

# Growth factors and platelet-rich plasma: promising biological strategies for early intervertebral disc degeneration

Shan-zheng Wang · Qing Chang · Jun Lu · Chen Wang

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**Abstract** Intervertebral disc degeneration (IDD) is a complex process with the mechanism not fully elucidated. The current clinical treatments for IDD are mainly focused on providing symptomatic relief without addressing the underlying cause of the IDD. Biological therapeutic strategies to repair and regenerate the degenerated discs are drawing more attention. Growth factor therapy is one of the biological strategies and holds promising prospects. As a promising bioactive substance, platelet-rich plasma (PRP) is considered to be an ideal growth factor “cocktail” for intervertebral disc (IVD) restoration. Results from many *in vitro* and *in vivo* studies have confirmed the efficacy of growth factors and PRP in IVD repair and regeneration. It is essential to advance the research on growth factor therapy and associated mechanism for IDD. This article reviews the background of IDD, current concepts in growth factor and PRP-related therapy for IDD. Future research perspectives and clinical directions are also discussed.

**Keywords** Intervertebral disc degeneration · Biological strategies · Growth factors · Platelet-rich plasma

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Shan-zheng Wang and Qing Chang have contributed equally to this work.

S.-z. Wang · Q. Chang · J. Lu · C. Wang  
Department of Orthopaedics, Zhongda Hospital, Medical School of Southeast University, 87 Ding Jia Qiao Road,  
Nanjing, Jiangsu 210009, People’s Republic of China

C. Wang (✉)  
Surgical Research Center, Medical School of Southeast University,  
87 Ding Jia Qiao Road, Nanjing, Jiangsu 210009, People’s Republic of China  
e-mail: wangchen\_seu\_edu@163.com

## Introduction

Intervertebral disc degeneration (IDD) is a major cause of low back pain (LBP) which is drawing increasing fiscal and societal concern [1]. The degenerated intervertebral disc (IVD) can severely prohibit the extension and flexion mobility of the spine. The common therapeutic strategies for IDD, including physiotherapy, anti-inflammatory medications and spinal surgery [2–4], are expected to provide relief of pain and degeneration, but they cannot reverse the degeneration and may even accelerate the degeneration of the adjacent discs.

Recent biological therapeutic strategies for IDD are enjoying more popularity in the field of IVD repair and regeneration [5]. Growth factor therapy, gene therapy, cell therapy and tissue engineering therapy are current promising biological therapeutic strategies. One of the widely studied strategies is growth factor therapy for early degenerated discs [6]. In the early stage of IDD, growth factors are expected to stimulate the proliferation and matrix accumulation of the remaining functional cells within the discs which further helps to restore the structure and function of degenerated IVDs. In the moderate or late stage of IDD, fewer functional cells are left to react to the growth factors, and the calcified endplates (EPs) of the degenerated IVD extremely restrain the nutrient and oxygen diffusion. As for these stages, other biological strategies may serve as better options for IVD regeneration.

Abundant investigations have proved the efficacy of growth factor therapy from *in vitro* and *in vivo* studies. As a natural carrier of multiple growth factors, platelet-rich plasma (PRP) is considered to be a promising strategy for IDD [7]. The aim of this review is to elaborate the role of growth factors and PRP in the regeneration of IDD and address directions for future research and clinical studies.

## Overview of IVD and IDD

An IVD is a weight-bearing organ, playing a significant role in maintaining the mobility and stability of the spine [8]. The IVD consists of three parts that differ histologically, biomechanically and physiologically. The outer region of the IVD is the annulus fibrosus (AF) which is mainly composed of bundles of type I collagen [9, 10]. The central region of the IVD is the nucleus pulposus (NP), containing type II collagen and aggrecan [11], but the network of the collagen is less organised compared with that of the AF. The functional cells within the NP produce the extracellular matrix (ECM) that binds to water, contributing to the maintenance of the swelling pressure of the NP and orientation of the lamellar structure of the inner AF [8]. Two thin cartilaginous plates that extend superiorly and inferiorly over the inner AF and NP are known as EPs. EPs can absorb the load transferred, prevent the collision between the vertebral bodies and supply nutrients and oxygen for the IVD by diffusion [12].

As an unbalanced state of the IVD, IDD often advances with aging. Besides living conditions, genetic factors and biomechanical loading are often related with IDD [13]. From the cellular microenvironment alteration to the structure breakdown, IDD is strongly associated with LBP, but the pathological mechanism is largely unknown. In the process of degeneration, IDD is often accompanied by the decrease of active cells and ECM, altered phenotype of disc cells and the presence of pro-inflammatory cytokines and mediators [14, 15]. Progression from different perspectives, including imaging examinations, new tissue examination techniques and trials of intervention, has advanced our understanding of the mechanism of IDD. The loss of water content and ECM of the degenerated discs can be suggested by a magnetic resonance imaging (MRI) T2-weighted scan (Fig. 1).

### The biology of the IVD: potential regenerative mechanism of growth factors and PRP

Current understanding of cellular components of the IVD

Cells play a significant role in synthesising and maintaining the ECM of the IVD, but the cellular biology of the IVD is not clear. Most cells in the adult NP are small chondrocyte-like cells and large vacuolated cells. Proliferating chondrocyte-like cells are often seen in degenerative discs and taken as an indicator of IDD. As there are no efficient phagocytes in the discs, dead chondrocyte-like cells would not be promptly cleared and would remain in the degenerated discs for a relatively long period. The large vacuolated cells have a notochord origin [16] and are often named notochordal cells. Human notochordal cells within the discs gradually disappear with aging, which is closely related to the process of IDD



**Fig. 1** MRI of IDD. MRI could confirm the changes of IDD, including the loss of water content, morphology and height of the discs. The loss of signal intensity at L4–5 (\*) was detected, compared with normal discs above and below (#)

[17]. Recently, some studies have confirmed the presence of progenitor/stem cells within the discs [18–21]. Compared with marrow stem cells, these progenitor/stem cells present in IVD tissues express a repertoire of similar membrane markers. To investigate and localise the progenitor/stem cells within the discs, Henriksson et al. [20] detected cell proliferation zones and label-retaining cells by *in vivo* 5-bromo-2'-deoxyuridine (BrdU) and confirmed the presence of BrdU-positive cells in comparable numbers in most regions of the AF and NP. In the AF border to the ligament zone and the perichondrium region, a stem cell niche-like pattern was determined. In a recent study, Sakai et al. [21] identified populations of progenitor cells that are Tie2 positive (Tie2+) and disialoganglioside 2 positive (GD2+) in the NP from mice and humans. They are clonally multipotent and induced reorganisation of NP tissue after transplantation. However, until now, few studies have elaborated on how the injected growth factors affect the progenitor/stem cells within the discs. These studies stated above offer insights for IDD biology and regenerative targets for future investigations.

IVD homeostasis: how growth factors play a role

The homeostasis of the IVD is a complicated process, which is associated with many growth factors, genes and proteinases. As bioactive proteins, many growth factors and corresponding receptors were discovered within discs, regulating the metabolism of the IVD. Transforming growth factor beta (TGF- $\beta$ ) consists of a series of peptides that are considered to be a significant relative factor associated with synthesis of collagen and proteoglycans, playing an important role in ECM accumulation [22]. Also, TGF- $\beta$  signalling is required for normal

EP growth at the postnatal stage [23]. Tolonen et al. [24] suggested that growth factors, including TGF- $\beta$ 1 and TGF- $\beta$ 2, basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF), create a cascade in IVD tissue, where they act and participate in cellular remodelling from the normal resting stage via disc degeneration to disc herniation. Bone morphogenetic proteins (BMPs), a family of growth factors that belong to the TGF- $\beta$  superfamily, are able to promote the proliferation and differentiation of multiple cell lines. The receptor of BMP could be found in the IVD, implying the IVD cells may react to the BMP superfamily to increase the synthesis of proteoglycan, upregulate the mRNA expression of type II collagen and serve as the mitotic agent of the IVD [25, 26]. The IVD is the largest avascular tissue in the human body, and vascular endothelial growth factor A (VEGF-A) expression in the NP is thus promoted by hypoxic conditions. The highly expressed VEGF-A plays an important role in NP survival in an autocrine/paracrine manner [27].

#### Regenerative mechanism of PRP: a growth factor “cocktail” therapy

PRP is defined as a fraction of the autologous plasma with high platelet concentration above the base line [28]. When activated, platelets are able to release multiple growth factors, including PDGF, TGF- $\beta$ , VEGF, epidermal growth factor (EGF) and insulin-like growth factor (IGF), among others [29]. PRP has been clinically used for its healing properties attributed to autologous growth factors and secretory proteins that accelerate the healing process on a cellular level [28]. Many growth factors present in PRP are associated with tissue repair and regeneration, and PDGF and TGF- $\beta$  are two of the more integral modulators [30]. Release of PDGF from platelets has a chemotactic effect on monocytes, neutrophils, fibroblasts, stem cells and osteoblasts [31]. This peptide is a potent mitogen for mesenchymal cells and involved in the three phases of wound healing, including angiogenesis, formation of fibrous tissue and re-epithelialisation [32, 33]. TGF- $\beta$  released from platelet alpha granules is a mitogen for multiple cell lines [34]. In addition, it promotes angiogenesis and ECM production. VEGF promotes cell migration, new blood vessel growth and anti-apoptosis of blood vessel cells [35]. EGF, another platelet-contained growth factor, is a mitogen and useful in healing chronic wounds [36]. IGF has been extensively studied for its potential to induce proliferation, differentiation and hypertrophy of multiple cell lines. IGF is also an important modulator of cell apoptosis and, when applied in combination with PDGF, can promote bone regeneration [37].

As a therapeutic strategy, PRP is consistently being used clinically in stimulation and acceleration of bone and soft

tissue healing [28]. In the process of localised delivery of PRP, a variety of biologically active growth factors released offer opportunities for treatment of IDD. PRP is superior to other growth factors for its autologous origin. As for clinical applications, autologous PRP avoids complex regulations, disease transmission and immunological reaction, while artificially synthesised growth factors are more costly with a short half-life. As a growth factor cocktail therapy, the combined application of multiple cytokines released from PRP may contribute to synergistic effects. A particular value of PRP is that these native growth factors present in PRP are in normal biological ratios. When PRP is activated, several cell adhesion molecules, including fibronectin, fibrin and vitronectin, are released. These adhesion molecules play an important role in cell migration and thus contribute to the potential of bioactivity of PRP [35]. The simplicity in preparation, potential cost-effectiveness, safety and permanent availability of PRP will promote its use in IVD regenerative medicine [7].

#### Current status of biological treatments for IDD: why early intervention by growth factors is more favoured?

Current therapeutic strategies for IDD mostly depend on physiotherapy, anti-inflammatory medications and spinal surgery to alleviate the symptoms, but these therapies cannot restore the degenerated IVD and even accelerate the degenerative progression of the adjacent IVD [38]. With the development of biotherapy, new methods have shed light on the repair and regeneration of the degenerated discs. Current biological strategies for IDD mainly focus on growth factor therapy, gene therapy, cell therapy and stem cell-based tissue engineering therapy [39, 40].

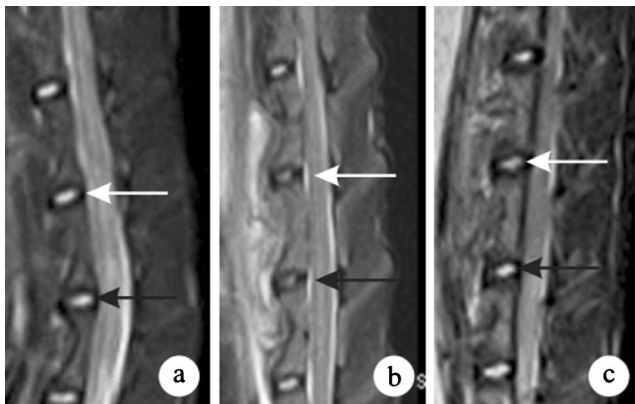
When designing any therapeutic strategies, the appropriate timing of interventions needs to be taken into serious consideration [41]. In the early stage of IDD, a large number of functional cells are retained and phenotypic changes of IVD cells are few. The injection of growth factors into the degenerated discs is a minimally invasive procedure, especially with fluoroscopic guidance (X-ray). The injected growth factors are able to promote the proliferation and ECM accumulation of IVD cells [42]. Thus, for the early degeneration of IDD, growth factor therapy is an ideal strategy to repair the degenerated discs.

As for moderate degeneration of the IVD, gene therapy and cell therapy may be more effective. When transfecting certain genes of active proteins to the IVD cells, these cells can stably produce the corresponding bioactive products to stimulate the remaining viable cells and upregulate the synthesis of ECM [43]. As a potential therapy, it should be noted that the safety issues and the efficacy of the genes to the starving cells need to

be carefully investigated. Cell therapy, especially using mesenchymal stem cells (MSCs) as seeds for transplantation, is proving to be a promising approach for IDD [44, 45]. MSCs have several theoretical and practical advantages over mature cells for cell therapy. As primitive cells, MSCs have better potential to survive and produce more matrix compared with terminally differentiated cells. Also, MSCs are available from many autologous sources and can be easily expanded in culture [46]. However, the long-term survival of the transplanted MSCs and whether the maintenance of targeted differentiation can be sustained within the degenerated discs are largely unknown.

In the late stage of IDD, few viable cells are left, and the calcified IVD holds limited potential to react to the biological intervention. In this stage, transplantation of a tissue-engineered disc might be the promising biological choice for IVD restoration [47]. However, tissue engineering therapy is more complicated compared with other biological therapies, because it requires a high standard of clinical research, optimal biocompatible scaffold and bioactive proteins as well as the complicated surgical procedures when applied in clinical use [48].

Therefore, intervention by active substances in the early stage of IDD is an ideal solution to retard or even reverse the degenerative trend of IDD. In this stage, even a single injection of biological growth factors could regulate the metabolic balance and homeostasis of the degenerated discs. Previously in our research, we induced IDD of rabbits by needle puncture of the AF [6]. Then we used PRP as multiple growth factor cocktail therapy to interfere in the early degenerated rabbit discs. The results suggested that even a single injection of PRP was potent to increase MRI signal intensity for the early degenerated IVD and confirmed the regenerative efficacy of PRP (Fig. 2).



**Fig. 2** Rabbit lumbar vertebral MRI observation at different time points. Compared with the sham group (without disc intervention) (a), 2 weeks after needle puncture, L4–5 (white arrow) and L5–6 (black arrow) in the experimental group (b) exhibited decreased T2 signal intensity. One week after PRP injection, T2 signal intensity of the experimental group (c) increased, indicating the regenerative process

## Growth factors and PRP: in vitro studies

Multiple growth factors, when applied to the stimulation of IVD cells, can effectively promote ECM production and cell proliferation (Table 1). TGF- $\beta$ 1 is one of the early applied growth factors which could stimulate the proliferation of IVD cells [49–51]. BMP-2 [51–53] and BMP-12 [52] were proved to upregulate ECM production. Besides, a study indicated that BMP-2 could facilitate expression of chondrogenic phenotype of human IVD cells [53]. As a member of the BMP superfamily, osteogenic protein 1 (OP-1) was proved effective when used to stimulate matrix metabolism of rabbit [54, 55] and human IVD cells [56]. Growth and differentiation factor 5 (GDF-5) (otherwise known as BMP-14), another member of the BMP family, exhibited a similar stimulatory effect on IVD cells, including increased proteoglycan and type II collagen expression [57]. Chujo et al. [58] confirmed the reparative capacity of GDF-5 on the IVD based on its effects of enhancing ECM production in vitro. IGF-1 was proved to be able to promote cell proliferation and matrix synthesis in many studies [49, 50, 59–61]. In a rat AF cell culture study, IGF-1 could also reduce the percentage of apoptotic cells [60]. PDGF was reported to be able to promote cell proliferation [61] and reduce the percentage of apoptotic AF cells which is similar to IGF-1 [60]. VEGF-A exhibited strong angiogenic activity and specific mitogenic and chemotactic actions, playing a significant role in the survival of NP cells [24]. bFGF [49, 61] and EGF [49] could both enhance IVD cell proliferation. In a gene transfection study, Liu et al. [62] reported that connective tissue growth factor (CTGF) enhanced collagen type II protein and proteoglycan synthesis of rhesus monkey lumbar NP cells.

With our deepening understanding of the biostimulative effect of an individually applied growth factor, the combined use of growth factors appears to be another choice. As a natural carrier of multiple growth factors, PRP has been introduced in the field of IVD restoration for its regenerative properties [63]. Until now, many studies have focused on how PRP affects the proliferation of the disc cells and the matrix produced in vitro [64–67]. Chen et al. [64] primarily proposed that PRP might be a therapeutic candidate for the prevention of IDD in a study of culturing NP cells together with PRP. In another study of this team, they confirmed PRP could effectively promote NP regeneration by upregulating levels of mRNAs involved in chondrogenesis and matrix accumulation by an in vitro IVD organ culture system [65]. A study from Akeda et al. [66] confirmed that PRP could stimulate the proliferation of porcine IVD cells cultured in alginate beads. In an in vitro culture study, Pirvu et al. [67] suggested that PRP preparations increased the matrix production and cell number of bovine AF cells. The results from these studies exhibited promising prospects for PRP application in IVD restoration.

**Table 1** Growth factors confirmed to have regenerative potential for degenerated IVDs: in vitro studies

Growth factor	Species	Cell types/tissues	Effect	Authors	Year
TGF- $\beta$ 1	Canine	IVD tissues	Increased matrix synthesis and cell proliferation	Thompson et al. [49]	1991
	E19 rat	AF cells	Pushing cells towards a fibrocartilaginous phenotype	Hayes et al. [50]	2011
	Rabbit	NP cells	Robust refurbishment of matrix	Lee et al. [51]	2012
BMP-2	Human	NP and AF cells	Increased newly synthesised PG and upregulated expression of aggrecan, collagen type I and collagen type II mRNA	Kim et al. [53]	2003
	Human	NP and AF cells	Increased matrix protein synthesis	Gilbertson et al. [52]	2008
	Rabbit	NP cells	Upregulation of aggrecan, collagen type I, and collagen type II mRNA expression	Lee et al. [51]	2012
BMP-12	Human	NP and AF cells	Increased matrix protein synthesis	Gilbertson et al. [52]	2008
OP-1	Rabbit	NP and AF cells	Increase in the total DNA, PG and collagen contents	Masuda et al. [54]	2003
	Rabbit	NP and AF cells	Stimulating matrix repair	Takegami et al. [55]	2005
	Human	NP and AF cells	Prevention of a decreased cell number during culture, significantly upregulated PG synthesis and accumulation	Imai et al. [56]	2007
GDF-5	Bovine	NP and AF cells	Increased PG synthesis and collagen synthesis	Chujo et al. [58]	2006
	GDF-5 (-/-) mice	NP and AF cells	Dose-dependent upregulation of the aggrecan and type II collagen genes	Li et al. [57]	2004
IGF-1	Mature canine	IVD tissues	Increased matrix synthesis and cell proliferation	Thompson et al. [49]	1991
	Bovine	NP cells	Increased PG synthesis	Osada et al. [60]	1996
	Human	AF cells	Significant reduction in the percentage of apoptotic cells	Gruber et al. [59]	2000
	Bovine	NP and AF cells	Improved proliferation	Pratsinis et al. [61]	2007
	E19 rat	AF cells	Upregulation of ECM	Hayes et al. [50]	2011
PDGF	Human	AF cells	Significant reduction in the percentage of apoptotic cells	Gruber et al. [59]	2000
	Bovine	NP and AF cells	Improved proliferation	Pratsinis et al. [61]	2007
VEGF	Vegfr-1lacZ/+ and Vegfr-2lacZ/+ mouse	NP cells	Promotion of NP survival	Fujita et al. [27]	2008
bFGF	Mature canine	IVD tissues	Increased matrix synthesis and cell proliferation	Thompson et al. [49]	1991
	Bovine	NP and AF cells	Enhanced proliferation	Pratsinis et al. [61]	2007
EGF	Mature canine	IVD tissues	Enhanced proliferation	Thompson et al. [49]	1991
CTGF	Rhesus monkey	NP cells	Enhanced synthesis of PG and collagen II	Liu et al. [62]	2010
PRP	Human	NP cells	Stimulation of NP proliferation and chondrogenic differentiation	Chen et al. [64]	2006
	Porcine	NP and AF cells	Proliferation of porcine IVD cells	Akeda et al. [66]	2006
	Porcine	IVD organ	Upregulated levels of mRNAs involved in chondrogenesis and matrix accumulation	Chen et al. [65]	2009
	Bovine	AF cells	Increased matrix production and cell number	Pirvu et al. [67]	2014

The in vitro studies listed above have confirmed the promising potential of multiple growth factors in the proliferation and ECM accumulation of IVD cells. IVD intervertebral disc, AF annulus fibrosus, NP nucleus pulposus, PG proteoglycan, TGF- $\beta$ 1 transforming growth factor  $\beta$ 1, IGF-1 insulin-like growth factor 1, PDGF platelet-derived growth factor, bFGF basic fibroblast growth factor, EGF endothelial growth factor, OP-1 osteogenic protein 1, GDF-5 growth and differentiation factor 5, CTGF connective tissue growth factor, PRP platelet-rich plasma

### Growth factors and PRP: in vivo studies

The injection of growth factors into the discs is a simple and minimally invasive technique, which helps to promote the production of ECM and restoration of disc height. Many studies in vivo have confirmed the efficacy of multiple growth

factors when applied in the repair and regeneration of degenerated discs. In an early murine IDD model, multiple growth factors, including GDF-5, TGF- $\beta$ , IGF-1 and bFGF, were proved to improve cellularity and stimulate cell proliferation [68]. In a rabbit IDD model, GDF-5 was also proved to restore disc height, improve MRI scores and histological

grading scores [57]. OP-1 is a widely studied growth factor for its regenerative effect on the degenerated discs [55, 56]. OP-1 could effectively increase the ECM accumulation and even contribute to the relief of pain-related behaviour in a rat IDD model [69].

PRP, a cocktail of multiple growth factors, has attracted increasing attention in regenerative medicine. Nagae et al. [70] injected PRP encapsulated in gelatin hydrogel microspheres into degenerated rabbit discs. The synergistic effect of multiple growth factors released from PRP successfully suppressed the degenerative trend of the IVD. An advanced study from this team indicated PRP could lead to the preservation of water content, upregulate expression of ECM and maintain disc height [71]. Gullung et al. [72] further suggested in their study that PRP could decrease the inflammatory cells. In a rat disc degeneration model, Obata et al. [73] indicated that PRP was able to proliferate the chondrocyte-like cells within the discs and restore disc height. A recent study from our group also confirmed the regenerative efficacy of PRP on the early degenerated rabbit discs [6].

### Future research and clinical directions

As a choice of biological strategies for IDD, growth factor therapy is a promising therapeutic strategy when applied in the regeneration of early degenerated discs [74]. However, the pathological mechanism of IDD still remains unclear and needs further investigations. The currently discovered progenitor/stem cells within the discs have advanced our knowledge of the cellular biology of the IVD and how these progenitor/stem cells could be related to IDD [21]. However, the cellular mechanism of how growth factors or PRP affect the endogenous progenitor/stem cells within the discs needs to be further studied. The early degenerative stage of IDD is considered to be the ideal time for growth factor therapy, and the short-term effect of growth factors and PRP have been proven effective based on animal studies [6, 55–57, 68–73]. However, data on the efficacy of the long-term effect are still scarce and the issue needs further research.

As for clinical applications, the major function of growth factor therapy for IDD is to prohibit the apoptosis of the disc cells and promote ECM production [75, 76]. However, until now, there is no consensus on the single dose and frequency of the injected growth factors to achieve the desired regenerative effect. Which therapeutic growth factors can be coapplied to reach the optimal results is largely unknown. Further, the short half-life and solubility of the growth factors may not serve as a long-term stimulus for IVD repair [77], which needs to be taken into serious consideration when designing an optimal therapeutic programme. In addition, whether IVD repair and regeneration induced by bioactive proteins can contribute to pain relief remains to be resolved, because matrix restoration

stimulated by growth factors or PRP may not necessarily alleviate the pain. Current studies mainly focus on the regenerative efficacy of growth factors and PRP, but the possibility of adverse effects for clinical applications needs to be further addressed.

### Summary

With our deepening studies of the cellular and molecular biology of IDD, we have a better understanding of growth factor therapy for IDD regeneration. Studies of growth factor therapy using growth factors or PRP have confirmed their promising efficacy in the treatment of IDD. However, these results are under limited conditions without clinical data. Future studies are required to solve multiple problems, including application conditions, technical and safety issues. The currently reported progenitor/stem cells within discs have provided a significant target for future IDD therapy. The advanced understanding of the cellular and molecular biology of IDD as well as the role of growth factors or PRP applied in IDD therapy are focal points of future research.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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