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INTERVERTEBRAL DISC DEGENERATION AND POSSIBILITIES OF TISSUE ENGINEERING: LITERATURE REVIEW

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Objective — to study the role of different structures of the intervertebral disc in its degeneration, as well as the possibilities of tissue engineering in the treatment of this disease. The most common clinical manifestation of intervertebral disc degeneration is back pain, which is often associated with the early disability of patients. Histological examination of intervertebral disc remains the most reliable method for diagnosing and studying the processes of its degeneration. The review considers a violation of the disc metabolism due to morphological changes in its structural components as a trigger for this process. It is shown that disc microcirculation disorders cause a change in the structure of the nucleus pulposus (fibrotization) and its extracellular matrix, which results in the disc destruction. The use of autologous disc-derived cells cultured in vitro and stem cells with their subsequent implantation can replenish the cell deficit and restore the structure of the matrix.
Key Words: intervertebral disc, endplate, matrix, angiogenesis, progenitor cells.

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Currently, degenerative processes of the intervertebral disc (IVD) are a global medical and social problem. Clinical manifestations of IVD degeneration include back pain, which is often associated with early disability. At the same time, more than 85 % of the world population older than 35 years suffer from back pain [1]. Back pain associated with IVD degeneration is the leading reason of disability benefit payments in the Social Security system. In the UK, 37 % of payments in the system of mandatory rehabilitation health insurance in 2012 was granted due to degenerative processes of the IVD. Even in such a small Western European country as the Netherlands, the total annual expenditures for back pain were evaluated to be 4.4 billion euros [2, 3]. In Russia, the incidence of chronic back pain is 26–33 % of the adult population. Notably, most of patients are working-age people (25–55 years) [4].

IVD is one of the most complex anatomical structures of the musculoskeletal system. Functionally, it is a form of continuous cartilaginous junctions, which are intermediate between synchondroses (strong synostoses) and the true joints [6]. Leveling of differences in loads and oscillating movements applied to the spine is the most important function of the IVD due to its structural characteristics. Human IVD belongs to avascular structures, and for this reason dosed load is an active stimulator of nutrient supply and this effect does not occur in static postures and/or during high loads [7].

During natural aging processes, IVD structure undergoes significant changes of the extracellular matrix, condition and density of cell populations. The incidence of spondylitis and osteoarthritis, which is the latest step of degenerative diseases of the IVD, steadily increases with age and reaches 100 % at the age of 80–90 years [8]. Nutrient supply to IVD through the endplates by diffusion, which will be described later, gradually decreases from the early childhood, which leads to decrease in cell density and formation of structural defects of the IVD [9]. Since IVD cells synthesize intercellular substances and maintain the constancy of the internal environment, investigation of their morphology and cell behavior may play a key role in preventing degenerative processes and activating IVD regeneration. However, maintenance of the structure and function of the IVD is determined not only by normal synthesis of the extracellular matrix, but also by regular arrangement of its proteins, mainly proteoglycans with glycosamine chains [10, 11]. During IVD degeneration, proteolysis of extracellular matrix proteins leads to cytoarchitecture disorders and normal arrangement of proteoglycans. Keratin sulphate to chondroitin sulphate ratio increases, their binding to collagen weakens, and IVD elasticity and stretching force decrease. Decrease in aggrecan level in the nucleus

pulposus results in reduced hydration along with impaired mechanical function, being one of the early signs of its degeneration [10].

The study was aimed at investigating the role of various structures of the IVD in its degeneration, as well as the possibilities of tissue engineering in the treatment of this disease. An integrated approach to the study of these issues will provide deep insight into the mechanisms of pathogenesis of IVD degeneration and determine new application points in the treatment of degenerative diseases of the spine.

Histological structure of the IVD

Cell composition. Cells account for only 1–2 % of IVD tissue volume. Despite the small amount of cells, they perform various regulatory functions, ensuring normal structure and functioning of the disc. The nucleus pulposus and annulus fibrosus differ in their cellular composition. In the outer part of the annulus fibrosus, cells have fibroblast-like structure (Fig. 1) and are arranged in parallel with collagen fibers. In the inner part of the annulus fibrosus, cells are more oval. Nucleus pulposus cells are oval in shape, immersed in a matrix, and discrete: about 5000 in 1000 mm^3. Some IVD cells both in nucleus pulposus and in annulus fibrosus are elongated up to 30 microns in length. It is believed that they play sensory communicative role in IVD [14].

The number of cells in the nucleus pulposus (Fig. 2) is on the average twice lower than in the annulus fibrosus (cell density of approximately 4106 cells/cm^3). Nucleus cells are round and/or oval and are classified as notochordal. These cells produce type II collagen, which forms fibrils the most suited to compression loads [15] and is the only type of collagen in the intact nucleus pulposus [16].

It is believed that these are the residual cells of the chord, which develops to form the IVD. In humans, the number of notochordal cells is maximal in newborns and then rapidly decreases during the first years of life. Adults have less notochordal cells. These cells are replaced with chondrocyte-like cells, which then transform into chondrocytes as a result of natural aging and degeneration. Chordal cells also produce proteoglycans and hyaluronic acid; decrease in their number or complete disappearance are associated with degenerative changes in IVDs [13].

Cell function is affected by mechanical and biochemical signals. Physiological and mechanical pressure on the IVD (3.5 MPa) has a stimulating effect on the synthesis of the extracellular matrix, while excessive pressure (7.5 MPa) has inhibiting effect [16]. For example, collagen content in IVDs of actively moving dogs is by 37 % higher than at rest. At the same time, periodically increasing pressure on the IVD significantly stimulates its degeneration [13]. Therefore, mechanical pressure significantly affects proliferation, differentiation, and functional activity of IVD cells.

Intercellular substance (matrix) of the IVD. Collagen, proteoglycans, elastin, and hyaluronic acid are the main components of the intercellular substance of the IVD. Collagen accounts for about 50% of the dry weight of the annulus fibrosus and 15–20 % of the weight of the nucleus pulposus. There are several collagen types in the IVDs (I, II, III, V, and XIV), whose ratio varies with age [12]. Type I and II collagens prevailing in IVDs account for more than 80 % of the total amount of collagen; type VI collagen accounts for 10–20 % of the total amount of collagen [17]. Proteoglycans are complex macromolecules consisting of protein and covalently bound glycosaminoglycans, which are physiologically active only when incorporated in this compound. Proteoglycans account for about 10 % of the dry weight of the annulus fibrosus, their content is higher in the central parts of the ring, and reaches 50 % in the nucleus pulposus. Hyaluronic acid is found in all areas of the IVD, but its concentration is significantly higher in the nucleus [18]. The studies of tissue culture and animal experiments have demonstrated that hyaluronic acid can activate aggregation of individual proteoglycan molecules and biosynthesis in chondrocytes [49].

Elastic fibers, which mainly consist of elastin protein, is another important IVD component. Elastin molecules are capable of considerable stretching. IVDs contain about 1–10 % of this structural protein. In the annulus fibrosus, elastic fibers are conjugated to the collagen fibrils and stabilize its lamellae with special bridges, while in nucleus tissue they are oriented randomly [20]. Additionally, IVDs contain many lipids, mainly in the form of cholesterol, triglycerides, and phospholipids. Lipofuscin (lipid pigment) and patholog-

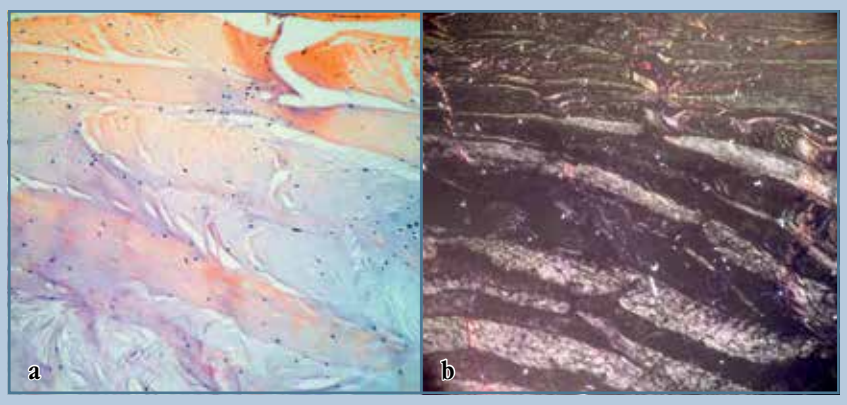

Fig. 1
The outer portions of the annulus fibrosus (our own case study of the patient with intervertebral disc herniation at L5–S1 level): **a** – H & E stain; **b** – polarizing microscopy, x100 magnification

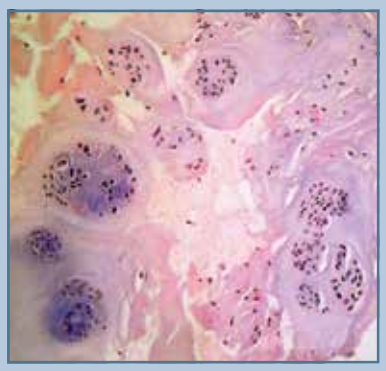

Fig. 2
The structure of the nucleus pulposus (our own case study of the patient with herniated intervertebral disc at L5–S1): the central part of the nucleus is represented by numerous chondrocyte clusters located in the intercellular substance; H & E stain, x200 magnification

ical amyloid protein are mostly detected in elderly and senile age [44].

Massive macromolecular aggregates with aggrecans and amorphous network of type II collagen are formed using hyaluronic acid. These aggregates can retain water molecules in the IVD, thereby forming an internal hydrostatic pressure, which enables nucleus pulposus to play a role of viscoelastic cushion, resisting axial compressive load in the spinal column [21].

Water molecules penetrate the nucleus pulposus by means of diffusion from the blood vessels of adjacent vertebral bodies through the endplate tubules. Increase in pressure inside the IVD results in pressure-gradient-driven water movement in the opposite direction outside the disc. For example, human IVDs loose about 25 % of water during daily physical activity and the same amount returns during night sleep. As a result, the spine is higher in the morning than at night by 1.5–2 cm [38]. The amount of water in IVDs decreases with age due to lower content of hydrophilic molecules (in particular, the reduced proteoglycan and collagenous fibers ratio). IVD degeneration process is associated with reduced synthesis of proteoglycans, which results in lowered hydrostatic pressure in the disc and loss of the elastic properties of the nucleus pulposus. This in turn leads to mechanical overload of the nucleus pulposus and annulus fibrosus and formation of breaks and cracks in the annulus fibrosus. Degenerative processes results in IVD replacement with connective tissue, which therefore cannot adequately fulfill its biomechanical function [22, 23].

Destruction of IVD matrix mainly occurs due to specific enzymes. These are aggrecanases, various types of matrix metalloproteinases (MMP), and other degrading enzymes. For example, MMP-3 (stromelysin) leads to destruction of type III, IX, and X collagen, proteoglycans, fibronectin; MMP-2 (gelatinase) degrades type IV collagen. Several other degrading enzymes are classified to ADAMTS family (adamalysin with thrombospondin module) [5, 41].

Nutrient supply to the IVD. IVDs are supplied by diffusion of substances from the blood vessels of adjacent structures. In the horizontal plane, these are vessels of the anterior and posterior longitudinal ligaments, in the vertical plane — lacunae of bone marrow, whereof nutrients are supplied to IVDs through special channels in the endplates. These channels are present throughout the entire length of the osteochondral junction, but the greatest density is observed in the nucleus pulposus. In the central part of the IVD, nutrient concentration is minimal, while concentration of metabolic products is maximal, which results in concentration-gradient-driven diffusion [41].

A.M. Zaydman et al. [5] studied in detail and described IVD microvasculature. They proved that tubules detected in the annulus fibrosus are connected to the tubules of the loose fibrous portion of the IVD and nucleus pulposus, which proves the existence of integrated transport and drainage system in IVD structures. Furthermore, tubules of the outer portion of the annulus fibrosus penetrate the vertebral body. In the medial regions of the lamella base, tubules permeate the endplate in the horizontal direction. Therefore, proteoglycans, collagen, and other extracellular matrix proteins synthesized in chondrocytes are exchanged and transported through the microtubule system. In turn, there is lymph drainage to the lymphatics of the outer portions of the annulus fibrosus, which receive the lymph from the tissue tubule, performing drainage from all structural components of the IVD.

Maintaining substances gradient between IVD center and periphery is an important condition for normal metabolism of the disc. Any factors that can alter this concentration gradient may affect vital functions of cells. Composition of the matrix wherethrough solutions diffuse play an important role in supplying the IVD. Condition of the endplates, which determines their permeability, is another factor important for normal nutrient supply to the IVD [24]. Any factor altering the contact area of the endplates leads to disturbance of their permeability. For example, calcification of the endplates or annulus fibrosus can impair transportation of metabolites.

The role of the endplates in IVD degenerative processes

Endplate is a hyaline cartilage having typical structural organization (Fig. 3). The endplate consists of chondrocytes and extracellular matrix, comprising mostly proteoglycans and type II collagen. Collagen fibers are arranged horizontally. IVD is separated from the bone tissue of the vertebral bodies by internal plate fibrils, which grow into endplate matrix [25, 27].

As mentioned above, IVDs are supplied due to diffusion of nutrients from the endplates. In these way, substances are synthesized in the cells and reverse outflow of metabolites occurs. However, recent studies of IVDs showed that a network of tiny tubules is located in the central part of the endplate. They are essential for nutrient supply to IVD structures. Its structures undergo reduction with age, which is associated with natural degeneration of the disc accompanied by calcification and ossification of the endplate [30].

Endplates are much thicker in children than in adults. According to Raj et al. [39], endplates of newborns are rich in tubules extending through the nucleus pulposus and annulus fibrosus. In adults, the endplates are much thinner (on the average, up to 1 mm), and the number of perforating vessels is low or they are absent. Degenerative processes in the endplate start in the area of its direct contact with the nucleus pulposus, which leads to an imbalance between diffusion of nutrients and metabolite outflow [47]. This results in nucleus pulposus dystrophy and cell death and accumulation of metabolic products in the nucleus pulposus followed by destruction.

In children aged 3–11 years, decrease in the amount of microtubules in the endplates is observed and there are solitary cracks [34]. It should be noted that these changes usually begin in the medial portion of the plate. In adolescence, there is sharp decrease in the number of tubules, which has negative effect on the structure of IVD matrix. Decrease in the number of microtubules correlates with decrease in cell density and significant increase in disorganization area of the endplate [36]. Morphological changes of the endplates in the age group 17–20 years are similar to those in children. Rudert et al. [42] noted the presence of supplying microtubules in the endplates of people under the age of thirty. Decrease in their number is accompanied by pronounced decrease in the thickness of the endplate and microscopic protrusions into the adjacent bone tissue of the vertebral bodies. From 20 to 30 years, structural changes in the endplates are almost identical to the changes occurring in younger groups, but their number is several times higher. Age period from 30 to 50 years is characterized by significant increase in the endplate destructions area. Disorganization of endplates is characterized by numerous micro-cracks and gaps. Later on, endplates are gradually replaced by fibrous cartilage with necrotic foci. In old age, degenerative processes of the endplates are caused by a sharp decrease in the number of tubules and cartilage replacement with bone tissue, which leads to significant disorders of nutrient diffusion and degeneration of the nucleus pulposus [28].

Aggrecan degradation as a triggering factor of IVD degeneration

Aggrecan is a structural proteoglycan, an essential component of IVD matrix. Maintenance of the osmotic pressure required for normal functioning of the IVD and resistance to mechanical load are the main functions of aggrecan. Aggrecan provides normal interaction between IVD cells and matrix due to binding to the collagen fibers and hyaluronic acid [48].

IVD degeneration reduces the amount of proteoglycans due to fragmentation and loss of glycosamine chains, which in turn reduces osmolality and worsens IVD hydration [48]. However, even with IVD degeneration, its cells are capable of synthesizing aggrecans having normal structure. Investigations have proved the key role of aggrecans in inhibiting growth of vessels and nerves in the cartilaginous tissues [50]. Normally, nerve fibers are detected only in the peripheral regions of the annulus fibrosus of the IVD. IVD degenerative processes are accompanied by growing nerves into the peripheral regions of the annulus fibrosus and the nucleus pulposus. Nerve growth factor receptors were observed on the ingrowing nerve fibers. It is known that nerve growth factor is expressed by chondrocytes of the annulus fibrosus and nucleus pulposus. There is also correlation between the severity of IVD degeneration and expression of nerve growth factor [32]. The development of discogenic pain is associated with ingrowth of nerve fibers into the IVD tissue.

It was proved that aggrecan can suppress both growth of nerves and endothelial cell migration [43]. The loss of aggrecan by IVD matrix is accompanied by ingrowth of vessels and nerves and its progressive degeneration. Aggrecan degradation is carried out by enzymes, matrix metalloproteinases and aggrecanase, which leads to disturbance of its structural organization and functioning. In particular, this process depends on glycosylation of keratan sulfates and chondroitin sulfates on aggrecan monomer [19, 31, 51].

The possibilities of tissue engineering in the treatment of IVD degeneration

Recent studies have shown that IVD structures contain progenitor cells. Richardson et al. [40] have shown the presence of stem cell populations in the outer region of the annulus fibrosus, which can be activated during IVD degeneration. Progenitor cells were detected using immunohistochemical markers (STRO-1, Ki-67). This results were supported by findings of Risbud and Blanco [32], who isolated stem-like cells from degenerated human IVD. In both cases, isolated cell populations were capable of differentiation into any mesodermal tissue, which confirms their pluripotent nature. In another study, Zhang et al. [52] isolated progenitor cell population from the annulus fibrosus of the non-degenerated IVD (age 13–15 years) and found that this

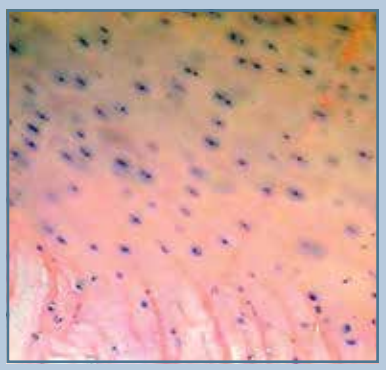

Fig. 3
The structure of endplate (our own case study of the patient with herniated intervertebral disc at L5–S1): endplate is represented by numerous layers of chondrocytes, located in the the large amount of eosinophilic extracellular matrix; H & E stain, x100 magnification

cell population has cellular CD-markers similar to those of mesenchymal stem cells, using the immunophenotyping method.

Advances in the use of IVD progenitor cells suggest that they can be a source of cells for therapy with subsequent implantation. Progenitor cells are the uncommitted pluripotent stem cells, which are found in various tissues and characterized by high plasticity and capability of multilineage differentiation. Several vector systems, which demonstrated high activity of expression of IVD extracellular matrix proteins, have been introduced to the progenitor cells [53]. However, transformation is not the only problem to be solved. Progenitor cells must be differentiated into chondrocyte-like cells before implantation. For this purpose, BMP growth factors were used [46]. More specific differentiation factor belonging to Sox family is also studied. Thus, Brachyury transcription factors also facilitate the required cell adhesion [33]. Nevertheless, it was recently found that culturing of mesenchymal cells with IVD cells is sufficient to induce disk-like phenotype [35]. Cultivation in a three-dimensional system also facilitates formation of chondrocyte-like phenotype. When collagen-gel-encapsulated mesenchymal cell system was implanted into degenerating IVD of rabbits, preservation of nucleus pulposus and the annulus fibrosus structure, prevention of proteoglycan synthesis reduction, and increase in IVD height was observed [26, 45]. Implanted cells survive and express the genetic markers of the nucleus pulposus and annulus fibrosus. Similar results were obtained with injection of progenitor cell suspension into IVDs of rabbits and gel-encapsulated progenitor cell suspension into coccygeal IVDs of rats [26]. The use of progenitor cells gave impetus to the development of autotransplantation methods in patients with IVD degenerative processes.

Currently, cell therapy methods based on the use of stem cells are being actively developed. Several types of adult stem cells have been described in cell therapy for IVD degeneration: mesenchymal cells derived from bone marrow; cells isolated from adipose tissue; cells obtained from muscle tissue; hematopoietic cells; olfactory membrane cells; synovial cells. Thus, Orozco et al. [37] conducted a pilot study on the implantation of mesenchymal stem cells into degenerated IVD in 2011. The concluding remark of this study states that patients reported significant decrease in the severity of pain and shorter disability time 3 months after administration of mesenchymal stem cells. According to MRI, there was increase in IVD hydration and height. Lu et al. [29] evaluated changes in gene expression in stem cells derived from the subcutaneous adipose tissue of a rabbit during their co-cultivation with cells of the annulus fibrosus and nucleus pulposus in vitro. The authors have shown that adipose stem cells have increased expression of type II collagen and aggrecan genes, when co-cultured with nucleus pulposus cells, but not with annulus fibrosus cells. Stem cell therapy is a modern and effective method to treat IVD degeneration.

Conclusion

IVD degeneration is a complicated staged process, where the key role is given to morphological changes in disc structures. Currently, histological examination of the IVD is the most reliable diagnostic method to study its degeneration. Joint action of the aforementioned pathological changes in the IVD results in disc degeneration. Impaired nutrient supply to the IVD due to structural changes in the endplate is the triggering mechanism of this process. Impaired nutrient supply to the IVD leads to decrease in cell populations, and, consequently, reduced synthesis of intercellular substance proteins. Neoangiogenesis and cell proliferation in the form of clusters prevent IVD degeneration only at the early stages. Later on, vessels growing into the nucleus pulposus and proliferating cell clusters disarrange matrix proteins and therefore the structure of the extracellular matrix of the nucleus itself, which eventually exacerbates IVD destruction. Therapies currently used in clinical practice are not able to restore the structure and biomechanical function of the IVD. The use of autologous IVD cells cultured in vitro followed by implantation is a promising method, which potentially can make up for deficiency of the cells and, as a consequence, the matrix. However, the viability and activity of the implanted material should be studied under the influence of various factors over a long period of time in order to implement this approach.

Therefore, an integrated approach to the study of the morphology of IVD degenerative processes will enable discovering new biological therapies aimed at restoring the microstructure and biomechanical function of the disc.

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The authors declare no conflict of interest.

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