

## Cell Therapy for Age-Related Intervertebral Disc Pathologies

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**Abstract**—Any spinal pathology is accompanied by a significant loss in the quality of life and a high disability rate. The rate of occurrence of spinal impairments increases with advancing age. Spinal impairments may be etiologically different and characterized by a wide range of significant and very diverse clinical symptoms. At the same time, the “lower back pain” (as well as the back pain) syndrome is the most common complaint. The main cause for the development of the syndrome is degenerative disc disease (DDD) of a nonspecific nature that leads to irreversible structural damage to intervertebral discs. Surgical DDD treatment methods, including those based on bone tissue autografting, are not radical; this compels researchers to search for alternative approaches. This review analyzes the principles of regenerative cellular and cell-replacement therapies that are based on the use of a variety of cell types, in the treatment of intervertebral pathologies and compares various cell substrates for these therapies.

**Keywords:** spine, intervertebral joints, age-related degeneration, cell therapy, stem cells

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Spinal pathologies occupy a particular place in the classification of locomotor system diseases in general, for a series of reasons. First of all, this situation is explained by the serious disability statistics of patients with spinal diseases and, apart from this, a significant loss of their quality of life in many other cases [52]. Spinal diseases and traumas account for up to 15% of the cases of disabilities in the developed countries; thus the economic significance of this group of impairments would be difficult to overestimate [27]. In Russian clinical practice, spinal diseases make up a sizeable share in the total number of neurological and orthopedic outpatient consultations. Spinal diseases affect people in different age groups, including relatively young patients, but the involvement of age- and sex-related factors is an obvious fact; the male population suffers from spinal impairments more frequently than females do [36]. Spinal deformations and osteochondrosis are the commonest complaints across elderly age groups. According to the available estimates based on different criteria, 16 to 39% of all people over the age of 70 suffer from spinal deformation [20, 22, 38] and about 24% suffer from osteochondrosis of the spine [39]. Spinal impairments may be of different etiologies and are characterized by a broad complex of significant and diverse symptoms. At the same time, the lower back pain (as well as back pain, BP) syndrome tops the list [23]. This syndrome is likely to be observed about equally often in men and women and affects up to 80% of all people during their lifetime with development of a chronic form occurring in 10% of all BP cases [50, 53, 54].

The etiological spectrum of the lower back-pain syndrome is very broad and includes such diverse causes, as urolithiasis and adnexitis (in women) [1]. Nevertheless, the main cause for the development of this syndrome is degenerative disc disease (DDD), an irreversible disc-related structural disorder of a non-specific nature, although its mechanism is incompletely clear thus far [26]. The combination of degenerative changes in intervertebral discs with clinical manifestations allows us to classify it as degenerative disc disease. DDD onset may be based on some genetic component [5, 12], while histological and biochemical changes that are observable in the nucleus pulposus (jelly-like) of an intervertebral disc during a generative process are characterized by great diversity; in particular, DDD is characteristic of a decrease in the proteoglycan and water content, as well, substitutions are reported to occur in the collagen types that are represented in the discs, etc. [3, 4, 34]. Such changes lead to structural disorders in intervertebral discs and, as a result, to their flattening.

The structural changes in the intervertebral discs are, certainly, an age-related process. Moreover, these changes may be secondary to an entire array of other spinal diseases. DDD-accompanying compression of nerve roots or the spinal cord leads to the emergence of a markedly expressed pain syndrome, which in severe cases forces physicians to prescribe traumatic bone plastic surgeries. It deserves to be particularly mentioned that DDD may be a result of failed surgical treatment of spinal disease and trauma, such as nucleotomy, a recently popularized and minimally-invasive method that is based on the removal of a disc fragment

that is pressing on a nerve. It is significant that a surgical intervention into even a single intervertebral disc may foster generative processes in the adjacent discs [14, 33].

In any case, the regeneration of the nucleus pulposus or annulus fibrosus in intervertebral discs is impossible. Endoscopic (transcutaneous) nucleotomy or discectomy, nucleoplasty, electrothermotherapy of intervertebral discs or their plastic surgery are indicated in severe and disabling lesions (the frequency rate of such an outcome may reach 10% of the total sum of clinical cases) for this disease [30].

One of the recognized therapeutic methods for the treatment of severe spinal impairments is spinal stabilization, an operation that consists in the rigid and permanent fixation of two or more vertebrae as one conglomerate [7, 48]. This surgery is indicated in severe forms of osteochondrosis, herniated intervertebral disc, spinal segment instability (this syndrome is also known as segmental instability, a condition where an extra range of movements may occur between two vertebrae), scoliosis, spondylolysis (a defect in the interarticular part of the vertebral arch) and its complication, which is called spondylolisthesis (where the top vertebral body slips forward onto the one below it). In the last two cases the bone tissue of the vertebra is filled with cartilage or connective tissue. Spinal stabilization surgery is usually combined with the decompression of nerve structures, which is aimed at relieving the spinal cord and nerve roots from compression due to a herniated disc, osteophytes, hypertrophied ligaments, or facet joints.

Historically, spinal stabilization surgery has consisted of removing a larger part of an intervertebral disc with replacement of the defect with an autograft, viz., a fragment of the patient's pelvic bone [28]. Supportive fixation was provided by a rigid jacket that the patient had to wear during many postoperative months. We should point that the efficacy this approach (i.e., the formation of bone conglomerate) was rather high. Spinal stabilization is performed today using fixing systems made of high-strength materials (titanium or titanium alloys). This allows clinicians to minimize a patient's postoperative activation period. So called "cages," i.e., intervertebral disc prostheses, have recently become more applicable [8, 9]. These prostheses resemble a hollow cage made of metal or composite materials that is filled with autologous bone powder.

Nevertheless, all these methods, despite their undeniable importance in the contemporary vertebral (spinal) surgical practice, are in no way radical. The treatment with such means is symptomatic, i.e., aimed at managing the manifestations rather than treating the nature of the degenerative process. The huge sums that are spent in developed countries on DDD treatment and DDD-related disability coverage (for example, by 2005 these costs had exceeded \$30 billion per year in the USA and 12 billion sterling pounds per year in

Great Britain [2]) compel these countries to actively search for alternative therapies in the management of this group of diseases.

The successful achievements of molecular medicine over the last decade are invaluable in view of this situation. The experimental study of the biology of intervertebral discs has allowed us to expand our knowledge about their structure, mechanics, and functions. As a result, an entire series of new approaches aimed at slowing degenerative processes in the intervertebral discs are under development and testing today. It is indicative that these approaches are based not only on creating novel medicinal preparations, but also on stimulating the production of cytokines and growth factors in the intervertebral discs and on genetic therapy. In particular, attempts are being made to perform DDD therapy with transforming growth factor- $\beta$ , fibroblast growth factor, growth/differentiation factor 5, and bone morphogenetic protein 2 [35, 51, 55]. Cell therapy has become a separate and one of the most important approaches to DDD treatment. Some time ago autografting of the nucleus pulposus cells to intervertebral discs was believed to be the most encouraging technique of this type. In particular, this technique was tested on the diurnal sand rat (*Psammomys obesus*), i.e., the gerbil, which is the recognized experimental models for age-related DDD [15]. However, this method has not achieved wide clinical recognition due to the fact that the chondrocyte-related sampling procedure is traumatic for an unaffected intervertebral disc and even the adjacent ones, a factor that accelerates the degenerative process in the adjacent intervertebral discs as well. Apart from this, this procedure requires more cells than are obtainable from one disc that is unaffected by the degenerative process.

As in the cases of Parkinson's disease or diabetes mellitus, the loss of a predominantly single cell population that is localized in a particular anatomical structure makes the development of cell replacement therapy-based approaches very attractive. The practical realization of such approaches requires the availability of a source of cellular material, while, as in the two other cases, the use of stem cells seems to be an attractive alternative to autologous, allogeneic, and xenogeneic tissue grafts.

One of the most advantageous factors that distinguish this methodology from the above examples (Parkinson's disease and diabetes mellitus) is the relative simplicity that characterizes the application of a patient's autologous somatic stem cells to DDD therapy. Both mesenchymal stem cells (MSCs) and hematopoietic stem cells isolated from bone marrow were tested as substrates for experimental grafting. We should note that red bone marrow is the largest niche of somatic stem cells in the human body, while bone marrow aspirations (in a small volume) are minimally invasive procedures that are routinely performed in specialized clinics for diagnostics and monitoring.

One of the key biological functions of MSCs is the reproduction of cartilage and bone-tissue cells. Since a considerable number of the cells from the nucleus pulposus and annulus fibrosus of intervertebral discs are of a cartilagenous nature, MSCs are a rather reasonable choice for use as a cell-replacement therapy substrate. In 2003 a group of Japanese clinicians performed the first MSC grafting to the degenerative intervertebral discs of model animals (rabbits, in this case) [46]. Moreover, atelocollagen, i.e., solubilized (liquefied or semi-liquefied) collagen, a hypoimmune material that is actively used in the manufacture of cosmetic products (such as creams) and is produced worldwide in the hundreds of tons, was chosen to serve as the MSC “carrier” to provide support. Further technological progress allowed researchers to finally confirm the significant efficacy of MSCs in DDD management. By lowering the grade of DDD in model animals, the same group of authors [44] made another large achievement in 2006. After 24 weeks had passed since MSC grafting, both the height of the lumbar spinal discs and their structure regenerated to a considerable degree (although incompletely) in model animals. The interest of the scientific community was also drawn to another experimental work, where fluorescent marker-stained MSCs mixed with hyaluronic gel (as a component of synovial fluid, hyaluron is actively used for the treatment of articular diseases) were injected into the intervertebral discs of rats. At 1 to 2 weeks after the injection, the count of dye-labeled living cells significantly decreased, but after 4 weeks the count returned to the initial level [11]. These data indicate that MSCs can actively proliferate in the conditions of a medium that is typical of intervertebral discs.

Practically simultaneously with the above-described pilot work, bone marrow stem cells were used in an experiment that was conducted by a group of American authors. Using dogs as model animals, they compared the efficacy of the standard bone prosthesis consisting of fragments of spongy bone tissue of donors (hound dogs) with a prosthesis that was inseminated with the autologous bone marrow aspirate of the recipients diluted in blood [32]. Despite the death of some of the experimental animals due to faults in the surgical equipment, the authors successfully demonstrated that it is possible to improve spinal stabilization efficacy through the application of bone-marrow cells. They also supposed that the effect of bone marrow and blood stem cells was in part due to physical and humoral factors, rather than due to cellular ones, e.g., due to the creation of both the support and the microenvironment for osteoprogenitor cells by a fibrinous clot and the release of numerous cytokines and growth factors (platelet-derived growth factor (*PDGF*), epidermal growth factor (*EGF*), fibroblast growth factor family members (*FGF*) and transforming growth factor  $\beta$  (*TGF- $\beta$* )) from the cells.

A similar experiment was simultaneously conducted by a team of researchers at Peking University [21]. Using rabbits, they studied the efficacy of ceramic materials inseminated with autologous bone marrow MSCs. With the ceramic + bone-marrow cell composite the success rate of spinal stabilization was even higher than that using an autologous bone tissue fragment (the iliac bone ridge) and significantly higher than when using a “hollow” ceramic intervertebral disc [21]. The stabilization efficacy increased even further when human recombinant bone morphogenetic protein 2 (*rhBMP-2*) was added to the application protocol.

Another experimental series has shown that the efficiency of the approach based on the use in prosthetic intervertebral discs of autologous bone marrow aspirates increases with their enrichment in cells (including cells with osteoprogenitor potential). Researchers initially used simple devices based on centrifugation for this purpose. However, the technical problems associated with the mandatory processing of cellular material in the operating room gave rise to an alternative approach that is based on the application of powdered and demineralized compact bone tissue [31]. Sedimentation of the adhesion-specific bone marrow cell types on bone-tissue particles leads to the formation of a so-called “cell matrix.” The use of such a substrate (as an alternative to a cell suspension) in tissue engineering in order to populate the pores of the bone prosthetic material according to the above-described technology has proven to be an interesting and attractive approach [31].

As has been reliably shown in some of the recently-accomplished experimental works (based on both rabbits and rats), stem cells can not only survive a grafting procedure but also actively initiate proliferation and successfully regenerate the proteoglycan composition of intervertebral discs. We should note that the enthusiasm of specialists working in this field fell somewhat after the report that was published by physicians from the MicroSpine Center (for minimally invasive vertebral surgery), DeFuniak Springs (USA), where allogeneic stem cells were grafted to the members of a group of ten voluntary patients (males and females in equal proportion). Endoscopic discectomy did not achieve a result in any of the subjects. An autologous bone marrow aspirate in the volume of 1 mL was introduced into their affected intervertebral discs with local anesthesia. The transplantation procedure was followed by a 2-week course of hyperbaric oxygenation therapy and a prescribed gentle loading regimen for the spine for the recipients [16]. However, by 1 year after the stem-cell grafting, none of the ten patients demonstrated any improvement: a majority of them chose to undergo considerable surgical interventions, including the use of a prosthetic intervertebral disc.

As in many other clinical situations, directly grafting undifferentiated stem cells during DDD treatment is only one possible approach. Considerable prospects

are associated with approaches that are based on the application of cells derived from in vitro stem-cell differentiation. According to some studies, there are certain factors that guide the differentiation of different types of stem cells (primarily, MSCs) into cells with many properties, which are characteristic of normal nucleus pulposus cells. Hypoxia is to be mentioned as one of these factors [42, 49]. A significant number of works contain reports about the importance of *TGF- $\beta$*  [10, 42]; other authors reported on growth/differentiation factor 5 (*GDF5*), dexamethasone, and ascorbic acid as additional factors for MSC differentiation into the chondrocytes in a similar manner to that of nucleus pulposus cells [6, 47, 49, 59]. These studies also indicated that both the cell proliferation and the proteoglycan synthesis efficiency increases with the culturing of cells in 3D structures, which are mainly based on a variety of gel and polymer types [40, 58, 60].

Actually, an important role in the success of cell therapies is played not only by the nature and the amount of the grafted cells, but also by their organization within their microenvironment. In some experiments MSCs have been immobilized on a skeleton of hyaluronic acid, collagen, or chytosan (a substance that structurally resembles cellulose). The cells fixed in the fibrous network turned out to be able to effectively differentiate, producing intercellular matrix components, cytokines, and other bioactive molecules [2].

An efficient variant for stimulating stem cell differentiation into nucleus pulposus cells is associated with their in vitro co-culturing with the cells of the nucleus pulposus itself [25, 29, 41, 56]. In this case, the co-culturing of the two cell types in a common nutrient (and in direct contact) is a strong factor for the induction/promotion of MSC differentiation in the direction of cells with the necessary properties. The mechanism of such an effect is unclear, but we can suggest that it is based on the joint action of soluble factors that are secreted by nucleus pulposus cells into a common nutrient and the signaling molecules that are expressed by them on the outer surface the cell membrane [25].

An approach that is based on sampling cell material from the affected vertebrae themselves is of particular interest. Considering the risk of adverse effects (including significant ones) from bone marrow aspiration during sternal or iliac bone ridge puncture, the researchers from the Thomas Jefferson University (USA) proposed to use the bone marrow cells of the vertebrae themselves, which are usually accessible in the course of a surgical procedure. By comparing the properties of the MSCs that were obtained from the bone marrow of the iliac bone ridge and that of the vertebral body in 15 patients with DDD, the authors concluded that they are practically identical in a set of key genetic and biochemical parameters, which gives them similar osteogenic properties [43]. Moreover, the bone marrow-isolated MSCs of the vertebral bodies have a more expressed proliferative potential. This makes such cells potentially usable as a substrate for

autografting with a lower risk of complications than during the puncturing an undamaged bone. However, the approach that is based on the application of MSC populations isolated from sources that are alternatives to bone marrow, in the first place, from subcutaneous fatty tissue, is more valuable as an initial substrate for a cell-based therapy for DDD [24, 25, 57].

It is interesting that attempts have been made in recent years to return to the use of cells obtained from the nucleus pulposus of intervertebral discs. However, instead of the direct transplantation of these cells from one (healthy) intervertebral disc into another/other discs (that are affected by the generative process), they are used as a substrate for cell expansion in vitro [17, 18]. The use of immortalized nucleus pulposus cell lines as a substrate for cell replacement therapy may be of practical significance. The first immortalized nucleus pulposus cell lines were obtained in 2003 using genetically modified (an adenoviral vector was used) nucleus pulposus cells from a 19-year old donor, who was a patient with a vertebral body fracture. The cell line, which is capable of active in vitro proliferation, was named *H-NPSV* (it is also known as *TUNPSV* [45]); active experimental work is now under way to work out approaches to the practical application of such cells in clinics, in particular, in decelerating degenerative processes in intervertebral discs [19].

There is no doubt that several more years will be required before stem cells are successfully and actively applicable in clinical practice. There are still many technological problems to be solved within this period: from the differentiation protocols for MSCs and other types of stem cells to the surgical equipment that will be used to support their successful attachment. It is known that the nucleus pulposus cell composition noticeably changes, not only in the course of disease, but also with age, which makes the task of its controlled regeneration even more complicated and, respectively, stem-cell differentiation protocols may differ depending on a patient's age, the severity of the damage to intervertebral discs, and other factors. In any case, this approach appears to be very promising during the current period of its passage from the experimental to the clinical stage. Elderly persons who suffer from severe forms of intervertebral pathologies are the main target group for the therapy. The encouraging results of ongoing clinical trials [13, 37] allow us to suggest that wide introduction of approaches based on the application of stem cells, primarily, autologous MSCs, to the treatment of age-related DDD will prove to be practical in the near future.

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