VIA MEDICA

REVIEW ARTICLE

Folia Morphol.
Vol. 82, No. 4, pp. 745–757
DOI: 10.5603/FM.a2022.0102

ISSN 0015–5659
eISSN 1644–3284
journals.viamedica.pl

Nucleus pulposus cells degeneration model: a necessary way to study intervertebral disc degeneration

Y.-X. Li, X.-X. Ma, Ch.-L. Zhao, J.-H. Wei, A.-H. Mei, Y. Liu

Department of Spine Surgery, The Affiliated Hospital of Qingdao University, Qingdao, People's Republic of China

[Received: 17 October 2022; Accepted: 9 November 2022; Early publication date: 30 November 2022]

The availability of an appropriate and reliable research model is helpful for researchers to understand the occurrence and development of diseases. Historically, animal models have been beneficial in the study of intervertebral disc degenerative diseases, but intervertebral disc degeneration (IDD) is a precise and complex process that needs to appear and occur in a specific tissue microenvironment, and animal degeneration models cannot fully simulate these parameters. These challenges must be overcome, especially when animal models cannot fully generalise the complex pathology of humans. In the past few years, the research on the cell disease model has made important progress, and the construction of the nucleus pulposus cell (NPC) degeneration model has become an indispensable step in studying the occurrence and development of IDD. Here, several different methods of constructing NPC degeneration models and indicators for testing the effect of modelling are introduced. The practical applications of cell models constructed by different methods are enumerated to screen and evaluate effective methods of establishing degenerative cell models and explore the mechanism of IDD. (Folia Morphol 2023; 82, 4: 745–757)

Key words: human cell model, intervertebral disc degeneration, disease modelling, nucleus pulposus cell, methods

INTRODUCTION

Intervertebral disc degeneration (IDD) is a multifactorial pathological process associated with lower back pain, which can lead to severe neurological dysfunction and disability [3, 95]. The intervertebral disc (IVD), as the joint connecting the vertebral body, is the most critical part of the spine's load-bearing system, and it is also the earliest tissue in the human body to develop degenerative changes [83]. The nucleus pulposus, located in the centre of the annulus fibrosus and between the upper and lower endplate, is gelatinous, can carry a large number of water molecules, and has strong toughness, helping to buffer axial pressure and ensure the flexibility of the spine. The nucleus pulposus is essential to maintain the balance and steady state of the IVD [37]. The main pathological features of IDD are considered to be phenotypic changes, dysfunctions, decreases in the number of active cells, and decreases in the extracellular matrix (ECM) content of nucleus pulposus cells (NPCs). These lead to a cascade event, which begins with changes in the local cellular microenvironment and progresses to damage to the structure and function of IVD [110]. A series of factors such as mechanical changes, imbalance of mitochondrial quality control, inflammation, and oxidative stress are involved

Address for correspondence: Y. Liu, PhD, Department of Spine Surgery, The Affiliated Hospital of Qingdao University, No. 16 of Jiangsu Road, Shinan District, Qingdao, 266003, China, tel: +86 13953296687, e-mail: liuyongly8@126.com

in promoting the occurrence of NPC degeneration [16, 22, 87, 99]. The new pharmacological strategy focuses on eliminating or reversing the degenerative cells in the degenerative IVD to prevent and treat intervertebral disc degenerative disease (IVDD) [27, 125]. Therefore, it is urgent to establish a reliable disease model to study the molecular basis of IVDD to reproduce what happens in the human body *in vitro*. Historically, models built by non-upright walking animals cannot effectively simulate the changes experienced in human IDD [42]. Primate models are expensive and ethically burdensome, which causes this author to consider economic and technological conveniences and find compromises between larger animals and humans. In recent years, the public has called for minimizing the use of animals in research laboratories, also promoting the improvement of the *in vitro* model.

The traditional severe degenerative NPCs isolated from patients' tissues are difficult to apply in a monolayer cell expansion culture. Although normal or mildly degenerative cells can be further cultured, the experimental results are not significant. Therefore, it is urgently needed to build an effective degenerative cell model through different induction methods, which can not only enhance the credibility of the experiment and the persuasive power of the experimental data but also help society to have a clearer understanding of the occurrence and development of IVDD. In the past few years, there have been many methods to induce NPC degeneration, but the model establishment lacks unified standards and has no systematic summary. Therefore, this paper will introduce several methods and model-effect evaluation indicators for inducing NPC degeneration to provide a reference for future screening and evaluation of effective methods for establishing degenerative cell models and exploring the mechanisms of IVD degeneration.

NUCLEUS PULPOSUS CELLS DEGENERATION MODEL ESTABLISHED BY A PHYSICAL METHOD

The IVD comprises the nucleus pulposus, endplates, and annulus fibrosus. The nucleus pulposus in the central part is gelatinous, which can effectively retain moisture and has strong flexibility [53]. The osmosis of the upper and lower endplates is the main way NPCs metabolise nutrients. The outer fibrous annulus, composed of a staggered distribution of elastin and type I collagen, has strong tension [2, 19, 95].

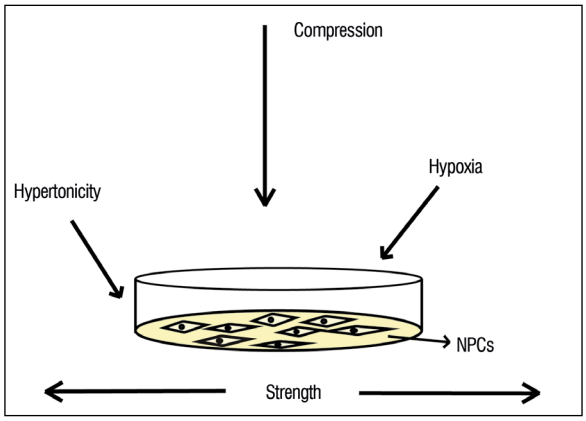

Figure 1. Simple schematic diagram of nucleus pulposus cells (NPCs) degeneration induced by commonly used physical methods.

These structures not only provide support for the IVD but also ensure the flexibility of the spine. However, this also exposes NPCs to various unfavourable environments, such as inadequate nutrient supply and long-term mechanical stress, including compression, shear stress, hydrostatic pressure, and tension [76, 78]. The long-term effects of these factors will cause changes in the structural and biochemical composition of the IVD, accompanied by a series of pathological changes, resulting in IVDD [32, 43]. It is particularly important to choose different modelling methods for different pathogeneses, according to which some researchers choose certain physical methods to induce NPC degeneration (axial compression, transverse stretching, hyperosmotic stress, hypoxia, etc., Fig. 1). In the axial compression method, NPCs are placed in a pressure device with good sealing to compress the air containing 5% carbon dioxide (CO_2) to provide different MPa pressure values and build NPC degeneration models [9, 44, 105]. In 2012, Ding et al. [15] used this method to construct a cell model and found that part of the pathological cause of IVDD induced by mechanical compression came from mitochondrial damage. Follow-up experiments showed that co-cultures with bone marrow mesenchymal stem cells could inhibit compression-induced NPC apoptosis [8]. The transverse periodic stretching method uses a transverse reciprocating stretching device to provide supracellular physiological tension for constructing a disease model [4, 14, 74, 100]. This method is also often used to build NPC degeneration models [115, 118]. In 2019, Yang et al. [107] used the Flexercell tension system to build an NPC degeneration model and found that abnormal mechanical stress promot-

ed NPC apoptosis and accelerated the occurrence of IDD, while autophagy helped to reverse apoptosis. When the biological stress exceeds the physiological expansion pressure of NPCs, whether the process is either axial compression or transverse stretching, the apoptosis pathway is activated, the expression of the polysaccharide-protein gene is down-regulated [12], and the osmotic and hydrostatic pressures increase. Some research teams have studied the effect of osmotic pressure on NPC biology because the change of osmotic pressure is a secondary change under biological stress. For example, in 2014, Dong et al. [18] established a rabbit NPC degeneration model by increasing the osmotic pressure of the culture medium and found that high osmotic pressure activated p38 mitogen-activated protein kinases (p38MAPK), mitogen-activated protein kinase 8/9 (JNK1/2), and mitogen-activated protein kinase 3/1 (ERK1/2) pathways in rabbit NPCs. The activated p38MAPK and JNK1/2 pathways induced NPC apoptosis, while the activated ERK1/2 pathway was beneficial to cell survival. The change in oxygen content is also closely related to NPC degeneration. In 2016, Choi et al. [10] successfully induced NPC degeneration by adjusting the oxygen concentration in an incubator from 21% to 1% (5% CO_2 and 94% nitrogen) in cultured cells for 24 h. In addition, reducing the pH value of the culture medium and creating an acidic environment can also be used to construct an NPC degeneration model [106].

NUCLEUS PULPOSUS CELLS DEGENERATION MODEL ESTABLISHED BY A CHEMICAL METHOD

Inflammatory reactions are important pathological mechanisms of IDD [84]. The overexpression of proinflammatory cytokines can destroy the ECM homeostasis of the IVD and induce degeneration and catabolism of the IVD [57, 71]. Neurotrophins are produced under the stimulation of inflammatory factors and promote nerve growth into the IVD, accelerating the IDD cascade [30]. In addition, inflammatory factors have a negative effect on reparative stem cells [55, 73, 89, 101]. The chemical method to construct the NPC degeneration model is to simulate the inflammatory environment in the early stage of lumbar pathology caused by chemical reagents and drugs to establish the cell model (Fig. 2). This type of cell model is highly important for promoting the research of IVDD. In 2006, Aota et al. [1] found that toll-like receptors in bovine NPCs are sensitive to the binding of bacterial lipopolysaccharides (LPSs) (a microbial component found in the outer membrane of Gram-negative bacteria). Toll-like receptors are important regulators of the nuclear factor kappa-B signalling pathway and are closely related to cellular inflammation and degeneration [75]. In 2013, Kim et al. [49] successfully established a degeneration model of bovine NPCs induced by LPS and used this cell model to prove that inhibition of the myeloid differentiation primary response 88 pathway can effectively inhibit inflammation and anti-catabolism. Since then, many scholars have chosen this modelling method [17, 60, 116, 121, 124], and the LPS induction method has since become the most used and popular modelling method. In addition, interleukin-1 beta and tumour necrosis factor-alpha are important pro-inflammatory factors involved in cell differentiation and apoptosis by regulating various pathways, so they are also often used to induce NPC degeneration [7, 31, 34, 47, 48, 117, 119, 123].

Some stressors can also be used to construct NPC degeneration models. These stressors strongly oxidize, which can destroy the normal redox state in cells, leading to the imbalance between the oxidation and antioxidant systems [81]. These stressors can also stimulate cells to produce harmful molecules, such as damaged nucleic acids, proteins, and lipids, which may lead to the occurrence and development of chronic degenerative disease [98]. Hydrogen peroxide (H_2O_2) is a commonly used induction reagent by researchers. As a type of reactive oxygen species, H_2O_2 can inhibit cell proliferation, cause oxidative damage to macromolecules in cells, and eventually lead to serious consequences, such as cell senescence, death, and mutation. Therefore, H_2O_2-induced oxidative stress cell models are widely used to explore the mechanism of free radical-mediated cell injury and the protection and repair mechanism of antioxidants on oxidative damage [35, 64, 72, 120]. In 2019, Tang et al. [93] used H_2O_2 to build a mouse NPC degeneration model to study the role of nuclear factor erythroid 2–related factor 2 (Nrf2) in NPC degeneration. It was found that Nrf2 can slow down NPC degeneration induced by oxidative stress by activating autophagy through feedback. Tert-butyl hydroperoxide (t-BHP) [70, 108, 122] can also be used to construct an NPC degeneration model. In 2016, Chen et al. [6] proved that metformin could inhibit the apoptosis and senescence of NPCs

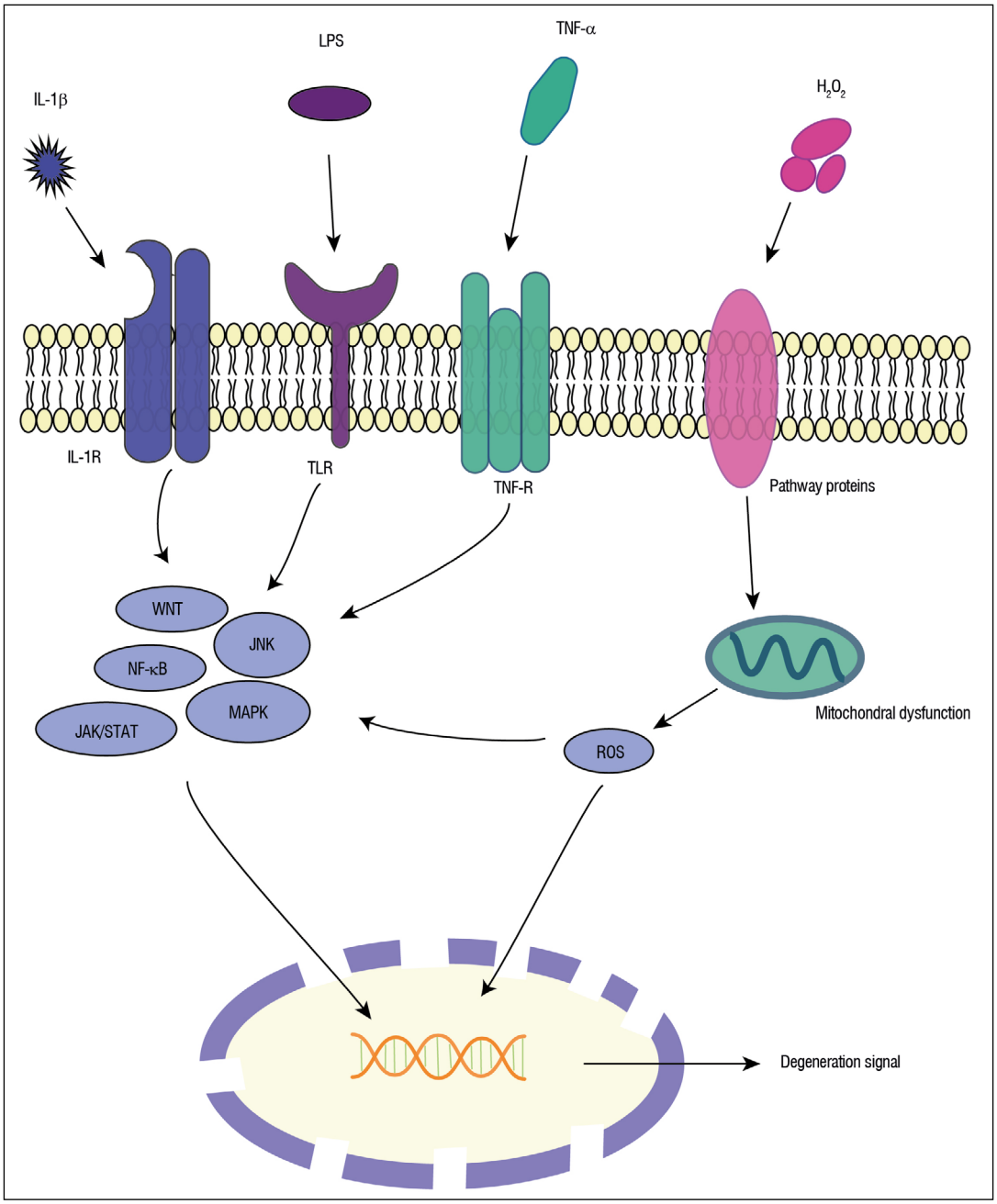

Figure 2. Simple schematic of nucleus pulposus cells degeneration induced by commonly used chemical methods; IL — interleukin; LPS — lipopolysaccharides; TNF — tumour necrosis factor; TLR — toll-like receptor; WNT — wingless-type MMTV integration site family; JNK — c-Jun N-terminal kinase; NF-κB — nuclear factor kappa-B; STAT — signal transducer and activator of transcription; MAPK — mitogen-activated protein kinases; ROS — reactive oxygen species.

induced by t-BHP by autophagy. In recent years, angiotensin II (AngII) has also been used to induce NPC degeneration [90]. The Ang II receptor type 1 (AT1) receptor (G protein-coupled receptor) is the main biological medium of AngII. The combination of AngII and AT1 can promote the production of reactive oxygen species and the accumulation of pro-inflammatory cytokines and classically activated macrophages, resulting in NPC degeneration [13, 88]. In addition, advanced oxidation protein products [104], stromal cell-derived factors [69], polymethyl methacrylate [24], nitroprusside [59], and cobalt chloride [23] can also be used to establish an NPC degeneration model.

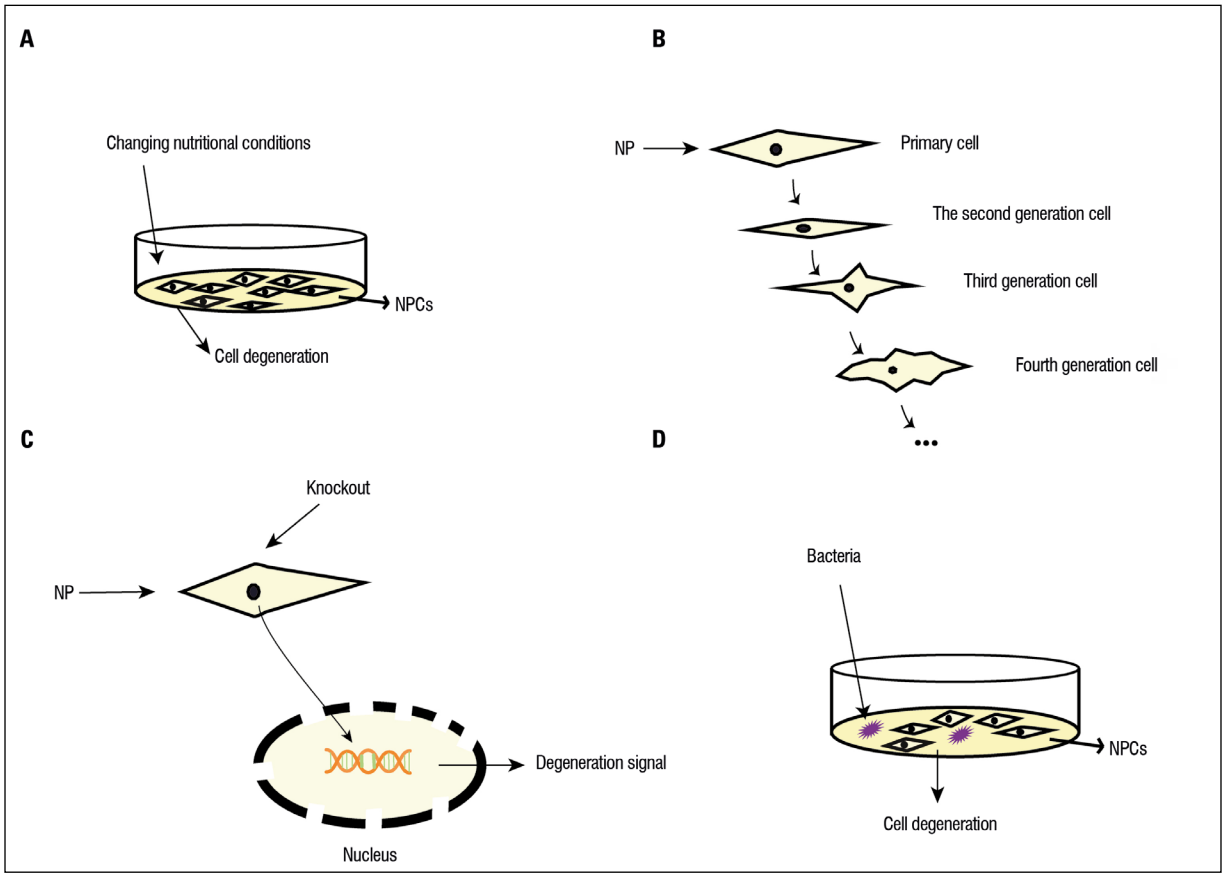

Figure 3. A–D. Simple schematic representation of biological or bioengineered methods for inducing nucleus pulposus (NP) cells (NPCs) degeneration.

NUCLEUS PULPOSUS CELL DEGENERATION MODEL ESTABLISHED BY BIOLOGICAL OR BIOENGINEERING METHODS

An NPC degeneration model can also be constructed by changing the nutritional environment, increasing replication algebra, gene knockout, microbial co-culture, etc. (Fig. 3). Nucleus pulposus cells exist in a unique environment regarding their nutritional supply. The IVD has no direct vascular supply, so nutrients are provided to NPCs by capillaries that penetrate the subchondral plate and terminate at the boundary of the cartilage endplate. The NPC microstructure changes, such as reduced capillary density [63, 113], endplate calcification [25, 112], and metabolic disorders [54], will cause the IVD to degenerate. In 2018, Wang et al. [97] successfully established an NPC degeneration model by incubating NPCs of normal mice in high glucose for 72 h. In 2017, Chang et al. [5] also successfully induced NPC degeneration by using a sugar-free medium. Amino acid and serum removal can also be used to construct an NPC degeneration model [61, 68]. The natural degeneration of NPCs is mostly related to age [51, 79]. In 2019, Gong et al. [28] constructed a degeneration model by repeated cell passage, which proved that bone morphogenetic protein 7 could reduce the senescence of human IVD NPCs induced by the passage of time by activating the phosphoinositide 3-kinase/protein kinase B pathway. Gene knockout and microbial co-culture are newer methods for NPC degeneration models. Kong et al. [52] constructed a degenerative cell model by down-regulating the hsa_circ_0059955 gene of normal mouse NPCs and inducing NPC apoptosis and cell cycle arrest. In 2020, He et al. [33] found that *Cutibacterium acnes* induce NPC degeneration by activating the NOD-like receptor thermal protein domain associated protein 3 (NLRP3)-dependent pathway.

MODEL EFFECT EVALUATION INDEX

After choosing different ways to construct the NPC degeneration model, it is necessary to further evaluate the success of the model, the state of cell activity, the degree of cell damage, etc. Morphological observation is a more intuitive way of detection

because degenerative cells will appear as swelling, flattening, with reduced nuclear volume, and have loose adhesion in the shape of a linear semi-adherent [15]. Transmission electron microscopes are used to observe the subcellular structure, nuclear shrinkage, a large number of apoptotic bodies, chromatin condensation, and cytoplasmic vesicles containing more dense vesicles [8]. However, there is no quantitative standard to judge the quality of cell morphology, so this method cannot be used as an evaluation index alone, and it is often necessary to use it alongside 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, cell counting Kit-8, or terminal deoxynucleotidyl transferase mediated nick end labelling assays, flow cytometry, etc. to further evaluate cell activity.

Type II collagen, polyproteoglycans, and cell matrix-degrading enzymes (matrix metalloproteinase [MMP] 3, MMP13, a disintegrin and metalloproteinase with thrombospondin motifs [ADAMTS] 4, and ADAMTS5) are often used as important indicators of NPC degeneration [58]. Type II collagen and polyproteoglycan are produced by NPCs and play an important role in maintaining the flexibility and compression function of the IVD. The decrease in its content is the initial factor of IDD because it changes the integrity of the biomechanical structure of the IVD and destroys the metabolic balance of the ECM. Under the stimulation of pathological conditions, inflammation-related pathways are activated, and matrix-degrading enzymes, whose main function is to degrade type II collagen and polyproteoglycans in NPCs, are promoted to secrete. In degenerative NPCs, the content of type II collagen and polyproteoglycan decreased, while the cell matrix-degrading enzyme content increased. The degree of change was related to the degree of cell degeneration.

In addition, the content of some apoptosis-related marker proteins can also be used to evaluate the degree of cell degeneration. Beta-galactosidase (β-galactosidase) (senescence-associated beta-galactosidase [SA-β-gal]) is found only in degenerative senescent cells and accumulates gradually with cell degeneration. Beta-galactosidase can hydrolyse β-galactosidase into monosaccharides. A blue stain precipitate will appear when β-galactosidase is present under acidic conditions. Cell degeneration can be further evaluated by observing the colour of the precipitate under a microscope [20]. There is a close relationship between caspase-3, B-cell lymphoma 2 (Bcl-2), Bcl-2-associated X protein (BAX), and apoptosis [109]. Caspase is a cysteine aspartic acid-specific protease in the cytoplasm, which is homologous to the suicide gene cell death protein 3 in nematodes and plays an important role in the process of cell senescence and degeneration [29]. B-cell lymphoma 2 contains two proteins: bcl-2 α and bcl-2 β. The bcl-2 α protein is an integrin of the mitochondrial membrane and plays a role in anti-apoptosis [85]. The BAX protein is inactive when it does not receive apoptosis stimulation. After it is activated, it can destroy the integrity of the mitochondrial membrane, antagonize the function of Bcl-2, and promote apoptosis [91]. The effect of establishing an NPC degeneration model can be evaluated by the content changes of the above indexes.

The cell oxidation ability can also be used to evaluate cell degeneration. Peroxidation often leads to cell dysfunction-induced degeneration, and malondialdehyde is one of the peroxidation products. The level of malondialdehyde content indicates the degree of cell damage and degeneration [38]. Superoxide dismutase and deacetylase are related to antioxidation [77, 114], and the level of these enzymes reflects the intracellular oxidation state and can be used as a detection index for the construction of an NPC degeneration model.

The key index to evaluating the modelling mode is to ensure the cell survival rate and successfully construct the degeneration model. The best way to induce degeneration is to have both the minimum cytotoxicity or damage and subsequently meet the needs of the experimental model. This makes it necessary to try different variables in the same way, such as intensity, concentration, time, etc., and use evaluation indicators to select the best treatment conditions. In this paper, different modelling methods are classified and summarised from the aspects of physics, chemistry, and biological engineering. The results of the same treatment methods and different treatment conditions are compared, and the best treatment conditions of the current research methods are selected (Table 1) [7, 9, 10, 17, 18, 28, 33–36, 39, 41, 44–46, 48, 50, 52, 56, 60, 62, 64–67, 70, 72, 80, 92, 102, 105, 107, 108, 111, 115–118, 120–122, 124].

CURRENT CHALLENGES

The physical modelling method is simple and controllable, causes less damage to cells, and is similar to the natural degeneration of NPCs, which is suitable for the pathological mechanism study. The modelling method is still not comprehensive, as treatments such

Table 1. Common methods of constructing nucleus pulposus cell degeneration model and optimal treatment conditions

Research style	Modelling approach	Optimum condition	References
Axial compression	The cells are placed in a pressurizing device that increases the pressure	Pressure value: 1 MPa; Treatment time was 36–48 h	[9, 36, 44, 45, 105]
Cyclic tension stretching	The cells are stimulated by periodic stretching	Tensile strength: 20%; Frequency: 1 Hz; Processing time: 4 to 6 h	[107, 115, 118]
Hypoxia-induce	Reduce the oxygen in the incubator	Oxygen content: 1%; The treatment time is 24 h	[10, 39, 50, 56]
Hypertonic-induce	Increase osmotic pressure of culture medium	Osmotic pressure: 550 mOsm/kg; Processing time: 3–7 days	[18, 41, 111]
LPS-induce	An appropriate amount of LPS was added to the culture medium	Concentration: 10 μg/mL; The processing time is 48 h	[17, 60, 62, 116, 121, 124]
IL-1β-induce	An appropriate amount of IL-1β was added to the culture medium	Concentration: 10 ng/mL; The treatment time is 24 h	[7, 34, 48, 80, 117]
H_2O_2-induce	An appropriate amount of H_2O_2 was added to the culture medium	Dose: 400 μmol; The treatment time is 24 h	[35, 64, 72, 102, 120]
TBHP-induce	An appropriate amount of TBHP was added to the culture medium	Dosage: 50 μmol; The treatment time is 24 h	[70, 46, 67, 108, 122]
Duplicator method	Cells were cultured by multiple passages	Passage times: 6 generations	[28]
Gene knockout	Knockdown of specific coding genes	Gene name: hsa_circ_0059955	[52]
Biological induction method	Co-culture with sustenance	Microorganism: P. acnes	[33, 65, 66, 92]

IL — interleukin; LPS — lipopolysaccharide; TBHP — tert-butyl hydrogen peroxide

as radiation, ultraviolet irradiation, current stimulation, low-temperature induction, and other cell modelling methods have not yet been applied. Chemical modelling has a variety of methods, short modelling times, and is practically simple. It is widely used to study the relationship between inflammation, oxidative stress, autophagy, and IDDs, but it causes varying degrees of damage to cells. A reasonable induction dose is particularly important for the success or failure of this modelling. The stability of the cell model constructed by a biological or bioengineering method is relatively poor, and it is not widely used at present. It is necessary to further explore the optimal conditions and optimize the induction method in the future.

Whether physical, chemical, biological, or bioengineering methods are used, the purpose of inducing cell degeneration can be achieved by simulating the changes in external stimulation conditions of NPCs. Some of the mechanisms of cell degeneration overlap; for example, hypoxia induction can cause cells to produce oxidative stress and degenerate, similar to using stressors to stimulate NPCs in chemical modelling methods. In the biological induction method, part of the reason for the construction of the NPC degeneration model with a microbial co-culture may be the inflammatory response of cells stimulated by microorganisms, which partially overlaps with the mechanism of the cell degeneration model constructed by inflammatory factors in chemical methods. Although there may be the same mechanisms in different induction methods, the occurrence and development of IDD involve multiple factors and stages. The *in vitro* model constructed by different methods can simulate the key sites or stages of specific lesions, which is conducive to a comprehensive and detailed study of IDD's aetiology and pathological mechanism.

Intervertebral disc degeneration resulting in pain and abnormal function of lower limbs, has become a serious problem in modern society, causing a huge economic burden [21]. Traditional surgery and drug therapy cannot satisfy the fundamental treatment goals. Changing IDD at the cellular, molecular, and genetic levels is the research goal for most researchers because *in vitro* experiments are controllable and accessible [26, 103]. The NPC degeneration model is an indispensable step in studying IDD's occurrence, so choosing a reliable, feasible, and appropriate modelling method is highly important to explore IDD's aetiology and pathogenesis.

CONCLUSIONS

Nucleus pulposus is located in the centre of annulus fibrosus and between the superior and inferior endplates. It has strong toughness and is an important part of maintaining the balance and stability of

IVD. The notochord cells originating from the mesoderm are wrapped in connective tissue derived from osteogenic knots and develop into nucleus pulposus tissue in the early stage of the embryo [82, 96]. Therefore, there are chordate cells with large shape and a large number of vesicles in the early nucleus pulposus. With the maturation of the IVD, the composition of the nucleus pulposus changed: the number of large vacuolar cells considered to be the origin of the notochord decreased and gradually transformed into small chondrocyte-like cells with smaller morphology and no vesicles [11, 86]. With the "disappearance" of notochord cells in human nucleus pulposus, some signs of early IDD, such as decreased water content of nucleus pulposus and microfissures of annulus fibrosus, began to appear. Based on this phenomenon, many scholars speculate that early IDD occurs with the disappearance or degeneration of notochord cells [94]. However, in some animals, such as rabbits, cattle and dogs, there are a large number of notochord cells in the nucleus pulposus, and there is almost no degeneration of the IVD [40]. Therefore, based on the fact that IVD cells are different from human IVD cells, there are some drawbacks when using animal models; they do not mimic the pathological changes of human degenerative IVD diseases.

Intervertebral disc degeneration is a multi-factor and complex process, and the changes in external stimuli and internal physical and chemical properties are the two core elements of IDD. A variety of provided animal and cell models assist greatly in in-depth studying and overcoming the mechanism of IDD. Compared with animal models, cell models have the advantages of short modelling cycles, economic factors, obvious effects, and a better ability to simulate the internal environment. In constructing an NPC degeneration model, the physical modelling method can be the most similar to the NPCs' natural degeneration, and the biological and chemical modelling methods can simulate the pathological environment to construct the model quickly. However, it is difficult to produce an *in vitro* model of NPCs induced by a single factor that meets the aetiology and pathogenesis of IVDD. Second, chemicals and microorganisms cause rapid degeneration through different degrees of cell damage. This is quite different from the degenerative cells in natural environments. Although the replicated model causes the least damage to the cells, the stability of the model is poor, and it is easy to generate apoptosis.

In summary, little is currently known about the cellular process of IDD's occurrence and development. Although the *in vitro* model cannot fully simulate the aetiology and pathological mechanism of IDD, researchers can choose a model reasonably according to the phenotype or pathogenic pathway due to the in-depth study of these *in vitro* models. To further optimise the construction mode and evaluation criteria of the cell model in the future, a suitable model can be constructed for pathological research or drug screening through differentiation analysis with natural degenerative cells to provide materials and basic data for exploring the mechanism and treatment strategy of IDD.

Acknowledgements

We are particularly grateful to all the people who have given us help on our article.

Funding

This study was supported by the "Clinical Medicine + X" scientific research project of the Affiliated Hospital of Qingdao University National Natural Science Foundation of China (81871804).

Conflict of interest: None declared

REFERENCES

1. Aota Y, An HS, Imai Y, et al. Comparison of cellular response in bovine intervertebral disc cells and articular chondrocytes: effects .of lipopolysaccharide on proteoglycan metabolism. Cell Tissue Res. 2006; 326(3): 787–793, doi: 10.1007/s00441-006-0225-1, indexed in Pubmed: 16788835.
2. Ashinsky B, Smith HE, Mauck RL, et al. Intervertebral disc degeneration and regeneration: a motion segment perspective. Eur Cell Mater. 2021; 41: 370–380, doi: 10.22203/eCM.v041a24, indexed in Pubmed: 33763848.
3. Bisson DG, Mannarino M, Racine R, et al. For whom the disc tolls: intervertebral disc degeneration, back pain and toll-like receptors. Eur Cell Mater. 2021; 41: 355–369, doi: 10.22203/eCM.v041a23, indexed in Pubmed: 33738788.
4. Campos LC, Ribeiro-Silva JC, Menegon AS, et al. Cyclic stretch-induced Crp3 sensitizes vascular smooth muscle cells to apoptosis during vein arterialization remodeling. Clin Sci (Lond). 2018; 132(4): 449–459, doi: 10.1042/CS20171601, indexed in Pubmed: 29437853.
5. Chang H, Cai F, Zhang Y, et al. Early-stage autophagy protects nucleus pulposus cells from glucose deprivation-induced degeneration via the p-eIF2α/ATF4 pathway. Biomed Pharmacother. 2017; 89: 529–535, doi: 10.1016/j.biopha.2017.02.074, indexed in Pubmed: 28254665.
6. Chen D, Xia D, Pan Z, et al. Metformin protects against apoptosis and senescence in nucleus pulposus cells and ameliorates disc degeneration in vivo. Cell Death Dis. 2016;

7(10): e2441, doi: 10.1038/cddis.2016.334, indexed in Pubmed: 27787519.
7. Chen F, Jiang G, Liu H, et al. Melatonin alleviates intervertebral disc degeneration by disrupting the IL-1β/NF-κB-NLRP3 inflammasome positive feedback loop. Bone Res. 2020; 8: 10, doi: 10.1038/s41413-020-0087-2, indexed in Pubmed: 32133213.
8. Chen S, Zhao L, Deng X, et al. Mesenchymal stem cells protect nucleus pulposus cells from compression-induced apoptosis by inhibiting the mitochondrial pathway. Stem Cells Int. 2017; 2017: 9843120, doi: 10.1155/2017/9843120, indexed in Pubmed: 29387092.
9. Chen S, Lv X, Hu B, et al. RIPK1/RIPK3/MLKL-mediated necroptosis contributes to compression-induced rat nucleus pulposus cells death. Apoptosis. 2017; 22(5): 626–638, doi: 10.1007/s10495-017-1358-2, indexed in Pubmed: 28289909.
10. Choi H, Merceron C, Mangiavini L, et al. Hypoxia promotes noncanonical autophagy in nucleus pulposus cells independent of MTOR and HIF1A signaling. Autophagy. 2016; 12(9): 1631–1646, doi: 10.1080/15548627.2016.1192753, indexed in Pubmed: 27314664.
11. Colombier P, Clouet J, Chariau C, et al. Generation of human nucleus pulposus cells from stem cells: First steps towards intervertebral disc regeneration. Osteoarthritis Cartilage. 2016; 24(Suppl. 1): S11–S12, doi: https://doi.org/10.1016/j.joca.2016.01.051.
12. Court C, Colliou OK, Chin JR, et al. The effect of static in vivo bending on the murine intervertebral disc. Spine J. 2001; 1(4): 239–245, doi: 10.1016/s1529-9430(01)00056-0, indexed in Pubmed: 14588327.
13. Dandona P, Dhindsa S, Ghanim H, et al. Angiotensin II and inflammation: the effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockade. J Hum Hypertens. 2007; 21(1): 20–27, doi: 10.1038/sj.jhh.1002101, indexed in Pubmed: 17096009.
14. Davidovich N, DiPaolo BC, Lawrence GG, et al. Cyclic stretch-induced oxidative stress increases pulmonary alveolar epithelial permeability. Am J Respir Cell Mol Biol. 2013; 49(1): 156–164, doi: 10.1165/rcmb.2012-0252OC, indexed in Pubmed: 23526210.
15. Ding F, Shao ZW, Yang SH, et al. Role of mitochondrial pathway in compression-induced apoptosis of nucleus pulposus cells. Apoptosis. 2012; 17(6): 579–590, doi: 10.1007/s10495-012-0708-3, indexed in Pubmed: 22392483.
16. Ding SL, Zhang TW, Zhang QC, et al. Excessive mechanical strain accelerates intervertebral disc degeneration by disrupting intrinsic circadian rhythm. Exp Mol Med. 2021; 53(12): 1911–1923, doi: 10.1038/s12276-021-00716-6, indexed in Pubmed: 34934193.
17. Dong Y, Liu L, Shan X, et al. Pilose antler peptide attenuates LPS-induced inflammatory reaction. Int J Biol Macromol. 2018; 108: 272–276, doi: 10.1016/j.ijbiomac.2017.11.176, indexed in Pubmed: 29208559.
18. Dong ZH, Wang DC, Liu TT, et al. The roles of MAPKs in rabbit nucleus pulposus cell apoptosis induced by high osmolality. Eur Rev Med Pharmacol Sci. 2014; 18(19): 2835–2845, doi: 10.1055/s-0034-1376584, indexed in Pubmed: 25339477.
19. Fearing BV, Hernandez PA, Setton LA, et al. Mechanotransduction and cell biomechanics of the intervertebral disc. JOR Spine. 2018; 1(3), doi: 10.1002/jsp2.1026, indexed in Pubmed: 30569032.
20. Feng C, Yang M, Zhang Y, et al. Cyclic mechanical tension reinforces DNA damage and activates the p53-p21-Rb pathway to induce premature senescence of nucleus pulposus cells. Int J Mol Med. 2018; 41(6): 3316–3326, doi: 10.3892/ijmm.2018.3522, indexed in Pubmed: 29512682.
21. Fiordalisi MF, Silva AJ, Barbosa M, et al. Intervertebral disc decellularisation: progress and challenges. Eur Cell Mater. 2021; 42: 196–219, doi: 10.22203/eCM.v042a15, indexed in Pubmed: 34613611.
22. Francisco V, Pino J, González-Gay MÁ, et al. A new immunometabolic perspective of intervertebral disc degeneration. Nat Rev Rheumatol. 2022; 18(1): 47–60, doi: 10.1038/s41584-021-00713-z, indexed in Pubmed: 34845360.
23. Gao XX, Liu CH, Hu ZL, et al. The biological effect of cobalt chloride mimetic-hypoxia on nucleus pulposus cells and the comparability with physical hypoxia . Front Biosci (Landmark Ed). 2021; 26(10): 799–812, doi: 10.52586/4989, indexed in Pubmed: 34719207.
24. Ge J, Yang H, Chen Y, et al. PMMA bone cement acts on the hippo/yap pathway to regulate CTGF and induce intervertebral disc degeneration. ACS Biomater Sci Eng. 2019; 5(7): 3293–3302, doi: 10.1021/acsbiomaterials.9b00146, indexed in Pubmed: 33405572.
25. Ge Q, Ying J, Shi Z, et al. Chlorogenic Acid retards cartilaginous endplate degeneration and ameliorates intervertebral disc degeneration via suppressing NF-κB signaling. Life Sci. 2021; 274: 119324, doi: 10.1016/j.lfs.2021.119324, indexed in Pubmed: 33711382.
26. Gianola S, Bargeri S, Del Castillo G, et al. Effectiveness of treatments for acute and subacute mechanical non-specific low back pain: a systematic review with network meta-analysis. Br J Sports Med. 2022; 56(1): 41–50, doi: 10.1136/bjsports-2020-103596, indexed in Pubmed: 33849907.
27. Gologorsky Y, Chi J. Bisphosphonate therapy for degenerative disc disease? Neurosurgery. 2013; 73(4): N12–N13, doi: 10.1227/01.neu.0000435113.33768.2d, indexed in Pubmed: 24051711.
28. Gong C, Pan W, Hu W, et al. Bone morphogenetic protein-7 retards cell subculture-induced senescence of human nucleus pulposus cells through activating the PI3K/Akt pathway. Biosci Rep. 2019; 39(3), doi: 10.1042/BSR20182312, indexed in Pubmed: 30787052.
29. Gröbner S, Adkins I, Schulz S, et al. Catalytically active Yersinia outer protein P induces cleavage of RIP and caspase-8 at the level of the DISC independently of death receptors in dendritic cells. Apoptosis. 2007; 12(10): 1813–1825, doi: 10.1007/s10495-007-0100-x, indexed in Pubmed: 17624595.
30. Gruber HE, Hoelscher GL, Ingram JA, et al. Genome-wide analysis of pain-, nerve- and neurotrophin-related gene expression in the degenerating human annulus. Mol Pain. 2012; 8: 63, doi: 10.1186/1744-8069-8-63, indexed in Pubmed: 22963171.
31. Guo Z, Gao WS, Wang YF, et al. MiR-502 suppresses tnf-alpha-induced nucleus pulposus cell apoptosis by tar-

geting TARF2. Biomed Res Int. 2021; 2021: 5558369, doi: 10.1155/2021/5558369, indexed in Pubmed: 33869626.
32. Haneder S, Ong MML, Budjan JM, et al. 23Na-magnetic resonance imaging of the human lumbar vertebral discs: in vivo measurements at 3.0 T in healthy volunteers and patients with low back pain. Spine J. 2014; 14(7): 1343–1350, doi: 10.1016/j.spinee.2014.01.031, indexed in Pubmed: 24472875.
33. He D, Zhou M, Bai Z, et al. Propionibacterium acnes induces intervertebral disc degeneration by promoting nucleus pulposus cell pyroptosis via NLRP3-dependent pathway. Biochem Biophys Res Commun. 2020; 526(3): 772–779, doi: 10.1016/j.bbrc.2020.03.161, indexed in Pubmed: 32265028.
34. He J, Yang J, Shen T, et al. Overexpression of long non-coding RNA XIST promotes IL-1β-induced degeneration of nucleus pulposus cells through targeting miR-499a-5p. Mol Cell Probes. 2021; 57: 101711, doi: 10.1016/j.mcp.2021.101711, indexed in Pubmed: 33722663.
35. He R, Cui M, Lin H, et al. Melatonin resists oxidative stress-induced apoptosis in nucleus pulposus cells. Life Sci. 2018; 199: 122–130, doi: 10.1016/j.lfs.2018.03.020, indexed in Pubmed: 29526797.
36. He R, Wang Z, Cui M, et al. HIF1A Alleviates compression-induced apoptosis of nucleus pulposus derived stem cells via upregulating autophagy. Autophagy. 2021; 17(11): 3338–3360, doi: 10.1080/15548627.2021.1872227, indexed in Pubmed: 33455530.
37. Hsieh AH, Twomey JD. Cellular mechanobiology of the intervertebral disc: new directions and approaches. J Biomech. 2010; 43(1): 137–145, doi: 10.1016/j.jbiomech.2009.09.019, indexed in Pubmed: 19828150.
38. Hu S, Zhang C, Qian T, et al. Promoting Nrf2/Sirt3-dependent mitophagy suppresses apoptosis in nucleus pulposus cells and protects against intervertebral disc degeneration. Oxid Med Cell Longev. 2021; 2021: 6694964, doi: 10.1155/2021/6694964, indexed in Pubmed: 34211633.
39. Huang Y, Wang Y, Wu C, et al. Elevated expression of hypoxia-inducible factor-2alpha regulated catabolic factors during intervertebral disc degeneration. Life Sci. 2019; 232: 116565, doi: 10.1016/j.lfs.2019.116565, indexed in Pubmed: 31251999.
40. Hunter CJ, Matyas JR, Duncan NA. The notochordal cell in the nucleus pulposus: a review in the context of tissue engineering. Tissue Eng. 2003; 9(4): 667–677, doi: 10.1089/107632703768247368, indexed in Pubmed: 13678445.
41. Jiao S, Li J, Liu B, et al. Nucleus pulposus cell apoptosis is attenuated by CDMP-2 through regulating oxidative damage under the hyperosmotic environment. Biosci Rep. 2018; 38(5), doi: 10.1042/BSR20181176, indexed in Pubmed: 30177520.
42. Jin L, Balian G, Li XJ. Animal models for disc degeneration: an update. Histology Histopathology. 2018; 33(6): 543–554, doi: 10.14670/HH-11-910, indexed in Pubmed: 28580566.
43. Johannessen W, Elliott DM. Effects of degeneration on the biphasic material properties of human nucleus pulposus in confined compression. Spine (Phila Pa 1976). 2005; 30(24): E724–E729, doi: 10.1097/01.brs.0000192236.92867.15, indexed in Pubmed: 16371889.
44. Kanda Y, Yurube T, Morita Y, et al. Delayed notochordal cell disappearance through integrin α5β1 mechanotransduction during ex-vivo dynamic loading-induced intervertebral disc degeneration. J Orthop Res. 2021; 39(9): 1933–1944, doi: 10.1002/jor.24883, indexed in Pubmed: 33049071.
45. Kang L, Liu S, Li J, et al. The mitochondria-targeted anti-oxidant MitoQ protects against intervertebral disc degeneration by ameliorating mitochondrial dysfunction and redox imbalance. Cell Prolif. 2020; 53(3): e12779, doi: 10.1111/cpr.12779, indexed in Pubmed: 32020711.
46. Kang L, Xiang Q, Zhan S, et al. Restoration of autophagic flux rescues oxidative damage and mitochondrial dysfunction to protect against intervertebral disc degeneration. Oxid Med Cell Longev. 2019; 2019: 7810320, doi: 10.1155/2019/7810320, indexed in Pubmed: 31976028.
47. Kang R, Li H, Rickers K, et al. Intervertebral disc degenerative changes after intradiscal injection of TNF-alpha in a porcine model. Eur Spine J. 2015; 24(9): 2010–2016, doi: 10.1007/s00586-015-3926-x, indexed in Pubmed: 25850392.
48. Kim H, Hong JY, Lee J, et al. IL-1β promotes disc degeneration and inflammation through direct injection of intervertebral disc in a rat lumbar disc herniation model. Spine J. 2021; 21(6): 1031–1041, doi: 10.1016/j.spinee.2021.01.014, indexed in Pubmed: 33460811.
49. Kim JS, Ellman MB, Yan D, et al. Lactoferricin mediates anti-inflammatory and anti-catabolic effects via inhibition of IL-1 and LPS activity in the intervertebral disc. J Cell Physiol. 2013; 228(9): 1884–1896, doi: 10.1002/jcp.24350, indexed in Pubmed: 23460134.
50. Kim JW, An HJ, Yeo H, et al. Activation of hypoxia-inducible factor-1α signaling pathway has the protective effect of intervertebral disc degeneration. Int J Mol Sci. 2021; 22(21), doi: 10.3390/ijms222111355, indexed in Pubmed: 34768786.
51. Kim YK, Kang D, Lee I, et al. Differences in the incidence of symptomatic cervical and lumbar disc herniation according to age, sex and national health insurance eligibility: a pilot study on the disease's association with work. Int J Environ Res Public Health. 2018; 15(10), doi: 10.3390/ijerph15102094, indexed in Pubmed: 30257414.
52. Kong D, Gu R, Zhang C, et al. Knockdown of hsa_circ_0059955 Induces Apoptosis and Cell Cycle Arrest in Nucleus Pulposus Cells via Inhibiting Itchy E3 Ubiquitin Protein Ligase. Drug Des Devel Ther. 2020; 14: 3951––3963, doi: 10.2147/DDDT.S253293, indexed in Pubmed: 33061300.
53. Kos N, Gradisnik L, Velnar T. A brief review of the degenerative intervertebral disc disease. Med Arch. 2019; 73(6): 421–424, doi: 10.5455/medarh.2019.73.421-424, indexed in Pubmed: 32082013.
54. Kritschil R, Scott M, Sowa G, et al. Role of autophagy in intervertebral disc degeneration. J Cell Physiol. 2022; 237(2): 1266–1284, doi: 10.1002/jcp.30631, indexed in Pubmed: 34787318.
55. Krock E, Rosenzweig DH, Haglund L. The inflammatory milieu of the degenerate disc: is mesenchymal stem cell-based therapy for intervertebral disc repair a feasible approach? Curr Stem Cell Res Ther. 2015; 10(4): 317–328, doi: 10.2174/1574888x10666150211161956, indexed in Pubmed: 25670061.

56. Kwon WK, Moon HJ, Kwon TH, et al. Influence of rabbit notochordal cells on symptomatic intervertebral disc degeneration: anti-angiogenic capacity on human endothelial cell proliferation under hypoxia. Osteoarthritis Cartilage. 2017; 25(10): 1738–1746, doi: 10.1016/j.joca.2017.06.003, indexed in Pubmed: 28647468.
57. Lee S, Moon CS, Sul D, et al. Comparison of growth factor and cytokine expression in patients with degenerated disc disease and herniated nucleus pulposus. Clin Biochem. 2009; 42(15): 1504–1511, doi: 10.1016/j.clinbiochem.2009.06.017, indexed in Pubmed: 19563795.
58. Li G, Song Yu, Liao Z, et al. Bone-derived mesenchymal stem cells alleviate compression-induced apoptosis of nucleus pulposus cells by N6 methyladenosine of autophagy. Cell Death Dis. 2020; 11(2): 103, doi: 10.1038/s41419-020-2284-8, indexed in Pubmed: 32029706.
59. Li K, Li Y, Mi J, et al. Resveratrol protects against sodium nitroprusside induced nucleus pulposus cell apoptosis by scavenging ROS. Int J Mol Med. 2018; 41(5): 2485–2492, doi: 10.3892/ijmm.2018.3461, indexed in Pubmed: 29436588.
60. Li Y, Li K, Mao Lu, et al. Cordycepin inhibits LPS-induced inflammatory and matrix degradation in the intervertebral disc. PeerJ. 2016; 4: e1992, doi: 10.7717/peerj.1992, indexed in Pubmed: 27190710.
61. Liang W, Ye D, Dai L, et al. Overexpression of hTERT extends replicative capacity of human nucleus pulposus cells, and protects against serum starvation-induced apoptosis and cell cycle arrest. J Cell Biochem. 2012; 113(6): 2112–2121, doi: 10.1002/jcb.24082, indexed in Pubmed: 22298321.
62. Liao Z, Li S, Liu R, et al. Autophagic degradation of gasdermin D protects against nucleus pulposus cell pyroptosis and retards intervertebral disc degeneration in vivo. Oxid Med Cell Longev. 2021; 2021: 5584447, doi: 10.1155/2021/5584447, indexed in Pubmed: 34239691.
63. Lin D, Alberton P, Delgado Caceres M, et al. Loss of tenomodulin expression is a risk factor for age-related intervertebral disc degeneration. Aging Cell. 2020; 19(3): e13091, doi: 10.1111/acel.13091, indexed in Pubmed: 32083813.
64. Lin J, Du J, Wu X, et al. SIRT3 mitigates intervertebral disc degeneration by delaying oxidative stress-induced senescence of nucleus pulposus cells. J Cell Physiol. 2021; 236(9): 6441–6456, doi: 10.1002/jcp.30319, indexed in Pubmed: 33565085.
65. Lin Y, Jiao Y, Yuan Y, et al. Propionibacterium acnes induces intervertebral disc degeneration by promoting nucleus pulposus cell apoptosis via the TLR2/JNK/mitochondrial-mediated pathway. Emerg Microbes Infect. 2018; 7(1): 1, doi: 10.1038/s41426-017-0002-0, indexed in Pubmed: 29323102.
66. Lin Y, Tang G, Jiao Y, et al. Induces intervertebral disc degeneration by promoting iNOS/NO and COX-2/PGE activation via the ROS-dependent NF-κB pathway. Oxid Med Cell Longev. 2018; 2018: 3692752, doi: 10.1155/2018/3692752, indexed in Pubmed: 30210652.
67. Liu J, Yu P, Dai F, et al. Tetrandrine reduces oxidative stress, apoptosis, and extracellular matrix degradation and improves intervertebral disc degeneration by inducing autophagy. Bioengineered. 2022; 13(2): 3944–3957, doi: 10.1080/21655979.2022.2031396, indexed in Pubmed: 35109761.
68. Liu J, Yuan C, Pu L, et al. Nutrient deprivation induces apoptosis of nucleus pulposus cells via activation of the BNIP3/AIF signalling pathway. Mol Med Rep. 2017; 16(5): 7253–7260, doi: 10.3892/mmr.2017.7550, indexed in Pubmed: 28944876.
69. Liu ZC, Wang ZL, Huang CY, et al. Duhuo Jisheng Decoction inhibits SDF-1-induced inflammation and matrix degradation in human degenerative nucleus pulposus cells in vitro through the CXCR4/NF-κB pathway. Acta Pharmacol Sin. 2018; 39(6): 912–922, doi: 10.1038/aps.2018.36, indexed in Pubmed: 29795361.
70. 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. 2021; 39(4): 467–481, doi: 10.1002/stem.3322.
71. Masuda K, Imai Y, Okuma M, et al. Osteogenic protein-1 injection into a degenerated disc induces the restoration of disc height and structural changes in the rabbit anular puncture model. Spine (Phila Pa 1976). 2006; 31(7): 742–754, doi: 10.1097/01.brs.0000206358.66412.7b, indexed in Pubmed: 16582847.
72. Mei L, Zheng Yi, Ma T, et al. (-)-Epigallocatechin-3-gallate ameliorates intervertebral disc degeneration through reprogramming of the circadian clock. Front Pharmacol. 2021; 12: 753548, doi: 10.3389/fphar.2021.753548, indexed in Pubmed: 34803694.
73. Mohanraj B, Huang AH, Yeger-McKeever MJ, et al. Chondrocyte and mesenchymal stem cell derived engineered cartilage exhibits differential sensitivity to pro-inflammatory cytokines. J Orthop Res. 2018; 36(11): 2901–2910, doi: 10.1002/jor.24061, indexed in Pubmed: 29809295.
74. Nagayama K, Fukuei T. Cyclic stretch-induced mechanical stress to the cell nucleus inhibits ultraviolet radiation-induced DNA damage. Biomech Model Mechanobiol. 2020; 19(2): 493–504, doi: 10.1007/s10237-019-01224-3, indexed in Pubmed: 31506862.
75. Narayanan KB, Park HHo. Toll/interleukin-1 receptor (TIR) domain-mediated cellular signaling pathways. Apoptosis. 2015; 20(2): 196–209, doi: 10.1007/s10495-014-1073-1, indexed in Pubmed: 25563856.
76. Nerurkar NL, Elliott DM, Mauck RL. Mechanical design criteria for intervertebral disc tissue engineering. J Biochem. 2010; 43(6): 1017–1030, doi: 10.1016/j.jbiomech.2009.12.001, indexed in Pubmed: 20080239.
77. Niu A, Bian WP, Feng SL, et al. Role of manganese superoxide dismutase (Mn-SOD) against Cr(III)-induced toxicity in bacteria. J Hazard Mater. 2021; 403: 123604, doi: 10.1016/j.jhazmat.2020.123604, indexed in Pubmed: 32781281.
78. O'Connell GD, Johannessen W, Vresilovic EJ, et al. Human internal disc strains in axial compression measured noninvasively using magnetic resonance imaging. Spine (Phila Pa 1976). 2007; 32(25): 2860–2868, doi: 10.1097/BRS.0b013e31815b75fb, indexed in Pubmed: 18246009.
79. Ohnishi T, Sudo H, Tsujimoto T, et al. Age-related spontaneous lumbar intervertebral disc degeneration in a mouse model. J Orthop Res. 2018; 36(1): 224–232, doi: 10.1002/jor.23634, indexed in Pubmed: 28631843.
80. Pei S, Ying J, Zhang Y, et al. RhTSG-6 inhibits IL-1β-induced extracellular matrix degradation and apoptosis by suppressing the p38, and JNK pathways in nucleus pulposus

cells. Folia Histochem Cytobiol. 2020; 58(3): 227–234, doi: 10.5603/FHC.a2020.0019, indexed in Pubmed: 32936927.
81. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. Eur J Med Chem. 2015; 97: 55–74, doi: 10.1016/j.ejmech.2015.04.040, indexed in Pubmed: 25942353.
82. Pourquié O, Coltey M, Teillet MA, et al. Control of dorsoventral patterning of somitic derivatives by notochord and floor plate. Proc Natl Acad Sci USA. 1993; 90(11): 5242–5246, doi: 10.1073/pnas.90.11.5242, indexed in Pubmed: 8506372.
83. Rider SM, Mizuno S, Kang JD. Molecular mechanisms of intervertebral disc degeneration. Spine Surg Relat Res. 2019; 3(1): 1–11, doi: 10.22603/ssrr.2017-0095, indexed in Pubmed: 31435545.
84. Risbud MV, Shapiro IM. Role of cytokines in intervertebral disc degeneration: pain and disc content. Nat Rev Rheumatol. 2014; 10(1): 44–56, doi: 10.1038/nrrheum.2013.160, indexed in Pubmed: 24166242.
85. Roset R, Ortet L, Gil-Gomez G. Role of Bcl-2 family members on apoptosis: what we have learned from knock-out mice. Front Biosci. 2007; 12: 4722–4730, doi: 10.2741/2421, indexed in Pubmed: 17485408.
86. Sakai D, Nakamura Y, Nakai T, et al. Exhaustion of nucleus pulposus progenitor cells with ageing and degeneration of the intervertebral disc. Nature Communications. 2012; 3(1): 1264, doi: 10.1038/ncomms2226.
87. Song Yu, Lu S, Geng W, et al. Mitochondrial quality control in intervertebral disc degeneration. Exp Mol Med. 2021; 53(7): 1124–1133, doi: 10.1038/s12276-021-00650-7, indexed in Pubmed: 34272472.
88. Sparks MA, Crowley SD, Gurley SB, et al. Classical renin-angiotensin system in kidney physiology. Compr Physiol. 2014; 4(3): 1201–1228, doi: 10.1002/cphy.c130040, indexed in Pubmed: 24944035.
89. Sullivan CB, Porter RM, Evans CH, et al. TNFα and IL-1β influence the differentiation and migration of murine MSCs independently of the NF-κB pathway. Stem Cell Res Ther. 2014; 5(4): 104, doi: 10.1186/scrt492, indexed in Pubmed: 25163844.
90. Sun K, Sun X, Sun J, et al. Tissue renin-angiotensin system (tRAS) induce intervertebral disc degeneration by activating oxidative stress and inflammatory reaction. Oxid Med Cell Longev. 2021; 2021: 3225439, doi: 10.1155/2021/3225439, indexed in Pubmed: 34413926.
91. Tan KO, Fu NY, Sukumaran SK, et al. MAP-1 is a mitochondrial effector of Bax. Proc Natl Acad Sci USA. 2005; 102(41): 14623–14628, doi: 10.1073/pnas.0503524102, indexed in Pubmed: 16199525.
92. Tang G, Han X, Lin Z, et al. Propionibacterium acnes accelerates intervertebral disc degeneration by inducing pyroptosis of nucleus pulposus cells via the ROS-NLRP3 pathway. Oxid Med Cell Longev. 2021; 2021: 4657014, doi: 10.1155/2021/4657014, indexed in Pubmed: 33603947.
93. Tang Z, Hu Bo, Zang F, et al. Nrf2 drives oxidative stress-induced autophagy in nucleus pulposus cells via a Keap1/Nrf2/p62 feedback loop to protect intervertebral disc from degeneration. Cell Death Dis. 2019; 10(7): 510, doi: 10.1038/s41419-019-1701-3, indexed in Pubmed: 31263165.
94. Tessier S, Risbud MV. Understanding embryonic development for cell-based therapies of intervertebral disc degeneration: Toward an effort to treat disc degeneration subphenotypes. Dev Dyn. 2021; 250(3): 302–317, doi: 10.1002/dvdy.217, indexed in Pubmed: 32564440.
95. Vergroesen PPA, Kingma I, Emanuel KS, et al. Mechanics and biology in intervertebral disc degeneration: a vicious circle. Osteoarthritis Cartilage. 2015; 23(7): 1057–1070, doi: 10.1016/j.joca.2015.03.028, indexed in Pubmed: 25827971.
96. Wang W, Deng G, Qiu Y, et al. Transplantation of allogenic nucleus pulposus cells attenuates intervertebral disc degeneration by inhibiting apoptosis and increasing migration. Int J Mol Med. 2018; 41(5): 2553–2564, doi: 10.3892/ijmm.2018.3454, indexed in Pubmed: 29436582.
97. Wang W, Li P, Xu J, et al. Resveratrol attenuates high glucose-induced nucleus pulposus cell apoptosis and senescence through activating the ROS-mediated PI3K/Akt pathway. Biosci Rep. 2018; 38(2), doi: 10.1042/BSR20171454, indexed in Pubmed: 29273676.
98. Wang X, Wang W, Li Li, et al. Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. Biochim Biophys Acta. 2014; 1842(8): 1240–1247, doi: 10.1016/j.bbadis.2013.10.015, indexed in Pubmed: 24189435.
99. Wang Y, Che M, Xin J, et al. The role of IL-1β and TNF-α in intervertebral disc degeneration. Biomed Pharmacother. 2020; 131: 110660, doi: 10.1016/j.biopha.2020.110660, indexed in Pubmed: 32853910.
100. Wang Y, Song Y, Zhong Q, et al. Suppressing ROS generation by apocynin inhibited cyclic stretch-induced inflammatory reaction in HPDLCs via a caspase-1 dependent pathway. Int Immunopharmacol. 2021; 90: 107129, doi: 10.1016/j.intimp.2020.107129, indexed in Pubmed: 33199234.
101. Wehling N, Palmer GD, Pilapil C, et al. Interleukin-1beta and tumor necrosis factor alpha inhibit chondrogenesis by human mesenchymal stem cells through NF-kappaB-dependent pathways. Arthritis Rheum. 2009; 60: 801–812, doi: 10.1002/art.24352, indexed in Pubmed: 19248089.
102. Xia C, Zeng Z, Fang B, et al. Mesenchymal stem cell-derived exosomes ameliorate intervertebral disc degeneration via anti-oxidant and anti-inflammatory effects. Free Radic Biol Med. 2019; 143: 1–15, doi: 10.1016/j.freeradbiomed.2019.07.026, indexed in Pubmed: 31351174.
103. Xia LM, Zhang AP, Zheng Q, et al. Quercetin-3-O-β-D-glucuronide inhibits mitochondria pathway-mediated platelet apoptosis via the phosphatidylinositol-3-kinase/AKT pathway in immunological bone marrow failure. World J Tradit Chin Med. 2022; 8(1): 115, doi: 10.4103/wjtcm.wjtcm_44_21.
104. Xiang Q, Cheng Z, Wang J, et al. Allicin attenuated advanced oxidation protein product-induced oxidative stress and mitochondrial apoptosis in human nucleus pulposus cells. Oxid Med Cell Longev. 2020; 2020: 6685043, doi: 10.1155/2020/6685043, indexed in Pubmed: 33381267.
105. Xiang Q, Kang L, Wang J, et al. CircRNA-CIDN mitigated compression loading-induced damage in human nucleus pulposus cells via miR-34a-5p/SIRT1 axis. EBioMedicine. 2020; 53: 102679, doi: 10.1016/j.ebiom.2020.102679, indexed in Pubmed: 32114390.
106. Xie ZY, Chen Lu, Wang F, et al. Endoplasmic reticulum stress is involved in nucleus pulposus degeneration and attenuates low pH-Induced apoptosis of rat nucleus

pulposus cells. DNA Cell Biol. 2017; 36(8): 627–637, doi: 10.1089/dna.2017.3736, indexed in Pubmed: 28622016.
107. Yang M, Feng C, Zhang Y, et al. Autophagy protects nucleus pulposus cells from cyclic mechanical tension-induced apoptosis. Int J Mol Med. 2019; 44(2): 750–758, doi: 10.3892/ijmm.2019.4212, indexed in Pubmed: 31173175.
108. Yang RZ, Xu WN, Zheng HL, et al. Involvement of oxidative stress-induced annulus fibrosus cell and nucleus pulposus cell ferroptosis in intervertebral disc degeneration pathogenesis. J Cell Physiol. 2021; 236(4): 2725–2739, doi: 10.1002/jcp.30039, indexed in Pubmed: 32892384.
109. Yang SD, Bai ZL, Zhang F, et al. Levofloxacin increases the effect of serum deprivation on anoikis of rat nucleus pulposus cells via Bax/Bcl-2/caspase-3 pathway. Toxicol Mech Methods. 2014; 24(9): 688–696, doi: 10.3109/15376516.2014.963772, indexed in Pubmed: 25224805.
110. Yang W, Yu XH, Wang C, et al. Interleukin-1β in intervertebral disk degeneration. Clin Chim Acta. 2015; 450: 262–272, doi: 10.1016/j.cca.2015.08.029, indexed in Pubmed: 26341894.
111. Yang Y, Wang X, Liu Z, et al. Osteogenic protein-1 attenuates nucleus pulposus cell apoptosis through activating the PI3K/Akt/mTOR pathway in a hyperosmotic culture. Biosci Rep. 2018; 38(6), doi: 10.1042/BSR20181708, indexed in Pubmed: 30459239.
112. Zehra U, Tryfonidou M, Iatridis JC, et al. Mechanisms and clinical implications of intervertebral disc calcification. Nat Rev Rheumatol. 2022; 18(6): 352–362, doi: 10.1038/s41584-022-00783-7, indexed in Pubmed: 35534553.
113. Zhan JW, Wang SQ, Feng MS, et al. Constant compression decreases vascular bud and VEGFA expression in a rabbit vertebral endplate ex vivo culture model. PLoS One. 2020; 15(6): e0234747, doi: 10.1371/journal.pone.0234747, indexed in Pubmed: 32584845.
114. Zhang GZ, Deng YJ, Xie QQ, et al. Sirtuins and intervertebral disc degeneration: Roles in inflammation, oxidative stress, and mitochondrial function. Clin Chim Acta. 2020; 508: 33–42, doi: 10.1016/j.cca.2020.04.016, indexed in Pubmed: 32348785.
115. Zhang K, Ding W, Sun W, et al. Beta1 integrin inhibits apoptosis induced by cyclic stretch in annulus fibrosus cells via ERK1/2 MAPK pathway. Apoptosis. 2016; 21(1): 13–24, doi: 10.1007/s10495-015-1180-7, indexed in Pubmed: 26467923.
116. Zhang Q, Weng Y, Jiang Y, et al. Overexpression of miR-140-5p inhibits lipopolysaccharide-induced human intervertebral disc inflammation and degeneration by downregulating toll-like receptor 4. Oncol Rep. 2018; 40(2): 793–802, doi: 10.3892/or.2018.6488, indexed in Pubmed: 29901170.
117. Zhang Y, He F, Chen Z, et al. Melatonin modulates IL-1β-induced extracellular matrix remodeling in human nucleus pulposus cells and attenuates rat intervertebral disc degeneration and inflammation. Aging (Albany NY). 2019; 11(22): 10499–10512, doi: 10.18632/aging.102472, indexed in Pubmed: 31772145.
118. Zhang YH, Shangguan WJ, Zhao ZJ, et al. Naringin inhibits apoptosis induced by cyclic stretch in rat annular cells and partially attenuates disc degeneration by inhibiting the ROS/NF-κB pathway. Oxid Med Cell Longev. 2022; 2022: 6179444, doi: 10.1155/2022/6179444, indexed in Pubmed: 35251479.
119. Zhao Y, Qiu C, Wang W, et al. Cortistatin protects against intervertebral disc degeneration through targeting mitochondrial ROS-dependent NLRP3 inflammasome activation. Theranostics. 2020; 10(15): 7015–7033, doi: 10.7150/thno.45359, indexed in Pubmed: 32550919.
120. Zheng J, Zhang J, Zhang X, et al. Reactive oxygen species mediate low back pain by upregulating substance P in intervertebral disc degeneration. Oxid Med Cell Longev. 2021; 2021: 6681815, doi: 10.1155/2021/6681815, indexed in Pubmed: 34093962.
121. Zhong H, Zhou Z, Guo L, et al. The miR-623/CXCL12 axis inhibits LPS-induced nucleus pulposus cell apoptosis and senescence. Mech Ageing Dev. 2021; 194: 111417, doi: 10.1016/j.mad.2020.111417, indexed in Pubmed: 33333129.
122. Zhou J, Liu Qi, Yang Z, et al. Rutin maintains redox balance to relieve oxidative stress induced by TBHP in nucleus pulposus cells. In Vitro Cell Dev Biol Anim. 2021; 57(4): 448–456, doi: 10.1007/s11626-021-00581-7, indexed in Pubmed: 33909255.
123. Zhu H, Sun B, Shen Q. TNF-α induces apoptosis of human nucleus pulposus cells via activating the TRIM14/NF-κB signalling pathway. Artif Cells Nanomed Biotechnol. 2019; 47(1): 3004–3012, doi: 10.1080/21691401.2019.1643733, indexed in Pubmed: 31322007.
124. Zhu J, Tang H, Zhang Z, et al. Kaempferol slows intervertebral disc degeneration by modifying LPS-induced osteogenesis/adipogenesis imbalance and inflammation response in BMSCs. Int Immunopharmacol. 2017; 43: 236–242, doi: 10.1016/j.intimp.2016.12.020, indexed in Pubmed: 28043032.
125. Zhu Y, Tchkonia T, Pirtskhalava T, et al. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell. 2015; 14(4): 644–658, doi: 10.1111/acel.12344.