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THE NUCLEUS PULPOSUS MICROENVIRONMENT IN THE INTERVERTEBRAL DISC: THE FOUNTAIN OF YOUTH?

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Abstract

The intervertebral disc (IVD) is a complex tissue, and its degeneration remains a problem for patients, without significant improvement in treatment strategies. This mostly age-related disease predominantly affects the nucleus pulposus (NP), the central region of the IVD. The NP tissue, and especially its microenvironment, exhibit changes that may be involved at the outset or affect the progression of IVD pathology. The NP tissue microenvironment is unique and can be defined by a variety of specific factors and components characteristic of its physiology and function. NP progenitor cell interactions with their surrounding microenvironment may be a key factor for the regulation of cellular metabolism, phenotype, and stemness. Recently, cell-transplantation approaches have been investigated for the treatment of degenerative disc disease, highlighting the need to better understand if and how transplanted cells can give rise to healthy NP tissue. Hence, understanding all the components of the NP microenvironment seems to be critical to better gauge the success and outcomes of approaches for tissue engineering and future clinical applications. Knowledge about the components of the NP microenvironment, how NP progenitor cells interact with them, and how changes in their surroundings can alter their function is summarised. Recent discoveries in NP tissue engineering linked to the microenvironment are also reviewed, meaning how crosstalk within the microenvironment can be adjusted to promote NP regeneration. Associated clinical problems are also considered, connecting bench-to-bedside in the context of IVD degeneration.

Keywords: Tissue engineering, regenerative medicine, intervertebral disc, nucleus pulposus, cell therapy, microenvironment, clinical research.

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List of Abbreviations

2D	2-dimensional
3D	3-dimensional
ADSC	adipose-derived stromal cell
AF	annulus fibrosus
AMPC	allogeneic mesenchymal precursor cell
API	recombinant human growth and differentiation factor-5 (aka. rhGDF-5)
ASC	adipose stromal cell
BMAC	bone marrow aspirate concentrate
BMP	bone morphogenetic protein
BMSCs	bone marrow/mesenchymal stem/stromal cells
CCL-5	chemokine (C-C motif)
CD202b	angiopoietin-1 receptor
CD24	cluster of differentiation 24
CEP	cartilaginous endplate
CFU-f	colony forming units-fibroblast
CT	computed tomography
CTGF	connective tissue growth factor
DDD	degenerative disc disease
DPQ	Dallas pain questionnaire
ECM	extracellular matrix
EQ5D	standardised measure of health-related quality of life developed
ES	embryonic stem
FGF	fibroblast growth factor
FRI	functional rating index
GAG	glycosaminoglycan

GD2	disialoganglioside 2
GDF	growth and differentiation factor
GLUT-1	glucose transporter 1
GMP	good manufacturing practice
HIF-1α	hypoxia-inducible factor 1α
IDCT	injectable disc cell therapy
IDD	intervertebral disc degeneration
IGF	insulin growth factor
IL	interleukin
iPSC	induced pluripotent stem cell
IVD	intervertebral disc
JOABPEQ	Japanese orthopaedic association back pain evaluation questionnaire
LBP	low-back pain
LDD	lumbar disc degeneration/disease
MB	methylene blue
MMP	matrix metalloproteinase
MODISC	Modic I discopathies
MPC	mesenchymal precursor cell
MRI	magnetic resonance imaging
MSC	mesenchymal stromal cell
MSV	GMP)-compliant expanded bone marrow MSC (MSV, PEI Num. 10-134)
Nanog	homeobox protein NANOG
NASS	North American Spine Society
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
Notch1	notch homolog 1, translocation-associated
NP	nucleus pulposus
NRS	numerical rating scale
Oct4	octamer-binding transcription factor 4
ODI	Oswestry disability index
PASS	patient acceptable symptom state
PDGF	platelet derived growth factor
PEG	polyethylene glycol
pNIPAM	poly(N-isopropylacrylamide)
PROMIS	patient-reported outcomes measurement information system
PRP	platelet-rich plasma
RCT	randomised-controlled trial
rhGDF-5	recombinant human growth and differentiation factor-5
RMQ	Roland-Morris questionnaire
SAPH	self-assembling peptide hydrogel
SF	short form
SIRT1	NAD-dependent deacetylase sirtuin-1
SIRT6	NAD-dependent deacetylase sirtuin-6
SLRP	small leucine-rich proteoglycan
SOX9	SRY-box transcription factor 9
TAA	triamcinolone acetonide
TGF-β1	transforming growth factor- β1
Tie2	TEK receptor tyrosine kinase
TNF-α	tumour necrosis factor-α
VAS	visual analogue scale
VAST	viable allograft supplemented disc regeneration treatment
WPAI	work productivity and activity index

Introduction

The IVD can be defined as a joint between adjacent vertebral bodies. It is composed of three primary tissues, the NP, the AF, and the CEP (Urban and Roberts, 2003). The cells within each of the regions of the IVD can not only be subjected to physical and biochemical stimuli from their ECM but also from their surrounding microenvironment (*i.e.* non-ECM related) (Baer *et al.*, 2003; Cao *et al.*, 2009; Cao *et al.*, 2011; Hsieh *et al.*, 2005; Hwang *et al.*, 2014; Jackson *et al.*, 2011a; Korecki *et al.*, 2008). Moreover, it is well documented that disc degeneration first occurs in the NP region of the IVD (Wang *et al.*, 2014; Zhao *et al.*, 2007). In this context, this full microenvironment (*i.e.* ECM and non-ECM related factors) of the IVD, and especially the NP microenvironment is believed to play a critical role in the regulation and maintenance of this tissue. It can potentially improve the repair and regeneration of the vertebral column joint (Fig. 1). However, the understanding of the NP microenvironment of the IVD is still incomplete.

In this review, papers reporting NP cell interactions with their surrounding microenvironment have been summarised to help improve future development of tissue engineering and regenerative medicine approaches. The first section covers what has been have learned from the current literature on:

- the "general" composition of the NP microenvironment with the description of essential components (non-ECM and ECM-related),
- how NP cells interact with their native microenvironment,
- how changes in the surrounding, particularly due to degeneration or disease, can alter NP cells.
- the state-of-the-art for tissue engineering and regenerative medicine approaches using this knowledge to treat IVD degeneration and LBP.
- clinical trials involving and targeting the NP microenvironment and how all the available expertise was used to bring bench and bedside closer together.

The "general" composition of the NP microenvironment

Non-ECM-related factors

The IVD is an avascular tissue. Therefore, the supply of nutrition to the disc primarily occurs through diffusion from its surrounding vasculature (Jackson *et al.*, 2011b). The variation between cellular consumption rates and nutrient transport (passive or active) leads to concentration gradients of these metabolites and nutrients throughout the IVD. Consequently, this markedly affects the viability, proliferation, and function of cells, and collectively will alter any subsequent potential regeneration or repair.

In human lumbar, thoracic, and cervical discs, oxygen levels vary considerably and do not appear to correlate with ageing or the degree of severity of pathological disease such as disc degeneration. Oxygen concentrations decrease from the AF across the disc inner structure (between 19.5 % and 0.65 %) with average normoxic levels in the central part of the NP being between 0.5 % and 10 % of oxygen

(Bartels *et al.*, 1998; Buckley *et al.*, 2018). These levels are mainly determined by the transport through the CEP, but also by the cellular density within the tissue, and the cellular consumption rates.

Another critical point of the microenvironment is the presence/absence of the most used "fuel" in the body, glucose. It is well recognised that NP cell viability is reduced if the microenvironment is glucose deficient. However, a low oxygen concentration within the microenvironment has not been shown to be detrimental to NP cell viability. Glucose level does play a critical role as a limiting factor for disc-cell survival (Urban *et al.*, 2004). A mathematical model has predicted a decrease in glucose concentrations, from around 5 mmol/L in the AF to approximately 0.8 mmol/L, in the NP tissue part of healthy IVDs (Selard *et al.*, 2003) that can even fall below critical levels with more substantial calcification of the CEP tissue (Jackson *et al.*, 2011b). Importantly, cell death can already occur when cells are subjected to glucose concentrations below 0.5 mmol/L for more than 3 d (Horner and Urban, 2001). Additionally, low cell viability correlates with low glucose concentrations, which has been shown in scoliotic discs (Bibby *et al.*, 2002).

Another important parameter in the NP tissue microenvironment is the pH. The pH ranges from 2 to 6, which is mainly due to the local production of lactic acid, as a result of glycolysis by the IVD cells (Bartels *et al.*, 1998). Several *in vivo* measurements revealed pH ranges from 5.7 to 7.5 (with a median at 7.0) (Bez *et al.*, 2018; Gilbert *et al.*, 2016; Nachemson, 1969). It is well established that the pH can significantly influence cell survival, negatively affect matrix synthesis rates (Horner and Urban, 2001; Ohshima and Urban, 1992), but may also increase the expression of pro-inflammatory cytokines and pain-related factors in the context of LBP (Gilbert *et al.*, 2016). Furthermore, scientists found that rates of metabolism (*e.g.* oxygen consumption) were sensitive and coupled in a non-linear way with the pH present in the microenvironment of bovine discs (Bibby *et al.*, 2005).

The physiological mechanical loading stress of the IVD tissue leads to constant exposure of the IVD cells to high osmolarity and, therefore, microenvironmental osmotic changes (Sadowska *et al.*, 2018). Aggrecan, as a cartilage-specific proteoglycan is essential for maintaining hydration and hence osmotic pressure in the IVD (Urban and Roberts, 2003). Negatively charged GAGs regulate the ionic balance of the IVD ECM (Johnson *et al.*, 2014). Aggrecan is linked to sulphated GAGs (*e.g.* keratan and chondroitin sulphate). They can create a negative charge and hence bind water within the tissue. If this is transposed to the tissue level, the generation of osmotic pressure in the IVD creates a water "flow" into the NP tissue. This water intake also leads to the load-bearing ability and the crucial swelling pressure of the IVD (Erwin and Hood, 2014; Urban and Maroudas, 1981; Urban *et al.*, 1979; Urban and Roberts, 2003). In a healthy state, the extracellular osmolarity can vary from around 430 mOsm/L (iso-osmotic pressure) to around 496 mOsm/L (hyper-osmotic pressure) (Ishihara *et al.*, 1997; van Dijk *et al.*, 2011). These values are in a physiological range for IVD cells but would be considered too high or too variable if compared to the osmotic pressure that most mammalian cells experience (Appelboom

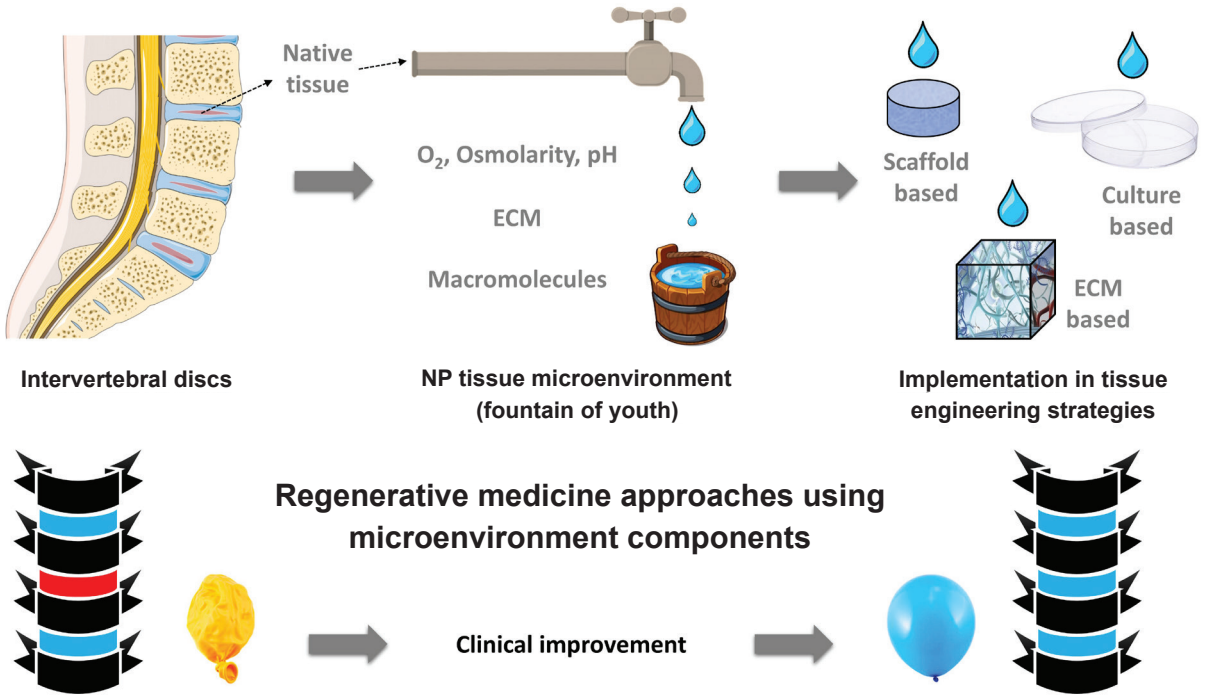

Fig. 1. The fountain of youth. How the NP microenvironment and how factors involved in it can implement tissue engineering approaches for the regeneration of the IVD and therefore enable future clinical improvement.

et al., 1956; Brocker *et al.*, 2012). As a reference, the osmotic pressure of healthy blood ranges between 280 mOsm/L and 320 mOsm/L (Neidlinger-Wilke *et al.*, 2012). In a diseased state (*e.g.* degenerated), the IVD osmolarity can even decrease so much that it reaches values of around 300 mOsm/L (hypo-osmotic pressure), due to a loss of proteoglycans (Wuertz *et al.*, 2007). As a result, IVD hydration can be compromised, and fibrosis may occur. However, this hypo-osmotic pressure would be considered physiological for cells of other tissues, especially in older people (Hooper *et al.*, 2015). Nevertheless, stem-cell-related research and associated publications discussing high osmolarities are still limited. Tao *et al.* (2013) pioneered the analysis of the effect of osmotic pressure on IVD progenitor cells. They found that high osmolarity could not only decrease the viability and proliferation of IVD cells but also affect the expression levels of collagen type II, SOX-9 transcription factor, and aggrecan in NP progenitor cells (Tao *et al.*, 2013). Additionally, a study showed the effects of high osmolarity on human ADSCs in the context of IVD regeneration (Liang *et al.*, 2012a). They concluded that high osmolarity in the IVD microenvironment is a deleterious factor that affects the survival and biological behaviour of ADSCs, making them less suitable for clinical applications. A recent bioinformatic analysis identified significant gene biomarkers of LBP caused by changes in the osmotic pressure of NP cells, providing a reference for future in-depth research (Zhao *et al.*, 2020).

In the context of regenerative medicine or tissue engineering, all 4 components of the microenvironment described above (oxygen, glucose, pH, and osmolarity) seem to have a different impact on the IVD cells (Fig. 2). On the one hand, oxygen concentration appears to have a pivotal role in regulating the biosynthesis and phenotype of targeted cells for therapeutic applications (Naqvi and Buckley, 2015). On the other hand, low glucose concentrations, osmolarity, and low pH levels can impair the survival and biological behaviour of progenitor cells (Li *et al.*, 2012; Naqvi and Buckley, 2016; Tao *et al.*, 2013; Wuertz *et al.*, 2009). All those parameters (non-ECM related) should be taken into account in the development of future approaches to tissue engineering and regenerative medicine targeting IVD and especially NP tissue.

ECM-related factors

The IVD ECM can be characterised as a scaffold rich in molecules that offer structural and biochemical support to the resident cells (Fig. 3). The structural, and indeed functional requirements, determine the mechanical properties of the ECM, which depend on the protein composition of the matrix – particularly the percentage and type of several collagens and elastin fibres (Mercuri *et al.*, 2014; Sivan *et al.*, 2014a). Furthermore, the biochemical and physiological relevance of these properties is illustrated by a mutual/reciprocal crosstalk between ECM and cells. Cells are capable of sensing surrounding ECM stiffness thanks to integrin-mediated interactions with the matrix (Gilchrist *et al.*, 2007). The mechanical properties of the ECM are then interpreted and can affect cell motility, proliferation, differentiation, and apoptosis (Newell *et al.*, 2019; Peng *et al.*, 2020). Hence, knowledge of the precise composition of IVD tissues is critical to the understanding of their physiology and physiopathology. For example, the most common pathological state of the IVD (degenerated) is often closely related to alterations in the composition and structure of the ECM. Degeneration of the IVD tissue is well known to be linked with excessive matrix catabolism (Roughley, 2004).

The protein composition of human IVD tissues

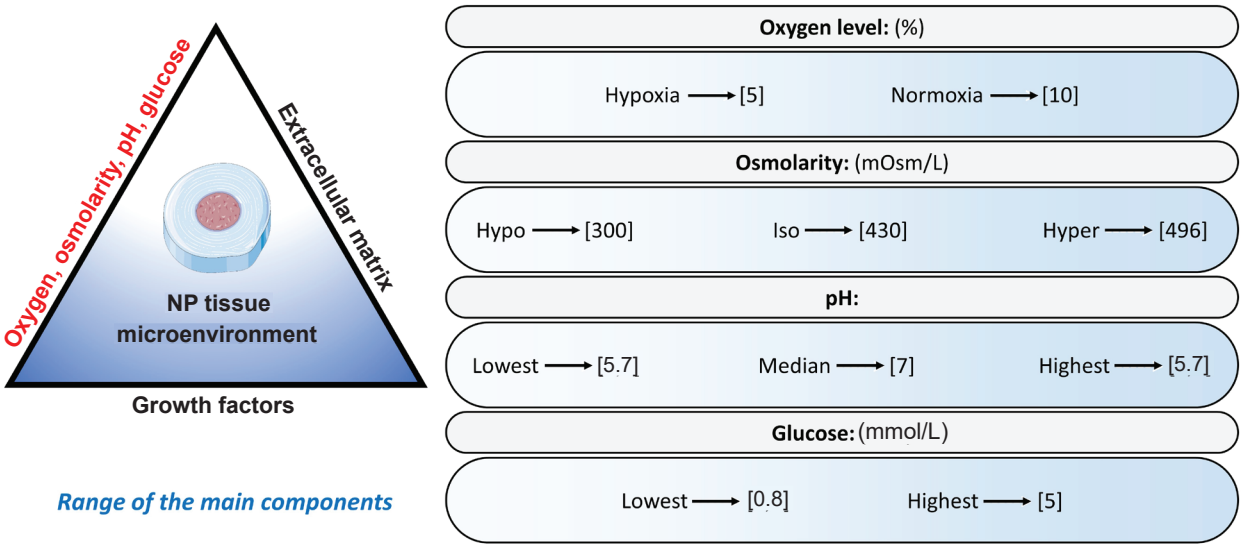

Fig. 2. Summary of non-ECM-related components and their ranges within the NP microenvironment. It can be characterised by 4 main aspects: (i) the oxygen level, (ii) the osmolarity, (iii) the pH, and (iv) the glucose concentration.

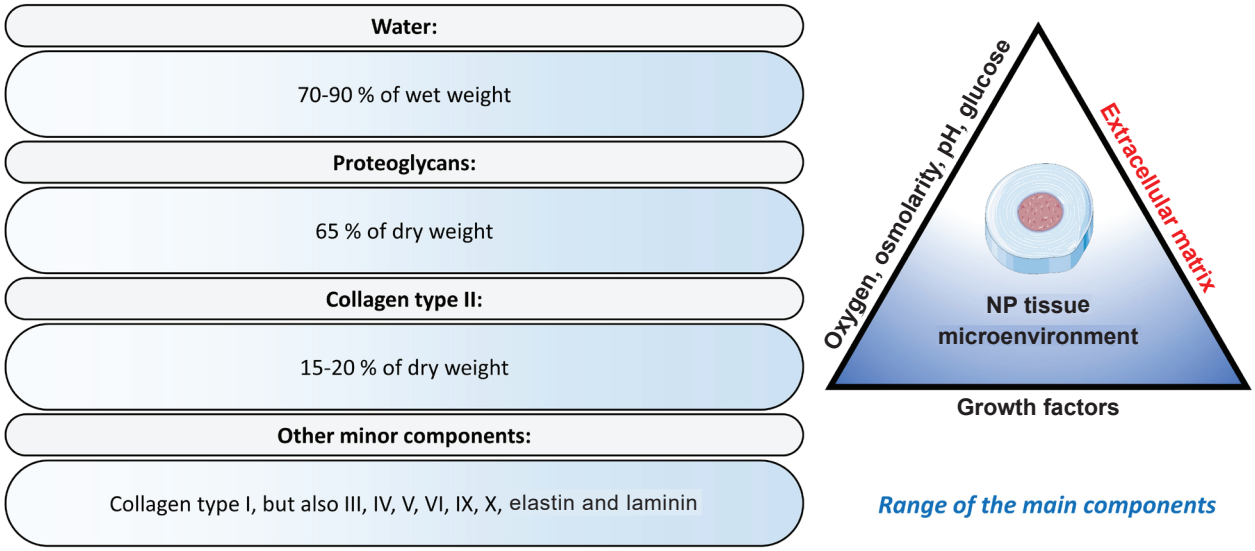

Fig. 3. Summary of ECM-related components and their ranges within NP microenvironment. It can be characterised by 4 main aspects: (i) water, (ii) proteoglycans, (iii) collagen type II, and (iv) other minor components.

will now be examined, to better estimate the amount of ECM proteins present both in a healthy and disease state. In terms of percentage, the ECM is mainly composed of water (70-90 % of wet weight), followed by proteoglycans (65 % of dry weight), and collagen type II (15-20 % of dry weight) (Eyre and Muir, 1977; Gower and Pedrini, 1969). Other minor components (though potentially critical functionally) of the NP ECM include elastin, small proteoglycans, other collagens (types III, VI, IX) (Melrose *et al.*, 2001; Roberts *et al.*, 1991; Yu, 2002), and laminins (Chen *et al.*, 2009; Gilchrist *et al.*, 2007; Nettles *et al.*, 2004). IVD-ECM contains 385 ± 35 μg of collagen (all types) per mg of dry tissue. Its abundance is not significantly affected by age or sex. But, the amount of collagen is slightly different between the AF and NP, with the AF tissue having significantly more collagen than NP tissue. All publications on the topic, conclude that the dry weight of the NP tissue has more proteoglycans than AF tissue (Choi, 2009; Mwale *et al.*, 2011). Concerning other main components of the IVD ECM, the total amount of proteoglycans in human tissue is 144 ± 16 μg per mg of dry tissue, and for elastin the amount is approximately 18 ± 4.1 μg per mg of dry tissue (McKee *et al.*, 2019). However, in a pathological context (*e.g.* scoliosis or disc degeneration), changes to ECM composition can occur, particularly in the percentage of collagen (McKee *et al.*, 2019). Furthermore, an increase in elastin is also observed in degenerated IVD (McKee *et al.*, 2019).

The protein composition of the ECM is a powerful regulator of cellular behaviour (Yue, 2014). Consequently, failure to adequately mimic the native extracellular environment during *in vitro* cell culture processes may lead to altered proliferation, adhesion, migration, polarity, differentiation, and apoptosis of the targeted cells. In the context of a clinical trial, *in vitro* results may not translate into the human patient (Edmondson *et al.*, 2014). To become closer to the native microenvironment, culturing on ECM-coated dishes, within 2D or even 3D culture, on biomimetic scaffolds, or organ-like/organoids culture has become a more and more common practice to overcome these unwanted artificial effects (Edmondson *et al.*, 2014; Guerrero *et al.*; Huch and Koo, 2015). By precisely summarising an "ingredient list" for the microenvironment it should be possible to recreate models that mimic it, and further improve knowledge through more efficient *in vitro* tools.

How NP progenitor cells interact with their microenvironment

IVD degeneration does not generally occur because of a single factor, but rather because of a variety of them. Usually, it is attributed to a complex interplay between environmental, genetic factors and mechanical damage (Dudli *et al.*, 2012; Kalichman and Hunter, 2008; Kamper *et al.*, 2016; Livshits *et al.*, 2011; Mayer *et al.*, 2013; Silva and Holguin, 2020). However, each of these factors leads to a final typical result of imbalance between the anabolic and catabolic microenvironment of the NP tissue in favour of catabolism.

During disc degeneration or just simply during ageing, significant changes can occur in the ECM composition and structure and, therefore, have an impact on IVD cell behaviour. All these changes that drastically affect the IVD microenvironment lead to an important decrease in cell density, and cell clustering (Boos *et al.*, 2002; Hastreiter *et al.*, 2001; Johnson *et al.*, 2001; Johnson and Roberts, 2003; Liebscher *et al.*, 2011; Roberts *et al.*, 2006). In the NP tissue, progenitor cells are embedded in a tissue-specific microenvironment that significantly influences the their biological and metabolic processes (Hu *et al.*, 2018). Low oxygen tension, hypertonicity, low pH, and reduced

nutrient supply are characteristics of the specialised IVD microenvironment. The level of specialisation and complexity of this microenvironment could be challenging for cell survival and health of endogenous progenitor or future implanted cells (Sakai and Andersson, 2015).

An indigenous progenitor cell population that expresses an endothelial specific marker, *i.e.* Tie2 (angiopoietin-1 receptor or CD202b), has been identified in human and murine NPs (Sakai *et al.*, 2012). These Tie2$^+$ cells were identified as the precursors of NP cells that further differentiate and start to express other surface markers, including GD2 and CD24 (Sakai *et al.*, 2012). Moreover, Tie2$^+$ cells have the ability of cell renewal (Sakai *et al.*, 2012), and multi-potency with differentiation towards neurogenic, osteogenic, chondrogenic, and adipogenic lineages. The number of Tie2 positive cells in the human (Sakai *et al.*, 2012) and mouse (Bach *et al.*, 2016) NPC populations also decreased, not only during ageing but also with the progression of IVD degeneration. The specific factors responsible for an *in vivo* decrease of NP cell clustering and a reduced percentage of NP progenitor cells with age, or during a degenerative state, are not fully understood. One of the advanced explanations is that cell mortality associated with decreased nutrient oxygen and glucose transport in IVD induces the decrease of both cell clustering and the percentage of Tie2$^+$ cells present in NP tissue (Urban and Roberts, 2003). Others have demonstrated that NP progenitor cells harvested from patients with degenerated IVDs still have similar cell colony-forming ability, proliferation rate, cell cycle, and trilineage differentiation properties to bone marrow stromal cells (Li *et al.*, 2017a).

Even NP progenitor cells derived from degenerated IVDs still retain their regeneration ability. They may be a promising cell candidate for cell-based regenerative medicine and tissue engineering for IVD degeneration. These results suggest that Tie2$^+$ NPCs could play a crucial role in IVD regeneration, knowing that – even if their microenvironment is disrupted – they do not seem to be affected (Sakai *et al.*, 2012). However, even if Tie2$^+$ NPCs seems to be the "ideal candidate", future strategies for NP tissue engineering and regenerative medicine should not only be focused on those cells as their numbers are very limited (around 1 %) in human IVD tissue (Hu *et al.*, 2018).

How changes in their surroundings can alter NP cells

Influences of non-ECM related factors on IVD progenitor cells

One intrinsic characteristic of progenitor disc cells is that they reside under hypoxic conditions due to a blood-supply shortage in the IVD and especially in the NP tissue. Under low-oxygen levels, the NP progenitor cells proliferate better than ASC, and their potential to differentiate on the chondrogenic lineage is increased (Li *et al.*, 2013). Physiological hypoxia (2-5 %) seems to be the normal and the physiological environment for optimal functioning of IVD progenitor cells (Chen *et al.*, 2014; Gantenbein *et al.*, 2014; Stoyanov *et al.*, 2011). However, invasion of blood vessels through fissures is generally observed during the process of IVD degeneration, which potentially increases the oxygen concentration and consequently aggravates the physiological hypoxic microenvironment of the IVD progenitor cells even further (Freemont *et al.*, 2002; Nerlich *et al.*, 2007). Therefore, in the context of cell therapy, restoring the hypoxic microenvironment may favour the use of NP progenitor cells.

Excessive mechanical loading and impaired biomechanics can be another crucial microenvironmental factor influencing NP progenitor behaviour (Chu *et al.*, 2018; Desmoulin *et al.*, 2020). For example, several studies showed an essential correlation between the presence and the severity of disc degeneration in obese or overweight adults (Lidar *et al.*, 2012; Samartzis *et al.*, 2012). Nevertheless, at a cellular level, the biomechanical factors display a wide range of impacts on the biological functions of NP progenitor cells. Recently, *in vitro* studies have shown that static compression stress induces mitochondrial apoptosis in NP progenitor cells (Li *et al.*, 2018; Yuan *et al.*, 2018). Apart from the induction of apoptosis and senescence, stress stimuli seem to be critical for the normal functioning of progenitor cells (Hosseini *et al.*, 2015; Kobayashi *et al.*, 1989; Zhao *et al.*, 2015). As an illustration of this, fluid-shear stress can give rise to overexpression of ECM components synthesised by AF progenitor cells (*e.g.* Collagen type I and MMP-1) (Chou *et al.*, 2016) – demonstrating the "double-edged sword" effects of applied forces on IVD cells and especially NP progenitor cells. To investigate these effects further, studies should focus on exploring various methods to protect IVD progenitor cells from excessive mechanical loading-induced dysfunction and increased cell death. Optimising mechanical stimulation of *in vitro* cultured IVD progenitor cells (also using Tie2$^+$) could be used to produce a matrix mimicking the microenvironment and to explore in more detail the relationship between ECM composition, mechanical forces, and cell behaviour.

Another non-ECM-related factor that can drastically influence NP progenitor cells (endogenous or implanted) seems to be osmolarity. Nevertheless, publications associating osmolarity and NP progenitor cells are still limited. Tao *et al.* (2013) pioneered the investigation of the influence of osmolarity on IVD progenitor cells. They found that high osmolarity in NP progenitor cells could decrease the viability, proliferation, and gene expression levels of not only the transcription factor SOX-9 but also aggrecan, and collagen type II, the two main components of the NP ECM.

Regarding the influences of non-ECM related factors on NP progenitor cells, it is necessary to examine the microenvironment pH. To have a comparison with already well-described cells, ASCs were used. Under low pH the proliferation and viability of the NP progenitor cells are better than those of ASCs (Han *et al.*, 2014). More recently, it was shown that the acid-sensing ion channels in NP progenitor cells play a vital role in low-pH induced apoptosis, and the downregulation of stem-cell-related genes (*e.g.* Oct4, Nanog, Jagged, Notch1) and ECM synthesis (*e.g.* aggrecan, SOX-9, collagen type I and collagen type II) (Liu *et al.*, 2017). It is also known, in connection with *Diabetes mellitus* or hyperglycaemia, that an excess of glucose has negative effects upon NP progenitor cell biology (Liu *et al.*, 2020). A comparison of NP progenitor cells cultured in low- or high-glucose media demonstrated that high glucose concentration significantly decreases cell proliferation, colony-formation ability, migration, and wound-healing capability. Moreover, NP progenitor cells from high-glucose culture also show significantly decreased expressions of a variety of markers, including SIRT1, SIRT6, HIF-1α, GLUT-1, and caspase-3. Altogether, these data show that high levels of glucose might drastically impact NP progenitor cell behaviour (Liu *et al.*, 2020).

Influences of ECM related factors on IVD progenitor cells

With the degeneration process of IVDs, the imbalance between ECM anabolism and catabolism intensifies the tissue dysfunction (Feng *et al.*, 2016; Kepler *et al.*, 2013). When investigating NP progenitor cells *in vitro*, the importance of the ECM in the original tissue is not usually considered. However, the crosstalk and interaction between cells and the ECM are essential in affecting not only the morphology and phenotype but also the function of progenitor cells, as shown in NP tissue (Guilak *et al.*, 2009; Rastogi *et al.*, 2009). Perlecan, the common component of many stem cell niches, is produced by progenitor cells located in the NP tissue (Brown *et al.*, 2018; Schlotzer-Schrehardt *et al.*, 2007). It was previously found to play a decisive role in the chondrogenic differentiation of the IVD mesenchymal progenitor cells (Shu *et al.*, 2013; Smith *et al.*, 2010). Most importantly, changes to ECM mechanical strength in degenerated discs are transmitted to the cell membrane and consequently, by means of perlecan or other components of stem cell niches, activate the IVD progenitor cells (Subramony *et al.*, 2013; Wang and Chen, 2013).

The Piezo1 ion channel, which acts as a mechanosensor between the ECM and cells, is found in human NP cells to be involved in the pathway that starts with mechanical force sensing and results in cell apoptosis. The signal is mediated through mitochondrial dysfunction and endoplasmic reticulum stress (Li *et al.*, 2017b). Nevertheless, many other aspects of mechanical sensing could potentially affect the behaviour of progenitor cells. How these IVD progenitor cells respond to them will require further investigation.

Specific components of the ECM seem to have a greater impact than the others on NP cells. Laminin recently attracted the attention of researchers, as it is present in healthy NP tissue, but absent in degenerating NP tissue (Chung and Mercurio, 2004; Foldager *et al.*, 2016; Foldager *et al.*, 2014; Mercurio *et al.*, 2001; Sun *et al.*, 2017). Moreover, laminin receptors (α6β1 and α6β4 integrins) are known to improve cell survival in response to microenvironmental changes such as low oxygen tension or serum-deprivation (Gu *et al.*, 2002), both relevant to NP tissue (Chen *et al.*, 2009).

Another critical aspect of the microenvironment that can drastically influence the behaviour of NP progenitor cells is the inflammatory process occurring during IVD degeneration. Cytokines, such as IL-1β and TNF-α, are generally considered to be the key mediators of the IVD degenerative process and LBP (Burke *et al.*, 2002; Risbud and Shapiro, 2014; Wang *et al.*, 2020b). They are also upregulated in the degenerative IVDs, and they are closely related to various pathological IDD processes, including the inflammatory response, matrix destruction, cellular senescence, autophagy, apoptosis, pyroptosis, and proliferation. Conversely, IL-1β suppression prevents disc degeneration (Genevay *et al.*, 2009; Le Maitre *et al.*, 2007). Therefore, anti-IL-1β and anti-TNF-α therapies may have the potential to stop this vicious cycle present in disc degeneration and LBP. Knowing that IL-1β and TNF-α inhibition have the potential to alleviate IDD, an in-depth understanding of the role of IL-1β and TNF-α in IDD will benefit the development of new treatment methods for disc degeneration.

The microenvironment of the native and, by extension, of degenerated IVD remains an important factor in the development of effective regenerative therapies (Baumgartner *et al.*, 2021), presenting a challenge not only for the application of cell therapies but also for interventions based on growth factors, or other biologicals such as drugs, and biomaterials.

Tissue engineering and regenerative medicine for NP tissue

A common way to improve or replace the function of biological tissue by tissue engineering is to use a scaffold (as a vehicle or a tissue replacement) in combination with cells that possess a regenerative potential, and/or additional components (chemicals, growth factors, oxygen priming, *etc.*). In the context of IVD tissue engineering, both the biological and mechanical properties of the scaffold will affect the biocompatibility within IVD tissue. Mimicking the microenvironment in the closest way possible will determine the *in vivo* function and regenerative potential of progenitor cells and determine the efficiency of IVD regeneration (Choi and Harley, 2012;

Engler *et al.*, 2007; Hsieh and Twomey, 2010; Seliktar, 2012). Utilising the natural molecules present in the IVD ECM could be an excellent starting point for the generation of scaffolding material, but synthetic polymers also seem to be efficient. Moreover, macromolecules present in the microenvironment could also be implemented or functionalised into an engineered scaffold in order to be even more similar to NP tissue. All those strategies will be presented in the following section.

Related to non-ECM factors

As discussed in the previous section, the components of the microenvironment from native and degenerative IVDs are well known to drive and affect the survival, behaviour, and fate of endogenous or transplanted NP cells. In the following part, non-ECM factors used for tissue engineering approaches for NP regeneration are described (Table 1).

One of the key components of the IVD microenvironment described above is the low pH. An acidic environment is vital for the maintenance of NP cells' activities. However, changes in the acidity of the environment on human NP cells can be detrimental. To overcome this problem, it has been shown that NP progenitor cells cultured in presence of amiloride, an acid-sensing ion channel blocker, could significantly improve their proliferation, expression of stem cell-related genes, and functional genes (Liu *et al.*, 2017).

As described above, the variation of osmolarity (osmotic concentration) can have a significant impact on the function and behaviour of NP cells. GAG has a key role in regulating osmolarity, therefore targeting this component of the microenvironment could be a critical element in improving tissue engineering approaches for NP repair and regeneration. For this reason, Sivan *et al.* (2014b) decided to develop a biomimetic GAG analogue-engineered scaffold based on sulphonate-containing polymers. The *in vivo* delivery of this biomimetic GAG, which can polymerise *in situ*, was used in a porcine and bovine degenerate explant model and, as a result, demonstrated the ability of the implanted hydrogel to restore NP tissue stiffness. Hydrogels or engineered scaffolds mimicking GAG function *in vivo* while maintaining disc hydration and height could be a solution for NP tissue repair or helping its regeneration by restoring part of the native microenvironment.

Another critical point for NP tissue engineering could be that cells *in vitro* primed in a low oxygen environment could then also promote an NP phenotype *in vivo*. As an example, Feng *et al.* (2014), cultured NP bovine cells for 3 weeks in a 3D environment with different oxygen tension levels (2 % to 20 %). They demonstrated that, even after 8 weeks of *in vivo* implantation, the hypoxic priming of NP cells resulted in the maintenance of GAG, collagen type II, aggrecan, and SOX-9 expression compared to normoxic priming. The maintenance of the NP phenotype was therefore achieved if cells were originally cultured under hypoxic conditions before implantation. In summary, combining hypoxia with a relevant scaffold could enhance NP function after implantation. Moreover, hypoxia induction of NP cells should be applied to most of the strategies to successfully regenerate NP tissue for IVD repair.

Researchers also found that SLRPs – bioactive components of the ECM – were associated with fibrillogenesis, cellular growth and apoptosis, as well as tissue remodelling of the IVD. They can support the survival of IVD progenitor cells under hypoxic conditions by the activation of specific hypoxia-inducible factors (Chen *et al.*, 2017). As a result, agents that can stimulate the production of SLRPs (*e.g.* biglycan or decorin) might help NP progenitor cells to survive under hypoxic conditions (Huang *et al.*, 2013) and could become critical components for the niche, providing new approaches to use the biology of the microenvironment as a toolbox (Rajasekaran *et al.*, 2021).

Recently, a new approach was developed for the sequential release of chemokines followed by growth factors, using a delivery system based on polysaccharide microbeads (Frapin *et al.*, 2020). In the pullulan-based scaffold, CCL-5 was first used to attract cells with a regenerative potential into NP tissue. After this first phase of recruitment, TGF-β1 and GDF-5 were chosen as growth factors to induce the synthesis of an ECM rich in collagen type II and aggrecan, *in vitro*. The authors then proceeded to *ex vivo* experiments using the same delivery system in degenerated ovine IVDs. As a result, after histological analysis, they observed an increase of NP cellularity and more collagen type II, and aggrecan. Moreover, the amount of $Tie2^{+}$ positive cells (NP progenitor) was higher compared to the control condition. The sequential delivery of macromolecules to repair and regenerate the ECM could be a strategy for functionalised engineered scaffolds in the future.

Concerning the GAG, trying to increase their expression *in vivo* after implantation can be one element to target – by the use of an engineered scaffold, as already described. However, trying to reduce its degradation *in vivo* can also be a way to improve the success rate after implantation. In this context, several *in vitro* studies have shown that glucosamine has an inhibitory effect on the degeneration of proteoglycans, providing the potential of a NP tissue engineering application in the context of IVD degeneration (Ilic *et al.*, 2003; Munteanu *et al.*, 2002; Samiric *et al.*, 2006). A novel deacetylated poly-N-acetyl glucosamine hydrogel, as a potential therapy for treating NP degeneration, has also been developed (Gorapalli *et al.*, 2012). Using primary human cells, they showed an increase in cell viability, metabolic activity, proteoglycan synthesis, and ECM protein expression (aggrecan and collagen type II). This hydrogel has promising *in vitro* characteristics and motivates further *in vivo* evaluation as a potential therapy for NP degeneration of the IVD.

The fabrication/development of scaffolds with chondroprotective supplementation could be an interesting approach to avoid straying any further from the microenvironment of the NP tissue, in case of degeneration. Using a combination of alginate and $CaCl_2$ (in a w/v ratio of 2 % and 0.025 mol/L, respectively), supplemented with glucosamine hydrochloride and chondroitin sulphate A, Foss *et al.* (2014) showed an improvement in both mechanical and biochemical properties of the engineered hydrogels. However, although the promising results come from a 28-day study with human NP cells, they are only *in vitro*. Therefore, *in vivo* studies appear as a necessity to confirm their validation for future applications in the field of regenerative medicine as applied to NP tissue.

Recently, many factors that are able to influence the native microenvironment by modulating IVD metabolism *in vivo* were listed (Yang and Li, 2009). Growth factors could be used as a biological treatment choice for intervertebral disc degeneration (Paesold *et al.*, 2007). Studies using IGF-1, PDGF, or BMP-7 for their effects on apoptosis inhibition in IVD cells can also be listed (Gruber *et al.*, 2000; Wei *et al.*, 2008). However, the purpose of adding single growth factors (Chujo *et al.*, 2006; Cui *et al.*, 2008; Gilbertson *et al.*, 2008; Hodgkinson *et al.*, 2020; Kawakami *et al.*, 2005; Li *et al.*, 2004; Masuda *et al.*, 2006; Tsai *et al.*, 2007; Walsh *et al.*, 2004) or a combination of them (Akyuva *et al.*, 2019; Henry *et al.*, 2017; Hou *et al.*, 2016; Liang *et al.*, 2012b; Sun *et al.*, 2021) is mostly to stimulate the IVD metabolism through GAG, collagen or non-collagen synthesis.

For successful implantation of tissue engineering scaffolds and/or cells within the NP tissue, another angle of attack could be to reduce the inflammation to get closer to the IVD anabolism and native microenvironment. To reach this target, the efficacy of intradiscal controlled release of TAA, an anti-inflammatory drug, in a preclinical degenerated IVD model has been evaluated (Rudnik-Jansen *et al.*, 2019). Another team, using the same approach but using

Table 1a. Tissue engineering strategies with non-ECM related factors for NP. Microenvironment modulation.

Target	Strategy	Component/ growth factor	Cells/ material	Results	Conclusion	Reference
pH/acidity	Blocking of acid-sensing ion channel	Amiloride	Human NP-MSCs	Improvement of NP proliferation, expression of stem cell-related genes, and functional genes	The amiloride may improve IVD degeneration by improving the activities of NP-MSCs	Liu *et al.*, 2017
Osmolarity	Increase the GAG content	Biomimetic GAG analogue engineered scaffold	Bovine and porcine IVD/ sulphonate-containing polymers	Appropriate fixed charge density, hydration and osmotic responsiveness	Can maintain disc hydration and height under the high and variable compressive loads encountered *in vivo*	Sivan *et al.*, 2014b
Oxygen level	Hypoxic cells priming prior to implantation	3 weeks of priming at 2 % O_2	Bovine NP cells	Maintenance of GAG, collagen type II, aggrecan, and SOX-9 expression compared to normoxic priming	Hypoxia enhanced the NP phenotype under experimental conditions both *in vitro* and *in vivo*	Feng *et al.*, 2014
Cell expansion/ ECM neosynthesis	Sequential delivery of chemokines and growth factors	Chemokine (C-C motif) ligand 5 followed by TGF-β1 and GDF-5	Human ADCs/ Pullulan-based scaffold	Proliferation of NP progenitor and induction of ECM synthesis, *in vitro*	The sequential delivery is efficient for the repair and regeneration of NP's ECM	Frapin *et al.*, 2020
GAG degradation	Inhibitory effect on the degeneration of proteoglycans	Deacetylated poly-N-acetyl glucosamine	Human disc cells/ hydrogel	Increase in cell viability, metabolic activity, proteoglycan synthesis, and ECM protein expression	Potential therapy for NP degeneration	Gorapalli *et al.*, 2012
GAG degradation	Chondroprotective supplementation	Glucosamine hydrochloride and chondroitin sulphate A	Human NP cells/ combination of alginate and $CaCl_2$	Increase in collagen type II content	Potential for a chondroprotective supplemented injectable scaffold in degenerative disc	Foss *et al.*, 2014
Modulation of progenitor cells survival under hypoxia	Implementation with native niche components	SLRPs/ biglycan/ decorin	Tissue-specific IVD progenitor cells from healthy Rhesus monkey	Reduction of the susceptibility of progenitor cells to hypoxia-induced apoptosis by promoting the activation/stabilisation of HIF-1α and HIF-2α	SLRPs are niche components of progenitor cells in IVD homeostasis, providing new insights in progenitor cell biology and niche factors under a hypoxic microenvironment	Huang *et al.*, 2013

celecoxib, obtained similar results. This prevented progression of IVD degeneration both *in vitro* by promoting the function of implanted progenitor cells, and *in vivo* by preserving water content and integrity of collagen fibres (Tellegen *et al.*, 2018). More recently, the use of ligustrazine showed interesting results in the reducing the expression of inflammatory factors by the suppression of TGF-β overactivation in NP cells (Liu *et al.*, 2019). Besides tipping the balance toward a healthier microenvironment, the anti-inflammatory factor related strategies can also be beneficial for pain relief in patients undergoing LBP (Fontana *et al.*, 2015).

Related to ECM factors

Biomaterials (scaffolds, hydrogels, *etc.*) based on native NP ECM components could provide the missing link for the functional support of exogenous or native cells. The different application, with their biological and mechanical influence on IVD cells (Table 2), is presented below.

Laminin seems to play a relatively important role in NP tissue, as NP cells are known to cluster and produce more GAG rich ECM when cultured on soft (< 0.5 kPa) laminin-rich substrates, compared to fibronectin or collagen enriched ECM (Hwang *et al.*, 2014). Therefore, laminin supplementation has already improved musculoskeletal regeneration in several models of disease and injury (Marcinczyk *et al.*, 2017). Implantation of a PEG hydrogel, functionalised with laminin, into the NP *in vivo* resulted in more implanted cells remaining after 14 d as well as an increased viability (Francisco *et al.*, 2013). Moreover, cultured NP cells within this PEG hydrogel and functionalised with laminin were also affecting cellular metabolism by stimulating the

Table 1b. Tissue engineering strategies with non-ECM related factors for NP. Single growth factors.

Target	Strategy	Component/ growth factor	Cells/ material	Results	Conclusion	Reference
Modulation of IVD metabolism	Inhibition of apoptosis	IGF-1	Human IVD cells	Exposure to 50 ng/mL IGF-1 significantly reduced the percentage of apoptosis	Selected cytokines can retard or prevent programmed IVD cell death *in vitro*	Gruber *et al.*, 2000
Modulation of IVD metabolism	Inhibition of apoptosis	PDGF	Human IVD cells	Exposure to 100 ng/mL PDGF significantly reduced the percentage of apoptosis	Selected cytokines can retard or prevent programmed IVD cell death *in vitro*	Gruber *et al.*, 2000
ECM synthesis	Stimulates synthesis of proteoglycans and collagen	BMP-7	Intradiscal injection in rats IVD	Content of the ECM was markedly increased	BMP-7 injection into degenerative intervertebral disc resulted in the enhancement of the ECM	Kawakami *et al.*, 2005
Modulation of IVD metabolism	Stimulates synthesis of proteoglycans and collagen		Intradiscal injection in rabbits IVD	The proteoglycan content of the NP was significantly higher in the BMP-7 treated group	The metabolic changes in the cells, following a single injection of BMP-7, induce long-term changes in disc structure	Masuda *et al.*, 2006
Inhibition of apoptosis	Incubation with growth factor		Human NP cells	Addition of BMP-7 resulted in inhibition of the apoptotic effects	BMP-7 prevented apoptosis of human disc cells *in vitro*	Wei *et al.*, 2008
Modulation of ECM protein synthesis	Incubation with growth factor	BMP-2	Human NP cells	BMP-2 increased NP proteoglycan, collagen, and non-collagen protein synthesis to 355 %, 388 %, and 234 % of control	BMP-2 increase human NP cell matrix protein synthesis while having minimal effects on AF cells	Gilbertson *et al.*, 2008
Modulation of IVD metabolism	Incubation with growth factor	BMP-12	Human NP cells	BMP-12 increased NP proteoglycan, collagen, and non-collagen protein synthesis to 140 %, 143 %, and 160 %	BMP-12 increase matrix protein synthesis in both NP and AF cells, making it a potential therapy for enhancing matrix production in the IVD	Gilbertson *et al.*, 2008
Modulation of NP cells growth and differentiation	Incubation with growth factor	FGF-2	Bovine NP cells/ alginate beads	FGF-2 treatment resulted in increased sulphated proteoglycan synthesis and lower aggrecan turnover	FGF-2 should be tested for its ability to maintain the reactivity of the NP cells to other morphogenic factors	Tsai *et al.*, 2007

Table 1c. Tissue engineering strategies with non-ECM related factors for NP. Multiple growth factors and anti-inflammatory strategies.

Target	Strategy	Component/ growth factor	Cells/material	Results	Conclusion	Reference
Multiple growth factors						
Potentiation of exogenous cells	Incubation with growth factor	BMP-2/PRP	Human BMSCs/ Rabbit IVD/PRP gel	The discs treated with BMP2-transduced BMSCs exhibited relatively well-preserved NP structure	The combined use of these two agents can significantly promote repair of the degenerated discs *in vivo*	Hou *et al.*, 2016
Modulation of IVD metabolism	Time control release of growth factor	BMP-2/IGF-1	Human IVD cells/polyvinyl alcohol-based polymeric scaffold	Increased in the number of cells and the degree of ECM development	Alternative method for intervertebral disc administration of growth factors	Akyuva *et al.*, 2019
Modulation of IVD metabolism	The *in situ* injection of growth factors targeting intervertebral disc degenerative process	TGF-β1 and GDF-5	Human ADCs/ Pullulan microbeads into a cellulose-based hydrogel	Sustained release of both growth factors, for up to 28 d	Biphasic system may be a promising candidate for the development of an innovative bioactive delivery system for IVD regenerative medicine	Henry *et al.*, 2017
Modulation of IVD metabolism	Incubation with growth factors	FGF-2/ dexamethasone	Rat MSCs/3D poly(lactide-co-glycolide) constructs/ heparin/ poly(L-lysine) nanoparticles	Expression of disc-matrix proteins was significantly higher and of osteogenic differentiation marker was decreased	Microspheres could be used as a scaffold to improve cells growth and differentiating into NP like cells	Liang *et al.*, 2012b
Regeneration of IVD	Anatomically correct IVD scaffold	3D Printing	CTGF/TGF-β3/ polydopamine nanoparticles/rat BMSCs	Fabricated and implanted IVD scaffold exhibited a zone-specific matrix that displayed the corresponding histological and immunological phenotypes of IVD	Clinical application potential of the dual growth factors-releasing IVD scaffold fabricated by 3D bioprinting	Sun *et al.*, 2021
Anti-inflammatory strategies						
Reduction of inflammation	Intradiscal delivery of nonsteroidal anti-inflammatory drug	Celecoxib (selective COX-2 inhibitor)	Preclinical canine IVD model/ polyesteramide microspheres	No evidence of adverse effects on CT and MRI or macroscopic evaluation of IVDs	Intradiscal controlled release of celecoxib from polyesteramide microspheres prevented progression of IVD degeneration	Tellegen *et al.*, 2018
Reduction of inflammation	Prolonging corticosteroid presence by controlled release from biomaterials	TAA	Preclinical canine IVD model/ poly(esteramide) microsphere	The low dosage of TAA microspheres significantly reduced nerve growth factor immunopositivity in degenerated NP tissue	Potential applicability for pain relief, although beneficial effects were absent on tissue degeneration	Rudnik-Jansen *et al.*, 2019
Modulation of IVD metabolism	Reduce the expression of inflammatory factors and TGF-β1	Ligustrazine (LIG)	Lumbar spinal instability mouse model	A dose of 10^{-5} M LIG could attenuated Smad2/3 phosphorylation in IVD *ex vivo* and suppressed pSmad2/3, CCN2, and ACAN expression in NP cells *in vitro*	Ligustrazine could prevents IDD by suppression of TGF-β overactivation in NP cells	Liu *et al.*, 2019

Table 2a. Tissue engineering strategies with ECM related factors for NP. Laminin and collagen related.

Target	Strategy	Component/ growth factor	Cells/ material	Results	Conclusion	Reference
Laminin related						
Laminin	Scaffold with the ability to promote or maintain an immature NP cell phenotype	Photo-crosslinkable poly(ethylene glycol)-laminin 111 (PEG-LM111) hydrogel	Immature porcine NP cells	Promotion of cell clustering and increased levels of sGAG production	LM111-functionalised hydrogels may promote or maintain the expression of specific markers characteristic of an immature NP cell phenotype	Francisco *et al.*, 2014
Laminin	Biomaterials that retain delivered cells, promote cell survival, and maintain or promote an NP cell phenotype *in vivo*	Injectable, laminin-111 functionalised poly(ethylene glycol)	Porcine NP cells	Higher NP cell retention in cultured IVD explants within a PEG-LM111 carrier compared to cells in liquid suspension	Injectable laminin-functionalised biomaterial may be an easy to use carrier for delivering cells to the IVD	Francisco *et al.*, 2013
Collagen related						
Collagen type II	Delivery system with genipin as the cross-linking agent	Collagen type II/chondroitin sulphate composite hydrogel	RatADSCs/ rat coccygeal vertebrae degeneration model	Disc height, water content, ECM synthesis, and structure of the degenerated NP were partly restored	Delivery system uses minimally invasive approaches to promote the regeneration of degenerated NP	Zhou *et al.*, 2018
Collagen type II	Influence the bioactivity of transplanted cells	Hydrogels composed of a mix of collagen types I and II	Human ADSCs	Expressions of SOX9, aggrecan, and collagen type II were increased in a collagen type II dependent manner	Collagen type II significantly ameliorates human ADSC differentiation into NP cells and promotes ECM synthesis	Tao *et al.*,2016
Collagen type II	Mimic the NP microenvironment and promote differentiation	Microgels of collagen type II and hyaluronan	Rabbit ADSCs	Higher expression of collagen type II, aggrecan, SOX9, and low levels of collagen type I	By tuning microgels' properties, it is possible to influence cells phenotype and differentiation ability	Fontana *et al.*, 2014
Cells carrier	Administration using minimal invasive surgery	Alginate-collagen type I composite porous scaffold	Porcine MSCs and AF cells	Alginate-collagen porous scaffolds supported cell proliferation and ECM deposition	Advantages of incorporating collagen to enhance cell migration and proliferation in porous scaffolds	Guillaume *et al.*, 2015
Restore native ECM	Repair the degenerative environment	Collagen type II - hyaluronan - chondroitin-6-sulphate tri-copolymer construct	Rabbit nucleotomy IVD model	Narrowing of the intervertebral disc space was significantly retarded by the cell-scaffold hybrids implantation	Maintenance of disc height and restoration using NP cell-seeded tri-copolymer implants	Huang *et al.*, 2011
Recreate the resilient and hydrophilic nature of the ECM	Construction of a chemically stabilised composite hydrogel	Elastin/GAG/ collagen type I	Human ADSCs	Cytocompatible and support the differentiation towards an NP cell-like phenotype	Successful for host cell infiltration and active remodelling after implantation	Mercuri *et al.*, 2014

Table 2b. Tissue engineering strategies with ECM related factors for NP. SAPH related and decellularised ECM.

Target	Strategy	Component/ growth factor	Cells/ material	Results	Conclusion	Reference
SAPH related						
Mimicking the natural ECM for cell delivery	3D injectable hydrogel	Short self-assembling peptide hydrogels	Bovine NP cells/ Graphene oxide	Promote high cell viability and retained cell metabolic activity in 3D over the 7 d of culture	These hybrid hydrogels harbour significant potential as injectable scaffolds for the *in vivo* delivery of NP cells	Ligorio *et al.*, 2019
Mimicking the natural ECM for cell delivery	3D injectable hydrogel	Short self-assembling peptide hydrogels	Bovine NP cells	Upregulation of KRT8, KRT18, FOXF1, higher cell viability, and increase in aggrecan and collagen type II deposition	SAPH had comparable strength to the native tissue, was injectable, restored the IVD cell phenotype and stimulated deposition of appropriate matrix components	Wan *et al.*, 2016
Mimicking the natural ECM	A link N nanofibre scaffold	Short self-assembling peptide hydrogels	Rabbit NP cells/mixing peptide solution of RLN and RADA16	Scaffold exhibited little cytotoxicity and promoted NP cells adhesion	Functionalised nanofibre scaffold had excellent biocompatibility and bioactivity with rabbit NP cells	Wang *et al.*, 2012
Decellularised ECM						
Creation of an appropriate micro-environment for long term cell survival	Decellularised ECM	Hydrogel	Bovine NP/rat ADSCs	Significant increase in NP marker genes expression without the addition of exogenous biological factors	Decellularised NP hydrogel has low toxicity and inducible differentiation, could serve as a bio-scaffold, bio-carrier, and three-dimensional culture system	Yu *et al.*, 2020
Rescue the degenerated IVD	Decellularised ECM	Biological scaffold	Porcine NP/ human MSCs	Good cytocompatibility *ex vivo* and decelerated the degeneration of the IVD *in vivo*	Naturally-derived ECM material that could induce MSCs into NP cells	Xu *et al.*, 2019
Replace degenerated IVDs	Decellularisation IVDs	Natural biological scaffold	Bovine IVD	Efficient cells removal and GAG retainment after decellularisation	Possible to create a cell-free human IVD biological scaffold with attached bone using decellularisation technology	Norbertczak *et al.*, 2020
Treat disc degeneration	Use of matrix niche factor for exogenous cells differentiation	NP cell-derived acellular matrix and collagen micro-encapsulation	Rabbit degenerative disc/human MSCs	Acellular matrix supported MSC survival and matrix production, and up-regulated the gene expression of NPC markers including collagen type II and glypican 3	Potential application of the NPC-derived matrix microsphere as a favourable cell carrier	Yuan *et al.*, 2013
IVD repair	Instruct the regenerative cell component to produce tissue-specific ECM	Injectable biomimetic disc derived self-assembled ECM hydrogels	Chondroitin sulphate and collagen type II/ porcine nasal chondrocytes	Increase in GAG production	Inclusion of chondroitin sulphate within material aids the preservation of a rounded cell morphology and enhances their ability to synthesise NP-like matrix	Borrelli and Buckley, 2020

Table 2c. Tissue engineering strategies with ECM related factors for NP. Natural and synthetic scaffolds.

Target	Strategy	Component/ growth factor	Cells/ material	Results	Conclusion	Reference
Natural scaffolds						
Restore functions of both the AF and NP	Biphasic biomaterial	Silk protein for the AF and fibrin/ hyaluronic acid gels for the NP	Porcine AF cells/porcine articular chondrocytes	Formation of AF band NP-tissue like within the hydrogel after 6 weeks of culture	This biphasic scaffold was effective in the formation of the total IVD *in vitro*	Park *et al.*, 2012
Restore physiological functionality of damaged IVD	Biomimetic scaffold	Alginate hydrogel encapsulating cells	Rabbit NP cells/dorsal space of nude mice	NP cells colonised in the alginate hydrogel	NP cells colonised the biomimetic scaffold and form ECM similar to native NP tissue	Du *et al.*, 2019
Mimics the mechanical properties of the human NP tissue	Thermo-sensitive hydrogel	Chitosan-based	Bovine NP cells/human lumbar IVDs	Mechanical properties similar to the human NP tissue	Provided suitable environment to maintain NP cells alive and active, and induced production of GAG by the encapsulated cells	Alinejad *et al.*, 2019
Mimics the mechanical properties of the human NP tissue	Chitosan hydrogel	Chitosan hydrogel	Nude mice/New Zealand rabbit NP cells	Mechanical property meets the requirement of the normal NP	Cells on the tissue engineered NP expressed ECM, which indicated that the cells maintained their biological function	Yuan *et al.*, 2019
Regeneration of IVD micro-environment	Acellular and cellular tissue-engineering strategies	Ionic- and photo-crosslinked methacrylated gellan gum hydrogel	Rat lung fibroblasts (L929 cells)	Non-cytotoxic *in vitro*	Promising biomaterials to be used in IVD tissue-engineering strategies	Silva-Correia *et al.*, 2011
Substitute the inner IVD part	Injectable hydrogel	Ionic- (iGG-MA) and photo-crosslinked (phGG-MA) methacrylated gellan gum hydrogels	Human BMSCs and nasal chondrocytes	No cytotoxicity with MSCs and nasal chondrocytes, and no inducion of pro-inflammatory responses in endothelial cells	Potential use of modified gellan gum-based hydrogel as a suitable material in NP tissue engineering	Tsaryk *et al.*, 2017
NP regeneration	Injectable biomaterials act as carriers of growth factors	Gelatine methacryloyl microspheres	Rat ADSCs/ GDF-5	Enhancement of the *in vitro* differentiation of cells into NP-like phenotypes	Attenuation of *in vivo* degeneration of rat IVD, maintenance of NP tissue integrity and acceleration of ECM synthesis	Xu *et al.*, 2020
Enhancing intra-NP residence time of therapeutic drugs	Bind with the intra-NP negatively charged groups	Positively charged avidin grafted branched dextran nanostructures	Bovine NP	Month-long retention of cationic nanostructures within the NP following intra-discal administration	Way for effective clinical translation of potential therapeutics	Wagner *et al.*, 2020
Minimally invasive repair of degenerative IVD	Injectable tissue engineered NP	Combination of PRP gel scaffold and cells	Rabbit ADSCs	The level of GAG, gene expression of HIF-1α, aggrecan, collagen type II was higher when cells were seeded with the scaffold	Feasible method for construction of autologous injectable tissue engineered NP	Zhang *et al.*, 2020
Synthetic scaffolds						
Increase survival and function of NP cells	Tissue stiffness	3D matrices of varying degrees of stiffness	Porcine NP cells	Matrices with a low shear storage modulus (G′ = 1 kPa) promoted significantly proliferation and chondrogenic differentiation	Effect of the matrix modulus on the fate of NP progenitor cells	Navaro *et al.*, 2015

expression of N-cadherin and cytokeratin 8 (Francisco *et al.*, 2014). Both studies provide evidence that the use of laminin in engineered scaffolds could positively influence NP cell proliferation and function and should be studied in more detail to improve NP tissue engineering strategies.

Another vital component of IVD ECM that can be used as scaffolding material is collagen and particularly collagen type II (Zhou *et al.*, 2018). This main ECM component promotes the differentiation of NP progenitor cells, especially into the chondrogenic lineage (Tao *et al.*, 2016). When collagen type II is used at high concentrations for the scaffolding material, ASCs express a high level of collagen type II, aggrecan, SOX9, and low levels of collagen type I in response (Fontana *et al.*, 2014; Tao *et al.*, 2016). Collagen (mainly type II) should be used as the only and main component of the scaffold. Its implementation at a significant level should be enough to generate advantages such as enhancing cell migration and proliferation in porous scaffolds, which could be used for implemented tissue repair strategies (Guillaume *et al.*, 2015; Huang *et al.*, 2011; Sakai and Grad, 2015; Sarker *et al.*, 2015; Yang and Li, 2009).

Merging/combining most of the components of the NP microenvironment – such as elastin, collagen, and GAG – within a unique engineered scaffold/hydrogel could be a huge step towards successful NP tissue engineering applications. A chemically-stabilised elastin-GAG-collagen composite hydrogel shows great *in vivo* biocompatibility potential, with host cell infiltration and active remodelling of the NP tissue, after 4 weeks of *in vivo* subcutaneous implantation in rats (Mercuri *et al.*, 2014). Studies should now investigate the feasibility of this composite hydrogel for the replacement and regeneration of the NP.

Very recently, short SAPHs have attracted significant interest as they can mimic the natural ECM of the targeted tissue, holding significant promise for the *ab initio* design of the cellular microenvironment (Ligorio *et al.*, 2019). Moreover, SAPHs are beneficial mechanically, as they present a strength comparable to the native NP tissue, are injectable, can restore the IVD cell phenotype, and stimulate the deposition of appropriate ECM components (Wan *et al.*, 2016). These hydrogels open up new possibilities as they can be perfectly integrated into the NP microenvironment and offer the possibility of being customised by functionalisation (Wang *et al.*, 2012).

Another approach, that has appeared recently, is the use of decellularised matrices instead of combining several main components. These matrices preserve the NP ECM biological components and microstructure and, therefore, should perfectly mimic the NP microenvironment (Yu *et al.*, 2020; Yuan *et al.*, 2013). A protocol for the decellularisation of porcine NP tissue has been developed, which results in a decrease in IVD degeneration following *in vivo* implantation (Xu *et al.*, 2019). This, naturally derived, bioactive ECM material could serve as a potential treatment option for degenerated discs (Borrelli and Buckley, 2020). Nevertheless, it still needs to be investigated in a human setting or for GMP rules. However, very recently and for the first time, the creation of decellularised whole human IVD with attached bone was achieved (Hodgkinson *et al.*, 2020). Further investigations are required before this technology can be taken forward as an implantable regenerative solution for IVD replacement in clinical applications.

In conclusion, key components of the NP microenvironment from an IVD play an important role in ongoing and future approaches for NP tissue engineering. The use of components that are already present in the native microenvironment seem to have a high rate of success. However, the development of scaffolds based on natural materials such as silk (Park *et al.*, 2012), alginate (Du *et al.*, 2019), chitosan (Alinejad *et al.*, 2019; Yuan *et al.*, 2019) or gellan gum (Silva-Correia *et al.*, 2011; Tsaryk *et al.*, 2017) also show good results but are more different from native components. Interesting and positive effects demonstrated by those different strategies, to promote IVD and NP regeneration, rely on the concept of optimally mimicking the microenvironment requiring treatment, and remain to be further elucidated (Guilak *et al.*, 2009).

Although synthetic scaffolds can provide functional support for progenitor cell transplantation, their mechanical influence on IVD progenitor cells should not be neglected (Melrose, 2016). The elasticity of a scaffold can influence the differentiation of progenitor cells (Zhu *et al.*, 2016); for example, a stiff synthetic matrix can promote osteogenesis and inhibit chondrogenesis of NP progenitor cells (Navaro *et al.*, 2015). Therefore, biomaterials should be designed with appropriate elasticity and stiffness to facilitate progenitor cell function.

The clinical problem – still in between bench and bedside

LBP compromises a complex of symptoms with varying underlying pathologies, including micro- and macro-instability, disturbing of neurological structures, and degeneration of facet joints, inflammatory conditions, neoplasm, and deformity. A widely accepted concept considers the IVD itself as the generator of pain, which is referred to as discogenic pain. However, the underlying biochemical or physical patho-mechanisms of LBP related solely to the IVD remain mostly unclear. Moreover, many patients with radiological abnormalities of the IVD do not suffer any clinical symptoms (Boden *et al.*, 1990). Several *in vitro* studies found an upregulation of inflammatory markers such as TNF-α and IL-1β in patients with suspected discogenic LBP. Histological studies revealed the ingrowth of nerves and vessels into the IVD. This could be the beginning of a degenerative cascade (Mosley *et al.*, 2017), with pain

signals transmitted continuously into the central nervous system. Thus, patients perceive pain because of the IVD-degeneration progression.

Despite these observations from *in vitro* and preclinical studies, the clinical diagnosis of discogenic pain is difficult. A possible approach to the diagnostic scheme for patients with suspected discogenic LBP is provocative discography. After puncturing the AF with a thin needle, saline and fluoroscopic agents are injected, increasing intradiscal pressure. Subsequently, the provocation of typical pain confirms the diagnosis of discogenic LBP. Drawbacks of provocative discography include the absence of objective findings with the positive result relying purely on subjective perception and response of the individual patient. Moreover, several studies demonstrated an acceleration of IVD degeneration following discography, likely as a result of iatrogenic AF injury during the procedure (Carragee *et al.*, 2009; Cuellar *et al.*, 2016). Therefore, the use of provocative discography as a diagnostic test has diminished recently.

In cases where discogenic pain is suspected to be the underlying cause of LBP in a clinical routine, patient treatment focuses on the management of symptoms and not on addressing the underlying pathomechanisms. First-line therapy of patients with pain related to IVD degeneration consists of conservative treatment modalities, including physiotherapy and pain management with non-steroid anti-inflammatory drugs, opioids, or epidural steroid injections (Airaksinen *et al.*, 2006). If conservative management fails, surgical treatment is often performed. The most common surgical intervention of degenerative disc disease is IVD removal, replacement with implants, and fusion using bone grafting to permanently connect two or more vertebrae of the spine.

Based on laboratory and preclinical findings, IVD-maintaining surgical approaches have been increasingly studied recently. Attempts to address IVD regeneration invasively comprise a variety of techniques that specifically aim at modulating the degeneration cascade of the IVD (Fig. 4). Access to the IVD to apply these techniques is either achieved by intradiscal injection or minimally-invasive or open-surgical approaches. However, only a few methods have made their way into clinical practice. A brief overview of ongoing clinical trials of the most promising strategies will now be provided, focussing on the ECM, and concentrating upon cell-based therapies, growth factors, and biomaterials.

Cell-based therapies

The cell-based therapy approach (Table 3) utilises the transplantation of cells into the IVD by puncturing the AF. The injected cells release anti-inflammatory factors/secretome and produce ECM (Wangler *et al.*, 2019). Theoretically, this leads to IVD regeneration, with reduced pain and improved function, for the patient.

There are mainly 5 categories of cell sources used to treat IVD degeneration: (1) IVD-derived cells, (2) chondrocyte-like cells, (3) MSCs, (4) iPSCs, and (5) ES cells (Sakai and Schol, 2017). By far, most published studies and ongoing clinical trials focus on MSCs (Table 3). However, any clinical evidence for positive effects still remains unclear. What has already been shown is that most of these procedures are safe (Orozco *et al.*, 2011; Wei *et al.*, 2014). The most often used donor site of autologous MSCs is the iliac crest, meaning an additional invasive procedure for the patient. The harvested cells are then either directly injected or grown in the lab and subsequently reinjected into the IVD. A meta-analysis of 6 studies (using either MSC or chondrocytes) showed an improvement of pain in the ODI as an outcome parameter for function (Wu *et al.*, 2018).

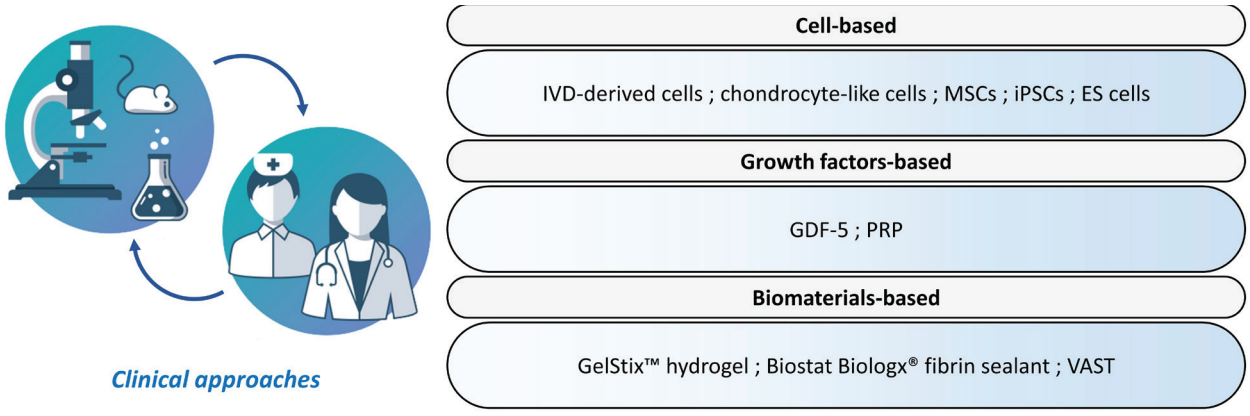

Fig. 4. Overview of the most recent applications used for clinical trials in the context of degenerative IVDs and LBP. Clinical therapies can be divided into 3 different strategies: (i) cell-based with the use of IVD-derived cells, chondrocyte-like cells, MSCs, iPSCs or ES cells, (ii) growth factors-based with the use of GDF-5 or PRP and to finish, (iii) biomaterials-based with the use of Gelstix™ hydrogel, Biostat Biologx® fibrin sealant and VAST.

Table 3. Recent clinical trials addressing cell-based materials for disc regeneration.

Cells	Study	Reference/ Web ref.	*n*	Inclusion criteria	Study design	Material	Outcome	Follow up	Conclusion/status	Clinical trial number
ASCs	Autologous adipose derived stem cell therapy for intervertebral disc	Han *et al.*, 2014 and Kumar *et al.*, 2017	10	19 and 70 years Pfirrmann's grade III–IV	Single centre, single-arm, phase I	Intradiscal injection combined hyaluronic acid derivative and AT-MSCs tissue fill	AS, ODI, Short Form-36 (SF-36), and imaging (lumbar spine X-ray imaging and MRI)	12 months	Combined implantation of AT-MSCs and HA derivative in chronic discogenic LBP is safe and tolerable. However, the efficacy of combined AT-MSCs and HA should be investigated in a randomised controlled trial in a larger population	NCT02338271
BMSCs	Efficacy of intradiscal injection of BM-MSC RESPINE	Web ref. 26	112	18 to 60 years Pfirrmann's score modified Griffith *et al.* (2007) grade 4 to 7	RCT, multicentre	Intradiscal injection, cell dose 20 ± 5 × 106 cells suspended in 2 mL of HypoThermosol isotonic transport solution	VAS pain, ODI	12 months	No results published yet Recruiting (until December 2021)	NCT03737461
	Treatment of DDD with allogenic mesenchymal stem cells (MSV)	Web ref. 4 and Noriega *et al.*, 2017	24	18 to 75 years Disc degeneration with loss of min 20 % disc height	Phase I-II trial	BMSCs expanded using the Valladolid IBGM procedure	VAS pain, ODI, SF-12	12 months	Allogeneic MSC therapy may be a valid alternative for the treatment of degenerative disc disease that is more logistically convenient than the autologous MSC treatment. The intervention is simple, does not require surgery, provides pain relief, and significantly improves disc quality	NCT01860417
	Bone marrow concentrate intradiscal injection for chronic discogenic LBP	Web ref. 15	60	18 to 55 years Chronic low back pain abnormal disc pathology in MRI or CT	RCT	Intradiscal injection of bone marrow concentrate	VAS pain, ODI	12 months	No results published yet. Recruiting (until January 2021)	NCT03340818
	Single intradiscal injection of BMAC	Web ref. 3	20	18 to 60 years lumbar disc height loss < 50 % (Modified Pfirrman grade ≤ 7) in MRI	Prospective study, single centre	Autologous BMAC is a cellular rich fraction of bone marrow aspirate	VAS pain, ODI, Cell viability testing, CFU-f assay	12 months	No results published yet Recruiting (until September 2021)	NCT03912454
	IVD repair by autologous mesenchymal bone marrow cells	Web ref. 15 and Orozco *et al.*, 2011	10	18 to 60 years lumbar disc height loss < 50 % (Modified Pfirrman grade ≤ 7) in MRI	Phase I trial	Autologous expanded bone marrow MSC injected into the NP	VAS pain, ODI, SF-36, MRI	12 months	MSC therapy may be a valid alternative treatment for chronic back pain caused by DDD. Advantages over current gold standards include simpler and more conservative intervention without surgery, preservation of normal biomechanics, and same or better pain relief	NCT03340818 EudraCT 2008-001191-68
MPCs	MPCs	Web ref. 11 and Amirdelfan *et al.*, 2021	100	18 years and older L1-S1 Disc degeneration confirmed by MRI	RCT, multicentre	Immuno-selected, culture-expanded, nucleated, allogeneic adult MPCs when combined with hyaluronic acid	VAS pain, ODI, WPAI, MRI	36 months	Evidence that intra-discal injection of MPCs could be a safe, effective, durable and minimally invasive therapy for subjects who have chronic LBP associated with moderate DDD	NCT01290367
	Rexlemestrocel-L alone or combined with hyaluronic acid in subjects with chronic LBP	Web ref. 12	404	18 years and older L1 to S1 modified Pfirrmann score 3 to 6 (MRI)	RCT, multicentre	Mesoblast's rexlemestrocel-L alone or combined with hyaluronic acid/AMPCs	VAS pain, ODI	24 months	Active, not recruiting	NCT02412735
Discogenic cells	Safety and preliminary efficacy of IDCT, a treatment for symptomatic LDD	Web ref. 8 and Silverman *et al.*, 2020	38	18 to 75 years L3 to S1 symptomatic disc degeneration	RCT, multicentre	Injection of IDCT discogenic cells + sodium hyaluronate vehicle	Adverse events, VAS pain, ODI, JOABPEQ	2 months	Preliminary Conclusion: That intradiscal injection of discogenic cells may be a viable treatment for human DDD. The cells produce ECM that may rebuild the depleting tissue within degenerating discs. The cells do not pose any significant safety concerns	NCT03955315
		Web ref. 7 and Silverman *et al.*, 2020	60				Adverse events, VAS pain, ODI,	24 months		NCT03347708
Autologous stem cells	A single dose of BRTX 100 for patients with chronic LDD	Web ref. 23	99	18 to 60 years Modified Pfirrmann score of 2 to 6 on MRI	RCT	Autologous hypoxic cultured MSCs collected from the patient's bone marrow and co-administered with an autologous biomaterial carrier (human platelet lysate)	VAS pain, ODI	12 months	Not yet recruiting. Estimated study completion August 2023	NCT04042844

A meta-regression analysis identified a significant preference for MSC over chondrogenic cells (Schol and Sakai, 2019; Wu *et al.*, 2018). Because of the need for high-quality clinical data, the RESPINE-project was funded by the European Union's funding programme for research and innovation, Horizon 2020. The project started in 2017 and aimed at verifying the efficacy of an allogeneic intervertebral MSC-based therapy by providing a high-level clinical trial, including 112 patients with pain related to disc degeneration. No results have yet been published.

Growth factors

Injection of growth factors could support homeostasis of IVD cells and, therefore, enhance disc regeneration. In addition to many *in vitro* and animal studies (Kennon *et al.*, 2018) there are only a few ongoing clinical trials (Table 4). A multicentre, randomised, blinded, and placebo-controlled trial, provided by DePuy Synthes, investigated the effect of rhGDF-5. The results, posted on clinicaltrails.org (Web ref. 17), showed a higher dosage of rhGDF-5 (2.0 mg/disc) with an improvement in pain and quality of life measurements but it also had a higher risk profile with more (serious) adverse events (Kennon *et al.*, 2018).

As a combination of different growth factors, PRP that has been already studied extensively for cartilage regeneration, has also been studied for LBP. Even though it seems to be a safe procedure (Levi *et al.*, 2016; Tuakli-Wosornu *et al.*, 2016), results from a prospective, non-randomised trial revealed a relatively low-success rate (pain and ODI) under 50 % (Levi *et al.*, 2016). Moreover, a prospective, randomised-controlled study on intradiscal PRP injection showed an improvement of pain and function after a one-year follow-up (Tuakli-Wosornu *et al.*, 2016). Nevertheless, adequately designed randomised controlled trials are still needed to clarify the safety as well as long-term benefits of intradiscal PRP injection (Buser *et al.*, 2019).

Drugs

With the various mechanisms associated with IVD degeneration in mind, some current research and clinical applications have focused on different approaches for pain treatment and/or reversing disc degeneration. One aspect is the application of drugs. In addition to analgesics, which with their low molecular weight are often used as a first resort to relieve LBP associated with IVD degeneration, various novel treatments are also being evaluated in preclinical models (Cherif *et al.*, 2020; Cunha *et al.*, 2020; Hu *et al.*, 2019; Wang *et al.*, 2020a). Among these, inflammatory cytokine and enzyme inhibitors are frequently used as therapeutic agents, but so far these agents have shown no or only limited effectiveness and an increased risk of adverse events (Tryfonidou *et al.*, 2020). However, several clinical trials are ongoing targeting different critical points of the diseased microenvironment (Table 5).

Biomaterials

One major problem that must be addressed for regenerative strategies of the IVD is the loss of the NP water content. The dehydration of the IVD tissue is an early sign of disc degeneration, seen as a so-called "black disc", with low water content and, therefore, a dark instead of a light NP, in MRI images (Pfirrmann *et al.*, 2001). Consequently, hydrophilic materials such as hydrogels that might compensate for the decreased water content have been extensively studied both in research (Buckley *et al.*, 2018) and clinical trials (Table 6).

The origin of the hydrogels can either be natural or synthetic. Natural hydrogels (including alginate, collagen or fibrin-clot) can serve as a cell-carriers (*e.g.* for MSCs) by providing a 3D cytocompatible environment. However, their inferior mechanical properties are a significant disadvantage (Frauchiger *et al.*, 2017; Peroglio *et al.*, 2013). Synthetic hydrogels, on the other hand, have better mechanical properties. Such gels are, for example, the thermo-responsive hydrogel pNIPAM, PEG or the very promising group of silk scaffolds. Frauchiger *et al.* (2017) reviewed the different hydrogels in detail. For clinical use, such a formulation needs to be injectable and has to be applied intradiscally by a minimally invasive approach. However, there are only a few studies on such injectable materials for humans (*e.g.* GelStix™, an intradiscal admitted hydrogel) (Ceylan *et al.*, 2019). A randomised, placebo-controlled, multicentre study indicated a significant reduction of pain, function, and high patient satisfaction (Ceylan *et al.*, 2019). Also, internal disruption of the disc can be found in degenerated IVDs. Therefore, a clinical study investigated the intradiscal injection of fibrin sealant (Biostat Biologx®) (Yin *et al.*, 2014). A prospective placebo-controlled pilot study with a two-year follow-up demonstrated a safe application and improved pain and function (Yin *et al.*, 2014). The VAST clinical trial aimed to safely supplement tissue loss to degenerate IVDs. By injection of a supplemental viable disc matrix, improved pain, and function at one-year was achieved (Beall *et al.*, 2020).

Despite all these increasing efforts to investigate degenerative processes in the IVD and straight-forward clinical trials, translation into everyday clinical practice with a direct impact on patient management is sparse. Moreover, care must be taken to measure the outcome parameters. Most of the studies mentioned above did not include any radiographic, especially MRI imaging, in their outcome measures. Therefore, it is not possible to assume the influence on the IVD microenvironment and especially restoration of the ECM.

Table 4. Recent clinical trials addressing growth-factors for disc regeneration.

Components	Study	Reference/ Web ref.	n	Inclusion critera	Study design	Material	Outcome	Follow up	Conclusion/status	Clinical trial number
PRP	PRP for LBP	Web ref. 13	30	20 to 60 years LDD confirmed by MRI	Single centre	Intradiscal injection of PRP	VAS pain, ODI,	4 months	No results published yet. Completed (Mar 2019)	NCT03197415
	Intra-discal Injection of PRP for LBP (MODI-PRP)	Web ref. 25	126	18 to 60 years lumbar (stages < 5 of Pfir-rmann's score) in MRI	RCT, single centre	Injection of 2 mL of autologous PRP into the median portion of the suspected disc under radiographic guidance.	ODI, RMQ, PASS, VAS pain, EQ5D, SF-36	3-12 months	No results published yet Recruiting (until Sep 2022)	NCT03712527
	Lumbar intradiscal PRP injections: A prospective, double-blind, randomised controlled study	Tuakli-Wosornu *et al.*, 2016	47	Grade 3 or 4 annular fissure as determined by discography. Concordant pain on discography	RCT	Single injections of autologous PRP into symptomatic degen-erative IVDs	FRI, NRS for pain, SF-36, modified NASS Outcome Questionnaire	2 months	Intradiscal PRP showed significant improvements in FRI, NRS Best Pain, and NASS patient satisfaction scores over 8 weeks compared with controls. Those who received PRP maintained significant improvements in FRI scores	N/A
	Intradiscal PRP injection for chronic discogenic LBP	Levi *et al.*, 2016	22	18 years and older positive discog-raphy	Prospective Trial	Injection of 1.5 mL of autologous PRP injected into the disc	VAS pain, ODI	6 months	Preliminary 6 month findings, using strict categorical success criteria, for intradiscal PRP as a treatment for presumed discogenic LBP	Sterling IRB, Trial No. 4143-001S
rhGDF-5	Intradiscal rhGDF-5 phase I/II clinical trial	Web ref. 16	32	18 years and older L3/L4 to L5/S1 discogenic pain confirmed by discography	Phase I/IIa, multicentre	Intradiscal injection of API (rhGDF-5), 0.25 mg and 1 mg	Neurological as-sessment, adverse events, ODI, VAS pain, SF-36,	12 months	Completed 2016, no peer-reviewed results available	NCT00813813
	Intradiscal rhGDF-5 for the treatment of early-stage LDD	Web ref. 19	40			Intradiscal injection of API (rhGDF-5), 1.0 mg or 2.0 mg				NCT01158924
	Intradiscal rhGDF-5 (single administration) for the treat-ment of early-stage LDD	Web ref. 17	24		RCT, multi-centre	Intradiscal injection of API (rhGDF-5), 1.0 mg or 2.0 mg				NCT01124006
	Intradiscal rhGDF-5 for the treatment of early-stage LDD	Web ref. 18	31			Intradiscal injection of API (rhGDF-5), 1.0 mg				NCT01182337

Table 5. Recent clinical trials addressing different drugs for disc regeneration.

Study	Reference/ Web ref.	n	Inclusion critera	Study design	Material	Outcome	Follow up	Conclusion/status	Clinical trial number
AMG0103 in subjects with chronic discogenic lumbar back pain	Web ref. 2	25	18 and 75 years L1 and S1 Pfirrmann score of 3 or 4 on MRI	RCT, multicentre	Single intradiscal injection of AMG0103 (Decoy oligonucleotides against NF-κB)	Saftey, VAS pain, ODI, RMDQ, SF-36, imaging (x-ray, MRI)	6 months	Active, not yet recruiting	NCT03263611
Assessment of the efficacy of an intradiscal injection of corticoids in Modic I discopathies. (MODISC)	Web ref. 24	50	18 and 80 years LBP associated with Modic I discopathy (MRI)	RCT, single centre	Patients will receive an intradiscal injection of hydrocortancyl.	VAS pain, SF-36, DPQ	6 months	Completed– no results published	NCT01694134
Effect of abaloparatide on LDD	Web ref. 27	109	18 years and older Modified Pfirrmann score of 2-3 (MRI)	RCT	Injection of 80 µg abaloparatide (parathyroid hormone) subcutaneously once daily for 90 d	NRS pain, PROMIS-29 score, MRI	12 months	Not yet recruiting	NCT03708926
A single ascending dose study of safety and tolerability of STA363 compared to placebo in 15 patients with chronic discogenic LBP	Web ref. 22	15	20 to 60 years L3/4 to L5/S1 disc degeneration Pfirrmann grade II-III	RCT	Intradiscal injection of different doses of STA363 (S-lactic acid) in a volume of 1.5 mL	VAS pain, ODI, MRI	3 months	Completed – no results published	NCT03055845
Efficacy and safety of STA363 at 2 concentrations (60 mg/mL and 120 mg/mL) compared to placebo in patients with chronic discogenic LBP		126	18 years and older disc degeneration Pfirrmann grade 2 to 3 on MRI at L2/3 to L5/S1	RCT, multicentre	Single intradiscal injection of STA363 into 1 or 2 IVDs	NRS pain, ODI, EQ5D, MRI	6 months	Ongoing	EudraCT 2019-004943-54 Web ref. 29
Safety, tolerability and efficacy of YH14618 following single intradiscal injection in patients	Web ref. 5	48	20 years and older L1-S1 disc degeneration degree 2~3 modified Thompson classification (MRI)	RCT	YH 14618 is a synthetic peptide acting as a transforming growth factor β (TGFβ) receptor antagonist	safety and tolerability, VAS pain, ODI	3 months	Completed	NCT01526330
Clinical trial of YH14618 in patients with degenerative	Web ref. 6	326	19 Years and older L1/L2 - L5/S1 disc degernaration Pfirrmann grade 2 to 4	RCT		VAS pain, ODI, EQ5D	6 months	Completed – no results published yet	NCT02320019
A study of the safety, tolerability, and pharmacokinetics of SM04690 injectable suspension following single intradiscal injection in subjects with DDD	Web ref. 10	6	25 to 55 years L4/L5 or L5/S1 discogenic pain	Phase I, non-randomired	Single intradiscal injection of 0.03 mg, 0.07 mg or 0.15 mg SM04690 (Wnt pathway inhibitor) under fluoroscopic guidance	Safety and tolerability	6 months	Terminated (Study stopped due to business reasons)	NCT03246399
Intradiscal MB injection treatment for chronic discogenic LBP. A prospective clinical series followed by a randomised placebo-controlled clinical trial	Web ref. 10 and Kim *et al.*, 2012	65	18 and 65 years discography Todd Wetzel classification grade 2 and 3 and a provoked pain of at least NRS 5	Phase I, non-randomised	Intradiscal methylene blue injection (methylthionium chloride)	NRS pain, adverse events	12 months	The intradiscal MB injection is a short-term effective minimally invasive treatment indicated for discogenic back pain but it may lose its effectiveness long-term.	NCT03246399 EudraCT 2010-022025-15

Table 6. Recent clinical trials addressing biomaterials for disc regeneration.

Study	Reference/ Web ref.	n	Inclusion critera	Study design	Material	Outcome	Follow up	Conclusion/status	Clinical trial number
VAST viable allograft supplemented disc regeneration in the treatment of patients with LBB with or without disc herniation	Web ref. 28 and Beall *et al.*, 2020	220	18 to 60 years L1 to S1 modified Pfirrmann grade 3 to 6 on MRI	RCT, multicentre	VIA disc matrix prepared from human NP allograft that contains min 6×10^6 allogeneic viable cells	VAS pain, ODI	12 months	Allograft was well tolerated and demonstrated the greatest amount of symptom relief, provided the most improvement in ODI. Allograft augmentation offered a safe and effective percutaneous treatment to decrease pain and improve function	NCT03709901
PerQdisc nucleus replacement device	Web ref. 21	15	22 and 60 years L1 to S1 Darkened disc on MRI in T2	Safety study	Artificial disc replacement	VAS pain, ODI, MRI for Modic changes, herniation and revision surgery	3 months	No results published yet. Recruiting until Dec 31, 2021	NCT04004156
LOPAIN1 lumbar operatively inserted PerQdisc artificial implant following nuclectomy	Web ref. 20	34	21 and 60 years L1 to S1 Darkened disc on T2-MRI	Prospective study, open-label, multicentre		VAS pain, ODI	6 months	No results published yet. Recruiting until Oct 30, 2022	NCT04141098
Treatment of symptomatic lumbar IDD with the **Biostat®** system	Web ref. 14	220	18 years and older L1 to S1 symptomatic lumbar IDD (positive discography)	RCT, multicentre	Use of 4 mL resorbable biological disc augmentation (Biostat Biologx fibrin®)	VAS pain, Roland-Morris Disability Questionnaire score	26 weeks	Intradiscal injection of Biostat Biologx fibrin sealant may improve pain and function in selected patients with discogenic pain	NCT01011816
Clinical results of intradiscal hydrogel administration (**GelStix**) in LDD	Ceylan *et al.*, 2019	29	18 years and older discogenic pain due DDD black disc on T2-MRI	Single centre, no control	Modified filamentous version of polyacrylonitrile (enlarges in volume after implantation)	VAS Pain, ODI	12 months	GelStix treatment is useful for pain relief in patients with DDD from the first month of treatment	N/A
Comparative evaluation of clinical outcome of DDD treated with **GelStix**	Web ref. 1	72	18 years to 65 discogenic pain > 3 months (NRS ≥ 5)	RCT, placebo-controlled, multicentre		VAS Pain, RMQ score	6 months	No results published yet. Recruiting until Aug 2021	NCT02763956

Conclusions

Healthy IVDs and especially their NP tissue are physiologically hypoxic, acidic, have a high osmolarity, and are poor in nutrient supplementation. These characteristics make this a very specific microenvironment in the human body but also sufficiently inhospitable for future implanted cells, if needed. This complex microenvironment can encounter many modifications/adjustments of non-ECM-related and ECM-related factors that, as a result, affect NP cells – inducing function impairment. During disc degeneration, NP cells that have lost their ability to form functional and stable cell-cell and cell-ECM crosstalk may also lose their ability to produce specific ECM and ECM-related proteins. This contributes to the inability of the NP tissue to regenerate and self-repair and, therefore, the IVD itself. The NP cells are in perpetual interaction with the surrounding microenvironment and receive cues that help to regulate and maintain the NP phenotype through the behaviour of NP progenitor cells.

Perfect knowledge of all the components of this microenvironment and how they interact with NP progenitor cells, could ultimately lead to the development of more efficient strategies able to reverse or at least slow down IVD degeneration. Being able to mimic, thus study and understand NP cell-ECM crosstalk gave rise to many new tissue engineering strategies with different rates of success. Partially recreating the microenvironment with one of the main ECM components (*e.g.* collagen type II, fibronectin, laminin, elastin, or GAG) did not show useful results for IVD repair or regeneration, as compared to engineered-scaffolds that combine many of them into one biomaterial. However, the best current approach –with outstanding potential for intervertebral disc regeneration – seems to be decellularised NP tissue, able to highly preserve structures and significant biochemical components of the native tissue.

There has been a dramatic improvement in the understanding and "mastering" in the use of potential key factors of the NP microenvironment for regenerative medicine approaches in the previous decade. The implementation of already existing culture methods, with key factors such as hypoxic conditions or ECM related proteins, close the gap towards successful *in vivo* applications. All the components needed for the "reconstruction" as well as the regeneration of the NP tissue seem to be already present within the IVD microenvironment. Therefore, the microenvironment of the NP tissue can be rightly considered as its own fountain of youth in the treatment of degenerative disease and LBP.

Understanding which factors are present in the IVD microenvironment and how they govern the IVD behaviour has not only scientific merit but also a huge translational value. New strategies, with the ability to harness these components/factors, have started to emerge and could provide a valid therapeutic option for future clinical management to treat DDD and more generally LBP. Future studies to further clarify the characteristics and functions of the IVD microenvironment in more detail and to mimic its function will facilitate IVD tissue engineering and the discovery of novel approaches for IVD regenerative medicine.

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Authors' contributions

J. Guerrero: study conception and design and drafted the manuscript.

S. Häckel drafted the manuscript.

A.S. Croft drafted and edited the manuscript.

S. Hoppe drafted the manuscript.

C.E. Albers drafted the manuscript and acquired funding.

B. Gantenbein drafted and edited the manuscript and acquired funding.

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Discussion with Reviewer

Jivko Stoyanov: In your review article you well describe the possible cell sources that can be used for the repair of degenerative disc disease (providing several references to published studies and clinical trials). If you had to choose one therapeutic cell type, which one would you select and why?
Authors: Various cell types are described in this review and have been used for IVD regeneration including IVD-derived cells, chondrocytes-like cells, mesenchymal stromal cells (MSCs), iPSCs, or ES cells. However, MSCs isolated from bone marrow or from adipose tissue have immunomodulatory functions, are more easily accessible for sampling, and can differentiate into cartilage. Therefore, they should be considered, on my point of view, as a potentially ideal cell source for IVD regeneration.

Jivko Stoyanov: Would you point the top three factors of the NP microenvironment on which regenerative medicine approach should be concentrated in the next decade?)
Authors: From our point of view and according to the large literature cited in this review, the top three factors/components that must be taken from the NP microenvironment for regenerative purposes are the following ones. First, the use of hypoxic conditions to pre-accommodate (prime) the cells used in the approach, secondly a biomaterial able to mimic mechanical and physiological aspects of the NP, ideally decellularised ECM, and thirdly the possible addition of the most promising growth factor, GDF-5.

Editor's note: The Guest Editor responsible for this paper was Zhen Li.