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Biological approaches in degenerative disc disease. Where are we now?

Maria Korompilia[1], Ioannis Gkiatas[2], Dimitrios Gelalis[3], Anastasios Korompilias[1], Emilios Pakos[1], Ioannis Gelalis[1]

[1]*Orthopaedic Department, School of Medicine, University of Ioannina, Ioannina, Greece*

[2]*Stavros Niarchos Foundation Complex Joint Reconstruction Center, Hospital for Special Surgery, New York, NY, USA*

[3]*School of Medicine, European University of Cyprus, Cyprus*

ABSTRACT

Intervertebral disc (IVD) disease consists one of the main chronic- age related diseases mostly in patients over 60 years old. IVD degeneration is considered a multifactorial process with interaction of genetic, nutritional and environmental factors. Any nutritional and compositional imbalance leads to disturbance in biochemical and structural integrity.

Unfortunately common therapeutic methods- conservative and surgical- focus mainly on the patients and rather to the pathology of disc degeneration. Biological treatment strategies approach the condition at a molecular level and according with the stage of degeneration are classified into biomolecular therapy, cell therapy and tissue-engineering (TE) therapy.

During the first stage of the disease, where there is damage to biomolecules, biomoleucular therapy is suitable for promoting extracellular matrix (ECM) synthesis. This is achieved through injection of protein solutions (bone morphogenic proteins, osteogenic protein-1, transforming growth factor superfamily), platelet-rich-plasma and gene therapy injection (viral or non-viral vectors). In the midstage of disease, with cell amount reduction, cell therapy through mesenchymal stem cells and chondrocyte transplantation forms the best option for production- differentiation of ECM components and disc repair. Lastly, as degeneration reaches the final stage, implantation of TE disc-like constructs is considered the most optional reconstruction therapy for disc repair.

Biological therapeutic strategies in IVD disease consists a revolutionary method, address not to symptoms but to pathophysiology of the degeneration with purpose to improve population's quality life

KEY WORDS: intervertebral disc disease, biological therapies, tissue engineering

CORRESPONDING AUTHOR, GUARANTOR

Ioannis D. Gelalis M.D, Ph.D, Professor of Orthopaedics
Spinal Surgery & Adult Reconstructive Surgery, Department of Orthopaedic Surgery and Traumatology, University of Ioannina, School of Medicine, Ioannina, Greece. President of the Hellenic Spine Society (2019)
Phone: +30 2410235199, mobile: +306977097871

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Introduction

Low back pain is one of the main chronic age-related diseases that burdens global health leading to a significant reduction in patients' quality of life [1]. Approximately, 90% of the general population over the age of 60s is more likely to suffer from low back pain due to degeneration of intervertebral disc disease (IVD) [2].

IVD is situated between two adjacent vertebrae with an outer fibrous annulus fibrosus (AF) enclosing a central gelatinous nucleus pulposus (NP) and the cartilaginous end plates (CEP) connecting discs to adjacent vertebral bodies. Discs are avascular, aneural tissues that exchange nutrients and metabolites through microvessels in the CEP and outer AF [3]. Thus, considering that IVD degeneration is a complex interaction between genetic, nutritional and environmental factors [4], any case of restriction in nutritional supply and compositional changes may lead to the disturbance of the structural integrity and biomechanical properties of the IVD as a respond to loads and injuries [5]

Conservative and surgical therapies are aiming at the symptoms and fail to address the underlying pathology, leading to higher rates of reoperation, adjacent segment disease and pseudarthrosis [6]. In order to surpass these restrictions our great interest is focused on the biological repair strategies as a feasible way to understand and treat pathologic disc segments. Biologic therapies approach the condition at a molecular level, in an attempt to alter the process cascade rather than treat patient's symptoms.

According with the stage of degeneration, biological strategies are classified into three categories: 1) biomolecular therapy, 2) cell therapy and 3) tissue-engineered disc like construction [7]. In early stages in which the disc still contains sufficient amount of cells, biomolecules are used, with the ability to enhance protein expression and facilitate extracellular matrix (ECM) synthesis. In midstage degeneration, where cells are now rapidly reduced and hypoactive, cell therapy is the choice through cell implantation. Lastly, during the terminal stage, with complete structural and functional disruption of the disc the most optimal method is the implantation of tissue-engineered (TE) IVD constructs for attempt of reconstruction of the disc segment [8].

In this review we focus on novel applications as therapeutical strategies for discogenic pathology, according with the stage of degeneration based on clinical and research trials.

Biological treatment strategies

Biomolecular Treatment

During the early stage of degeneration, there is damage to biomolecules (DNA- proteins) due to inflammatory and oxidative stress so the disc undergoes an imbalance of anabolic and catabolic factors leading to degradation of ECM [9]. In that stage recombinant proteins and genes can regenerate expression of the targeted molecules by increasing anabolic or decreasing catabolic factor production and thus promoting ECM synthesis.

Protein solution injection

It has been shown that injection of protein solutions into discs can trigger cell growth, shift cellular metabolism to the anabolic state thus restoring its biochemical properties reversing degeneration process. The mostly used proteins are bone morphogenic proteins (BMPs), osteogenic protein-1 (OP-1), transforming growth factor (TGF)-β superfamily [10]. Gruber et al. proved that the addition of TGF-β triggers the synthesis of proteoglycans (PGs) and stimulates cell proliferation of human AF [11]. BMP family has been found to increase PG synthesis and metabolism of IVD cells and stimulates production and formation of ECM [12]. Wehling P proved that the use of autologous growth factors (IL-1 receptor antagonist, IGF-1) may reduce the rate of apoptosis and the production of IL-1, inflammatory cytokines [13]. In 2015, Liu et al studied that Mineralization Protein-1 (LMP-1) suppresses TNF-a induced IVD degeneration, by maintaining pro-

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duction of NP and ECM [14].

The only limitation here is that a direct injection into IVD requires many repeated doses due to chronicity of the condition and the short biologic half-lives of these factors, thus limited therapeutic effect. Many proposals have been made for development of slow-release carriers or gene-based delivery [8].

Platelet- Rich Plasma (PRP)

As a therapeutic strategy, PRP is consistently being utilized in stimulation and acceleration of bone and soft tissue healing, with many studies proving their increased efficacy in osteoarthritis, cartilage damage and recently in the treatment of DDD [16]. These platelets release a variety of growth factors, such as platelet- derived growth factor (PDGF), TGF-β1, vascular endothelial growth factor (VEGF). PRP seems to be an effective stimulator of cell proliferation and PG and collagen synthesis in porcupine NP and AF cells [17]. Clinical evidence for PRP treatment of discogenic low back pain in humans has been reported since 2011, by Akeda et al, who injected autologous PRP in 6 patients with chronic low back pain [18]. At 6 months follow-up, patients showed a remarkable decrease in mean pain score and adverse effects after the injection were reported. Cho et al. demonstrate that PRP can decrease the expression of proteolytic matrix metalloproteinases and increase synthesis of ECM in a in vitro porcine model [19]. Gelalis et al. proved that intradiscal PRP treatment in DDD provokes the maintenance of the disc's basic morphological characteristics in rabbit IVD [20]. Autologous PRP therapy has the benefit of avoidance disease transmission and immunological reaction in comparison with artificially synthesized GF [16]. Finally, PRP when used in the early stage of degeneration can better enhance disk height and hydration [21].

Gene therapy

Gene therapy has been used for several years, through gene mapping, nucleic acid modification and is widely used in the therapeutic strategies for DDD. The selected genes are delivered through viral (adenovirus, lentivirus) or non-viral vectors which are then injected into the tissue or transferred into cells in vitro and then transplanted into viable tissue [22]. Many in vitro and in vivo studies have shown that viral delivery of BMP-7, TGF-β3 improves IVD extracellular environment with increased synthesis of type II collagen, and glycosaminoglycan [23]. Although, there is an increased rate of immunogenicity, toxicity and insertional mutagenesis through viral vectors, which is why there is a great interest toward non-viral gene delivery systems [24]. However, those delivery systems are limited due to their low transfection efficiency.

Cell therapy

As degeneration progresses, the amount of cells that respond to biomolecular therapy start to reduce, which makes cell therapy the optimal treatment for midstage degeneration.

Mesenchymal Stem cells (MSCs)

Attention has been posed on stem cells as a potential source of cells to regenerate the IVD. There are a large number of potential sources of MSCs [25], including adipose tissue, bone marrow, embryonic and fetal stem cells, which are pluripotent cells with a potential to differentiate into any body tissue. These cells are able to differentiate into any type of tissue thus making the ideal method for disc repair and also due to their ability to produce the required proteoglycan and collagen for disc's ECM [8]. Although is more technically demanding process than PRP, is easy to collect and post-collecting algorithm is simple, leading to its popularity as therapeutic option for DDD. Yoshikawa et al. in 2010 analyzed the regenerative restoration ability of autologous MSCs in degeneration of IVDs in 2 patients with chronic low back pain, leg pain, and numbness [26]. bMSCs were isolated coupled with colla-

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gen sponges and grafted percutaneously to the degenerated IVD. After a 2 year follow-up both patients had significant symptomatic reliefs and MRI results showed high NP hydration without progressive degeneration. Pettine and colleagues in 2015 injected autologous bMSCs in 26 patients with discogenic back pain. It was observed clinical improvement with pain relief, functional and imaging improvement at a two-year follow up [27].

Adipose stem cells (ASCs) have been the focus of recent studies in autologous biologic research due to a number of promising characteristics [16]. In fact, ASCs are easier to harvest, contain a higher frequency of stem cells, are more potent immunomodulator than bMSCs and they are characterized by their ability to differentiate into NP- like phenotype [28]. For all these, aMSCs make an attractive single-step therapeutic method for DDD. In vitro experiments show that ASCs may provide mechanical protection by decreasing degradation enzymes and inflammatory factors and increasing expression of genes and proteins involved in maintenance of ECM integrity [29].

Chondrocytes transplantation

Implantation of chondrocytes can produce the appropriate amount of ECM components (proteoglycans, collagen type I-II) under nutritional stress and hypoxia and meet the increased cellular and metabolic demands of the disc [30]. Ganey et al. through canine model proved that implantation of chondrocyte in NP disc contributes to ECM regeneration and halt further disc degeneration [31]. Unfortunately, no matter how promising this technique is there are some limitations such as, donor site morbidity, immunocompatibility complications and disease transmission.

Tissue-engineering Therapy

TE was defined 25 years ago by Langer and Vacanti in 1993 as an interdisciplinary field of research that applies the effort towards the development of biological substitutes that restore, maintain and improve tissue function [32]. Since the inception of this concept, many attempts have been made for the construction of functional substitutes for damaged disc tissues. As the degeneration process reach the terminal stage implantation of TE disc-like constructs is considered the most optional reconstruction therapy. It is very important to understand the combining role of stems cells, absorbable scaffolds, bioactive molecules like growth factors and mechanical stimuli.

Scaffolds

Injection of scaffold can provide structural support to MSCs injected in to intervertebral space. The content of scaffold must be similar to the natural ECM in composition and physical properties [33]. Examples are natural proteins of alginate, collagen and synthetic polymers. In this hypoxic and nutrient-poor environment of the IVD these method assist cellular survival by enhancing adhesive strength and providing a healthier ECM microenvironment [16].

Tissue-engineered constructs

In recent years, advanced TE enables whole IVD construction, through the combination of constructed tissue engineered AF and NP, in vitro which can be implanted in vivo. In 1976, Mizuno et al. were the first to construct whole IVDs consisting of sheep AF and NP cells seeded on polyglycolic acid and calcium alginate matrices [34]. The disc implants were implanted in the subcutaneous space of the dorsum of athymic mice. Gross morphology and histology of the constructs strongly resembled those of the native IVDs. TE AF was rich in type I collagen but NP contained type II collagen similar to the native. Moriguchi et al constructed TE-IVD components using adult canine AF and NP cells seeded into collagen and alginate hydogels. After cervical spine discectomy implantation of TE-IVD was performed. Implanted TE-IVDs maintained their

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position, structure and hydration as well as disc height over 16 weeks in vivo [35].

The construction of whole disc implants through tissue engineering consists of a revolutionary progress in the treatment of DDD with extensive biological and functional challenges in vivo.

Conclusion and Perspectives

Over the past two decades there has been a significant development in the conservative and surgical treatment of spinal disorders. Unfortunately, all these methods affect the symptom rather the underlying pathology, as there is still limited understanding of the biology of the IVD thus limited understanding of DDD pathogenesis and progression. Therefore, scientists focused on the value of biological treatments for DDD.

It is crucial to select the proper therapeutic protocol according with the patient's profile and the stage of degeneration. Injection of biomolecules, genes and cellular therapy can attenuate the degenerative process at the early to mid-stages of the disease progression. Until now, some first clinical trials with recombinant proteins are underway. Cellular therapy seems to be effective, according with animal and human studies, in treating pain in patients in middle stage of degeneration. TE-IVD is useful in the terminal stage of degeneration, where there is complete structural and functional disruption of the IVD, through regeneration disc morphology and functionality postimplantation. Until now, only two studies have demonstrated the in-vivo transplantation of TE-IVDs.

We anticipated future research in the field of biological therapy for identify the ideal solution for each special pathogenesis and for each individual.

Conflict of interest

The authors declare no conflicts of interest.

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