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Endplate role in the degenerative disc disease: a brief review

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Abstract

The degenerative disease of the intervertebral disc is nowadays an important health problem, which has still not been understood and solved adequately. The vertebral endplate is regarded as one of the vital elements in the structure of the intervertebral disc. Its constituent cells, the chondrocytes in the endplate, may also be involved in the process of the intervertebral disc degeneration and their role is central both under physiological and pathological conditions. They main functions include a role in homeostasis of the extracellular environment of the intervertebral disc, metabolic support and nutrition of the discal nucleus and annulus beneath and the preservation of the extracellular matrix. Therefore, it is understandable that the cells in the endplate have been in the centre of research from several viewpoints, such as development, degeneration and growth, reparation and remodelling, as well as treatment strategies. In this article, we briefly review the importance of vertebral endplate, which are often overlooked, in the intervertebral disc degeneration.

INTRODUCTION

The degenerative disease of the intervertebral disc is a chronic condition and it is frequently encountered in clinical practice. Intervertebral disc degeneration may result from numerous factors and has been regarded as one of the most important reasons of both disability and elevated expenses in healthcare among adult population [1,2]. The disc-

generated pain and pain from chronic instability of the affected spine segments may in long-term lead to significant functional disability in both genders, therefore considerably affecting living quality, especially in the young and active population. The accurate pathomorphological mechanism for the degeneration process of the intervertebral disc is remaining mysterious. There have been some risk factors identified as a cause for the degeneration, in addition to smoking, age, diabetes and obesity, also occupational, psychosocial and genetic causes. These known and unknown aspects of the disk disease are complex and often involve synergistic interactions between physical and biological mechanisms, eventually leading to the intervertebral disc degeneration [3-7].

Low back pain, which is one of the main direct consequences of intervertebral disc degeneration, varies widely in its incidence among literature reports. It affects 7.6 % to 37 % of patients and represents the fifth most common cause of the patient visit to the medical services. Up to 10 % of patients may experience movement difficulties aggravated by long-lasting pain [8-10]. The tissues of the intervertebral disc begin to degenerate earlier than other skeletal and muscular tissues. The degeneration is in many cases progressive and often asymptomatic. The early degenerative process of the intervertebral disc may start already in teenage years when around 20 % of adolescents experience minor form of the symptoms [11-13]. The incidence rises with age. Later, about 10 % of the population over the age of 50 years is affected and after the age of 70 years, as many as 50 % of people can experience the symptoms. Some literature reports state that the intervertebral disc degeneration may exist in 90 % of population, although many people do not exhibit clinical signs of the disease or they may be mild and insignificant [9,12-15].

The degenerative process of the intervertebral disc is a progressive and chronic disorder. The thickness or height of the intervertebral disc gradually diminishes [11,12]. Consequently, the dynamics in the affected spine segment change. This results in the accelerated deterioration of other, adjacent spine segments, in addition to other spinal structures, including spinal joints, ligaments and even paraspinal muscles. In the long period, the spinal canal narrows, resulting in the spinal stenosis, thus compressing the

neural tissues. This is one of the leading causes of pain, particularly in the elderly people. With the rise of the elderly population, this clinical problem is becoming increasingly important [11-14].

The most important characteristics discernible on clinical imaging comprise the visible alterations in the pulposus nucleus of the disc. This is the first structure affected. The degenerative cascade of the disc matrix and cellular death first start in the centre, which is the innermost part of the pulpous nucleus. During this process, the nucleus pulposus changes in its integrity and loses height. These alterations are also discernible on magnetic resonance imaging (MRI) as the signal intensity alteration. Because of simultaneous annular failure, the fibrous band around the nucleus fails and disc herniations of different grades may occur. When exploring the mechanisms of the degenerative process of the intervertebral disc, the mainstream of research has been focused on the annulus fibrosus and nucleus pulposus, which are two most commonly affected structures. On the other and, the vertebral endplate's role in these settings has still not been studied and described in detail. Many reports dealing with the intervertebral disc pathophysiology have frequently overlooked the degeneration processes. Recent studies have revealed that in the degeneration cascade, the disc endplate is at least equally important, as are the annulus and nucleus [16-19]. The aim of this article is to discuss the significance of the vertebral endplate, its function in the degenerative cascade of the intervertebral disc.

THE PATHOPHYSIOLOGICAL PROCESS OF THE INTERVERTEBRAL DISC DEGENERATION

The intervertebral disc is regarded as an avascular structure, composed of cartilage and fibrous tissue. The fibroblast-like cells reside in the extracellular matrix. They constitute most of the intervertebral disc structure [11]. The cells and disc matrix are essential for the normal function of the disc [11,20]. Numerous mechanical factors, which vary in severity, type, duration and direction of load, may influence and affect the condition of the

intervertebral disc and consequently the biological reaction to these aspects [11,21,22]. The degenerative processes involve the structural damage of the intervertebral disc and the alterations in the number and composition of cells. Not only is the centre of the intervertebral disc damaged in this process, but also the endplate suffers damage, further potentiating the loss of cells in the nucleus and the annulus and the alteration of the intervertebral disc composition [23,24].

The nucleus pulpous is the first and the most sensitive stricture in the intervertebral disc, which suffers damage. With ageing and advancing degeneration, the nucleus is therefore primary affected. Its composition changes in that it becomes more fibrous and less elastic. In the outer part of the intervertebral disc, tiny concentric breaks emerge and they extend into the nucleus [14,25]. As a result of the reparative process, the quantity of fibrous tissue starts rising and the composition and the amount of proteoglycans changes, as well as the number of cells due to apoptosis. Various factors, including traumatic, mechanical, genetic and nutritional may influence the path of the degenerative cascade [21,22,24]. As the collagen fibres in the annulus fibrous become progressively disoriented, the network, which is made of collagen and elastin fibres, deteriorates steadily. The cells in the nucleus pulposus are being lost due to the apoptotic process and later in the course of the disc degeneration by necrosis. In order to counterbalance the loss, their proliferation is excessive. These degenerative processes are common and in mature disc, up to 50% of intervertebral disc cells may be necrotic [26,27]. In parallel, the matrix of the nucleus pulposus deteriorates [12,14,26,27]. With matrix degeneration, both the collagen quantity and its composition are associated. The most affected are the fibre orientation, location and composition or types of the collagen fibres. On the other hand, the total quantity of collagen is affected to a lesser degree [12,14]. Already early in the degenerative process, the old collagen fibres become denatured and the new ones are being synthesised to replace the loss. Enzyme activity greatly influences the process of denaturation, leading the breakdown of proteoglycans, collagen and fibronectin. The most important enzymes, among others, include cathepsins and matrix metalloproteinases [12,14,28].

Loss of proteoglycans represents the main aspect of the intervertebral disc degeneration. Proteoglycans are large molecules and are truncated into smaller fragments [29,30]. Thus, proteoglycans are lost from the intervertebral disc tissue [30]. Consequently, the osmotic pressure in the intervertebral disc matrix falls and water molecules are lost from the matrix, affecting the mechanical features of the intervertebral disc. The degenerated intervertebral discs hold lower quantity of water and therefore exhibit lesser abilities for sustaining pressure. As a result, they bulge and lose height [29,31,32]. Proteoglycan degradation affects also the traffic of other molecules into the disc matrix and of it. For example, the cytokines and proteins from serum diffuse into the disc matrix and accelerate the degeneration process by affecting the cells there [12,30,32].

For spinal stability and function, other anatomical structures are also important. With intervertebral disc degeneration, other nearby structures can deteriorate. These include intervertebral joints, ligaments and even vertebral muscles. Functional changes and higher susceptibility to injuries ensues [12,14]. For the reason of overloading, the height of degenerated intervertebral disc is lower in comparison to a normal one. Apophyseal joints need to stand higher loads, leading to osteoarthritic degeneration [28]. The yellow ligaments decrease in strength, resulting in hypertrophy and protrusion into the lumen of the spinal canal, which consequently narrows and neural structures become compressed [33]. The reasons for pain during the course of the intervertebral disc degeneration are multifactorial. In many circumstances, there is a combination of mechanical and structural deformation in addition to the damage exerted by the activity of inflammatory mediators. In the degenerative cascade, the radices of spinal nerves are commonly involved, leading to chronic pain because of their compression and partly because of ingrowths into the degenerated disc of minute neural endings and their activation owing to the continuous action of inflammatory mediators [12,34].

FACTORS INDUCING THE DEGENERATION OF THE INTERVERTEBRAL DISC

Prolapsed or herniated discs are common causes for patient referral to the neurosurgeon or orthopaedist. A herniation is defined as a protrusion of the intervertebral disc owing

to a complete or even partial rupture of the outer annulus fibrosus. The bulging of the disc may include various directions; this is anterior, posterolateral or posterior course, hence various clinical pictures [34]. Especially the last two directions have clinical significance as they may result in compression of the neural structures in the spinal canal [34,35]. Spontaneous resorptions of the herniated intervertebral disc fragments may occasionally take place, leading to weakening or even cessation of lumbar pain. According to the literature, about 85 % of such fragments may be partially resorbed or in the course of ten to twelve months [36]. Despite the fact that the herniation of the intervertebral disc is most frequently caused as a result of mechanical injury and following rupture of the fibrous annulus, some extent of preliminary intervertebral disc degeneration is essential to be present. This is one important factor contributing to the herniation of the nucleus into the vertebral canal through the fibrous bands of degenerated and ruptured annulus. A rupture of a healthy disc is uncommon and an enormous force is needed [33-35]. In many instances, the terminal endplate of the vertebrae fails sooner with a massive force than the fibrous belt [27,37,38]. The most common factors taken into account when talking about intervertebral disc degeneration include genetic predisposition, mechanical load and stress, and nutritional disorders, wakening the intervertebral disc structures. Other factors in intervertebral disc degeneration include enzymatic changes, inflammatory processes and microtrauma, age-related changes, and structural changes (Table 1) [39-43].

Genetic factors

For the degenerative process of the intervertebral disc, the genetic basis is very important. Several genes, such as THBS2, CLIP, ASPN, PARK2, CHTS and single nucleotide polymorphisms (SNP), including COL1A1, COL9A3, COL11A2, and COL11 have been identified and linked to the process of the intervertebral disc degeneration since their expression increases the possibility of the intervertebral disc disease. These genes are involved in matrix synthesis [43-45]. Certain genetic polymorphisms determining the

matrix molecules also outline the extracellular matrix in the intervertebral disc and these polymorphisms may affect the path of the degenerative cascade [46,47]. Mutations in genes encoding the matrix molecules result in the changes of the matrix morphology, therefore disturbing the flow of biochemical processes in the intervertebral disc and its function [46,48]. It is important to stress, however, that solely genes and genetic factors are not the only causes for intervertebral disc degeneration. The environmental factors are equally important, leading to the fact that intervertebral disc degeneration is almost certainly a multifactorial disease [49-51].

Mechanical stress

Wear and tear that affects both acellular and cellular components, resulting from unceasing microscopic injuries and anomalous mechanical loads may lead to intervertebral disc degeneration. This results in the pathophysiological process outlined above. Chronic pain is the most common clinical consequence [52,53]. The principal risk factors that are preventable to some extent consist of obesity, heavy physical labour (especially when non-physiological movements are exerted), smoking (accelerated atherosclerosis damages minute vessels supplying the terminal plates), inappropriate flexed posture and physical inactivity [30,52,54-56].

Nutritional disorders in the intervertebral disc degeneration

For normal intervertebral disc structure and function, the intervertebral disc cells need an adequate nutritional supply. Nutritional disorders of the intervertebral disc are also among the significant explanations for the intervertebral disc degeneration [57,58]. Being an avascular structure, the nutrient availability of the intervertebral disc is mainly the subject of diffusion. Capillaries that arise in the bodies of the vertebras cover only the subchondral area of the disc terminal plate (ie. to the vertebral endplate). In order to reach the cells, nutrients and gasses have to diffuse through the extracellular matrix. A reduction in nutritional supply results in a decrease in oxygen availability and an increase in lactate concentration, affecting the pH in the disc and thus the cell function and extracellular matrix synthesis. The importance of the vertebral endplate is evident also

from the point of intervertebral disc nutrition. The failure of the vertebral endplate may initiate the slow and long-lasting cascade of the degenerative process [57-60].

THE VERTEBRAL ENDPLATE ANATOMY

The intervertebral disc is a fibrocartilaginous and avascular structure, which is separates the vertebral bodies, provides load transmission and enables flexibility throughout the vertebral column [33]. Three distinctive layers are the main structural parts of the intervertebral disc: I) the nucleus pulposus with its outer and inner region lies in the centre of the disc, II) the annulus fibrous made predominantly of collagen encircles the nucleus in the outer area and III) the cartilaginous endplates or terminal plates, which divide the nucleus pulposus and annulus fibrosus form the vertebral bodies [61-63].

The endplates consist of two parts. The outer part is the bony endplate and the inner part is made of cartilage [63]. On one side, the bony endplate is intimately connected with the vertebral bone and on the other, the cartilaginous component of the vertebral endplate encloses the intervertebral disc, that is the nucleus and the annulus. The structural function of the cartilaginous part is the separation of the intervertebral disc from the adjacent vertebrae and contains the nucleus pulposus. The cartilage endplate is composed of semi-porous thickened cancellous bone of 0.6 mm to 1 mm in thickness that is arranged in layers and of hyaline cartilage of 0.2 mm to 0.8 mm in thickness. The thickness of the human endplate diminishes toward the centre. In the cartilaginous part of the endplate, the most copious constituent of extracellular substance includes mainly water proteoglycans. Aggrecan and type II collagen are its main constituents. The fibres of collagen, which are located in the cartilaginous portion of the endplate, run mostly in parallel to the surface of the vertebra. On the contrary to the pattern of collagen alignment found in the articular cartilage, where the fibres are located erratically. Water content in the extracellular matrix is high and varies during the lifetime. After birth it is close to 80 % and then diminishes gradually to below 70 % after 15 years of age [11,12].

At the junction of the vertebral endplate and fibrous annulus, the structure of the endplate is more complicated. In the outer part of the annulus, the vertebral border

consists of fibrocartilaginous bondage. Here, the annular fibres are inserted into a region of calcified cartilage, which is attached to the subchondral bone [19,63,64]. The collagen fibres, which are positioned in the lamellae of the innermost portion of the fibrous annulus, are oriented in continuity with the collagen fibres in the endplate. This arrangement is important for minimising the concentration of mechanical stress during complex loading, which comprises pressure, compressive forces and shear forces. As the cartilaginous portion of the endplate is not secured into the bony portion, this border may be detached or divided easily [65-68].

The cartilage endplate incorporates the superior and inferior borders of the intervertebral disc. The bony endplate extends into the bone marrow compartment of the vertebra where a myriad of thin-walled capillaries, haematopoietic cells, fat cells and nerves are located [69-71]. The endplate also forms intervertebral disc's main nutrient supply network. The vertebral nerves and capillaries entering into the basivertebral foramen at the posterior part of the vertebral cortex supply this zone through tiny pores positioned in the cortical shell. In the centre, the capillaries form an arterial network, which then branches and terminates nearby the cartilaginous endplate. These vessels and sinusoid venous channels make a continuous vascular bed across the bone-disc interface. This enables disc nutrition by diffusion from the vessels in the vicinity. Because nutrient supply is one of the factors associated with the degenerative disease of the intervertebral disc, it is reasonable to think that the alterations in the cartilage endplate also have a profound effect on the course of interverebral disc degeneration [62,66].

The nerve supply of the vertebral endplate is comparable to that of the intervertebral disc *per se*. The nerve endings are located mainly in the outer layers of the endplate and spread to its central part. In a healthy disc, they extend approximately to the three outermost lamellae of the annulus fibrosus. Ninety per cent of the nerves consist of sympathetic afferent fibres and are branches of the sinuvertebral nerves. In pathological conditions, their concentration is increased in the areas of endplate damage. These nerves can send nociceptive impulses to the sympathetic nervous system that may cause a form of visceral-like pain, which may be similar to the enteric structures [11,62,67,72].

THE DEGENERATIVE PROCESS OF THE VERTEBRAL ENDPLATE

Intervertebral disc degeneration is many times related to low back pain [63,66]. The nearby tissues are included in the maintenance of the mechanical and biochemical homeostasis, both being disturbed during the course of degenerative events. In addition to the intervertebral disc, included are also the cartilaginous endplate, bony vertebral endplate and the neighbouring vertebral bodies, which are in contact through the endplates [67]. The transmission of mechanical loads on the intervertebral disc is largely influenced by vertebral bodies and bone in the vertebral endplate. This load transmission, therefore, depends on both the morphological properties of the bone and its composition. The former includes the properties and strength of the cortical and trabecular bone and the latter the bone mineral density. As a result, the preservation of disc health is dependent on the structure and composition of these surrounding tissues, since the changes in surrounding tissues may induce cellular, molecular and structural disorders in the intervertebral disc [62,72-76]. The aging significantly affects the extracellular matrix and disc cells due to the biological changes connected to the degenerative process. Loss of proteoglycans is the main factor. As these large molecules are degraded into their minor fragments, they are gradually lost from the disc tissue. Therefore, the osmotic pressure in the intervertebral disc matrix drops and consequent loss of water molecules follows. The mechanical properties of the intervertebral disc depend on these events, which result in disc bulging and loss of its height [65,77,78].

The degenerative processes of the intervertebral disc, endplate and adjacent bone marrow are highly associated [77,79]. It is well recognised that degenerative disease of the intervertebral disc has been related with the alterations in the disc endplate. Both the morphological changes of the bone and changes in its composition can be observed. Nevertheless, the precise causative relationship and the connection between the evolution of the degenerative disease of the intervertebral disc and pathomorphological alterations in the endplate have not yet been fully understood. Various variations in the morphology of the endplate resulting from degenerative disc disease have been

documented. Some studies described increased endplate porosity, loss of tissue strength and thinning of endplate layers. Other researchers have reported that with the increasing degree of disc degeneration, the mineral density of the vertebral bone also increased, resulting in the thickening and calcification of the endplate in the course of degenerative cascade of the intervertebral disc [79-81]. During progression of the intervertebral disc degeneration, the extracellular matrix breakdown in the cartilage endplate is one of the main processes. The course of degeneration of the intervertebral disc, in addition, includes other neighbouring structures, eventually affecting and damaging the vertebral bone and the vertebral endplate, since the bone marrow and endplate are closely connected [80-84].

Similarly to the macromolecule breakdown in the nucleus pulposus, the degradation of aggrecan and collagen II is viewed as a central feature in the damage of the cartilage endplate during the course of degeneration. Here, the matrix metalloproteinases are the principal enzymes for collagen breakdown [85-87]. The degenerated cartilage endplate presents a source of inflammatory mediators, such as tumour necrosis factor (TNF)- α; interleukin- 6, interleukin- 1β and macrophage inhibition factor. In addition, the loss of proteoglycans influences the transport of other molecules from and to the extracellular matrix. Cytokines and serum proteins diffuse into the matrix, damaging the cells there and quickening the progression of disc degeneration (Table 2). The changes in the bone of the vertebral body have also been observed, in addition to the endplate morphology alterations. The variations in vertebral trabecular architecture depend on the severity of the disc degeneration. The intervertebral disc is therefore not the only and the most important structure in the spinal degenerative process. According to these facts, the role of the vertebral endplate in the degenerative cascade of the intervertebral disc and its health is becoming progressively important [88-92].

A GLANCE AT THE TREATMENT OF THE DEGENERATIVE DISC DISEASE

During the cascade of intervertebral disc degeneration, the endplate plays an indispensable role. During the intervertebral disc wear and tear, the cells that are most

prone to degeneration are the chondrocytes in the endplates. Other cell types in the intervertebral disc, such as annulus fibrosus and nucleus pulposus cells, are less sensitive. This is the reason that in the degenerative disc disease the endplate degeneration is getting more and more important. Several biological and mechanical characteristics in a well-controlled mechanical and physiological environment can be addressed on these cells in the *in vitro* situation. Human endplate chondrocytes can be obtained in higher numbers relatively easily from vertebral endplate that has been removed during various lumbar or cervical operations, addressing the degenerative spinal and intervertebral disc pathology in various *in vitro* models, studying the degenerative processes [93-95].

Besides conservative treatment, the most common currently available treatment for degenerative disc disease remains operative [96-99]. This includes various procedures such as discectomy, spinal fusion, disc arthroplasty and epidural steroid injections. These options are in general considered interventional and none has been shown to reverse the degeneration cascade. The suppression of accelerated senescence and excessive apoptosis of disc cells may be another option to tackle disc degeneration. Among current biologic therapies, gene-based therapy has been tried, as well as the use of mesenchymal stem cells, anti-catabolic factors, biomaterials and intradiscal infiltration of plasma rich in growth factors. When taking into account biological therapy to repair or regenerate the degenerated disc, nutrient and biomechanical factors should always be kept in mind, since they are the major causes of the biological changes in the disc environment. The majority of these approaches remain experimental and are not currently approved for everyday clinical use practice [100-106].

The importance of tissue engineering and vertebral endplate cell isolation for intervertebral disc regeneration

In addition to well-recognised and clinically implemented therapeutic modalities for the treatment of degenerative disc disease, which include both conservative and surgical methods, recent advances in the techniques of tissue engineering and regenerative medicine have offered new possibilities to tackle this problem. The *in vitro* organ systems, which may act as a replacement choice for experimental animals, are getting increasingly

interesting [107,108]. In recent years, great advances have been made in the techniques of the *in vitro* cell cultures. Several cell models that involve isolated cells allow the study of physiological processes and pathophysiological mechanisms devoid of experimental animals. For the experimentation studying human pathobiology and live cells in the *in vitro* organ systems, human cell cultures are getting more attractive, since they are more suitable, in comparison to animal ones. Even though most intervertebral disc cells used in the laboratory practice have been obtained from animal tissue, it is not possible to convey the experimental results from animals to humans directly [16,107,108].

The endplate has an indispensable part in the course of intervertebral disc degeneration [37]. During intervertebral disc deterioration, chondrocytes in the endplates are the first cells that are prone to degeneration. Only then, the cells of annulus fibrosus and nucleus pulposus follow. This is the reason that in the course of degenerative cascade of the intervertebral disc, the endplate degeneration is getting more and more significant, both in clinical practice and in research. On these cells, several biological and mechanical characteristics in a well-controlled physiological and mechanical environment or so called the *in vitro* setting can be studied [104-106,109-112].

When talking about the regenerative approaches itself, two possibilities exist, including I) the approaches with intervertebral disc replacement and II) the methods aiming at the reconstruction or regenerative methodologies for the intervertebral disc. The former include surgical approaches with decompression of the spinal cord or cauda equine and spinal nerved, depending on the clinical situation, with or without spinal stabilisation and with the insertion of an artificial implant (intervertebral cage) or artificial disk. All these surgical approaches have their advantages and limits [109-111]. They have been used according to the clinical indications, the experience of the surgeon and hospital policy. The approaches targeting the reconstruction or regeneration of the intervertebral disc, on the other hand, are still evolving, thus being in the experimental phase of clinical use, mainly in the *in vitro* settings. Here, the tissue engineering strategies encompass the use of implantable biomaterials, nanofibers and implantable cells, since the degenerative processes are not limited to the nucleus or annulus only but also include the terminal

plate [112-114]. The knowledge of the intervertebral disc biomechanical properties, ageing and degeneration and the design of novel treatment strategies are crucial for the design of a therapeutic approach. Below we summarise the tissue engineering methods and the challenges we are facing in their design.

The tissue engineering of the disc scaffold

In the *in vitro* setting, the individual intervertebral disc scaffolds can be manufactured of synthetic and/or natural biomaterials. Several approaches have been developed that involve the use of such artificial scaffolds, which exhibit the biomechanical properties of the intervertebral disc, are biocompatible with the tissue and are structurally similar to the intervertebral disc acellular components [112-115].

An important step in the development of such *in vitro* model is the choice of a suitable material for the scaffold. Collagen is the material of choice. It is ubiquitous in the human body, relatively easy obtainable in larger quantities and with low immunogenicity. At the same time, atelocollagen can be acquired from collagen by preparation with the enzyme pepsin [114-116]. The atelocollagen has excellent bioimmunological properties and is therefore one of the most commonly used materials for tissue matrices. The honeycomb-like organisation of the atelocollagen also offers excellent mechanical stability and provides a scaffold for optimal cell ingrowth in proliferation [114,115].

Another valuable material for scaffold components of intervertebral disc implants is fibroin. Fibroin is a protein product of the silkworm. It is a component of silk, one of the strongest natural fibres, sporting a high compressive strength. The scaffolds manufactured from fibroin, therefore, exhibit a high compressive and tensile strength as well as slow degradation and facilitate disk cell ingrowth and population [113,117,118]. The fibroin-made scaffold may for that reason offer not only a regenerative environment but also performs a biomechanical function. The protein structure of the scaffold also allows the covalent binding of other peptides that would serve as anchors for the cellular network [112,113,118].

The polymers chitosan and alginate have the potential to be used as scaffold building blocks. Chitosan, resulting from the modification of chitin, is a biodegradable and

biocompatible material with a beneficial antimicrobial effect. Scaffolds from chitosan are highly porous and soft and they support cell adhesion and growth. Alginate, a product of algae and some bacteria, is widely used in biomedicine due to its excellent biocompatibility properties [112,114-116]. In addition to natural biopolymers, synthetic biodegradable polymers can also be considered potential building blocks for intervertebral disc regeneration. The most commonly utilised are polylactide, polycaprolactone; polyurethane and polyglycolide. These materials are suitable due to their predictable properties, and low immunogenicity and can be easily synthesised and modelled into desired structures and implants. They are commonly associated with applications in tissue regeneration from AF (Table 3) [112,113,115,117].

Cellular integration

In tissue engineering strategies for annulus fibrous and nucleus pulpous development, cellular integration is a crucial aspect. Mesenchymal stem cells, which represent a useful link in the intervertebral disc tissue engineering chain, can be used very efficiently as a basis for the *in vitro* development of the intervertebral disc components [117,118]. The mesenchymal stem cells are capable of differentiating into a variety of connective tissue cells, they allow prolonged self-renewal and are relatively easy to obtain because they can be found in many convenient donor sites of the body, such as the dermis, adipose tissue, muscle, the bone marrow and the umbilical cord. Clinically effective applications may be conceivable in several ways: I) as cells producing growth factors, II) directly as cells that differentiate into the cellular structure of the intervertebral disc and III) as cells, which may modulate the inflammatory response in the intervertebral disc tissue. Accordingly, their potential applications depend on these properties. Moreover, the mesenchymal stem cells may also be integrated with various biomaterials into the individual components of the intervertebral disc. The animal models performed in rabbits have suggested the use of these methods for intervertebral disc regeneration strategies [112-114,117,118].

CONCLUSION

Since intervertebral disc is the biggest avascular organ, it has some typical features. Nutrition of the cells in the pulpous nucleus is one of them. This process depends only on diffusion, which is conducted through the myriad of tiny capillary vessels and capillary spouts spreading out from the adjacent vertebral body. The cartilaginous endplate also takes part in the angiogenesis. Injury to the cartilaginous surface of the endplate may initiate alterations in the metabolism of the matrix, which gradually lead to intervertebral disc deterioration. Taking part in such significant roles, the endplate chondrocytes are in the centre of research and represent a key target for both basic neuroscience and translational investigations, principally for the *in vitro* cell models, concerned with processes of intervertebral disc degeneration. In addition to regular conservative and surgical therapy, various techniques of regenerative treatment are becoming very encouraging, although at the moment of writing they are still in the experimental stage. The goals of regenerative therapy include restoration of the degenerated intervertebral disc matrix by two approaches: I) employing the agents that act as cytokine inhibitors, normally causing matrix loss and II) using growth factors, which may encourage and enhance the synthesis of extracellular matrix by the intervertebral disc cells. Thus, the preservation of the structural integrity and the function of the vertebral endplate may protect the disc against degeneration and is becoming an intensive area of research.

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