 8-12 a.m., to avoid the influence of memorial reflex. Data from scalded rats should be eliminated to avoid bias.	
	Hot algesia (tail flick test)	Animal are calmed by enclosing their heads with a towel on the apparatus, and acclimate to the test environment for 30 minutes.	314, 655
		Radiant heat is applied to the tail 5 cm from the tip using a tail-fick analgesia meter.	
		Record baseline latencies of the animals. Test the animals' tail-flick response using a tail-flick apparatus, and adjust the intensity of the heat source to produce tail-flick latencies of 3 to 4 seconds. For mice, focus the light beam ~15 mm from the tip of the tail. For rats, stimulate an area ~50 mm from the tip of the tail. In the absence of a withdrawal reflex, set the stimulus cutoff to 10 seconds to avoid possible tissue damage.	
		Record the time for the animal to show a tail-flick response, or assign a value of 10 seconds (cutoff time) if no tail-flick is observed.	
		After sufficient data collection (n = 8 per group and dose), perform statistical analysis and calculate the means and standard errors for data presentation.	
	Cold algesia (hindpaw and back)	The total duration of acetone-evoked behaviours (e.g. flinching, licking or biting) are measured in seconds for 1 minute after a drop of acetone (25 μL) is applied to the plantar surface of the hindpaw using a blunt needle connected to a 1 mL-syringe.	30, 657, 662
		Increased behavioural response to acetone suggests the development of cold hypersensitivity.	
		The grades are recorded as follow: 0, static; 1, slow flinching or paw movement; 2, fast flinching with paw shaking; 3, fast flinching, biting and paw remaining off the ground.	
	Cold algesia (tail)	Animals were placed individually in the test chamber for 60 minutes prior to testing.	256
		Half of the length of the tail was dipped into the cold water, and the latency to tail withdrawal was measured.	
		A maximum cut-off of 30 seconds was set to avoid tissue damage.	

Additional Table 5. Movement-evoked hypersensitivity measurement in rodent model

Method	Protocol	Reference
Grip Force assay	The mice grip a metal bar attached to a Grip Strength Meter (Stoelting Co., Wood Dale, IL, USA) with their forepaws.	256, 657
	The mice are slowly pulled back by the tail, exerting a stretching force.	
	The peak force in grams at the point of release is recorded twice at a 10 minutes interval.	
	A decrease in grip force is interpreted as a measure of hypersensitivity to axial stretching.	
Tail suspension	Mice are suspended individually underneath a platform by the tail with adhesive tape attached 0.5 to 1 cm from the base of the tail and are videotaped for 180 seconds.	256, 657
	The duration of time spent in (a) immobility (not moving but stretched out) and (b) escape behaviours (rearing to reach the underside of the platform, extending to reach the floor, or self-supported at the base of the tail or the suspension tape) are determined.	
	The duration of immobility reflects the animal's willingness to stretch its main body axis.	
	Deceased immobility is indicative of axial discomfort.	
FlexMaze assay	The FlexMaze apparatus consists of a long (8 cm × 80 cm) transparent corridor with regularly spaced staggered doors and neutral (beige) 15 cm × 15 cm compartments with 6 cm × 6 cm openings on either side	256
	The FlexMaze apparatus is placed in a quiet room illuminated with white light.	
	Mice are placed into one of the neutral compartments and are allowed to explore the apparatus freely for 10 minutes.	
	Videotapes are analyzed for total distance covered and average velocity.	

Additional Table 6. Pfirrmann et al.'s classification of disc degeneration

Grade	Structure	Distinction of nucleus and annulus	Signal intensity	Height of intervertebral disc
I	Homogeneous, bright white	Clear	Hyperintense, isointense to cerebrospinal fluid	Normal
II	Inhomogeneous with or without horizontal bands	Clear	Hyperintense, isointense to cerebrospinal fluid	Normal
III	Inhomogeneous, gray	Unclear	Intermediate	Normal to slightly decreased
IV	Inhomogeneous, gray to black	Lost	Intermediate to hypointense	Normal to moderately decreased
V	Inhomogeneous, black	Lost	Hypointense	Collapsed disc space

Note: The classification is widely applied for intervertebral disc degeneration grading.[729]

Additional Table 7. Nomura et al.'s histological grading system

Grade	Annulus fibrosus	Nucleus pulposus
0	Normal structure	Normal structure
1	Mildly serpentine appearance of the annulus fibrosus	No proliferative connective tissue but a honey-comb appearance of the extracellular matrix
2	Moderately serpentine appearance of the annulus fibrosus with rupture	As much as 24% of the nucleus pulposus occupied by proliferative connective tissue
3	Severely serpentine appearance of the annulus fibrosus with mildly reversed contour	25% to 50% of the nucleus pulposus occupied by proliferative connective tissue
4	Severely reversed contour	More than 50% occupied by proliferative connective tissue
5	Indistinct	Complete replacement of normal architecture by proliferative connective tissue

Note: The grading system contained grades of only nucleus pulposus and annulus fibrosus tissues.[760]

Additional Table 8. Masuda et al.'s histological grading scale

Grade	Structure	Scale
I	Annulus fibrosus	1. Normal pattern of fibrocartilage lamellae (U-shaped in the posterior aspect and slightly convex in the anterior aspect), without ruptured fibers and a serpentine appearance anywhere within the annulus
		2. Ruptured or serpentine patterned fibers in less than 30% of the annulus
		3. Ruptured or serpentine patterned fibers in more than 30% of the annulus
II	Border between the annulus fibrosus and nucleus pulposus	1. Normal
		2. Minimal interruption
		3. Moderate or severe interruption
III	Cellularity of the nucleus pulposus	1. Normal cellularity with large vacuoles in the gelatinous structure of the matrix
		2. Slight decrease in the number of cells and fewer vacuoles
		3. Moderate/severe decrease (> 50%) in the number of cells and no vacuoles
IV	Morphology of the nucleus pulposus	1. Normal gelatinous appearance
		2. Slight condensation of the extracellular matrix
		3. Moderate/severe condensation of the extracellular matrix

Note: Histological grading scale based on 4 categories of degenerative changes, with scores ranging from a normal disc with 4 points (1 point in each category) to a severely degenerated disc with 12 points (3 points in each category).

Additional Table 9. Han et al.'s histological grading scale

Grade	Structure	Scale
I	Cellularity of the annulus fibrosus	1. Fibroblasts comprise more than 75% of the cells
		2. Neither fibroblasts nor chondrocytes comprise more than 75% of the cells
		3. Chondrocytes comprise more than 75% of the cells
II	Morphology of the annulus fibrosus	1. Well-organized collagen lamellae without ruptured or serpentine fibers
		2. Inward bulging, ruptured or serpentine fibers in less than one-third of the annulus
		3. Inward bulging, ruptured or serpentine fibers in more than one-third of the annulus
III	Border between the annulus fibrosus and nucleus pulposus	1. Normal, without any interruption
		2. Minimal interruption
		3. Moderate or severe interruption
IV	Cellularity of the nucleus pulposus	1. Normal cellularity with stellar shaped nuclear cells evenly distributed throughout the nucleus
		2. Slight decrease in the number of cells with some clustering
		3. Moderate or severe decrease (> 50%) in the number of cells with all remaining cells clustered and separated by dense areas of proteoglycans
V	Morphology of the nucleus pulposus	1. Round, comprising at least half of the disc area in midsagittal sections
		2. Rounded or irregularly shaped, comprising one-quarter to half of the disc area in midsagittal sections
		3. Irregularly shaped, comprising less than one-quarter of the disc area in midsagittal sections

Note: The scale is based on five categories of degenerative changes, with scores ranging from 5 points (1 in each category) for a normal disc to 15 points (3 in each category) for a severely degenerated disc. [313]

Additional Table 10. Thompson et al.'s description of morphologic grades

Grade	Nucleus	Annulus	Endplate	Vertebral body
I	Bulging gel	Annulus	Hyaline, uniformly thick	Margins rounded
II	White fibrous tissue peripherally	Discrete fibrosus lamellas	Thickness irregular	Margins pointed
III	Consolidated fibrous tissue	Mucinous material between lamellas	Focal defects in cartilage	Early chondrophytes or osteophytes at margins
IV	Horizontal clefts parallel to endplate	Extensive mucinous infiltration; loss of annular-nuclear demarcation	Fibrocartilage extending from subchondral bone; irregularity and focal sclerosis in subchondral bone	Osteophytes less than 2 mm
V	Clefts extend through nucleus and annulus	Focal disruptions	Diffuse sclerosis	Osteophytes greater than 2 mm

Note: The grading system widely employed for histological grading of human discs, distributing equal weights to the nucleus, annulus, endplates, and vertebral body. [767]

Additional Table 11. Boos et al.'s variables of macroscopic and histological assessment

Global disc appearance	Grade
Macroscopic assessment IVD, endplate, and adjacent bone)	Grade 1 = normal juvenile disc; Grade 2 = normal adult disc; Grade 3 = mild disc degeneration; Grade 4 = moderate disc degeneration; Grade 5 = severe disc degeneration
IVD	
Cells (chondrocyte proliferation)	0 = no proliferation; 1 = increased cell density; 2 = connection of two chondrocytes; 3 = small size clones (several chondrocytes, grouped together, 3–7 cells); 4 = moderate size clones (8–15 cells); 5 = huge clones (> 15 cells)
Multiple chondrocytes growing in small, rounded groups or clusters sharply demarcated by a rim of territorial matrix	
Granular changes	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Eosinophilic-staining amorphous granules within the fibrocartilage matrix	
Mucous degeneration	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Cystic, oval, or irregular areas with an intense deposition of acid mucopolysaccharides (i.e., sulfated glycosaminoglycans) staining dark blue with Alcian blue/PAS	
Edge neovascularity	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Newly formed blood vessels with reparative alteration	
Rim lesions	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Radial tears adjacent to the endplates	
Concentric tears	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Tears after the orientation of collagen fiber bundles in the annulus fibrosus	
Radial tears	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Radiating defects extending from the nucleus pulposus to the outer annulus lamellae parallel or oblique to the endplate (clefts)	
Notochordal cells	0 = absent; 1 = present
Embryonic disc cells	
Cell death	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Altered phenotype	
Scar formation	0 = absent; 1 = present
Amorphous fibrosus tissue without any differentiation	
Tissue defects	0 = absent; 1 = present
Voids within the tissue (e.g., resulting from tissue resorption, probably filled with fluid *in vivo*)	
Endplate	
Cells	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Number of cells (chondrocyte clusters)	
Structural disorganization	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Focal disorganization of the cartilaginous matrix with clumping of chondrocytes	
Clefts	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Tears in the endplate	
Microfracture	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Disruption of the subchondral bone	
Neovascularization	0 = absent; 1 = rarely present; 2 = present in intermediate amounts of 1 to 3; 3 = abundantly present
Vessels penetrating from the bone marrow into the endplate in conjunction with microfractures	
New bone formation	0 = absent; 1 = present
Bone islands within the cartilage	
Bony sclerosis	0 = absent; 1 = present
Formation of new bone	
Physiologic vessels	0 = absent; 1 = present
Obliterated vessels	0 = absent; 1 = present
Scar formation	0 = absent; 1 = present
Amorphous fibrosus tissue without any differentiation	
Tissue defects	0 = absent; 1 = present
Voids within the tissue (e.g., resulting from tissue resorption, probably filled with fluid *in vivo*)	

Note: IVD: intervertebral disc; PAS: Periodic acid–Schiff.

Additional Table 12. Boyd et al.'s grading for intervertebral disc and endplate regions

Criteria	Range
Intervertebral disc region	
Chondrocyte proliferation/density	0–6
Mucous degeneration	0–4
Cell death	0–4
Tear/cleft formation	0–4
Granular changes	0–4
Vertebral endplate region	
Cell proliferation	0–4
Cartilage disorganization	0–4
Cartilage cracks	0–4
Microfracture	0–2
New bone formation	0–2
Bony sclerosis	0–2

Note: A grading system was formed by Boyd et al.[232] with extracted 11 criteria.

Additional Table 13. Methods for the evaluation of adhesive properties

	Method	Protocol	Reference
In vitro	ASTM F2256-05 (T-Peel by Tension Loading)	At least 10 specimens of each type are to be tested.	773
		Tissue specimen thickness should be uniform and less than 5 mm.	
		The specimen width is 2.5 ± 0.1 cm, and the specimen length is 15 ± 0.2 cm (2.5 cm unbonded, 12.5 cm bonded).	
		A bond force of 5–10 N is applied until the experimental adhesive sets.	
		The specimens are conditioned for 1 hour ± 15 minutes in phosphate buffered saline at 37 ± 1°C.	
		After conditioning, samples are acclimated to the test temperature for 15 minutes.	
		The sample apparatus is loaded into the tensile test machine and at a constant cross-head speed of 250 mm/min.	
		The load as a function of displacement and the type of failure (percentage cohesive, adhesive, or substrate failure) are recorded	
	ASTM F2258-05 (Tension)	At least ten specimens of each type are to be tested.	773
		The bond area of 2.5 ± 0.005 cm by 2.5 ± 0.005 cm.	
		A bond force of 1–2 N is applied until the experimental adhesive sets.	
		The specimens are conditioned for 1 hour ± 15 minutes in phosphate buffered saline at 37 ± 1°C.	
		After conditioning, samples are acclimated to the test temperature for 15 minutes.	
		The sample apparatus is loaded into the tensile test machine and at a constant cross-head speed of 2 mm/min.	
		The load at failure (maximum load sustained) and the type of failure (percentage cohesive, adhesive, or substrate failure) are recorded.	
	ASTM F2255-05 (Lap-Shear by Tension Loading)	At least 10 specimens of each type are to be tested.	774
		The length of the tissue substrate attached to each specimen holder should be 1.5 times the length of the bond area (1.0 ± 0.1 cm).	
		The tissue specimens should be 1–2 mm thick.	
		A bond force of 1–2 N is placed on the bond area between the two tissue specimens (1.0 ± 0.1 cm by 2.5 ± 0.1 cm) until the experimental adhesive sets.	
		The specimens are conditioned for 1 hour ± 15 minutes in phosphate buffered saline at 37 ± 1°C.	
		After conditioning, samples are acclimated to the test temperature for 15 minutes.	
		The sample is loaded into the testing apparatus such that the load coincides with the long axis of the sample.	
		The sample is loaded to failure at a constant crosshead speed of 5 mm/min. The load at failure (maximum load sustained) and the type of failure (percentage cohesive, adhesive, or substrate failure) are recorded.	
	ASTM F2458-05 (Wound Closure Strength of Tissue Adhesives and Sealants)	At least ten specimens of each type are to be tested.	775
		Two tissue samples of identical size (10 ± 0.2 cm by 2.5 ± 0.1 cm) are bonded using the experimental adhesive on the 2.5 cm side, with a bonding length of 0.5 cm on either side of the join line.	
		The thickness of the specimens should be uniform and less than 5 mm.	
		The specimens are conditioned for 1 hour ± 15 minutes in phosphate buffered saline at 37 ± 1°C.	
		After conditioning, samples are acclimated to the test temperature for 15 minutes.	
		The sample is loaded into the testing apparatus such that the load coincides with the long axis of the sample.	
		The distance from the grip to the midline of each sample is 5 cm, with the remaining 5 cm held between the grips.	
		The specimen is loaded to failure at a constant speed of 50 mm/min.	
		The time from application to testing (cure time), force at failure (maximum force required to disrupt substrate), and the type of failure (percentage cohesive, adhesive, or substrate failure) are recorded.	
	ASTM F2392-04 (Burst Strength of Surgical Sealants)	At least 10 specimens of each type are to be tested.	776
		This test employs an apparatus that clamps down on a substrate to prevent leakage.	
		The thickness of the tissue should be uniform and not exceed 5 mm.	
		Tissue samples should be circles 3.0 ± 0.1 cm in diameter, in which a 3.0 mm diameter hole is created using a biopsy punch.	
		The specimens are conditioned for 1 hour ± 15 minutes in phosphate buffered saline at 37 ± 1°C.	
		After conditioning, samples are acclimated to the test temperature for 15 minutes.	
		This test uses a stationary fixture containing test substrate and the sealant to be tested.	
		A 1.0 mm thick PTFE mask with a 15 mm diameter hole is secured over the sample, with the hole in the mask centered with the hole in the sample.	
		Saline is pumped into the fixture at a constant rate of 2 mL/min, and pressures are measured at all time points.	
		Peak pressure and failure type (cohesive, adhesive, or substrate) are recorded.	
Ex vivo (risk of herniation)	Ramp-to-Failure Testing	Herniation risk was evaluated through failure testing using a MTS Bionix Servohydraulic Test System (MTS, Eden Parairie, MN, USA).	117
		Specimens were placed on the MTS stage at an offset of 5° from the normal axis, with the postero-lateral portion of the disc at the outside of the bend to simulate postero-lateral flexion.	
		A force of ~20 N was applied as a pre-load.	
		The samples were then compressed in displacement-control mode using a ramp function at 2 mm/min until failure.	
		Biomechanical output measures that quantitatively describe IVD herniation risk include failure strength, failure strain, subsidence-to-failure, maximum stiffness, work-to-failure, yield strength, ultimate strength, and the ratios of the ultimate or yield strength to the failure strength of the motion segment.	
	Fatigue Endurance Testing	The fatigue loading protocol consisted of cyclic eccentric compression between 50 N and 300 N at 1 Hz and at an offset of 20 mm to induce a physiological bending moment of 6 N·m.	117
		The loading indenter cyclically rotated from −135° to +135° from the axis opposite of the incision site at 15° increments with 1 minute of cyclic loading at each location.	
		This test setup was considered to mimic the "worst-case scenario" as loading opposite of the injury site was expected to aggravate NP extrusion.	
		Failure was defined by significant NP protrusion greater than 2 mm.	
		The main outcome measure from the fatigue tests was cycles-to-failure, which was indicative of fatigue endurance.	

Note: IVD: intervertebral disc; MTS: material test system; NP: nucleus pulposus; PTFE: polytetrafluoroethylene.

Additional Table 14. A paradigm for testing intervertebral disc mechanical properties.

Items	Purpose	Reference for protocals
Adhesion evaluation (*in vitro* and *ex vivo*)	To determine the tissue integrating strength after implantation	Additional Table 13
Tension/compression/shear evaluation (*in vitro*)	To determine whether the mechanical properties of biomaterials match with that of human tissue	386
Swelling (*in vitro*)	To determine whether biomaterials will swelling and its potential damage to surrounding tissue	538
Gelation kinetics (*in vitro*)	To determine whether the gelation time is suitable for clinical application	464, 538
Failure test & fatigue failure test (*ex vivo*)	To determine the herniation risk under extensive and prolonged mechanical loadings	778, 779
Biomechanics test (*ex vivo*)	To determine whether biomaterials will maintain the motion segment biomechanics	601, 780
Compressive/torsional/tensile stiffness; creep displacement; torque range; axial range of motion (*ex vivo*)	To determine the biomechanical reparative effects of implanted biomaterials	452, 461, 462, 601

Additional Table 15. Recommended parameters for disc regeneration

Parameter	Recommended value
Disc pressure, after implantation	1.50 MPa
Disc pressure, maximal (till failure)	2.30 MPa
Tensile modulus, axial	0.5–1 MPa
Compressive/tensile strain	28%/65%
Axial stiffness of restored intervertebral disc	1.5–2 kN/mm
Torsional stiffness of restored intervertebral disc	3.2 N·m/°
Tensile modulus, circumferential	11–29 MPa
Aggregate modulus	0.4–6 MPa
Shear modulus	0.1–0.28 MPa

Note: The recommended parameters for mechanical properties after biomaterials implantation were from Long et al.[28]