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Review Article

New strategies for the treatment of intervertebral disc degeneration: cell, exosome, gene, and tissue engineering

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Abstract: Low back pain (LBP) caused by intervertebral disc (IVD) generation (IVDD) has always been an important problem that cannot be ignored. Traditional therapies have many deep-rooted and intractable complications that promote their treatment mode transfer to new therapies. This article mainly summarizes the shortcomings of traditional treatment methods and analyzes the research status and future development direction of IVDD treatment. We outlined the most promising IVDD therapies, including cell, exosome, gene, and tissue engineering therapy, especially tissue engineering therapy, which runs through the whole process of other therapies. In addition, the article focuses on the cellular, animal, and preclinical challenges faced by each therapeutic approach, as well as their respective advantages and disadvantages, to provide better ideas for relieving the IVDD patients' pain through new treatment methods.

Keywords: Intervertebral disc degeneration, treatments, cell therapy, exosome therapy, gene therapy, tissue engineering therapy

Introduction

Low back pain (LBP) is an important problem affecting the quality of life of middle-aged and older adults and can lead to disability. It has become a severe medical and social problem worldwide. The leading cause of LBP is intervertebral disc (IVD) generation (IVDD). With the growth of the global aging trend, the incidence of LBP caused by IVDD is increasing yearly, which has driven a substantial economic burden on society and families [1].

IVD has a complex structure. Macroscopically, it is composed of three parts: lateral annulus fibrous (AF), central nucleus pulposus (NP), and upper and lower sides cartilaginous endplate (CEP) [2], which is equivalent to the "buffer zone" between the vertebrae (**Figure 1**). Microscopically, it comprises AF cells (AFCs), NP cells (NPCs), CEP cells (CEPCs), and many extracellular matrix (ECM) components [3]. These components are interdependent to jointly maintain the standard physiological mechanism and ensure the regular exercise of biological functions of IVD. When the change of any part breaks through the self-healing ability of IVD, IVD will degenerate to a certain extent, including IVD structure damage and the IVD cell numbers and ECM composition changes.

With the continuous achievement of regenerative medicine and biomaterials in IVDD, which aims to reverse or replace the injured IVD through regeneration pathways such as cells, exosomes, and genes, as well as the construction of artificial IVDs with scaffold materials, these emerging therapies are expected to become a new method. In this review, we focus on the research status and future application prospects of cell therapy, exosome therapy, gene therapy, and tissue engineering to lay a theoretical foundation for preventing and improving the clinical treatment effect of IVDD patients.

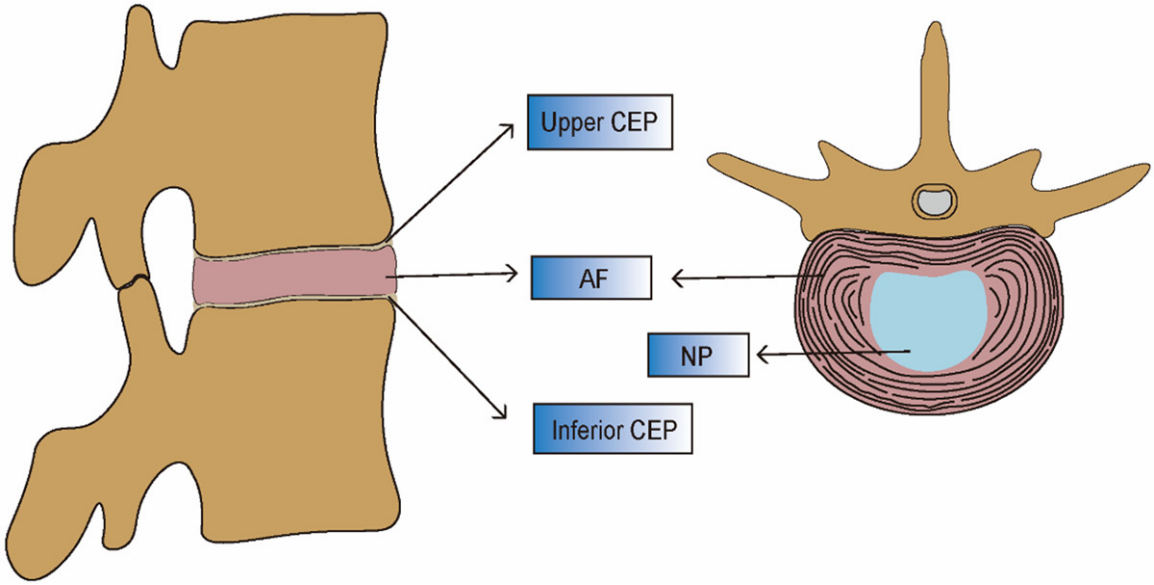

Figure 1. Structure composition diagram of intervertebral disc (IVD) generation (IVDD). This figure shows that the IVD is composed of the nucleus pulposus (NP), annulus fibrous (AF), and cartilaginous endplate (CEP), which are located between two adjacent vertebral bodies and play the role of a connection, mechanical force buffer, etc.

Traditional treatment

Currently, the treatment methods of IVDD include conservative and surgical treatment, and the technology has become mature. Most IVDD patients can obtain good short-term results, but the long-term efficacy is poor. The purpose of conservative treatment is to relieve patients' pain and improve patients' health. For patients with the first attack and most light and medium-sized IVDD diseases, priority should be given to conservative treatment, including physical strengthening, physical therapy, oral drugs, analgesic needles, and other symptomatic treatments [4-8]. In recent years, acupuncture and traditional Chinese medicine have also aroused many scholars' research interest. It plays a particular role in relieving pain in conservative treatment and has an excellent short-term curative effect [9, 10]. Conventional treatment is often ineffective for LBP patients with acute nervous system deterioration, cauda equina syndrome, and chronic LBP. For most patients, surgical treatment has become the final choice, such as simple decompression surgery, fusion surgery, IVD replacement surgery, and endoscopic resection of diseased IVD tissue or decompression, fusion, and IVD replacement [11-15].

Nevertheless, it is prone to various complications after several years. In addition, the robot's application in IVDD disease surgery has been gradually recognized, and there are few relevant research reports. Although domestic and foreign scholars have conducted extensive and far-reaching research on the traditional treatment of IVDD, most IVDD patients have received good treatment to a certain extent. However, these current treatment methods are symptomatic treatments. Many complications, such as IVD inflammation, adjacent vertebral lesions, and infection, cannot fundamentally restore the normal anatomical function of IVD [16]. Therefore, there is an urgent need for new therapies to restore the structure and function of IVD.

Promising treatment strategies

Cell therapy

The loss of IVD cells is one of the most significant pathological changes in IVDD. Supplementing the lost IVD cells has always been considered the most direct and effective method of IVDD molecular treatment [17]. Cell therapy is to transport the patient's autologous (or allogeneic) adult cells (or stem cells) to degenerative IVD in a specific way to supplement the lost IVD cells and increase proteoglycan content and collagen. This restores the typical tissue structure and biomechanical function of the IVD.

Table 1. The role of cells in repairing damaged IVD

Cell type	Source	Experiment	Target	Function	Ref.
NPCs	Dog or human	Cell, animal or human	NPCs/ECM	Replenish lost NPCs and ECM.	[21]
BMSCs	Rat	Cell	NPCs	Directionally induce BMSCs to differentiate into NPCs and promote regeneration.	[30]
ADMSCs	Human or rat	Cell or animal	NPCs	Induce ADMSCs to differentiate into NPCs and supplement the lost ECM.	[31-33]
NPMSCs	Rat	Cell or animal	NPCs/ECM	Induce NPMSCs to differentiate into NPCs and supplement the lost ECM.	[34, 35]
UCMSCs	Rabbit	Cell or animal	NPCs/ECM	Promote the regeneration of NPCs and ECM.	[37]
IPSCs	Pig	Cell or animal	Notochord cell/NPCs	Induce IPSCs to differentiate into NPCs and promote NP regeneration.	[38, 39]

IVD: Intervertebral Disc; Npcs: Nucleus Pulposus Cells; ECM: Extracellular Matrix; Bmscs: Bone Marrow Mesenchymal Stem Cells; Admscs: Adipose-Derived Mesenchymal Stem Cells; Npmscs: Nucleus Pulposus Mesenchymal Stem Cells; Ucmscs: Umbilical Cord Mesenchymal Stem Cells; Ipscs: Induced Pluripotent Stem Cells.

Additionally, cell therapy does not cause additional IVD damage [18].

Cell therapy is most widely used in clinical research and the transformation of clinical achievements. First, the selection of cell types follows the first choice of autologous healthy stem cells or adult cells, followed by homologous cells, and cross-species cell transplantation is not recommended. Second, the specially treated cell components comply with ethical principles and significantly affect the treatment of diseases. They can be used in clinical trials after review and approval [19, 20]. Cell therapy development has gone through many stages, including selecting cell sources and exploring biological mechanisms and application methods. Here, we focus on the possible role of cell sources and their functions in treating IVDD diseases. At present, cells commonly used in IVDD disease treatment research include NPCs, bone marrow mesenchymal stem cells (BMSCs), adipose-derived mesenchymal stem cells (ADMSCs), NP mesenchymal stem cells (NPMSCs), umbilical cord mesenchymal stem cells (UCMSCs) and pluripotent stem cells (IPSCs) (**Table 1**).

The first cells used in cell therapy were autologous NPCs. The study found that NPCs extracted from canine IVDs can be transplanted into autologous IVDs after amplification in vitro. Transplanted NPCs can usually survive, proliferate, and secrete ECM [21]. A further clinical trial evaluation found that patients who received NPC transplants could experience significant improvement in pain symptoms, and the IVD water content could be increased considerably. However, NPC transplantation has many defects, such as limited cell sources, poor quality, and premature aging [22-24]. Therefore, NPCs cannot be used as the ideal cell source for IVD repair.

Mesenchymal stem cells (MSCs) are pluripotent and can differentiate into osteoblasts, chondroblasts, and NPCs. They have the function of repairing damaged IVDs. They are the most widely used stem cells in IVDD diseases [25]. First, BMSCs in a hypoxic environment and transforming growth factor (TGF)-β can differentiate into NPCs with a similar phenotype, activate endogenous NPCs, and increase growth factors and ECM [26, 27]. At the same time, BMSCs can reduce the expression of β-galactosidase and matrix metalloproteinase (MMP)-9, and downregulation of TGF-β/NF-κB signal transduction increases the number of type II collagen and NPCs to reduce inflammation-induced IVD cell aging and restore the activity and function of degenerative NPCs [28, 29]. Second, ADMSCs can differentiate into NPCs under the stimulation of type II collagen and promote the regeneration of NPCs. Combined transplantation with hydroxyapatite derivatives has higher safety and tolerance, can alleviate patient's pain and increase ECM in IVD [30, 31]. Other studies confirmed that a smoothed agonist and TGF-β3 combination could increase the ECM synthesis and secretion of ADMSCs and improve the expression level of NP-specific marker genes and proteins, restoring the height water content and biological structure of degenerative IVD [32]. NPMSCs,

compared with MSCs from other sources, have better differentiation ability of NPCs and adaptability to the IVD microenvironment. They have obvious advantages for cell transplantation and NPC regeneration. It was found that NPMSCs isolated from rat tail IVD could survive for at least 30 days and significantly increase the number of NPCs and ECM content [33, 34]. Clinical trials are ongoing, and many clinical trials are still needed to verify the safety and feasibility of IVDD.

In addition, Perez cruet et al. [35] transplanted UCMSCs into the degenerative IVD model of rabbits and observed that the histological structure of NP was enhanced, and the proteoglycan and water content were significantly improved. IPSCs are like embryonic stem cells. In vitro experiments found that they can differentiate into notochord cells in a porcine NP tissue matrix, which allows mass production of high-quality notochord cells to achieve IVD degeneration [36]. In addition, IPSCs can also be induced to differentiate into NP-like cells in vitro, which also has the potential to regenerate IVD [37]. UCMSCs have the advantages of lower immunogenicity, noninvasive acquisition, and easy expansion in vitro. The most important thing is its ability to survive and present a chondrocyte-like phenotype when injected into rabbit IVD. At the same time, it secretes type II collagen, which is expected to reverse the degenerative IVD and restore the microenvironment composition of IVD [38, 39].

Cell therapy to change the degenerative IVD and reshape the microenvironment for IVD cells is an important research direction in treating IVDD-related diseases. There are various common problems of cell transplantation in cell therapy; for example, cell senescence in advance, the low survival rate after transplantation, requirement of differentiation, isolation, and extraction in the advanced laboratory, storage and transportation difficulties, etc.

Exosome therapy

Exosomes, also called extracellular vesicles (EVs), are the products of cells that exercise the biological function instructions of cells. Its diameter is between 40 and 100 nm [40, 41]. The mechanism of exosome formation is complex. After stimulating specific biological information, the cell membrane invaginates to form "formatted" early endosomes, receives various substances from the plasma membrane and Golgi apparatus, and regulates cell signals through the downregulation of internalization receptors [42]. At this time, early endosomes containing specific biological function information mature and separate to form free multiple internal vesicles. Late internal vesicles are also called multivesicular bodies. Then, multivesicular bodies are further processed and modified by the Golgi and other organelles. Finally, most multivesicular bodies are transported outside the cell in cell budding to form exosomes with specific functions, and only a tiny portion is degraded by lysosomes [43] (**Figure 2**). In addition, the biogenesis of exosomes involves the strict regulation of various factors and cellular signals, including neutral sphingomyelinase-2 and ATP [44]. It participates in cell regeneration and apoptosis under normal and pathological conditions. It can also replicate all genetic and functional information of mother cells. Compared with IVD used for repairing degeneration by stem cell or adult cell transplantation, it can be a carrier of small molecular substances, with more stable properties, no immunogenicity, and more convenient storage and transportation. Therefore, exosome transplantation after human intervention may be better for reversing or regenerating degenerated IVD cells than cell transplantation.

The specific mechanism of exosomes in the treatment of IVDD is unclear. This may be related to inhibiting the inflammatory response, inhibiting apoptosis, promoting the transformation of chondroid NPCs, and regulating miRNA expression. Exosomes secreted by MSCs (MSCs-Exo) probably play an anti-inflammatory role by reducing the expression of inflammatory factor cyclooxygenase-2, inducible nitric oxide synthase, and inflammatory body thioredoxin interacting protein/nucleotide-binding and leucine-rich repeat protein 3 and mRNA to reduce the expression of ECM degradation protease and slow down the catabolic reaction of ECM to delay the process of IVDD [50]. In addition, exosomes can reduce the expression of endoplasmic reticulum stress-related proteins in an NPC apoptosis model and inhibit the activity of critical enzymes in the process of apoptosis and the manifestation of apoptosis-related proteins caspase-3 and 12 to block IVD cell apoptosis induced by

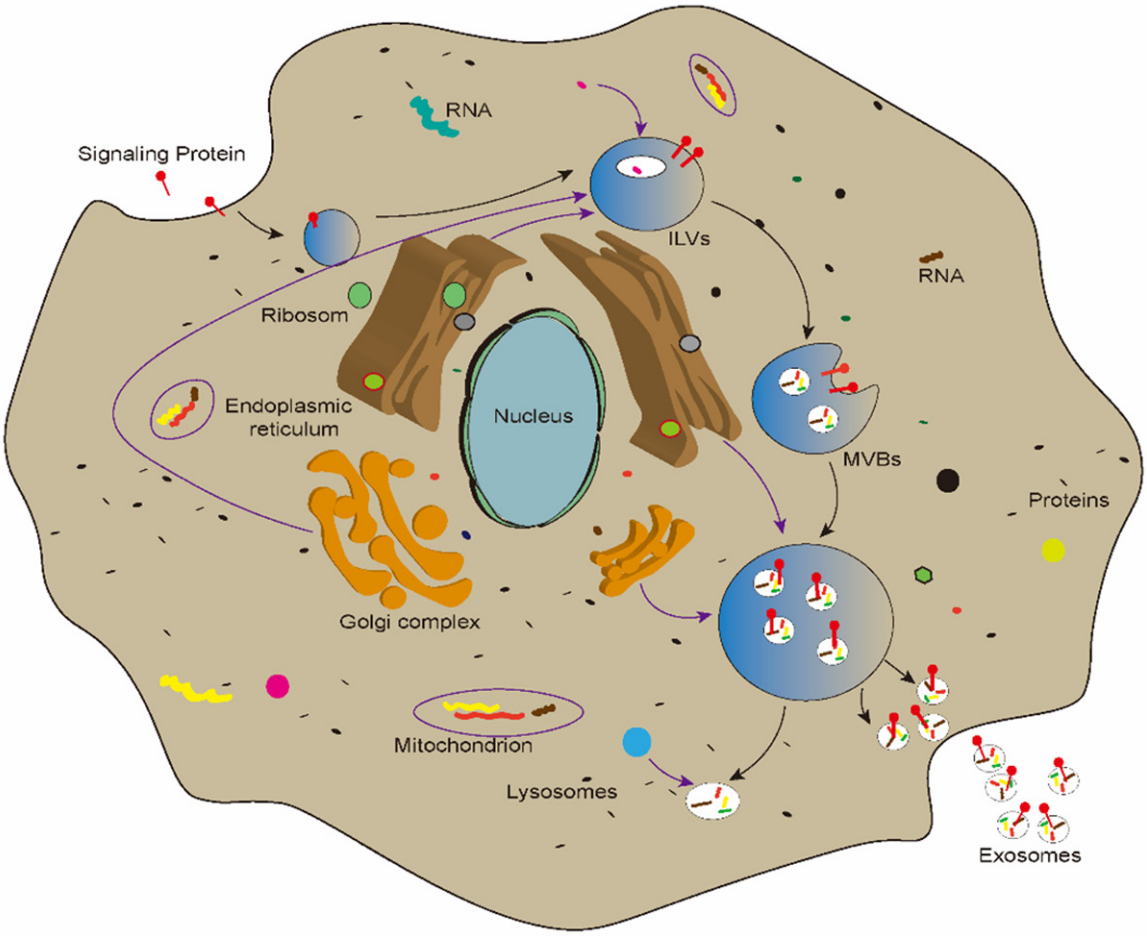

Figure 2. Biosynthesis of exosomes. First, when foreign signals stimulate the cells, the cell membrane collapses and selectively carries specific functional signal molecules (proteins, DNAs, RNAs, and cell-matrix) under the joint action of the Golgi apparatus, endoplasmic reticulum, and other organelles and forms early endosomes. Second, the early endosomes were further modified and processed by the endoplasmic reticulum and Golgi apparatus to form late endosomes. Finally, exosomes are excreted as cell budding, cells play specific biological functions, and intracellular lysosomes catabolize some of them.

endoplasmic reticulum stress [51, 52]. In addition, normal exosomes secreted by NPC (NPC-Exos) promote the differentiation of stem cells into chondroid NPCs and increase the expression of ECM components such as proteoglycans and type II collagen to supplement NPCs and maintain the homeostasis of the ECM environment [53]. Meanwhile, exosomes may also indirectly regulate IVD tissue repair by carrying miRNAs, such as miRNA-27a, miRNA-532-5p, miRNA-142-3p, and miRNA-21/-155 [54-58].

Exosomes come from a wide range of sources, and the application of exosomes from MSCs is the most studied. In the same medium, exosomes secreted by BMSCs (BMSC-Exos) were cocultured with degenerative NPCs. Degenerative NPCs showed an NP-like phenotype, and a certain number of regenerated normal NPCs could be detected. In this process, the miRNA-142-3p carried by exosomes effectively blocked the mitogen-activated protein kinase pathway, reduced the expression of apoptotic genes, and prevented the further degeneration of NPCs [54, 55]. In addition, exosomes can promote the production and secretion of ECM components such as proteoglycans, aggrecans, and type II collagen in IVD, which is conducive to restoring the microenvironment of degenerative IVD [54, 59-61]. Exosomes containing miRNA-21/-155 can act on phosphatase, tensin homolog, and Bach1, inhibiting p53 and the phosphatidylinositol 3-kinase/Akt pathway and upregulating heme oxygenase-1

to prevent IVD cell degeneration [57, 58]. The specific proteins carried by BMSC-Exos can restore the damage to mitochondria in NPCs, maintain the stability of the mitochondrial internal environment, reduce the participation of mitochondria in the oxidative stress response and inhibit the formation of inflammatory bodies by reducing the inflammatory response in IVD tissue [50]. Exosomes are also involved in inhibiting IVDD caused by autophagy. Exosomes can block the inflammatory response induced by interleukin (IL)-1 by acting on the critical factor of the inflammatory response, IL-1 [62].

Similarly, exosomes from ADMSCs are round or oval capsules with precise edges and an average diameter of 90 nm, with a larger diameter than BMSCs [63]. It was found that the miRNAs presented by BMSC-Exos and exosomes secreted by ADMSCs (AMSC-Exos) can inhibit the further occurrence of IVD cell degeneration by regulating and clearing inflammatory factors in degenerative IVDs to restore the normal biological microenvironment in IVDs [64-67]. In addition, both types of exosomes can specifically express CD63 and 70-kDa heat shock proteins, which is conducive to the early diagnosis of IVDD [67]. ADMSC-Exos also showed a similar effect to BMSCs in inhibiting IVD cell apoptosis, but they can also reduce the calcification of CEP [68, 69]. Recent studies have found that exosomes secreted by NPMSCs (NPMSC-Exos) are a channel to realize the biological function of NPMSCs, which can maximize their parental cells' natural process in the degenerative IVD microenvironment. NPMSC-Exos could induce the directional differentiation of NPMSCs, and the expression levels of type II collagen, proteoglycan, Sox9, CD24, and Krt-19 increased significantly [53]. In addition, healthy exosomes secreted by CEP stem cells (CEPSCs) (CEPSC-Exos) can also enhance the occurrence of autophagy and inhibit NPC apoptosis by activating the phosphatidylinositol 3-kinase/Akt signaling pathway, effectively improving the microenvironment of IVD and delaying IVDD to a certain extent, which may be related to anti-apoptotic proteins [70]. In addition, relevant studies have confirmed that CEPSC-Exos can overexpress GATA-binding protein 4, promote TGF-β, and accelerate the invasion, migration, and differentiation of CEPSCs into NPCs, which is conducive to repairing degenerative IVD tissue [71].

Although initial results have been achieved in the source, extraction, and culture of exosomes, the biological mechanisms of exosomes are still unclear, and how to realize the application of exosomes in diseases is still the most prominent and urgent problem.

Gene therapy

Gene therapy provides a new idea for treating IVDD. The technique is to insert the foreign gene into the IVDD patients' appropriate receptor cells through gene transfer technology. The foreign gene can express critical products, such as NPCs and extracellular matrix, to treat IVDD diseases. Gene therapy can also include therapeutic measures and new technologies taken at the DNA level.

There are three approaches for IVDD diseases: viral vectors, non-viral vectors, and gene editing. The advantage of a virus vector is that it can replicate and proliferate efficiently in cells, but its safety needs to be confirmed. This virus includes retrovirus, lentivirus, adenovirus, adeno-associated, and baculovirus [72-76]. Non-viral vectors have high safety but low transfection efficiency, mainly polylysine, polyethyleneimine, inorganic nanoparticles, silicon nanoparticles, natural polymer nanoparticles, and RNA interference [77-84]. DNA nuclease gene-editing technology can achieve accurate and efficient gene editing in normal eukaryotic cells [85, 86]. The above three gene therapy approaches are commonly used for the treatment of IVDD and are the most promising clinical application methods (**Figure 3**). One or more genes control the expression of matrix synthesis factors, catabolic, growth factors and receptors, inflammatory factors and receptors, and intracellular regulatory factors related to IVDD. Their expression may be realized by genetic modification.

Viral vectors are widely studied, and the technology is relatively mature. They are suitable biological vectors for gene therapy for IVDD. For example, the retrovirus is a single-stranded RNA virus that can efficiently transfect IVD cells and express the target gene product in host cells. Wehling et al. [86] successfully produced an IL-1 receptor antagonist protein by transferring the complementary DNA of the bacterial lacZ gene and human IL-1 receptor antagonist into chondrocytes isolated from bovine tail CEP

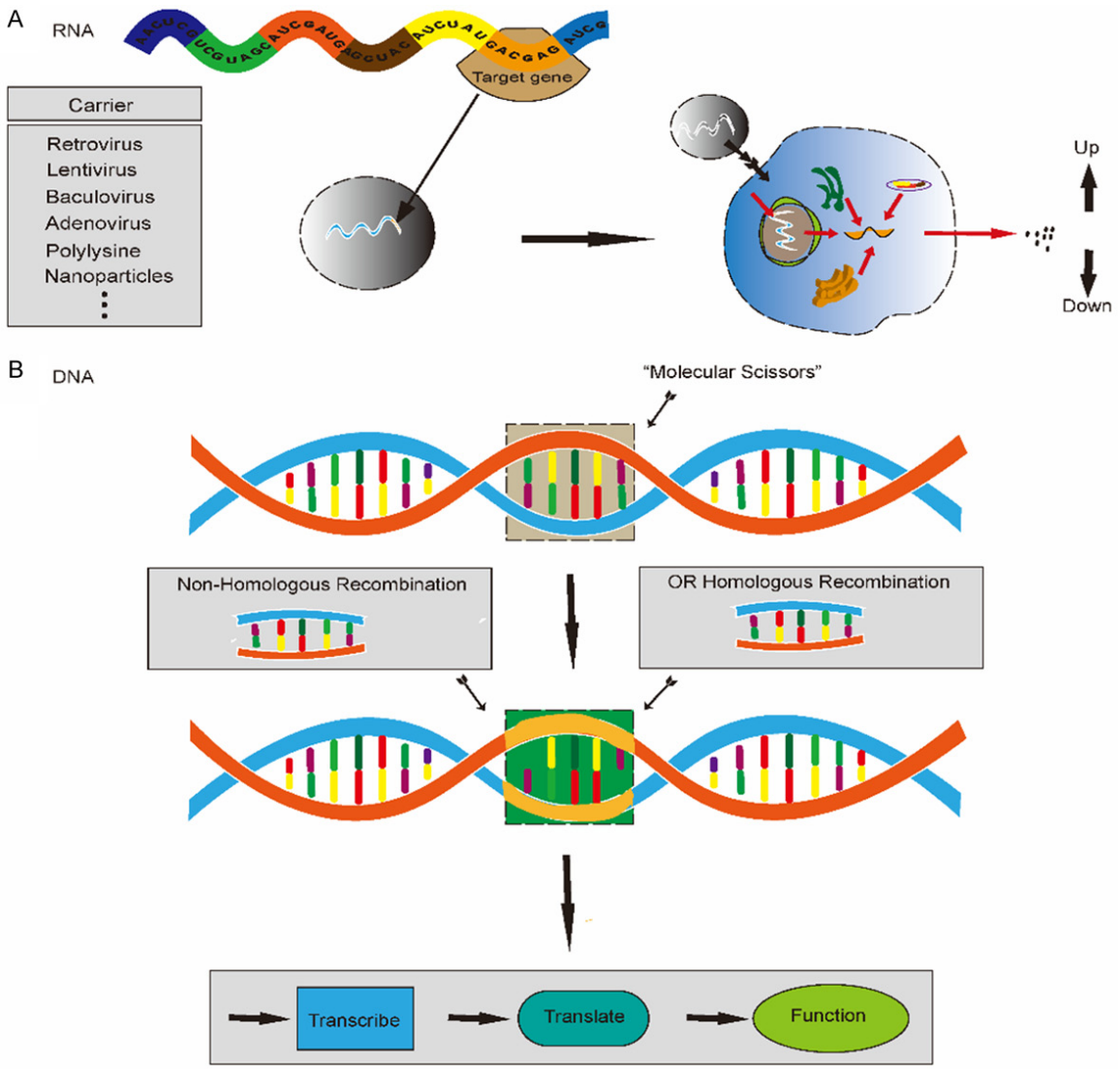

Figure 3. The transcription, translation, and function of foreign genes after they enter the target cell through gene editing technology. A. The target gene is inserted into RNA through gene editing technology. It enters the receptor cell through vectors for transcription and translation to form functional proteins and perform biological functions outside the cell. B. First, the target DNA fragment is removed by "molecular scissors". Then, nonhomologous or homologous recombination is completed and replaced by gene editing. Finally, functional proteins are formed through transcription and translation to perform biological functions.

through a retroviral vector. Reinecke et al. [87] detected similar results in rat IVD cells. These results show the potential value of local gene therapy for IVDD and are a new method of exogenous gene therapy for IVDD. The characteristic of lentivirus is that it can carry a relatively large gene load and a large genome. It has obvious advantages in the multigene expression system. It can efficiently transfect cells in mitotic and nonmitotic stages [88]. It was also found that the contents of type II collagen and proteoglycan in the lentivirus vector gvi15-mediated caspase-3 siRNA-transfected NPCs of human degenerative IVD were significantly higher than those in the control group for one week [73]. In addition, lentivirus-mediated survivin-transfected NPCs of degenerative IVD could restore the morphology of degenerative NPCs but did not affect the apoptosis rate. At the same time, similar studies have shown that the lentivirus vector β3 transduces TGF. Connective tissue growth factor and tissue inhibitor of metalloproteinase (TIMP)-1 can also upregulate proteoglycans and type II collagen [89]. TIMP-1 mediated

by adeno-associated virus alone can also delay the progression of IVDD, and its immunogenicity is lower, which is not related to any known diseases in mammals [90, 91]. In addition, the adenovirus vector has the characteristics of in vitro stability and easy purification. It is not integrated into the host cell to avoid the possibility of insertion gene mutation. Relevant studies have found that adenovirus as a gene delivery vector can prolong the expression of growth differentiation factor (GDF)-5, significantly increase the content of mucopolysaccharide and hydroxyproline in NPCs, and promote the synthesis of ECM, which is very important for restoring the IVD cell survival environment [92]. There are few studies on gene therapy for IVDD mediated by a baculovirus vector. Only a few studies have shown that baculovirus can successfully express the green fluorescent protein gene in IVDD without symptoms [76]. Nevertheless, its effectiveness and availability need to be confirmed by further research.

Compared with viral vectors, nanoparticle vector-mediated gene therapy has unique advantages, including a small volume, no cytotoxicity, no immunogenicity, and high gene transduction efficiency. It is widely used in experimental research on gene therapy for IVDD disease [81]. It was found that these nanoparticles could downregulate the gene expression of matrix protein aggrecan/type I collagen and type II collagen and upregulate MMP-3 expression [80]. This is a discovery for gene therapy in IVDD in vivo. RNA interference is mainly used for specific gene silencing and can be used as gene therapy for IVDD. Moreover, knocking down caspase-3 in rabbit IVD cells by siRNA technology effectively prevented NPC apoptosis and delayed IVDD development. SiRNA can also reduce the IVD cell response to IL-β and effectively reduce or inhibit the inflammatory response stimulation to IVD [84]. In recent years, nanoparticle carriers have been a research hotspot for gene modification and have shown good application prospects.

Unlike viral and nanoparticle vectors, gene editing technology is a new and popular technology for site-specific genome modification. It was developed for the third-generation clustered regularly interspaced short palindromic repeats/Cas-9 system. This technology is becoming more mature, the cost is gradually reduced, and the scope of application is expanding [85]. The latest research found that gene-editing technology can directly upregulate cartilage tissue protein aggrecan and type II collagen to enhance the regeneration phenotype without inducing other growth factors [93, 94]. Therefore, gene therapy shows excellent potential application value in treating IVDD diseases and provides a method to accurately control the phenotype of stem cells for treating IVDD diseases.

Gene therapy, as a targeted therapy that has been pursued for a long time, still has various challenges, including long-term safety, the mass production of gene drugs, the controllability of genes expressed in vivo, the inability of foreign genes to be stably expressed in vivo for a long time, the low efficiency of target gene transfer, changes in the biological characteristics of target cells, ethical problems, etc.

Tissue engineering therapy

Tissue engineering was first proposed at the Washington Science Foundation in 1987 and officially confirmed and defined in 1988. It applies engineering and life science principles to develop biological substitutes for restoring, maintaining, and improving injured IVD function [95]. In particular, tissue engineering technology may be the most critical step in developing cell, exosome, and gene therapy. Tissue engineering materials can provide a suitable carrier and minimally invasive surgery for cell transplantation, exosome transplantation and genetic modification. At present, research on tissue engineering technology mainly focuses on regenerative IVD cells, bionic IVDs, and solving the complications caused by traditional therapies (encapsulating regenerative factors to promote fusion surgery and eliminate inflammatory reactions) [60, 96, 97] (**Figure 4**).

First, tissue engineering therapy has stringent requirements for materials, including low immunogenicity, reasonable encapsulation rate, nontoxicity, degradability, and specific mechanical support properties. The most popular biomaterials in clinical research include natural collagen, chitosan, and hyaluronic acid, as well as synthesized carbon fibers, hydrogels, polylactic acid, polyglycolic acid, and polylactic-co-glycolic acid [98]. Especially for IVD regeneration and artificial IVD construction, a single biomaterial

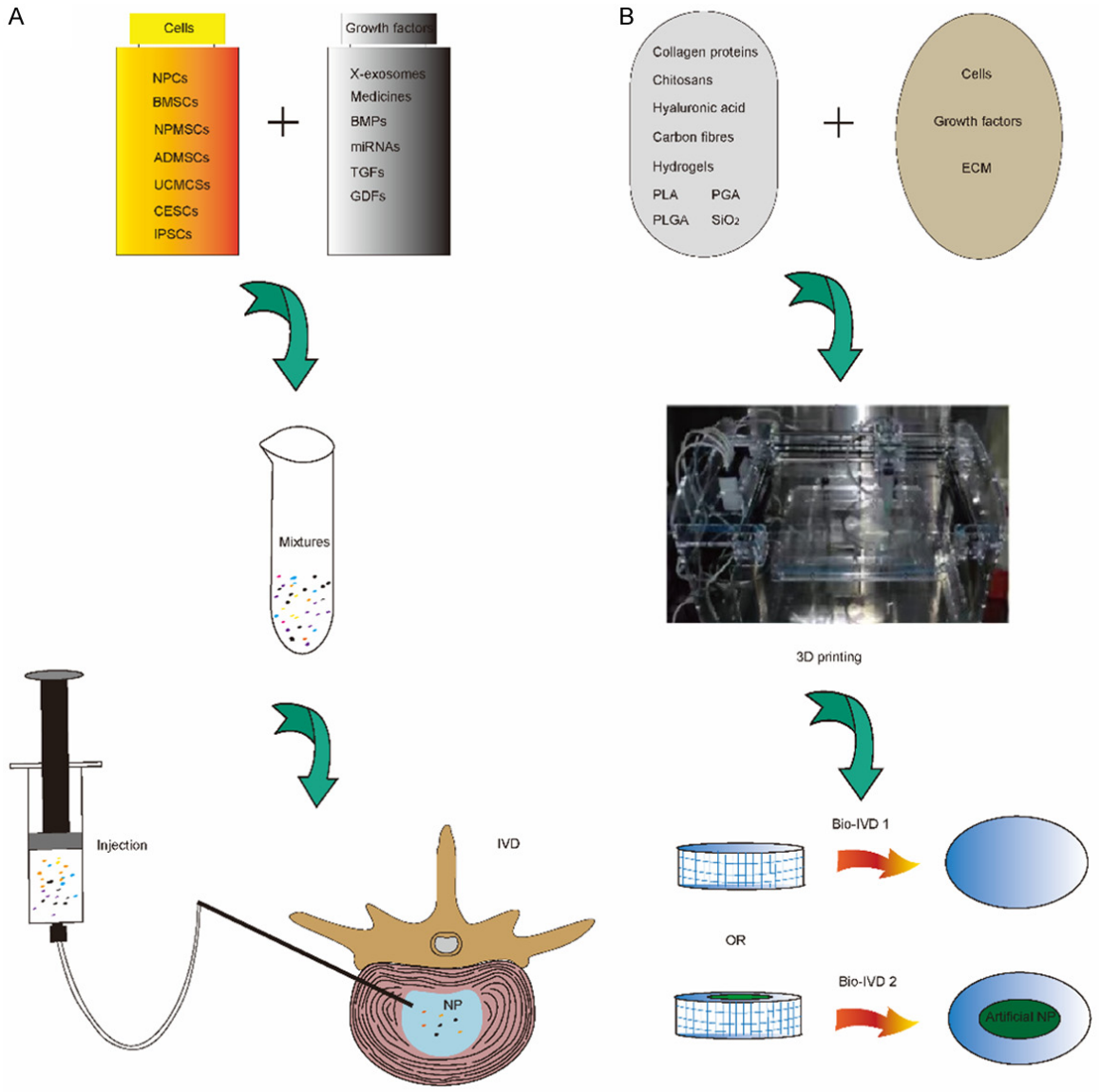

Figure 4. Cells combined with biological factors promote nucleus pulposus (NP) regeneration or 3D-printed biological intervertebral discs (IVDs) through tissue engineering technology. A. Cells are combined with growth factors and transplanted into degenerative IVDs through a specific delivery system. Growth factors can directionally induce some cells to differentiate into NPCs to promote the regeneration of NPCs. Among them, these cells have the function of NP forming or directional differentiation into NP cells (NPCs), including NPCs, bone marrow mesenchymal stem cells (BMSCs), nucleus pulposus mesenchymal stem cells (NPMSCs), adipose-derived mesenchymal stem cells (ADMSCs), umbilical cord mesenchymal stem cells (UCMSCs), cartilaginous endplate stem cells (CEPSCs) and pluripotent stem cells (IPSCs). Currently, the commonly used differentiation-inducing factors include exosomes, drugs, bone morphogenetic proteins (BMPs), miRNAs, transforming growth factor (TGFs), and growth differentiation factors (GDFs). B. Biomaterials, cells, and biological factors are printed in different proportions by a special 3D printing machine [138] to obtain the target biological IVD. These biomaterials are mainly collagen proteins, chitosana, hyaluronic acid, carbon fibers, hydrogels, polylactic acid (PLA), polyglycolic acid (PGA), poly(lactic-co-glycolic acid) (PLGA), and SiO_2. At present, there are mainly two kinds of biological IVDs. One is the bio-IVD, which can withstand certain horizontal, vertical, and torsional stresses. Another is that in addition to the above characteristics, the bio-IVD with NP-like tissue in the center has the function of the natural IVD to distribute mechanical force evenly.

often cannot meet the requirements of complex IVD regeneration. It often needs to hybridize two or more materials to meet the needs of IVD tissue. For example, nanofiber chitosan solution can be used to enhance NP for IVD regeneration and repair [99]. The former mainly

Table 2. Biological factors for GelMA hydrogel clinical disease research

Biological factor	Target	Organization	Function	Ref.
GDF-5 and adipose-derived stem cells	Rats	IVD	Maintain NP tissue integrity and accelerate ECM synthesis	[60]
Aspirin	Rabbits	IVD	Regulation of inflammation after discectomy	[97]
Human dental pulp stem cell and human umbilical vein endothelial cells	Cells	Dental pulp tissue	Lead to ameloblast and odontoblast differentiation	[108]
Human BMSCs	Cells	Bone tissue	Stem cell osteogenic differentiation	[109]
BMSCs	Cells	Bile ducts	Construct biologically active artificial bile ducts	[110]
Human amniotic mesenchymal stromal cells and stromal-derived factor-1alpha	Rats	Brain tissue	Promote stem cell differentiation and repair focal brain injury	[111]
ECM and human cardiac progenitor cell	Rats	Myocardial tissue	Repair damaged myocardium	[112]
NPCs	Cells	IVD	Promote NPC regeneration	[113]
Collagen	Cells	Vascular tissue	Promote angiogenesis	[115]
EVs	Rats	Cartilage tissue	Stimulate chondrogenesis and heal cartilage defects	[116]
Exosomes secreted by human umbilical vein endothelial cells	Rats	Skin tissue	Accelerate wound healing	[117]
Exosomes and ECM	Rabbits	Bone and cartilage tissue	Supplement mitochondria-related proteins and promise osteochondral defects regeneration	[118]
Riboflavin	Cells	Bone tissue	Promote bone regeneration	[119]
Induced pluripotent stem cell-derived neural stem cells	Mice	Spinal cord	Repair injured spinal cord	[120]
14-3-3ε protein	Cells	Bone tissue	Promote osteoblast differentiation and osteogenesis	[121]
Vascular endothelial growth factor	Cells	Vascular tissue	Promote angiogenesis	[122]
Doxorubicin	Mice	Skin tissue	Sustained delivery of drugs	[123]
Angiogenic growth factor	Cells	Vascular tissue	Delivery of vascular growth factors and promotion of angiogenesis	[124]
Transposase-470	Cells	Vascular tissue	Inhibit tumor angiogenesis	[125]
Sinomenium	Mice	Bone and joint tissue	Delay surgery-induced osteoarthritis	[126]
Ciprofloxacin	Cells	Oral tissue	Ablation for oral infection	[127, 128]
Puerarin	Rabbits	Pelvic tissue	Anti-inflammatory and promoting tissue regeneration	[129]
Calcium peroxide	Cells	Cartilage tissue	Promote chondrocyte regeneration	[130]
Metformin	Cells	Bone tissue	Promote osteoblast proliferation	[131]
Octacalcium phosphate	Cells	Bone and vascular tissue	Promote osteogenesis and angiogenesis	[132]
Tumor necrosis factors	Cells	soft tissue	Promote wound healing	[133]
Decellularized liver matrix	Cells	Liver tissue	Elevate liver functions	[134]
Platelet	Cells	Bone and cartilage tissue	Promote bone and cartilage regeneration	[135]
6-deoxy-aminocellulose derivatives	Cells and rats	Skin and soft tissue	Accelerate wound healing	[136]
Hydroxyapatite	Rats	Bone tissue	Fill bone defect	[137]

Gelma: Gelatin Methacryloyl; GDF: Growth Differentiation Factor; IVD: Intervertebral Disc; Npcs: Nucleus Pulposus Cells; Evs: Extracellular Vesicles; ECM: Extracellular Matrix; Bmscs: Bone Marrow Mesenchymal Stem Cells.

strengthens the mechanical properties, which are similar to those of silicon dioxide. The latter mainly enhances the biocompatibility of the tissue, which is similar to that of hydrogel and polylactic-co-glycolic acid [100-102]. This must meet the standards of biomaterial transplantation, such as being nontoxic and having low immunogenicity. In addition, hydrogels with certain photosensitivity, such as gelatin methacryloyl (GelMA) hydrogel and sericin methacryloyl hydrogel can meet the requirement of IVD regeneration by injection at a certain light time. All the biological properties of GelMA hydrogels conform to the requirement for NPC regeneration. It can induce ECM production, induce NPC regeneration, and reduce inflammatory factors

via biological factors. Liu et al. [97] transplanted aspirin-GelMA hydrogel into IVDs with active inflammatory factors. They found that the expression of the inflammatory factor, MMP-3 and a disintegrin-like metalloproteinase with thrombospondin-4/-5 could be significantly inhibited and the inflammatory cycle could be shortened. In addition, celecoxib/polyesterimide microspheres have been anti-inflammatory in canine degenerative IVD tissue [103].

Second, it should be pointed out that bionic IVDs, besides having good biocompatibility with the host, have rigorous requirements on the mechanical properties of tissue engineering scaffold materials, such as longitudinal compression force, torsion force and elastic retraction force [104]. Vicente et al. [105] used 20% wt $BaSO_4$ as the contrast agent for the preformed hydrogel injected into the bovine NP chamber. The axial compression tensile cycle test is carried out at different frequencies. The results show that the treated gelatin-treated IVD has good mechanical properties. The significance of single imitation NP transplantation is low for IVD diseases in which NP and AP are damaged simultaneously. However, there is no bionic IVD to meet the requirements of clinical trials. For example, natural biomaterials lack specific mechanical properties, while synthetic materials lose particular biological activity and degrade. These are still unsolved problems in constructing artificial IVDs [106, 107]. Therefore, simulating the overall cell and matrix structure of IVD remains a severe challenge.

In addition, tissue engineering technology also has a strict selection of molecular substances carried, mainly including cells [108-113], proteins [60, 114-122], and medicine [97, 123-131]. In addition to the above, it also includes stromal-cell derived factor-1α [111], octacalcium phosphate [132], cellulose nanofibrils [133], decellularized liver matrix [134], platelets [135], 6-deoxy-aminocellulose derivatives [136] and gelatin-hydroxyapatite [137]. These small molecules can be encapsulated by GelMA hydrogels and transplanted into clinical disease (**Table 2**).

Tissue engineering technology can provide a new approach to cell, exosome and gene therapy. At the same time, this technology has great potential for future medical regeneration and could provide a better strategy for clinical disease treatment. However, the current clinical application of tissue engineering technology is not mature, and the safety of the material needs to be further explored, which has delayed the clinical application of tissue engineering to some extent.

Conclusions

In conclusion, there are still many unresolved complications in conservative, surgical, and minimally invasive treatments, and it is impossible to cure IVDD fundamentally. Tissue engineering technology crosses the entire cell, exosome and gene therapy spectrum. It is expected to restore the normal physiological structure of IVD at the molecular level. Although there are many problems to be solved in these emerging IVDD therapies, from long-term research, these therapies for IVDD diseases at the etiological level are of great clinical research value.

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Disclosure of conflict of interest

None.

Abbreviations

LBP, Low Back Pain; IVD, Intervertebral Disc; IVDD, IVD Degeneration; AF, Annulus Fibrous; NP, Nucleus Pulposus; CEP, Cartilaginous Endplate; Afcs, AF Cells; Npcs, NP Cells; Cepcs, CEP Cells; ECM, Extracellular Matrix; Bmscs, Bone Marrow Mesenchymal Stem Cells; Admscs, Adipose-Derived Mesenchymal Stem Cells; Npmscs, Nucleus Pulposus Mesenchymal Stem Cells; Ucmscs, Umbilical Cord Mesenchymal Stem Cells; Ipscs, Induced Pluripotent Stem Cells; Mscs, Mesenchymal Stem Cells; TGF, Transforming Growth Factor; GDF, Growth Differentiation Factor; MMP, Matrix Metalloproteinase; Evs, Extracellular Vesicles; Mscs-Exo, Exosomes Secreted By Mscs; NPC-Exos, Exosomes Secreted By Npcs; BMSC-Exos, Exosomes Secreted By Bmscs; IL, Interleukin; ADMSC-Exos, Exosomes Secreted By Admscs; NPMSC-Exos, Exosomes Secreted By Npmscs; Cepscs, CEP Stem Cells; CEPSC-Exos, Exosomes Secreted By Cepscs; TIMP,

Tissue Inhibitor Of Metalloproteinase; Gelma, Gelatin Methacryloyl; PLA, Polylactic Acid; PGA, Polyglycolic Acid; PLGA, Poly (Lactic-Co-Glycolic Acid).

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