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Repair and reconstruction of intervertebral disc degeneration

From tissue engineering to organoid and assembloid construction

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Abstract: Intervertebral disc degeneration (IDD) is the primary pathological driver of debilitating low back pain, representing a massive global socioeconomic burden. Current clinical management relies on palliative analgesia or destructive surgical fusion, which fails to halt degeneration and risks adjacent segment disease. The irreconcilable conflict between palliative clinical management and escalating degenerative burden underscores the imperative for biologically rational reconstruction strategies. This review synthesizes recent advances in regenerative approaches for IDD, progressing from targeted tissue engineering towards the emerging paradigm of organoid and assembloid construction. Conventional tissue engineering strategies focus on mitigating the pathological microenvironment (inflammation, oxidative stress, hypoxia, and acidosis) and restoring the structural integrity of degenerated disc components. For the nucleus pulposus, injectable biomaterials deliver anti-inflammatory/antioxidant agents, modulate immune cell infiltration, enhance hypoxia tolerance, and serve as biomechanical surrogates. Annulus fibrosus repair employs suturing techniques, biomimetic angle-ply patches, adhesive hydrogels, and scaffolds modulating the local niche to promote endogenous repair and extracellular matrix integration. Cartilaginous endplate strategies aim to enhance nutrient transport and combat degeneration via matrix modification or stem cell-derived exosome delivery. While promising, these approaches often face limitations in achieving native-equivalent biomechanics, seamless interfacial integration, and replicating the disc's complex heterocellular composition. Organoid technology, recapitulating developmental processes, offers a transformative solution. Significant progress has been made in generating nucleus pulposus-like cells from induced pluripotent stem cells via defined signaling pathways, differentiating induced pluripotent stem cells towards annulus fibrosus-like fates, and constructing cartilage organoids for cartilaginous endplate modeling. These self-organizing 3D structures better mimic native tissue microarchitecture, cellular diversity, and matrix composition than traditional scaffolds. Furthermore, "assembloid" strategies, involving the fusion of distinct organoids or their combination with specialized scaffolds, present a revolutionary framework for holistic disc reconstruction, overcoming the limitations of ad hoc component assembly. Despite the immense potential of organoid/assembloid platforms for creating developmentally inspired, functional disc replacements, key challenges remain: standardization of protocols, biomimetic engineering of critical disc-vertebral interfaces, and achieving physiological biomechanical competence. Future translation necessitates GMP-compliant biomanufacturing, advanced material integration, and optimized maturation protocols. The evolution from palliative care and reductionist tissue engineering towards organoid/assembloid-based reconstruction heralds a new era in biologically rational IDD therapy.

Keywords: assembloid, intervertebral disc degeneration, organoids, regenerative medicine, tissue engineering

1. Introduction

Low back pain (LBP) constitutes the predominant global cause of years lived with disability among musculoskeletal disorders. Epidemiological analyses of disease burden data further corroborate LBP as the foremost disabling musculoskeletal condition.[1] It is well-established that approximately 80% of individuals will experience LBP during their lifetime, with a substantial subset requiring medical intervention and incurring productivity losses due to work absenteeism.[2] In the United States, the American Academy of Pain Medicine (2006) documented estimated annual costs for chronic pain ranging from $560 to $635 billion USD, notably attributing

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53% of the chronic pain population to LBP—affecting an estimated 31 million Americans at any given time.[2–4] While LBP etiology remains incompletely understood and multifactorial, intervertebral disc degeneration (IDD) is mechanistically implicated. Discogenic disorders associated with LBP affect ~632 million individuals worldwide, constituting nearly 40% of all LBP cases.[1]

Contemporary clinical management of IDD and resultant LBP predominantly employs palliative strategies centered on pharmacologic analgesia and symptomatic control. These approaches primarily utilize nonsteroidal anti-inflammatory drugs as foundational analgesics, augmented by muscle relaxants and herbal stasis-resolving compounds (e.g., traditional Chinese medicine formulations) to alleviate patient-reported numbness and stiffness. Crucially, such interventions neither arrest disease progression nor modify its natural history. As degeneration advances—typically manifesting as spinal stenosis and severe radicular compression—surgical intervention frequently becomes necessary. This generally entails decompressive procedures coupled with instrumented segmental fusion, a "destructive" stabilization strategy that achieves spinal fixation through deliberate sacrifice of segmental mobility. Regrettably, this approach predisposes to adjacent segment degeneration, consequently accelerating further loss of spinal function.

To address this challenge, the scientific community pursues 2 primary strategies (1) Elucidating the precise mechanisms underlying IDD to develop disease-modifying antidegenerative drugs capable of slowing disease progression; (2) Advancing tissue engineering approaches to create functional biological implants for repairing or reconstructing severely degenerated disc components—including the nucleus pulposus (NP), annulus fibrosus (AF), and cartilaginous endplate (CEP). Notably, however, no clinically validated disease-modifying antidegenerative drugs for IDD have yet been developed. Consequently, given the global demographic aging trend, a substantial population of patients with end-stage IDD is anticipated to emerge, creating an urgent clinical need for tissue-engineered grafts capable of structural and functional restoration.

2. Nucleus pulposus degeneration and tissue engineering repair and reconstruction strategies

2.1. Brief introduction of nucleus pulposus and nucleus pulposus degeneration

Centrally positioned within the intervertebral disc (IVD), the NP derives its nomenclature from the Latin *nucleus pulpaōsus* ("pulpy core")—a descriptor precisely reflecting its physical properties. Healthy NP tissue exhibits high water content and a translucent, gelatinous gross morphology. It is confined superiorly and inferiorly by CPE and circumferentially enveloped by the AF. Significantly, removal of the endplates induces immediate NP extrusion, demonstrating its physiological maintenance under substantial hydrostatic pressure.[5] Histologically, the NP comprises a highly hydrated, proteoglycan-rich extracellular matrix (ECM) (predominantly aggrecan) interspersed with collagen and elastic fibers. Collagen fibers exhibit random orientation, while elastic fibers demonstrate radial organization originating from the NP center. Functionally, the NP primarily absorbs axial spinal loads and distributes compressive forces radially. Embryologically, it originates from endoderm-derived notochordal tissue. Residual notochordal cells undergo postnatal involution, with mature NP homeostasis sustained by chondrocyte-like cells.[6]

The healthy NP is physiologically avascular. During IVD development, transient vasculature initially supplies the primordial CEPs and develops disc tissues. Upon skeletal maturation, these vessels undergo obliteration through scarring and regression. Concurrently, capillaries penetrating adjacent vertebral growth plates terminate at the CEP surfaces without perfusing the disc proper. Consequently, the IVD becomes the largest avascular structure in humans. Cells within the outermost AF derive nutrients and exchange metabolites via vasculature in adjacent soft tissues (e.g., longitudinal ligaments). In contrast, the NP and inner AF rely exclusively on small-molecule diffusion for metabolic sustenance. This avascular architecture chronically exposes the NP and inner AF to a hostile microenvironment characterized by hypoxia and acidic pH. Such conditions further compromise intrinsic reparative mechanisms during disc degeneration.[7,8]

NP degeneration represents the core pathological driver of IDD. The term "intervertebral disc degeneration" itself originated from characteristic magnetic resonance imaging T2-weighted findings—specifically diminished NP hydration and disc height reduction. The Pfirrmann grading system, currently the gold standard imaging classification for IDD, principally evaluates degeneration severity based on NP T2 signal intensity parameters. NP degeneration constitutes a multifaceted pathological process characterized by 3 interconnected hallmarks[9–12] (1) Hydration deficit: age-related and stress-induced depletion of NP proteoglycans progressively compromises tissue hydration capacity, critically impairing disc viscoelasticity; (2) ECM catabolism: disc cells overexpress matrix metalloproteinases and A Disintegrin and Metalloproteinase with Thrombospondin Motifs. These enzymes degrade essential ECM components (e.g., aggrecan and collagens), disrupting structural integrity while concomitantly suppressing ECM anabolism and reparative capacity; (3) Inflammatory activation and oxidative stress: degenerative niches exhibit localized inflammation and reactive oxygen species (ROS) accumulation, particularly in herniated or injured discs. Upregulated pro-inflammatory cytokines—including tumor necrosis factor-alpha and interleukins (ILs)—synergize with ROS to accelerate ECM degradation and drive a self-sustaining degeneration cascade.

Tissue engineering-based strategies for NP repair represent promising therapeutic interventions for NP degeneration.[13] These approaches target core pathological drivers—including the dysregulated inflammatory milieu, ROS accumulation, and deteriorating niche conditions—with the dual objectives of arresting NP degeneration progression and promoting functional tissue regeneration. This review synthesizes recent advances by examining biomaterial-pathology interactions across 5 strategic dimensions.

2.2. Tissue engineering strategies targeting the inflammation and reactive oxygen species environment

Recent research identifies intradiscal inflammation and ROS accumulation as pivotal pathogenic drivers in IDD. Inflammatory dysregulation and ROS excess directly mediate ECM degradation, compromise disc microarchitecture, and precipitate accelerated cellular senescence/apoptosis. Furthermore, inflammation facilitates aberrant neural ingrowth and sustained nociceptive signaling, thereby perpetuating a degenerative cascade. The disc's inherent avascularity severely limits systemic anti-inflammatory drug efficacy, while repeated local injections risk iatrogenic structural compromise. Biomaterial-based delivery platforms consequently represent a promising disease-modifying approach for spatiotemporal modulation of inflammatory pathways in IDD.[14]

High molecular weight hyaluronic acid (HA) hydrogels represent promising biomaterials for NP regeneration, exhibiting intrinsic anti-inflammatory properties while fostering an NP-conducive microenvironment. Isa et al.[15] developed a polyethylene glycol-crosslinked HA hydrogel that demonstrated therapeutic efficacy through IL-1R1/MyD88 pathway suppression and concomitant downregulation of *NGF* and *BDNF* gene expression, thereby establishing a regenerative niche for NP tissue repair. Additionally, they established a novel rat model of disc injury-induced pain, with phenotypic validation via morphine responsiveness. Assessment of HA hydrogel efficacy demonstrated significant attenuation of nociceptive behaviors, correlating with downregulated nociception markers and suppressed hyperinnervation. Moreover, the hydrogel modified glycosylation patterns and modulated key inflammatory/regulatory signaling pathways, culminating in reduced inflammation and regulated ECM composition. These findings position HA hydrogel as a viable clinical therapeutic for disc degeneration-mediated back pain.[16]

Excessive inflammatory responses accelerate the senescence of nucleus pulposus progenitor cells (NPPC), decreasing its regeneration potential. Strategies to remodel the regeneration capacity of senescent NPPC are urgently needed. To overcome this challenge, Zhao et al. engineered NPPC-targeted lipid-thymine nanoparticles for *in situ* regenerative reprogramming. Results showed that NPPC-targeted lipid-thymine nanoparticles could enhanced NPPC regenerative potential. Mechanistically, customized lipid nanoparticles efficiently delivered *Klotho* circular RNA (circRNA) into NPPCs, scavenged chemokines (MCP-1/IL-8), modulated inflammatory microenvironments, inducing a rejuvenated phenotype while rebalancing ECM synthesis/catabolism *in vitro* and *in vivo*.[17]

Furthermore, Bian et al. demonstrated that injectable "peptide-cell-hydrogel" microspheres—constructed through covalent conjugation of APETx2 followed by NP cell encapsulation—effectively suppressed localized cytokine storms *in vitro*. This intervention restored ECM metabolic homeostasis, establishing a novel regenerative strategy for inflammatory microenvironment management.[18] Additionally, numerous natural compounds possess potent anti-inflammatory properties. Zhu et al. proposed a new functionalization strategy using vanillin, a natural molecule with anti-inflammatory and antioxidant properties, to develop multifunctional gelatin methacrylate (GelMA) microspheres for local delivery of transforming growth factor-β3 (TGF-β3) toward IVDD treatment. *In vitro* and *in vivo* studies showed that such a functionalized platform plays roles in alleviating inflammation and oxidative stress, preserving the water content of NP and disc height, and maintaining intact structure and biomechanical functions, thereby promoting the regeneration of IVD.[19]

ROS accumulation accelerates NP cell apoptosis, thereby exacerbating IDD. To address this, Bu et al. engineered an injectable dynamic hydrogel (HA-NCSN/Cu) through reductive chelation between thiourea-grafted hyaluronic acid (HA-NCSN) and Cu^{2+}. The abundant thiourea moieties within this hydrogel effectively scavenged ROS, ameliorating inflammatory stress in NP cells. Experimental results demonstrated that the HA-NCSN/Cu hydrogel promoted both structural and functional restoration of degenerated IVD.[20]

Besides, Bai et al. engineered a rapamycin-loaded ROS-scavenging scaffold (Rapa@Gel), establishing a novel paradigm for modulating the local inflammatory microenvironment to enhance IVD regeneration. This therapeutic platform—comprising an ROS-degradable injectable hydrogel—precisely targets degenerated disc sites while enabling ROS-responsive release kinetics. Scaffold-mediated ROS depletion significantly attenuates inflammatory cascades. Crucially, Rapa@Gel treatment in rat models induced macrophage phenotypic repolarization, characterized by increased M2-polarized macrophages and concurrent reduction of M1-polarized subsets. This immunomodulatory shift, coupled with inflammation resolution, effectively promoted structural and functional IVD restoration.[21]

2.3. Tissue engineering strategies targeting the hypoxic and acidic microenvironment

In mature, healthy IVDs, vascularization is restricted exclusively to the outer AF and CEPs. The inner AF and NP rely on passive, concentration gradient-driven solute exchange via simple diffusion through the CEPs. This transport mechanism establishes a distinctive hypoxic and acidic microenvironment within the NP.

Although physiologically hypoxic, IDD exacerbates intranuclear oxygen deprivation, thereby inhibiting disc regeneration. This suggests that therapeutic oxygen modulation may promote regenerative processes. Zhen et al. incorporated perfluorotributylamine (PFTBA) as an oxygen-regulating agent within alginate scaffolds for *in vitro* and *in vivo* disc regeneration. Their findings demonstrate that PFTBA enhances NP cell viability and proliferation, optimally restores ECM composition toward native disc architecture while preserving NP phenotype markers, and significantly rescues disc height as well as ECM integrity in murine degeneration models, establishing PFTBA's ameliorative potential against disc degeneration through oxygen-mediated regenerative mechanisms.[22]

During IDD, inflammatory factor accumulation within the NP elevates lactate, exacerbating the inflammatory microenvironment via a positive feedback loop. To address this, Zhan et al. engineered CAP-sEXOs@Gel, a $CaCO_3$/chitosan hydrogel incorporating cartilage endplate cell (CEPC)-targeted exosomes loaded with salvianolic acid A. Upon CEPC uptake, CAP-sEXOs inhibit HIF-2α/TfR1 expression, reducing intracellular iron and ROS. This preserves mitochondrial function and decreases mtDNA leakage, suppressing the cGAS-STING pathway. Simultaneously, the hydrogel's $CaCO_3$ neutralizes H^+, mitigating NP cell inflammation.[23]

2.4. Tissue engineering strategies targeting the microenvironment of immune cell infiltration

IVD was originally in immune privilege prevented the attack of immune cells (mainly effector T cells and macrophages).[24] During IDD, progressive loss of disc integrity occurs alongside gradual immune cell infiltration. Immune regulatory and inflammatory factors released by effector T cells, macrophages, and the IVD further aggravate IVDD.[25,26] Elevated IL-1β, tumor necrosis factor-alpha, and other inflammatory mediators significantly drive degenerative cascades, thereby chemotactically recruiting neutrophils to the disc microenvironment. Consequently, biomaterial-based interventions that block immune cell migration and restore ECM synthesis-catabolism homeostasis represent a promising therapeutic paradigm for IVD management.[27]

Zhou et al. developed an immunomodulatory hydrogel microsphere system integrating biomimetic cell-membrane coating and surface functionalization strategies. This platform grafts neutrophil membrane-coated poly(lactic-co-glycolic acid) nanoparticles (T-NNPs) encapsulating transforming growth factor-β1 (TGF-β1) onto methacrylated gelatin microspheres (GM) via amide bonding. The resulting GM@T-NNP complex demonstrated sustained release of biofunctional T-NNPs over 36 days as well as effective sequestration of pro-inflammatory cytokines. In rat IDD models, GM@T-NNPs significantly rescued disc height deterioration and restored NP ultrastructure and biomechanical competence. This synergistic integration of biomimetic nanotechnology and drug delivery paradigms expands the therapeutic application space for cell-membrane engineered materials while establishing a novel translational framework for IDD intervention.[28]

Furthermore, inspired by the natural mucin coating that enables living organisms to evade immune recognition, Wang et al. utilized mucin-derived gels (Muc-gels). These gels form *in situ* at the surgical site following microdiscectomy. Encapsulating IVDs within the Muc-gels prevented fibrous encapsulation and macrophage infiltration in a mouse subcutaneous model. Moreover, injection of Muc-gels inhibited IVD degeneration in a rat tail model for up to 24 weeks postoperatively. Mechanistic investigations revealed that Muc-gels attenuate immune cell infiltration into the NP, thereby providing durable protection against immune attack after microdiscectomy.[29]

Discogenic pain secondary to IDD represents a prevalent therapeutic challenge. Macrophage infiltration and ectopic neurite ingrowth—consequences of structural disruption within the disc—constitute primary pathomechanisms underlying this condition. To address this, Wang et al. elucidated how metal-polyphenol nanoparticles synergistically modulate the immune microenvironment and mitochondrial dynamics to alleviate discogenic pain. This antioxidant nanoparticle system sequentially reprogrammed macrophage polarization states, enhanced mitochondrial transfer efficiency, and facilitated sustained mitochondrial delivery to compromised cells. *In vivo*, the formulation preserved disc height, maintained NP structural integrity, and restored nociceptive thresholds—demonstrating comprehensive therapeutic efficacy.[30]

2.5. Tissue engineering strategies for rebuilding normal structure

The ultimate therapeutic objective in IDD management is *in situ* regeneration of the degenerated NP. Tissue engineering strategies represent a cornerstone approach for achieving this regenerative goal in IDD treatment.

Injectable hydrogels represent a prominent therapeutic strategy for IDD due to their ECM-mimetic properties that support cellular functions and compatibility with minimally invasive delivery. Jia et al. engineered a shear-thinning hydrogel (GPG) through glycerol-mediated crosslinking of poly(vinyl alcohol) chains, which exhibited a high biosafety profile, mechanical adaptability to disc microenvironment, and significant energy dissipation capacity under cyclic loading. In addition, *in vivo* evaluation demonstrated sustained therapeutic efficacy in both IDD and nucleotomy (NPD) models, establishing GPG's translational promise for minimally invasive clinical management of disc degeneration.[13]

Furthermore, inspired by the GPG work, Wang et al. fabricated a novel injectable and self-crosslinking adhesive organohydrogel, designated GPG-AG. This material possesses viscoelastic properties closely resembling those of the native NP tissue and exhibits strong adhesion to the IVD, thereby mitigating the risk of postinjection leakage under dynamic loading. Notably, results from the *in vivo* study demonstrated that the GPG-AG-treated group achieved a significantly higher IVD height index following needle puncture compared with both the previously reported GPG and the control group. Taken together, this organohydrogel represents a promising candidate for managing IDD.[31]

Furthermore, ferroptosis of NPCs induced by discectomy contributes to the pathogenesis of postoperative lumbar disc herniation recurrence and IDD. To address this challenge, Wang et al. developed synthetically engineered exosomes derived from NPPCs, exhibiting enhanced miR-221-3p expression and improved NPC uptake efficiency. These exosomes were integrated into an injectable hydrogel based on ECM-mimetic analogues. This ECM-mimetic hydrogel (HACS) functions as a biomimetic filler for postoperative management of herniated discs, facilitating facile injection into the NP cavity created by discectomy for localized therapy. Sustained, HACS-mediated *in situ* release of exosomes within the NP cavity achieves significant inhibition of ferroptosis in NPCs.[32]

Conley et al. engineered a dynamic multifunctional nanohybrid peptide hydrogel through hierarchical self-assembly of peptide amphiphiles incorporating enzyme-mimetic biodegradable 2D nanomaterials. This construct significantly enhanced NP cell differentiation and attenuated pain in rat nucleotomy models, demonstrating its therapeutic efficacy for disc regeneration.[33] Also, Tao et al. covalently conjugated 3 distinct bone morphogenetic protein (BMP) 7-derived functional peptides to the C-terminus of RADA16-I. *In vitro* evaluation demonstrated excellent biocompatibility of all functionalized self-assembling peptide hydrogels (RAD-SNV, RAD-KPS, and RAD-KAI) with human degenerated nucleus pulposus cells. Notably, both RAD-SNV and RAD-KPS scaffolds exhibited significant potential for NP tissue engineering applications, with RAD-KPS demonstrating superior efficacy relative to RAD-SNV.[34]

3. Annulus fibrosus injury and tissue engineering reconstruction strategies

3.1. Brief introduction of annulus fibrosus and annulus fibrosus injury

The term "anulus" (often spelled "annulus" in English) derives from the Latin "*anus*," meaning "ring," and the English term directly translates to "fibrous ring." This word aptly describes and defines the AF: it is a fibrous structure encircling the NP and CEP of the IVD, tightly enveloping the NP akin to a "bandage." The AF also determines the overall shape and size of the entire IVD.

A typical AF is composed of 15 to 25 concentric layers (lamellae) of thin collagen fibers. Within adjacent layers, the fiber orientation runs parallel in the sagittal plane but forms an angle of approximately 60° to each other in the coronal plane projection. The outer annulus connects to the anterior and posterior longitudinal ligaments and the adjacent vertebral bodies above and below. The inner annulus connects to the superior and inferior CEPs via elastic fibers. These elastic fibers run perpendicular to the collagen lamellae and traverse them longitudinally, forming Sharpey's fibers. This complex crossed-fiber grid structure of collagen and elastic fibers significantly enhances the stiffness of the AF.

Acting like a tight bandage around the NP, this dense and robust AF prevents excessive deformation and posterior protrusion (herniation) of the NP when it bears axial loading from the spine. The outer annulus is primarily populated by elongated, "fibroblast-like" cells aligned parallel to the fibrous tissue. The inner annulus contains more rounded, "chondrocyte-like" cells. Interestingly, the annulus also harbors cells with multiple long processes, morphologically suggestive of mechanoreceptor cells.

Biochemical composition studies reveal that the AF contains approximately 65% water. The remaining dry weight consists mainly of ~55% collagen, ~20% proteoglycans, and ~10% elastic fibers. Further research indicates the presence of both type I and type II collagen fibers within the annulus, along with smaller amounts of type III, V, VI, and IX collagen. Compared with type II collagen, type I collagen possesses greater stiffness and is predominantly located in the outer annulus, providing the histological basis for its strong mechanical support. Conversely, the more compliant type II collagen is mainly found in the inner annulus, affording greater deformability at the interfaces with the NP and CEP.

After skeletal maturity, a distinct boundary exists between the NP and the AF. The AF also relies on the integrity of the NP to support its inner fibers, preventing their inward collapse and deformation.[35] The AF's gradient in water content and Col II from its inner to outer layers facilitates a smooth mechanical transition. Moreover, its layered architecture contributes to the AF's nonlinear and anisotropic mechanical properties.

3.2. Reconstruction of annulus fibrosus structural integrity by suturing and repair patches

When AF integrity is compromised, surgical discectomy constitutes the primary clinical intervention for symptomatic relief. However, this approach neither halts degenerative progression nor prevents adjacent segment deterioration, and may accelerate secondary pathologies. Consequently, reconstructing AF structural continuity has emerged as a critical strategy to mitigate surgical complications and impede IDD advancement. Hydrogels and anisotropic fibrous scaffolds represent promising biomaterial platforms for achieving functional AF restoration.

In 2016, Bateman et al. conducted a study utilizing 15 *ex vivo* porcine spinal motion segments. Following simulated discectomy, the annular defect was repaired using a 2–0 nonabsorbable ultrahigh molecular-weight polyethylene suture secured with a Dines knot. Their findings demonstrated the potential utility of a novel suture closure device for AF repair after surgical discectomy. Specifically, a Dines knot configuration incorporating 4 half-hitches yielded optimal performance. Subsequent *in* *vivo* studies further validated the feasibility of AF defect closure, maintained over a 4-week period. These results provide the basis for a planned clinical study aimed at assessing the feasibility, safety, and efficacy of this device in patients undergoing surgery for lumbar disc herniation.[36]

Furthermore, repair patches offer a more effective strategy for treating large AF injuries where suturing is challenging or impractical. Borem et al. developed a novel collagen-based, multilaminate AF repair patch designed to mimic the angle-ply architecture and fundamental tensile properties of the native human AF. Results demonstrated the patch's mechanical suitability for implantation into the mechanically demanding spinal environment, while also illustrating its biologic functionality in supporting IVD cell activity.[37]

In addition, the acute inflammation that arises following AF injury can further compromise disc integrity and contribute to long-term, disc-wide degeneration. To address this issue, Peredo et al. designed and fabricated tension-activated repair patches (TARPs) for AF repair and local delivery of the anti-inflammatory agent anakinra, a recombinant interleukin-1 receptor antagonist. The researchers assessed the therapeutic efficacy of TARP-mediated annular repair combined with anakinra delivery in a goat model of cervical spine annular injury. Results demonstrated that TARPs effectively integrated with host tissue, providing structural reinforcement at the injury site and preventing aberrant disc-wide remodeling caused by loss of tensile integrity in the AF. Moreover, local anakinra delivery via TARP implantation enhanced matrix deposition and retention at the injury site while improving the maintenance of disc ECM. Additionally, anakinra attenuated the inflammatory response associated with TARP implantation, reducing osteolysis in adjacent vertebrae and preserving disc cellularity and matrix organization throughout the AF.[38]

To address poor tissue adhesion and herniation risks during spinal loading, DiStefano et al. engineered a dual-modified glycosaminoglycan strategy. Oxidized-methacrylated hyaluronic acid chemically anchors an injectable fibrin-conjugated polyethylene glycol diacrylate (PEGDA) hydrogel to AF tissue. Oxidized-methacrylated hyaluronic acid demonstrated superior tissue adhesion versus modified chondroitin sulfate, with oxidation degree dictating adhesive strength more than methacrylation. *In ex vivo* bovine discectomy models, lower molecular weight PEGDA (20 kDa) produced hydrogels with optimized modulus that reduced herniation risk—surpassing discectomy-only controls. This approach covalently bonds hydrogels to disc ECM proteins, effectively sealing defects while offering tunable mechanical properties for enhanced IVD repair.[39]

3.3. Reconstruction of annulus fibrosus structural integrity by microenvironment modifying hydrogels

Beyond direct mechanical repair strategies (e.g., suturing or patches) for AF defects, emerging research focuses on postinjury microenvironmental remodeling. These approaches employ bioactive factors to modulate the local niche, aiming to mobilize endogenous stem cells or recruit exogenous stem cells for functional AF defect repair. Meng et al. engineered high-strength PDA/GelMA microneedles (MNs) for minimally invasive AF penetration. These near-infrared-responsive MNs enable remotely controlled drug release and photothermal therapy. Loaded with diclofenac sodium, they extracellularly neutralize inflammatory microenvironments while intracellularly upregulate cytoprotective heat shock proteins, achieving dual "offensive-defensive" effects. *In vitro*, synergistic photothermal-anti-inflammatory treatment reduced inflammation, suppressed apoptosis, and enhanced ECM synthesis. *In vivo*, MNs attenuated inflammation, promoted ECM deposition, reduced apoptosis, and restored IVD biomechanics in rats. This system provides a novel strategy for disc repair through microenvironmental modulation.[40]

To improve the poor integration between implanted scaffolds and AF tissue. Zhao et al. engineered a multifunctional scaffold delivering lysyl oxidase (LOX) plasmid DNA via exosomes with MnO_2 nanoparticles. LOX enhances ECM crosslinking while MnO_2 scavenges ROS, synergistically preventing ECM degradation. The scaffold promoted seamless integration with AF tissue without fibrotic encapsulation, enabling cellular infiltration. This functional integration significantly improved mechanical properties and inhibited vascular invasion, demonstrating critical scaffold-tissue synergy for effective AF repair.[41]

To facilitate the migration of AF cells, Wei et al. engineered a photocurable injectable hydrogel by combining PEGDA and decellularized annulus fibrosus matrix (DAFM) for AF repair. PEGDA significantly enhanced DAFM hydrogel mechanical strength while preserving porosity. Incorporated TGF-β1 exhibited sustained release, promoting AF cell migration *in vitro*. The PEGDA/DAFM/TGF-β1 composite supported AF cell adhesion, proliferation, and ECM production. In rat AF defect models, implanted hydrogels effectively sealed defects, prevented NPs atrophy, maintained disc height, and partially restored disc biomechanics. Histology revealed cellular infiltration resembling AF cells and seamless tissue integration. These findings demonstrate TGF-β1-enhanced DAFM hydrogels as a promising AF repair strategy.[42]

Similar to the NPs, localized inflammatory responses intensify within damaged AF tissue, accompanied by substantial ROS accumulation. These pathological alterations collectively exacerbate AF degeneration and structural compromise. Han et al. engineered an antioxidant/anti-inflammatory composite hydrogel incorporating ceria-modified mesoporous silica nanoparticles loaded with TGF-β3 into GelMA/HAMA matrices. This design scavenges ROS and polarizes macrophages toward anti-inflammatory M2 phenotypes, releases TGF-β3 to recruit AF cells and stimulate ECM secretion, and enables *in situ* solidification within defects. *In vivo*, the hydrogel effectively repaired rat AF defects through dual mechanisms:

oxidative stress modulation and regenerative microenvironment improvement.[43]

Mobilization of locally resident AF progenitor cells represents a pivotal approach for annular repair. Zhou et al. engineered poly (ether carbonate urethane) urea scaffolds with tunable fiber diameters to mimic AF microstructure and direct annulus fibrosus stem cells (AFSC) differentiation. Scaffold topography regulated AFSC morphology and zonal-specific gene expression: large fibers upregulated outer-AF markers while small fibers enhanced inner-AF markers, recapitulating native tissue biochemistry. Increased fiber diameter promoted cell spreading, Yes-associated protein (YAP) nuclear translocation, and focal adhesion maturation. Crucially, Caveolin-1 (CAV1) mediated YAP mechanoresponses to substrate topography. These findings demonstrate fiber diameter-dependent activation of the CAV1-YAP mechanotransduction axis controls AFSC shape, adhesion complex formation, and ECM expression, providing mechanistic insights for designing topographically instructive scaffolds for AF regeneration.[44]

4. Repair and reconstruction strategies for cartilaginous endplate tissue engineering

4.1. Brief introduction of cartilaginous endplate and cartilaginous endplate degeneration

The CEP constitutes a ~1 mm hyaline cartilage lamina positioned on opposing superior/inferior vertebral body surfaces. Its composition primarily comprises type II collagen fibers, GAGs, and water. Mature CEPs are predominantly populated by chondrocytes responsible for matrix homeostasis maintenance. These superior and inferior endplates form a tri-laminar architecture enclosing the AF and NP within the IVD. Functionally, the CEP demarcates the disc-vertebral body interface while providing structural support and biomechanical protection.

Histologically, the CEP lacks direct collagenous integration with adjacent vertebral bodies. Instead, it interfaces via fibrocartilaginous integration with vertebral bone. Collagen fibers within the CEP exhibit predominantly parallel rather than perpendicular orientation relative to the vertebral surface. Crucially, given the continuity between CEP matrix fibers and AF fibers, the CEP is histologically classified as an integral disc component rather than vertebral tissue.

The CEP's matrix composition underpins its biomechanical function. Its interlaced collagen meshwork restricts NP herniation into vertebral bodies while dissipating axially transmitted compressive loads. The CEP additionally functions as a semipermeable membrane, facilitating selective molecular transport between vertebral bodies and the disc. Proteoglycan content within the CEP – and their associated water molecules – directly underpins tissue permeability. This permeability enables essential molecular exchange between the disc and endplate. Reduced CEP proteoglycan content precipitates impaired transport capacity, with studies confirming diminished CEP permeability strongly correlates with disc degeneration.[45,46]

Degenerated CEP exhibits increased stiffness, decreased permeability, and reduced hydration. These alterations disrupt substance transport and compromise mechanical function within the IVD, accelerating IDD. Inadequate nutrient supply through the CEP represents a recognized pathogenic precursor to IDD, while CEP degeneration frequently associates with Modic changes—common magnetic resonance imaging-visible vertebral bone marrow alterations during IDD progression.

4.2. Tissue engineering strategies for the cartilaginous endplate target restoration of its critical barrier function and nutrient transport capacity through matrix-modifying interventions or stem cell-derived exosome delivery

Recent advances in tissue engineering and regenerative medicine offer novel strategies to mitigate CEP degeneration. Pioneering work by Boyd and Carter first established that full CEP decalcification enhances solute transport into the IVD.[47] Subsequently, Dolor et al. demonstrated that matrix metalloproteinase treatment of human cadaveric lumbar spines selectively degrades diffusion-barrier matrix components, thereby augmenting nutrient diffusion through the CEP.[48]

To enable chondrocyte-targeted delivery within the CEP, Lin et al. engineered exosomes (CAP-Nrf2-Exos) surface-functionalized with chondrocyte-affinity peptide (CAP) and loaded with the antioxidant transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2). Following subendplate injection, CAP-Nrf2-Exos significantly enhanced Nrf2 expression, promoted its nuclear translocation, and augmented endogenous antioxidant defenses in CEP cells under inflammatory conditions.[49]

The CEP serves as a critical structural interface between the IVD and adjacent vertebral bodies. While substantial research has enhanced CEP matrix anabolism and reduced catabolism to some extent, current tissue-engineered grafts still exhibit limited biomechanical strength. These constructs cannot withstand physiological loading during daily activities, nor achieve bio-osseointegration with adjacent vertebrae—thus failing to fulfill their critical function in solute transport between disc and vertebral compartments.

The aforementioned tissue engineering studies primarily focus on delaying degeneration of the CEP itself. Given the CEP's critical role in overall IVD functional performance and metabolic exchange, emerging efforts have evaluated CEP-derived stem cell transplantation to mitigate degeneration of the NP and AF.

Liwen Luo et al. developed a therapeutic strategy for IDD involving subendplate injection of ECM-modified hydrogels (ECM-Gels) loaded with cartilage endplate stem cells (CESCs). Following *in vivo* administration in rats, ECM-Gels encapsulating *Sphk2*-overexpressing CESCs (Lenti-*Sphk2*-CESCs) generated *Sphk2*-engineered exosomes (Lenti-*Sphk2*-Exos). These exosomes penetrated the AF and delivered *Sphk2* to NPCs, activating both the phosphatidylinositol 3-kinase/p-AKT pathway and intracellular autophagy, ameliorating IDD pathology.[50]

5.Organoids: a novel strategy for disc reconstruction

The aforementioned tissue engineering-derived biomaterials represent a promising upstream regenerative strategy for managing IDD. By incorporating active seeding cells, functional bioactive agents, and engineered scaffolds, these approaches enable precisely tailored interventions that target the heterogeneous pathological mechanisms underlying IDD. This paradigm holds significant potential to transform the future therapeutic landscape and clinical outcomes in IDD management.

However, the selection of the traditional 3 elements of tissue engineering—seed cells, scaffolds, and bioinductive factors—often lacks a theoretical foundation in developmental biology. Merely screening seed cells and bioinductive factors *in vitro*, mimicking mature tissue morphology, and designing scaffolds to achieve superficial resemblance overlooks the heterogeneity of internal cellular composition and neglects microstructural biomimicry. Consequently, it becomes difficult to attain the correct cellular composition and matrix components. Furthermore, reproducing biomechanical properties consistent with natural tissues remains an elusive goal.

5.1. Development and establishment of organoids

In-depth research on organ development and regeneration has provided novel insights and methodologies for the conceptualization of organoids and the advancement of related technologies. By recapitulating organ developmental processes, organoid technology enables the *in vitro* induction of pluripotent stem cells (PSCs) or adult stem cells to self-organize into target tissues. This strategy replicates natural tissue development, recreating organ structure and function under physiological conditions, thereby pioneering new pathways for organ regeneration research at a fundamental level.

In 2007, Barker et al.[51] identified *Lgr5*$^+$ intestinal stem cells using lineage tracing. In 2009, the same research group successfully induced a single murine *Lgr5*$^+$ intestinal stem cell to self-organize *in vitro* into an intestinal organoid featuring crypt-villus structures.[52]

Since organoid construction recapitulates the differentiation and development of progenitor cells, the resulting organoids exhibit high similarity to native tissues in cellular composition, matrix components, and tissue microstructure. Moreover, when generated from stem cells carrying specific disease-causing genetic mutations, organoids can serve as *in vitro* models of genetic disorders. Leveraging these dual advantages alongside large-scale production techniques, organoids have rapidly found applications in disease mechanism exploration, high-throughput drug screening, and beyond.[53,54]

In addition, bioprinted organoids merge bioprinting techniques with organoid science, allowing *in vitro* reconstruction of both human tissue architecture and physiological function.[55] This integrated approach offers distinct advantages for organoid fabrication, particularly enhancing structural precision, microstructural fidelity, and functional maturation. By combining the complementary strengths of these technologies, bioprinted organoids facilitate the creation of personalized, architecturally controlled models that more accurately mimic organogenesis, physiological dynamics, and disease pathogenesis.

5.2. Organoids for locomotor system repair and reconstruction

Gradually, the applications of organoids have expanded into the field of tissue repair and reconstruction.[56] Their biomimetic microenvironment preserves cell-matrix interaction characteristics, endowing them with the potential to promote proliferation, differentiation, and functional recovery. Furthermore, organoids possess intrinsic self-renewal and self-organization capabilities, enabling sustained tissue repair and regeneration. Compared with traditional biomaterials, organoids offer high customizability to match individual patient needs. By regulating tissue type, structural properties, and repair outcomes, they facilitate personalized therapeutic strategies.[57]

Simultaneously, continuous advancements in bioengineering technologies have yielded multiple optimized strategies for organoid construction and cultivation. Utilizing bioreactors,[58,59] microfluidic systems,[60–62] and 3D bioprinting[55] technologies, these approaches not only provide highly controllable platforms for large-scale production but also precisely recapitulate the *in vivo* microenvironment. This offers ideal support for organoid development, maturation, and functional realization in tissue repair contexts.[63]

Despite bone's remarkable self-repair and regenerative capacity, severe defects present persistent challenges including poor repair outcomes, prolonged healing periods, and frequent complications—constituting a significant clinical and research hurdle. Bone organoids are tissue-engineered constructs formed via 3D self-organization of bone-related stem cells under *in vitro* induction within biomimetic microenvironments. These organoids emulate the 3D architecture, biomechanical properties, and genetic profiles of native bone tissue.

Fracture healing represents a highly complex and coordinated process. During repair, locally recruited mesenchymal stem cells proliferate and differentiate into chondrocytes, progressing through soft callus formation, hard callus development, and continuous remodeling—culminating in restored bone morphology and biomechanical integrity. As a specialized postnatal bone formation process, fracture healing remarkably recapitulates embryonic skeletal development, making bone one of few tissues capable of scarless regeneration.

The process of chondrogenesis forming the fracture callus represents one of the most critical steps in initiating bone repair. Tam et al. directed the differentiation of induced pluripotent stem cells (iPSCs) into mesodermal lineage cells, followed by chondrogenic differentiation, culminating in the self-assembly of cartilage organoids (CORGs). Through systematic validation in both ectopic and orthotopic bone formation models, they demonstrated the tissue-forming capacity of these organoids, successfully achieving critical-sized bone defect repair in immunocompromised mouse models.[64] Nilsson et al. utilized human periosteum-derived cells to mass-produce “callus organoids” that recapitulate the fracture callus formation process. These organoids simulated long bone development and healing *in vivo*, facilitating functional osteogenesis.[65] Concurrently, organoid construction via 3D bioprinting offers promise for treating large-segment bone defects. Pitacco et al. employed a developmental biology-inspired bone tissue engineering strategy to fabricate mechanically reinforced cartilage templates using 3D bioprinting. Their evaluation of these templates in femoral critical-sized defects confirmed 3D bioprinting as an effective strategy for scalable production of biomimetic developmental templates.[66]

Beyond endochondral ossification strategies, direct transplantation of bone organoids with self-organization capacity and mineralization potential represents another viable therapeutic approach. To address the clinical challenges of large-segment bone defects, Professor Su Jiacan’s team at Shanghai University employed a novel gelatin methacryloyl/sodium alginate methacryloyl/hydroxyapatite (GelMA/AlgMA/HAP) bioink to construct highly biomimetic large-scale bone organoids. These organoids achieved long-term culture and progressive maturation while demonstrating autonomous mineralization and establishing bidirectional integration with host cells—effectively promoting bone regeneration.[67] Furthermore, they also developed dynamic DNA/gelatin methacryloyl hydrogels that recapitulate critical biochemical and mechanical features of the bone ECM. This platform provides a supportive microenvironment for bone organoid construction, offering a novel strategy for bone tissue regeneration.[68]

Cartilage tissue possesses limited intrinsic capacity for self-regeneration and repair. In recent years, CORGs constructed from PSCs or chondroprogenitor cells via 3D culture technology have enabled highly biomimetic recapitulation of cartilage matrix architecture, garnering significant scientific interest. A research team from Osaka University developed iPSC-derived CORGs and evaluated their transplantation efficacy in a primate model. Results demonstrated that allogeneic iPSC-derived CORGs not only survived and integrated with host tissue after implantation in a primate knee cartilage defect model, but also exhibited promising clinical translation potential.[69] Meanwhile, a team at Nanjing Medical University targeted the senescent chondrocyte phenotype in osteoarthritis patients, creating senescence-targeting synovial mesenchymal stem cell organoid hydrogels for cartilage repair. Using a rat model, they validated the cartilage reparative efficacy of these organoids *in vivo*, thereby laying the groundwork for future clinical applications.[70]

5.3. Review of disc developmental biology

Organoid construction recapitulates tissue and organ development. Elucidating developmental trajectories of tissues/organs and cellular differentiation pathways serves as the essential theoretical foundation for building organoids.[71,72]

The IVD comprises the NP, CEP, and AF, each derived from distinct embryonic origins. Vertebral bodies and IVDs develop through the integration of notochordal and sclerotomal tissues. Ultimately, the notochord gives rise to the NP,[73–76] while the sclerotome differentiates into vertebral bodies, AF, and CEP.[77] During early vertebrate development, segmented blocks of paraxial mesoderm form along the embryonic craniocaudal axis, known as somites. Each somite consists of 3 compartments: sclerotome (precursor to axial skeleton), dermatome (dermal precursors), and myotome (muscle precursors). Human disc development initiates at approximately 4 to 5 weeks of gestation. Sclerotomal cells proliferate and expand, forming 3 substructures: ventral, lateral, and dorsal sclerotomes. Pax1 + mesenchymal cells within the ventral sclerotome ultimately differentiate into vertebral bodies and AF.[78]

Beyond dorsoventral patterning, the sclerotome exhibits pronounced craniocaudal heterogeneity. This morphological gradient is particularly evident in the ventral sclerotome, where caudal regions display compact cellular organization while cranial regions remain loosely structured. Developmentally, the compact caudal portion contributes to the superior half of the subjacent vertebral body, whereas the dispersed cranial portion forms the inferior half of the adjacent superior vertebra. A transverse groove—eponymously named von Ebner’s fissure after Austrian anatomist Victor von Ebner – demarcates the boundary between compact caudal and dispersed cranial sclerotomal regions. This specialized structure later develops into the passage for spinal nerves between vertebrae. Cells flanking von Ebner’s fissure ultimately differentiate into the AF. While notochordal segments surrounded by vertebral bodies regress, the intervertebral portions persist and mature into the NP.[6]

5.4. Organoids and assembloids: novel strategies for disc reconstruction

Organoid-based engineering of NP, AF, or CEP prostheses necessitates comprehensive understanding of their developmental trajectories and cellular evolution pathways. CEP cells exhibit a hyaline cartilage-like phenotype, with iPSC-derived cartilage organoid technology demonstrating notable progress.[69,70]

The AF originates from mesodermal development. Our group previously decoded murine AF cellular composition via single-cell RNA sequencing, achieving preliminary region-specific differentiation of inner/outer AF cells from AF-derived stem cells.[79] Also, Peredo et al. reported an initial approach to guide human iPSCs toward an AF-like fate for cellular delivery strategies.[80] These establishes a foundational theoretical framework for AF organoid engineering.

The NP derives embryologically from notochordal tissue. Multiple studies have established protocols for differentiating notochordal-like NP cells from induced iPSCs, thereby paving the way for NP organoid construction.

Zhang et al. reported that notochord-like and NP-like cells can be derived from human PSCs using a NOTO-eGFP reporter system and a chemically defined protocol. These derived NP-like cells exhibit high similarity to/closely resemble adolescent human NP cells and attenuate injury-induced IDD after transplantation.[81] Subsequently, Tong et al. generated 2 human iPSC lines harboring an ACAN-2A-mScarlet reporter and functionally validated this reporter during notochordal and chondrogenic differentiation.[82] Warin et al. further defined the precise balance of canonical Wnt/β-catenin, Nodal/Smad2/3, and BMP signaling required to enhance hiPSC differentiation into notochord-like cells, achieving robust 20% efficiency on average. This was accomplished by replacing Matrigel with laminin coating—a major notochordal basement membrane component advancing GMP-compliant protocols—alongside using modified synthetic mRNA on day 2 to boost transfection efficiency for transient NOTO expression. Concurrent TGF-β pathway inhibition with SB-431542 restricted endodermal specification, while BMP signaling blockade via NOGGIN minimized intermediate mesoderm induction.[83]

It is foreseeable that iPSC-derived NPs, AF, and CEP organoids are anticipated to constitute a novel strategy for the reconstructive repair of IDD.

The IVD comprises 3 distinct tissues with divergent characteristics and anatomical structures: NP, AF, and CEP. Degeneration or damage to any single component precipitates IDD, leading to restricted spinal mobility and motor dysfunction. As understanding of spinal degeneration pathology has advanced, researchers recognize that isolated replacement therapies targeting individual IVD components (NP, AF, or CEP) fail to address the complexity of holistic disc degeneration. To overcome these limitations, pioneering investigators have developed combined reconstruction strategies targeting both NP and AF.

Sun et al. employed 3D bioprinting to fabricate an anatomically precise IVD scaffold integrating biomaterials, cells, and growth factors. Specifically, connective tissue growth factor and TGF-β3 were loaded onto polydopamine nanoparticles, which were subsequently combined with bone marrow mesenchymal stem cells to regenerate NP and AF structures. *In vitro* studies confirmed spatially controlled release of connective tissue growth factor and TGF-β3 from the scaffold, directing bone marrow mesenchymal stem cell differentiation into NP-like and AF-like cells. Following implantation into nude mouse dorsum subcutaneous tissue, the reconstructed IVD exhibited zone-specific extracellular matrices: a core region rich in type II collagen and glycosaminoglycans, surrounded by a peripheral zone expressing type I collagen—recapitulating native histological and immunological phenotypes.[84]

Subsequently, researchers have engineered integrated tissue-engineered implants replicating the tri-layered architecture of the IVD—incorporating NP, AF and CEP. Gullbrand et al. engineered tissue-derived, endplate-modified disc-like angle ply structures scaled for rat caudal and goat cervical spines that faithfully replicate native disc hierarchy. These constructs demonstrated functional maturation and host integration in both rat caudal and goat cervical disc replacement models, establishing the clinical translatability of tissue-engineered disc replacement for advanced degeneration.[85]

These pioneering studies established the conceptual framework for "holistic biological disc replacement," demonstrating engineered implants with significant biointegration and biomimetic properties. They thereby substantiated the feasibility and advantages of integrated biological disc reconstruction. Nevertheless, ad hoc assembly of NPs, AF, and CEP components fails to achieve adequate interfacial stability between tissue layers, critically compromising the construct's mechanical performance.

The conceptualization and evolution of assembloid technologies have propelled novel paradigms for integrated reconstruction of complex tissues such as IVD.[86] Assembloids are 3D preparations formed by the fusion and functional integration of different organoids with 1 another or with other specialized cell types.[87] Originally, this technology was deployed to investigate complex interactions between carcinoma cells and tumor tissues,[86] as well as among diverse neural cell populations.[87–89]

Nevertheless, researchers rapidly recognized the significant translational potential of assembloid technologies for reconstructing architecturally complex tissue defects. Rossi et al. established a 3D *in vitro* model for evaluating functional dynamics of human neuromuscular systems interacting with engineered skeletal muscle (SkM) constructs. Using decellularized SkM scaffolds and human neuromuscular organoids to form graft-host assembloids, the study documented myogenic cell migration and neural axon invasion from neuromuscular organoids into engineered SkM, culminating in functional neuromuscular junction formation. Notably, these assembloids demonstrated regenerative capacity after acute injury, exhibiting SkM reconstruction and functional recovery. Although lacking immunocompetent cells and vasculature, this platform provides a robust tool for *in vitro* assessment of innervated human SkM responses to tissue-engineered grafts.[90]

In addition, Peng et al. pioneered a novel strategy employing melt electrowriting (MEW)-assembloids—hybrid constructs integrating MEW scaffolds with cartilaginous microtissues—to augment bone regeneration. Bucket-shaped MEW scaffolds (OMesh/CMesh) were

Table 1

Repair and reconstruction of intervertebral disc degeneration: from tissue engineering to organoids and assembloids.

Target tissue	Pathological focus	Tissue engineering strategy	Key examples/approaches	Platform/ delivery system	Key findings/limitations
Nucleus pulposus (NP)	Inflammation and ROS	Anti-inflammatory biomaterials; ROS-scavenging hydrogels; RNA delivery	- HA hydrogels (IL-1R1/MyD88 suppression) (Mohd Isa et al.[16]) - Klotho circRNA-loaded NPs (Zhao et al.[17]) - APETx2-peptide-cell hydrogels (Bian et al.[18]) - Vanillin-functionalized GelMA (Zhu et al.[19]) - Thiourea-grafted HA/Cu^{2+} hydrogel (Bu et al.[20]) - Rapamycin-loaded ROS-degradable hydrogel (Bai et al.[21])	Injectable hydrogels; nanoparticles	Reduced inflammation/ROS; enhanced ECM synthesis; pain alleviation. Limitations: limited long-term biomechanical stability
	Hypoxia and acidity	HIF1α overexpression; oxygen-delivery scaffolds; neutralizer of H$^+$	- HIF1α@NPs (NP membrane-coated plasmid delivery) (Li et al.[93]) - PFTBA-alginate oxygenating scaffolds (Sun et al.[22]) - CAP-sEXOs@Gel containing CaCO$_3$/chitosan hydrogel (Zhan et al.[23])	Nanoparticles; scaffolds; engineered exosomes	Enhanced hypoxia tolerance; neutralizing of H$^+$; improved NP cell viability/ECM restoration
	Immune cell infiltration	Immune-modulating biomaterials; neutrophil membrane coating	- TGF-β1-loaded neutrophil-mimetic NPs (GM@T-NNPs) (Zhou et al.[28]) - Mucin-derived gels (Muc-gels) (Wang et al.[29]) - Metal-polyphenol nanoparticles (Wang et al.[30])	Hydrogel microspheres; coatings	Blocked immune cell infiltration; reduced inflammation; restored disc height/nociceptive thresholds
	Structural rebuilding	Injectable biomechanical surrogates; ferroptosis inhibition	- Shear-thinning PVA hydrogel (GPG) (Jia et al.[13]) - Self-crosslinking adhesive organohydrogel (GPG-AG) (Wang et al.[31]) - miR-221-3p exosomes in ECM-mimetic hydrogel (Wang et al.[32]) - Nanohybrid peptide hydrogel (NHPH) (Conley et al.[33]) - BMP7-peptide conjugated SAPs (Tao et al.[94])	Injectable hydrogels; Exosomes	Restored disc height/biomechanics; minimized leakage; inhibited ferroptosis. Limitations: incomplete replication of native NP heterogeneity/biomechanics
Annulus fibrosus (AF)	Structural integrity	Suturing; biomimetic repair patches; adhesive hydrogels	- UHMWPE suture closure (Bateman et al.[36]) - Angle-ply collagen patches (Borem et al.[37]) - Anakinra-loaded tension-activated repair patches (TARPs) (Peredo et al.[38])	Patches; sutures; hydrogels	Mechanical reinforcement; reduced inflammation; enhanced matrix deposition. Limitations: poor integration with native tissue under dynamic load
	Microenvironment modification	Anti-inflammatory/antioxidant delivery; stem cell recruitment; topographical cues	- oMeHA-fibrin-PEGDA adhesive hydrogel (DiStefano et al.[39]) - PDA/GelMA microneedles + phototherapy (Meng et al.[40]) - LOX-DNA/MnO$_2$-exosome scaffold (Zhao et al.[41]) - PEGDA/DAFM/TGF-β1 hydrogel (Wei et al.[42]) - CeO$_2$-MSNA/TGF-β3 in GelMA/HAMA (Han et al.[43]) - PECUU scaffolds with tuned fiber diameter (Chu et al.[44])	Microneedles; scaffolds; hydrogels	Enhanced AF cell migration/ECM integration; ROS scavenging; M2 macrophage polarization. Topographical scaffolds directed AFSC differentiation
Cartilaginous endplate (CEP)	Barrier function and transport	Matrix modification; targeted exosome delivery; stem cell therapy	- MMP-8 treatment (Dolor et al.[48]) - CAP-Nrf2-Exos (Lin et al.[49]) - ECM-Gels with Sphk2-engineered CESCs (Luo et al.)[50]	Enzymes; exosomes; hydrogels	Enhanced nutrient diffusion; antioxidant protection; improved ECM synthesis. Limitations: poor biomechanical strength and bio-osseointegration with vertebrae
Holistic disc reconstruction	Component integration	Multi-zone bioprinting; Tissue-derived angle-ply structures	- CTGF/TGF-β3-loaded BMSC bioprinted disc (Sun et al.[84]) - Endplate-modified disc-like angle ply structures (eDAPS) (Gullbrand et al.)[85]	3D Bioprinting; decellularized tissue	Zone-specific ECM deposition; host integration in animal models. Limitations: weak interfacial stability between NP/AF/CEP

(Continued)

Table 1

Continued

Target tissue	Pathological focus	Tissue engineering strategy	Key examples/approaches	Platform/ delivery system	Key findings/limitations
Emerging paradigms	Organoids	iPSC-derived tissue-specific organoids	- iPSC → Notochordal/NP-like cells (Zhang et al., Tong et al., Warin et al.[81–83]) - iPSC → AF-like cells (Peredo et al.[80]) - Cartilage organoids (CORGs) for CEP (Abe et al. and Sun et al.[69,70])	3D cell culture; iPSC differentiation	Recapitulated native microarchitecture/cellular diversity. Limitations: lack standardized protocols; immature biomechanics
	Assembloids	Fusion of organoids or organoid-scaffold hybrids	- Neuromuscular assembloids (Rossi et al.[90]) - MEW-scaffold + cartilaginous microtissues (Peng et al.[91]) - Conceptual application for IVD: Fusion of NP/AF/CEP organoids	Organoid fusion; bioprinted scaffolds	Enabled complex tissue integration; functional tissue formation. Potential for IVD: solve interfacial instability via developmentally inspired fusion. Limitations: vascularization; scale-up; biomechanical maturation
Future challenges	1. Standardization: GMP-compliant biomanufacturing of organoids 2. Interfaces: Biomimetic engineering of disc-vertebral junctions 3. Biomechanics: Achieving native-equivalent load-bearing via maturation protocols and stimuli-responsive scaffolds				

AFCS = annulus fibrosus stem cells, BMSC = bone marrow mesenchymal stem cells, BMP = bone morphogenetic protein, CORGs = cartilage organoids, CTGF = connective tissue growth factor, DAFM = decellularized annulus fibrosus matrix, ECM = extracellular matrix, GelMA = gelatin methacrylate, IVD = intervertebral discs, LOX = lysyl oxidase, MEW = melt electrowriting, MMP-8 = matrix metalloproteinase-8, NPs = nucleus pulposus, oMeHA = oxidized-methacrylated hyaluronic acid, PECUU = poly (ether carbonate urethane) urea, PEGDA = polyethylene glycol diacrylate, PFTBA = perfluorotributylamine, PVA = poly(vinyl alcohol), ROS = reactive oxygen species, TGF-β1 = transforming growth factor-β1, UHMWPE = ultrahigh molecular-weight polyethylene.

engineered to optimize microtissue retention and integration, with OMesh demonstrating superior structural integrity postseeding. For *in vivo* translation, elongated MEW scaffolds (EMesh) derived from OMesh configurations were developed to form EMesh-assembloids. Subcutaneous implantation revealed robust endochondral ossification and mineralization capacity. Furthermore, tubular MEW stabilizers orthotopically implanted with EMesh-assembloids in critical-sized murine tibial defects induced substantial neobone formation and near-complete bridging at 8 weeks.[91]

6. Concluding remarks

This evolving technological trajectory—from reductionist tissue engineering toward developmentally inspired organoid/assembloid paradigms—heralds a transformative shift in disc reconstruction philosophy (Summarized in Table 1).

A primary objective of tissue engineering is to engineer implantable *in vitro* tissues that functionally replace diseased or damaged structures.[92] Recent advances in IVD reconstruction have adopted multipronged approaches: modulating degenerative microenvironments, mobilizing endogenous disc stem/progenitor cells, and deploying biomechanical surrogates. Nevertheless, the inherent developmental divergence and tissue heterogeneity across IVD compartments present fundamental barriers to engineering integrated disc transplants. Representing a paradigm-shifting technological advance, organoid-derived assembloid platforms now furnish innovative conceptual frameworks and experimental paradigms to surmount regenerative challenges in IVD reconstruction.

To advance the clinical translation of organoid/assembloid-based IVD transplants, 3 strategic imperatives require resolution: First, standardized protocols remain elusive. Current organoid systems lack unified frameworks, resulting in significant inter-laboratory variability in culture conditions and procedures that impede cross-study validation and compromise reliability. Second, interfacial tissue engineering challenges persist. Deciphering the developmental mechanisms of critical interfaces—particularly the vertebral body – CEP junction and AF-endplate insertion sites—is prerequisite for biomimetic assembloid reconstruction, representing the cornerstone for functional IVD prosthetic development. Third, biomechanical constraints in IVD organoid engineering demand resolution. Conventional tissue engineering fails to recapitulate spatiotemporal heterogeneity, microstructural complexity, and load-bearing capacity. While organoid/assembloid technologies enable heterogeneous cellular organization and microarchitectural fidelity through developmental principles, current constructs cannot provide native-equivalent biomechanical strength due to abbreviated maturation periods and absence of macroscale tissue architecture.

Future progress will converge on establishing GMP-compliant, automated closed-production systems to standardize organoid biomanufacturing, eliminating batch variability. Concurrently, material innovations integrated with assembloid platforms will enable stable reconstruction of critical intradisc and disc-vertebral interfaces. Through optimized maturation protocols and stimuli-responsive graded scaffolds, organoid/assembloid-based implants will achieve transformative biomechanical competence—paving the way for paradigm-shifting advances in regenerative medicine.

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Ethical statement

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Conflicts of interest

The authors have no conflicts of interest to disclose.

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Author contributions

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References

[1] Hoy D, March L, Brooks P, et al. The global burden of low back pain: estimates from the Global Burden of Disease 2010 study. Ann Rheum Dis. 2014;73:968–74.
[2] Driscoll T, Jacklyn G, Orchard J, et al. The global burden of occupationally related low back pain: estimates from the global burden of disease 2010 study. Ann Rheum Dis. 2014;73:975–81.
[3] Maher C, Underwood M, Buchbinder R. Non-specific low back pain. Lancet. 2017;389:736–47.
[4] Peng BG. Pathophysiology, diagnosis, and treatment of discogenic low back pain. World J Orthop. 2013;4:42.
[5] Mizuno HL, Kang JD, Mizuno S. Effects of hydrostatic pressure, osmotic pressure, and confinement on extracellular matrix associated responses in the nucleus pulposus cells ex vivo. Matrix Biol. 2024;134:162–74.
[6] Williams S, Alkhatib B, Serra R. Development of the axial skeleton and intervertebral disc. Curr Top Dev Biol. 2019;133:49–90.
[7] Mao J, Wang D, Wang D, et al. SIRT5-related desuccinylation modification of AIFM1 protects against compression-induced intervertebral disc degeneration by regulating mitochondrial homeostasis. Exp Mol Med. 2023;55:253–68.
[8] Wang D, Shang Q, Mao J, et al. Phosphorylation of KRT8 (keratin 8) by excessive mechanical load-activated PKN (protein kinase N) impairs autophagosome initiation and contributes to disc degeneration. Autophagy. 2023;19:2485–503.
[9] Peng P, Wang D, Xu X, et al. Targeting clock-controlled gene Nrf2 ameliorates inflammation-induced intervertebral disc degeneration. Arthritis Res Ther. 2022;24:181.
[10] Mei L, Zheng Y, Gao X, et al. Hsa-let-7f-1-3p targeting the circadian gene Bmal1 mediates intervertebral disc degeneration by regulating autophagy. Pharmacol Res. 2022;186:106537.
[11] Wang D, Peng P, Dudek M, et al. Restoring the dampened expression of the core clock molecule BMAL1 protects against compression-induced intervertebral disc degeneration. Bone Res. 2022;10:20.
[12] Xu X, Wang D, Zheng C, et al. Progerin accumulation in nucleus pulposus cells impairs mitochondrial function and induces intervertebral disc degeneration and therapeutic effects of sulforaphane. Theranostics. 2019;9:2252–67.
[13] Jia H, Lin X, Wang D, et al. Injectable hydrogel with nucleus pulposus-matched viscoelastic property prevents intervertebral disc degeneration. J Orthop Translat. 2022;33:162–73.
[14] Srivastava A, Isa ILM, Rooney P, Pandit A. Bioengineered three-dimensional diseased intervertebral disc model revealed inflammatory crosstalk. Biomaterials. 2017;123:127–41.
[15] Isa ILM, Srivastava A, Tiernan D, et al. Hyaluronic acid based hydrogels attenuate inflammatory receptors and neurotrophins in interleukin-1β induced inflammation model of nucleus pulposus cells. Biomacromolecules. 2015;16:1714–25.
[16] Mohd Isa IL, Abbah SA, Kilcoyne M, et al. Implantation of hyaluronic acid hydrogel prevents the pain phenotype in a rat model of intervertebral disc injury. Sci Adv. 2018;4:eaaq0597.
[17] Zhao D, Zhang J, Chen C, et al. Rejuvenation modulation of nucleus pulposus progenitor cells reverses senescence-associated intervertebral disc degeneration. Adv Mater. 2025;37:e2409979.
[18] Bian J, Cai F, Chen H, et al. Modulation of local overactive inflammation via injectable hydrogel microspheres. Nano Lett. 2021;21:2690–8.
[19] Zhu Z, Yu Q, Li H, et al. Vanillin-based functionalization strategy to construct multifunctional microspheres for treating inflammation and regenerating intervertebral disc. Bioact Mater. 2023;28:167–82.
[20] Bu P, Peng R, Zhang J, et al. A one-stone-two-birds strategy for intervertebral disc repair: constructing a reductive chelation hydrogel to mitigate oxidative stress and promote disc matrix reconstruction. Adv Mater (Deerfield Beach, Fla.). 2025;37:e2411290.
[21] Bai J, Zhang Y, Fan Q, et al. Reactive oxygen species-scavenging scaffold with rapamycin for treatment of intervertebral disk degeneration. Adv Healthc Mater. 2020;9:e1901186.
[22] Sun Z, Luo B, Liu Z, et al. Effect of perfluorotributylamine-enriched alginate on nucleus pulposus cell: implications for intervertebral disc regeneration. Biomaterials. 2016;82:34–47.
[23] Zhan J, Cui Y, Zhang P, et al. Cartilage endplate-targeted engineered exosome releasing and acid neutralizing hydrogel reverses intervertebral disc degeneration. Adv Healthc Mater. 2025;14:e2403315.
[24] Sun Z, Liu B, Luo ZJ. The immune privilege of the intervertebral disc: implications for intervertebral disc degeneration treatment. Int J Med Sci. 2020;17:685–92.
[25] Koroth J, Chitwood C, Kumar R, et al. Identification of a novel, MSC-induced macrophage subtype via single-cell

sequencing: implications for intervertebral disc degeneration therapy. Front Cell Dev Biol. 2024;11:1286011.

[26] Risbud MV, Shapiro IM. Role of cytokines in intervertebral disc degeneration: pain and disc content. Nat Rev Rheumatol. 2014;10:44–56.

[27] Ye F, Lyu FJ, Wang H, Zheng Z. The involvement of immune system in intervertebral disc herniation and degeneration. JOR Spine. 2022;5:e1196.

[28] Zhou L, Cai F, Zhu H, et al. Immune-defensive microspheres promote regeneration of the nucleus pulposus by targeted entrapment of the inflammatory cascade during intervertebral disc degeneration. Bioact Mater. 2024;37:132–52.

[29] Wang H, Chen S, Liu Z, et al. Preserving the immune-privileged niche of the nucleus pulposus: safeguarding intervertebral discs from degeneration after discectomy with synthetic mucin hydrogel injection. Adv Sci (Weinh). 2024;11:e2404496.

[30] Wang X, Guo Z, Chen L, et al. Synergistic modulating of mitochondrial transfer and immune microenvironment to attenuate discogenic pain. Adv Sci. 2025;12:e2500128.

[31] Wang Y, Wang D, Gao C, et al. Development of a mechanical adaptable, moisture retention capable, injectable and adhesive organohydrogel for nucleus pulposus repairing. Regen Biomater. 2025;12:rbaf047.

[32] Wang W, Cheng Z, Yu M, et al. Injectable ECM-mimetic dynamic hydrogels abolish ferroptosis-induced post-discectomy herniation through delivering nucleus pulposus progenitor cell-derived exosomes. Nat Commun. 2025;16:3131.

[33] Conley BM, Yang L, Bhujel B, Luo J, Han I, Lee KB. Development of a nanohybrid peptide hydrogel for enhanced intervertebral disc repair and regeneration. ACS Nano. 2023;17:3750–64.

[34] Mokdad AH, Bisignano C, Hsu JM, et al. Burden of disease scenarios by state in the USA, 2022–50: a forecasting analysis for the global burden of disease study 2021. Lancet. 2024;404:2341–70.

[35] Bron JL, Helder MN, Meisel HJ, Van Royen BJ, Smit TH. Repair, regenerative and supportive therapies of the annulus fibrosus: achievements and challenges. Eur Spine J. 2009;18:301–13.

[36] Bateman AH, Balkovec C, Akens MK, et al. Closure of the annulus fibrosus of the intervertebral disc using a novel suture application device—in vivo porcine and ex vivo biomechanical evaluation. Spine J. 2016;16:889–95.

[37] Borem R, Madeline A, Walters J, Mayo H, Gill S, Mercuri J. Angle-ply biomaterial scaffold for annulus fibrosus repair replicates native tissue mechanical properties, restores spinal kinematics, and supports cell viability. Acta Biomater. 2017;58:254–68.

[38] Peredo AP, Gullbrand SE, Friday CS, et al. Tension-activated nanofiber patches delivering an anti-inflammatory drug improve repair in a goat intervertebral disc herniation model. Sci Transl Med. 2023;15:eadf1690.

[39] DiStefano TJ, Shmukler JO, Danias G, et al. Development of a two-part biomaterial adhesive strategy for annulus fibrosus repair and ex vivo evaluation of implant herniation risk. Biomaterials. 2020;258:120309.

[40] Meng Q, Xie E, Sun H, et al. High-strength smart microneedles with "offensive and defensive" effects for intervertebral disc repair. Adv Mater Deerfield Beach Fla. 2024;36:e2305468.

[41] Zhao R, Han F, Yu Q, et al. A multifunctional scaffold that promotes the scaffold-tissue interface integration and rescues the ROS microenvironment for repair of annulus fibrosus defects. Bioact Mater. 2024;41:257–70.

[42] Wei Q, Liu D, Chu G, et al. TGF-β1-supplemented decellularized annulus fibrosus matrix hydrogels promote annulus fibrosus repair. Bioact Mater. 2023;19:581–93.

[43] Han F, Tu Z, Zhu Z, et al. Targeting endogenous reactive oxygen species removal and regulating regenerative microenvironment at annulus fibrosus defects promote tissue repair. ACS Nano. 2023;17:7645–61.

[44] Zhou P, Chu G, Yuan Z, et al. Regulation of differentiation of annulus fibrosus-derived stem cells using heterogeneous electrospun fibrous scaffolds. J Orthop Translat. 2021;26:171–80.

[45] Crump KB, Alminnawi A, Bermudez-Lekerika P, et al. Cartilaginous endplates: a comprehensive review on a neglected structure in intervertebral disc research. Jor Spine. 2023;6:e1294.

[46] Moon SM, Yoder JH, Wright AC, Smith LJ, Vresilovic EJ, Elliott DM. Evaluation of intervertebral disc cartilaginous endplate structure using magnetic resonance imaging. Eur Spine J. 2013;22:1820–8.

[47] Boyd LM, Carter AJ. Injectable biomaterials and vertebral endplate treatment for repair and regeneration of the intervertebral disc. Eur Spine J. 2006;15:414–21.

[48] Dolor A, Sampson SL, Lazar AA, Lotz JC, Szoka FC, Fields AJ. Matrix modification for enhancing the transport properties of the human cartilage endplate to improve disc nutrition. PLoS One. 2019;14:e0215218.

[49] Lin Z, Xu G, Lu X, et al. Chondrocyte-targeted exosome-mediated delivery of Nrf2 alleviates cartilaginous endplate degeneration by modulating mitochondrial fission. J Nanobiotechnology. 2024;22:281.

[50] Luo L, Jian X, Sun H, et al. Cartilage endplate stem cells inhibit intervertebral disc degeneration by releasing exosomes to nucleus pulposus cells to activate akt/autophagy. Stem cells (Dayton, Ohio). 2021;39:467–81.

[51] Barker N, Van Es JH, Kuipers J, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature. 2007;449:1003–7.

[52] Sato T, Vries RG, Snippert HJ, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature. 2009;459:262–5.

[53] Schutgens F, Clevers H. Human organoids: tools for understanding biology and treating diseases. Annu Rev Pathol. 2020;15:211–34.

[54] Li G, He J, Shi J, et al. Bioprinting functional hepatocyte organoids derived from human chemically induced pluripotent stem cells to treat liver failure. Gut. 2025;74:1150–64.

[55] Huang J, Jia S, Liang R, et al. Construction of organoids using bioprinting technology: a frontier exploration of cartilage repair. J Orthop Translat. 2025;54:37–50.

[56] Yuan X, Wu J, Sun Z, et al. Preclinical efficacy and safety of encapsulated proliferating human hepatocyte organoids in treating liver failure. Cell Stem Cell. 2024;31:484–98.e5.

[57] Zhang J, Chen X, Chai Y, et al. 3D printing of a vascularized mini-liver based on the size-dependent functional enhancements of cell spheroids for rescue of liver failure. Adv Sci. 2024;11:e2309899.

[58] Qian X, Nguyen HN, Song MM, et al. Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. Cell. 2016;165:1238–54.

[59] Qian X, Jacob F, Song MM, Nguyen HN, Song H, Ming GL. Generation of human brain region-specific organoids using a miniaturized spinning bioreactor. Nat Protoc. 2018;13:565–80.

[60] Carvalho MR, Yan LP, Li B, et al. Gastrointestinal organs and organoids-on-a-chip: advances and translation into the clinics. Biofabrication. 2023;15.

[61] Hong ZX, Zhu ST, Li H, et al. Bioengineered skin organoids: from development to applications. Mil Med Res. 2023;10:40.

[62] Quintard C, Tubbs E, Jonsson G, et al. A microfluidic platform integrating functional vascularized organoids-on-chip. Nat Commun. 2024;15:1452.

[63] Kim W, Gwon Y, Park S, Kim H, Kim J. Therapeutic strategies of three-dimensional stem cell spheroids and organoids for tissue repair and regeneration. Bioact Mater. 2023;19:50–74.

[64] Tam WL, Freitas Mendes L, Chen X, et al. Human pluripotent stem cell-derived cartilaginous organoids promote scaffold-free healing of critical size long bone defects. Stem Cell Res Ther. 2021;12:513.

[65] Nilsson Hall G, Mendes LF, Gklava C, Geris L, Luyten FP, Papantoniou I. Developmentally engineered callus organoid bioassemblies exhibit predictive In vivo long bone healing. Adv Sci. 2020;7:1902295.

[66] Pitacco P, Sadowska JM, O'Brien FJ, Kelly DJ. 3D bioprinting of cartilaginous templates for large bone defect healing. Acta Biomater. 2023;156:61–74.

[67] Wang J, Wu Y, Li G, et al. Engineering large-scale self-mineralizing bone organoids with bone matrix-inspired hydroxyapatite hybrid bioinks. Adv Mater (Deerfield Beach, Fla.). 2024;36:e2309875.

[68] Zhu M, Zhang H, Zhou Q, et al. Dynamic GelMA/DNA dual-network hydrogels promote woven bone organoid formation and enhance bone regeneration. Adv Mater. 2025;37:e2501254.

[69] Abe K, Yamashita A, Morioka M, et al. Engraftment of allogeneic iPS cell-derived cartilage organoid in a primate model of articular cartilage defect. Nat Commun. 2023;14:804.

[70] Sun Y, You Y, Wu Q, Hu R, Dai K. Senescence-targeted MicroRNA/organoid composite hydrogel repair cartilage defect and prevention joint degeneration via improved chondrocyte homeostasis. Bioact Mater. 2024;39:427–42.

[71] Huch M, Koo BK. Modeling mouse and human development using organoid cultures. Development. 2015;142:3113–25.

[72] Miao Y, Pek NM, Tan C, et al. Co-development of mesoderm and endoderm enables organotypic vascularization in lung and gut organoids. Cell. 2025;188(16):4295–313.e27.

[73] Lawson LY, Harfe BD. Developmental mechanisms of intervertebral disc and vertebral column formation. Wiley Interdiscip Rev Dev Biol. 2017;6.

[74] Choi K, Cohn MJ, Harfe BD. Identification of nucleus pulposus precursor cells and notochordal remnants in the mouse: implications for disk degeneration and chordoma formation. Dev Dyn. 2008;237:3953–8.

[75] Harfe BD. Intervertebral disc repair and regeneration: insights from the notochord. Semin Cell Dev Biol. 2022;127:3–9.

[76] Lawson L, Harfe BD. Notochord to nucleus pulposus transition. Curr Osteoporos Rep. 2015;13:336–41.

[77] Bruggeman BJ, Maier JA, Mohiuddin YS, et al. Avian intervertebral disc arises from rostral sclerotome and lacks a nucleus pulposus: implications for evolution of the vertebrate disc. Dev Dyn. 2012;241:675–83.

[78] Tani S, Chung UI, Ohba S, Hojo H. Understanding paraxial mesoderm development and sclerotome specification for skeletal repair. Exp Mol Med. 2020;52:1166–77.

[79] Wang H, Wang D, Luo B, et al. Decoding the annulus fibrosus cell atlas by scRNA-seq to develop an inducible composite hydrogel: a novel strategy for disc reconstruction. Bioact Mater. 2022;14:350–63.

[80] Peredo AP, Tsinman TK, Bonnevie ED, et al. Developmental morphogens direct human induced pluripotent stem cells toward an annulus fibrosus-like cell phenotype. Jor Spine. 2024;7:e1313.

[81] Zhang Y, Zhang Z, Chen P, et al. Directed differentiation of notochord-like and nucleus pulposus-like cells using human pluripotent stem cells. Cell Rep. 2020;30:2791–806.e5.

[82] Tong X, Poramba-Liyanage DW, Van Hoolwerff M, et al. Isolation and tracing of matrix-producing notochordal and chondrocyte cells using ACAN-2A-mScarlet reporter human iPSC lines. Sci Adv. 2024;10:eadp3170.

[83] Warin J, Vedrenne N, Tam V, et al. In vitro and in vivo models define a molecular signature reference for human embryonic notochordal cells. Iscience. 2024;27:109018.

[84] Sun B, Lian M, Han Y, et al. A 3D-bioprinted dual growth factor-releasing intervertebral disc scaffold induces nucleus pulposus and annulus fibrosus reconstruction. Bioact Mater. 2021;6:179–90.

[85] Gullbrand SE, Ashinsky BG, Bonnevie ED, et al. Long-term mechanical function and integration of an implanted tissue-engineered intervertebral disc. Sci Transl Med. 2018;10:eaau0670.

[86] Kim E, Choi S, Kang B, et al. Creation of bladder assembloids mimicking tissue regeneration and cancer. Nature. 2020;588:664–9.

[87] Onesto MM, Kim JI, Pasca SP. Assembloid models of cell-cell interaction to study tissue and disease biology. Cell Stem Cell. 2024;31:1563–73.

[88] Wu SR, Nowakowski TJ. Exploring human brain development and disease using assembloids. Neuron. 2025;113:1133–50.

[89] Zhu Y, Zhang X, Sun L, Wang Y, Zhao Y. Engineering human brain assembloids by microfluidics. Adv Mater (Deerfield Beach, Fla.). 2023;35:e2210083.

[90] Rossi L, Auletta B, Sartore L, et al. Engineering assembloids to mimic graft-host skeletal muscle interaction. Adv Healthc Mater. 2025;14:e2404111.

[91] Peng L, Chandrakar A, Nilsson Hall G, et al. Structurally defined cartilaginous MEW-assembloids for critical-size long bone healing. Biomaterials. 2025;319:123202.

[92] Langer R, Vacanti JP. Tissue engineering. Science. 1993;260:920–6.

[93] Li K, Lin H, Yu Y, et al. Nucleus pulposus cell-mimicking nanoparticles for cell-specific HIF1A editing to modulate SASP-mediated disc inflammation via autophagy activation. Acta Biomater. 2025;197:357–73.

[94] Tao H, Wu Y, Li H, et al. BMP7-based functionalized self-assembling peptides for nucleus pulposus tissue engineering. ACS Appl Mater Interfaces. 2015;7:17076–87.