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REVIEW ARTICLE

The effects of dynamic loading on the intervertebral disc

Samantha C. W. Chan · Stephen J. Ferguson · Benjamin Gantenbein-Ritter

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Abstract Loading is important to maintain the balance of matrix turnover in the intervertebral disc (IVD). Daily cyclic diurnal assists in the transport of large soluble factors across the IVD and its surrounding circulation and applies direct and indirect stimulus to disc cells. Acute mechanical injury and accumulated overloading, however, could induce disc degeneration. Recently, there is more information available on how cyclic loading, especially axial compression and hydrostatic pressure, affects IVD cell biology. This review summarises recent studies on the response of the IVD and stem cells to applied cyclic compression and hydrostatic pressure. These studies investigate the possible role of loading in the initiation and progression of disc degeneration as well as quantifying a physiological loading condition for the study of disc degeneration biological therapy. Subsequently, a possible physiological/beneficial loading range is proposed. This physiological/beneficial loading could provide insight into how to design loading regimes in specific system for the testing of various biological therapies such as cell therapy, chemical therapy or tissue engineering constructs to achieve a better final outcome. In addition, the parameter space of 'physiological' loading may also be an important factor for the differentiation of stem cells towards most ideally 'discogenic' cells for tissue engineering purpose.

Keywords Intervertebral disc · Disc generation · Dynamic loading · Axial compression · Hydrostatic pressure · Mechanobiology

S. C. W. Chan · S. J. Ferguson · B. Gantenbein-Ritter (✉)
ARTORG Center for Biomedical Engineering, Spine Research Center, Institute for Surgical Technology and Biomechanics, University of Bern, Stauffacherstrasse 78, 3014 Bern, Switzerland
e-mail: benjamin.gantenbein@artorg.unibe.ch

Introduction

The intervertebral disc (IVD) is a cushion-like structure that transmits loads and provides flexibility to the spine [44]. Degeneration of the disc is often associated with ageing, which is characterised by a loss of disc height, a decrease in proteoglycans and water content [2]. Genetic, nutritional, mechanical, environment and behavioural factors (e.g. nicotine [6]) influence this degenerative process [34]. The mechanical load acting on the IVD is particularly important for maintaining a healthy IVD. The normal day-to-day mechanical loading generates biophysical signals to the cells, which regulate the physiologic functions of the disc such that the matrix is continuously maintained and remodelled according to these external mechanical stimuli.

In recent years, our understanding on the influence of loading in disc degeneration and regeneration has been significantly increased. There is evidence that a spinal loading, which comprises the diurnal variation in IVD pressure from a low magnitude (around 0.2 MPa) to a high magnitude (around 0.6 MPa) dynamic loading at a physiological frequency (0.2–1 Hz), is essential to maintain the health and function of the non-pathological IVD. Dynamic loading of a certain magnitude, frequency and duration has been shown to maintain the matrix balance within the disc [70, 71, 122, 127] and even provide a synergistic effect on the differentiation of stem cells towards the chondrocytic lineage under the influence of growth factors [14, 21, 40, 41, 48, 54, 66, 83, 90, 112, 113]. Conversely, prolonged exposure to hypo- or hyper-physiological loading can be harmful to the disc. In an unloaded condition, the IVD swells and starts to lose proteoglycans [118]; static loading causes cell death and induces disc degeneration [4, 67, 68, 89, 110]; a hyper-physiological magnitude and frequency of dynamic loading induces signs of disc degeneration

[45, 56, 70, 71, 122, 127]. A comprehensive review on the effect of hydrostatic pressure, osmotic pressure, tensile loading and static compression on the IVD has been reported by Setton and Chen [106]. This review focuses on recent studies of dynamic compressive loading and hydrostatic pressure on the IVD and its implications for IVD tissue engineering using stem cells.

IVD and compression

The IVD is composed of three specialised structures. The nucleus pulposus (NP) is the central part of the IVD and is rich in poly-anionic proteoglycans. The high fixed negative charges of the proteoglycans impart a high osmotic pressure on the NP [114]. The high water content of the NP contributes to the absorption of spinal loads and transmission to the surrounding tissue. The annulus fibrosus (AF) consists of elastic collagen fibres, which encompass the NP and are anchored within to the upper and the lower endplates (EP). The EP form a porous, semi-permeable barrier between the vertebrae and disc and regulate the transport of nutrients into and out of the disc centre.

The IVD is the largest avascular structure of the body; it exchanges nutrients, and metabolic by-products with the surrounding vasculature mainly through diffusion and convection [114, 117]. Changes in interstitial pressure, balanced against osmotic swelling pressure, provide a pumping action for the convective transport of larger solutes such as growth factors, cytokines and enzymes [24, 51, 117, 119, 130].

The IVD experiences a diurnal change in intradiscal pressure according to the variation in day and night activity. High loads are generated by up-right postures and load-bearing activities; lower loads are applied in the supine position. Under high loading, the IVD deforms, the hydrostatic pressure inside the disc increases and fluid is squeezed out of the disc slowly. The loss of fluid increases the disc proteoglycans concentration and the fixed charge density and results in a higher osmolality and a lower pH [114]. In the supine position, pressure is released and hence fluid is drawn back into the disc [1, 60, 73, 76]. Around 20–25% of the disc's water content is expressed and re-imbibed during -each diurnal cycle [108].

Other than the diurnal fluctuation in load, various daily activities exert different magnitudes and frequencies of load on the spine. Short running duration of 1 h has caused greater decrease in disc height (greater loss of fluid) compared with 7.5 h of static activity in humans [125]. Diurnal cyclic compression enhanced transport of a 3 kDa dextran solute into the disc through the endplates in an ovine disc culture model [28]. Dynamic compression (0.1 Hz, 10% strain, 200 cycles) increased oxygen concentration and decreased lactate concentration in the disc than the unloaded samples in a study using a finite element model [39]. These results imply that dynamic loading may improve the transport of solutes through the disc and hence affecting the metabolism of the disc.

Mechanobiology of the IVD

Loading affects the IVD cell metabolism and fate directly and indirectly. First, the direct mechanical stimulation applied to the matrix results in cell and nucleus deformation, cell volume change, cell membrane stretch, cytoskeletal strain and altered polarisation [107]. Second, the changes in the physical environment around the cells caused by the loading, including fluid content, concentration of proteoglycans, fixed charge density, osmolarity and pH, lead to alterations in the concentration of nutrients, bioactive factors, and metabolic waste products within the disc.

Direct stimulus

Although cellular mechanobiological response is generally cell-type-dependent, studies on the relation between loading and chondrocyte deformation may offer reference information on the IVD cells' response. Studies on cartilage explants or isolated chondrocytes embedded in agarose or alginate showed that chondrocytes deform under compression, resulting in cell, organelles and nucleus flattening [33, 55, 62, 111]. Cell membrane stretch due to cell flattening activates ion channels and induces alterations in membrane potential and hence affects the cell metabolism [11, 81]. The stretching of rabbit AF cells (5% elongation or 15% area change) has been shown to increase nitric oxide production, to decrease proteoglycan production and to increase apoptosis of AF cells [8, 96, 97]. However, a 2% cyclic strain applied on human AF cells was shown to increase aggrecan and decrease matrix metalloproteinase (MMP) gene expression [85]. Inside the cytosol, mechano-transduction is mediated through cytoskeleton components such as vimentin intermediate filaments, actin microfilaments and microtubules to the nucleus [47]. Such modifications in the cytoskeleton are believed to be involved in the activation of ion channels and intracellular calcium signalling [47]. Compression might also reduce the cell volume, due to cellular confinement and the increase in extracellular osmolality [26].

Indirect stimulus

A mechanism of how loading alters the physical environment of the disc, and its effect on the IVD cells'

biosynthetic activity, has been explained in detail by Urban [114]. She concluded that if the disc deforms under loading, the hydrostatic pressure increases inside the disc. The high pressure forces fluid to flow out of the disc. This results in an elevated proteoglycan concentration, fixed charge density and osmotic pressure in the disc, and an increased osmolality and decreased pH of the disc. On the other hand, the increased intercellular osmolality draws water out of the cells and the cell volume is reduced. Therefore, loading alters the physical environment of the disc matrix, which leads to changes in the water and chemical composition of the cell and its volume. Cell volume changes could affect various membrane ion transporters and alter cell metabolism, resulting in a different biosynthetic activity of the cell [35, 116]. Furthermore, studies have shown that osmolality and pH directly affect IVD cell metabolism [12, 18, 27, 37, 115, 129].

Static, diurnal and dynamic compression

To test the biological response of the IVD to different loading regimes, various in vivo and in vitro models have been developed. Static loading, static diurnal loading or a continuous dynamic loading results in different responses of the IVD [19, 57, 58, 72, 75, 123]. Studies comparing the different axial loadings (static, diurnal or dynamic compression) are summarised in Table 1.

In vivo studies

Running exercise studies on animals compared the effect of dynamic impact loading with static physiological loading. Running has been found to be beneficial to disc cells with increased matrix production in a rat model [13] and a dog model [104]. However, these in vivo results may also be attributed to other unknown physical factors, such as the increased production of cytokines by exercise, or a systemic increase in circulatory capacity, but not just to cyclic loading.

In vivo studies using a model with an external loading device on a rat tail allowed the evaluation of the loading itself without other physical factors. It was found that immobilisation provoked a degeneration pattern in the IVD, such as a down-regulation of anabolic gene expression [72] and a decrease in GAG content [19], whereas dynamic loading resulted in an up-regulation of anabolic gene expression [19]. However, one study suggested that a threshold exists for detrimental loading levels, whether static or dynamic. In this study, both a 72-h static loading and a 2-h dynamic loading, inducing an intradiscal pressure of 1 MPa, lead to a down-regulation of anabolic gene expression and up-regulation of catabolic gene expression [72]. It is apparent that the magnitude, frequency and duration of the loading are crucial factors that influence disc health.

In vitro organ culture

Organ culture studies offer a simulated physiological environment for testing the influence of specific factors in a controlled fashion, while preserving the disc cells in their native matrix. In a bovine disc organ culture model, with the endplates removed, it has been demonstrated that diurnal loading was better to maintain GAG content than static loading but little beneficial effect was found with dynamic loading in a human physiological range (0.2–1 MPa disc pressure, 1 Hz) [57, 58]. It is likely that the removal of endplates from the IVD affected the cell viability and the biosynthetic activity of the disc cells [61]. In another study, using a rabbit disc explant model with endplates, anabolic gene expression in the NP of the dynamic loading group was found to be higher than in the static loading group [123]. In general, a dynamic loading pattern is more likely to promote an anabolic response in an IVD organ culture model than static loading. It is possible that a well-designed diurnal dynamic loading protocol may benefit the synthetic activity and anabolic response of the disc, even in the in vitro organ culture condition.

In vitro cell culture study with hydrostatic pressure

The IVD experiences varying magnitudes of hydrostatic pressure during the diurnal cycle. The influence of static hydrostatic pressure has been studied on isolated human and animal disc cells [42, 43, 65] (Table 3). The application of a constant hydrostatic pressure has been shown to affect disc cell metabolism in a magnitude- and duration-dependent manner. A constant hydrostatic pressure of 0.3–1 MPa increased matrix synthesis in bovine caudal disc tissue, dog IVD cells in alginate and even human pathological disc tissue but hydrostatic pressure higher than 3 MPa was found to induce a catabolic response of the disc cells [36, 42, 43, 46, 65].

In summary, static compression loading of >1 MPa could induce degeneration pattern of the disc, whereas dynamic loading in the form of direct compression or hydrostatic pressure could be beneficial and the response of disc cells to dynamic loading is magnitude, frequency and duration dependent.

Factors affecting IVD responses to loading

Dynamic loading could be beneficial to the biosynthesis of disc cells, but this is magnitude, frequency and duration dependent. It has been shown in various in vivo and in vitro

Table 1 Comparison between static loading and diurnal or dynamic loading

Objective	Species/model	Load magnitude/ frequency/duration	Findings
Static vs. dynamic compression [72]	In vivo rat tail model	Static: 72 h immobilization Cyclic: 1 MPa; 0.2 Hz; 2 h	**AF** Immobilization or dynamic loading: ↓ *COL1A1*, *COL2A1*; ↑ *MMP-13*, *MMP-3* gene expression **NP** Static: ↓ *COL1A1* Static or dynamic: ↑ *ADAMTS-4* Static followed by dynamic: up-regulated *MMP-3*, *MMP-13* gene expression
Repeated static vs. dynamic compression [19]	In vivo rat tail model	Static: 0.69 MPa Cyclic: 0.44–0.94 MPa; 0.5, 1.5, 2.5 Hz; 1 h × 14 days	↓ Proteoglycan in static group but not in dynamic loading group ↑ Proteoglycan content at 0.5 Hz within all dynamic compression group
Static vs. dynamic compression [123]	Rabbit lumbar IVD with endplates organ culture	Static: 0.5 or 1 MPa; 6 h Cyclic: 0.5, 1 MPa; 0.1 or 1 Hz; 6 h	Most significant cell death at 1 MPa static loading with disorganized AF lamellar, disassociated NP cells **NP** ↑ *COL1A1* and *COL2A1* gene expression in dynamic loading
Constant vs. diurnal static compression [57]	Bovine (1.5- to 2-year-old) caudal IVD without endplates organ culture	Constant static: 0.2 MPa Diurnal static: 0.1 MPa, 0.3 MPa in 12 h shift; 4 or 8 days	GAG content: diurnal > static Cell viability: static > diurnal
Static vs. cyclic compression [75]	In vivo rat tail model	Static: 0.575 or 1 MPa; 9, 90, 900 min Cyclic: 0.15–1 MPa; 1 Hz; 900 min	Disc height loss: cyclic > static Water loss in AF: cyclic > static
Repeated static and dynamic compression at various magnitude [58]	Bovine (1.5- to 2-year-old) caudal IVD without endplates organ culture	Static: 0.2 MPa; 1 h × 5 days *Cyclic* LOW: 0.2–1 MPa, HIGH: 0.2–2.5 MPa; 1 Hz; 1 h × 5 days	**AF** ↓ *COL1A1* gene expression in LOW **NP** ↑ *COL1A1* and *MMP-13* gene expression in LOW

COL1A1 collagen 1, *COL2A1* collagen 2, *ACAN* aggrecan, *ADAMTS-4* a disintegrin and metalloproteinase with thrombospondin motifs-4, *MMP* matrix metalloproteinase, *GAG* glycosaminoglycan

models that a low magnitude of dynamic compression [70, 122] and static/dynamic hydrostatic pressure [31, 36, 43, 46, 50, 65, 86, 98, 124] at a physiological frequency could increase anabolic response, but a high magnitude of dynamic loading causes early signs of disc damage and catabolic remodelling (Tables 2, 3).

Magnitude response: axial compression

The effect of the magnitude of dynamic compression was compared [58] in an in vitro culture system using bovine discs cultured without endplates. It was suggested that dynamic compression of 0.2–1 MPa, for 1 h/day for 5 days, increases the disc's metabolic rate and enhances anabolic remodelling, whereas compression of 0.2–2.5 MPa induces catabolic response in the disc. Another study using an in vivo rat tail model showed a higher amount of apoptotic cells in the NP when the disc was subjected to dynamic compression of greater than 1.3 MPa [122], whereas 0.9 MPa [122] or 1 MPa [70] dynamic compression was favourable for anabolic responses.

Response to amplitude modulation: hydrostatic pressure

Similar to the findings on axial compression, applying hydrostatic pressure of greater than 2.5 MPa magnitude resulted in a degenerative response of the IVD cells. Decreased anabolic gene expression and increased catabolic gene expression were found on isolated porcine NP

Table 2 List of studies comparing influences of magnitude, frequency and duration of the dynamic compression acting on the IVD

Objective	Species/model	Loading magnitude/ frequency/duration	Findings
One time loading at different magnitude and frequency [70]	In vivo rat tail model	Cyclic: 1 or 0.2 MPa; 1, 0.2, 0.01 Hz; 2 h	Largest ↓ in disc height at 1 Hz **AF** ↑ Catabolic genes at all frequency of 0.2 MPa More sensitive to magnitude of load than frequency Suggested at 0.2 MPa and 0.2 Hz do not alter disc metabolism **NP** ↑ Anabolic gene expression at 1 MPa, 0.01 Hz More sensitive to frequency of loading
Magnitude and frequency response of repeated compression [122]	In vivo rat tail model	0.9, 1.3 MPa; 0.1, 0.01 Hz; 6 h × 7 days	Higher proteoglycan content in all group except at 0.9 MPa, 0.1 Hz **AF** Increased apoptotic cells at low frequency, low stress Higher *COL2A1* expression at 1.3 MPa **NP** Higher apoptotic cells at 1.3 MPa, little effect on frequency
Duration response of a single loading event [71]	In vivo rat tail model	1 MPa; 1 Hz; 0.5, 2, 4 h	**AF** Larger up-regulation in catabolic gene at 4 h loading than 0.5 or 2 h 2 h or 4 h loading up-regulated both anabolic and catabolic gene **NP** ↑ Anabolic gene at 0.5 or 2 h Largest ↑ catabolic genes at 2 h
Duration response of compression [127]	In vivo rat tail model	1 MPa; 1 Hz; 1.5 h × 14 days or 8 h × 14 days or 8 h × 56 days	↓ Disc height, ↑ in water content, increase GAG in NP, ↓ GAG in AF **AF** ↑ Anabolic gene and ↓ in catabolic genes at 1.5 h × 14 days **NP** Up-regulation of anabolic gene at 8 h × 14 days
Age of the samples and their response to frequency of the compression [56]	Bovine young (4–6 m), mature (18–24 m); NP, AF cells in alginate	Axial compression; 2–12%; 0.1, 1, 3 Hz; 2 h × 7 days	*Age response* **AF** Lower GAG/DNA, *COL1A1* and *ACAN* gene expression in young < mature Higher *MMP-3* expression young > mature **NP** Lower GAG/DNA in mature animal Lower *COL1A1*, *COL2A1* gene expression in young animal *Frequency response* **AF** Higher *COL1A1*, *MMP-3* at high frequency > static control Lower *COL2A1*, *ACAN* at higher frequency > static control **NP** Higher *MMP-3* at 0.1 and 1 Hz > static control

Table 2 continued

Objective	Species/model	Loading magnitude/ frequency/duration	Findings
Repetitive dynamic compression at various frequency vs. glucose supply [45]	Ovine (2–5 years) caudal IVD with endplates organ culture	0.6 MPa ± 0.2 × 16 h (0.2 or 10 Hz) + 0.2 MPa × 8 h Glucose level: 2 or 4.5 g/L	↑ Cell death at high frequency, high glucose, and low glucose, low frequency conditions Exacerbated cell death and up-regulation in *MMP-13* gene expression in high frequency, low glucose condition

COL1A1 collagen 1, *COL2A1* collagen 2, *ACAN* aggrecan, *ADAMTS-4* a disintegrin and metalloproteinase with thrombospondin motifs-4, *MMP* matrix metalloproteinase, *GAG* glycosaminoglycan

Table 3 Effect of Intermittent hydrostatic pressure (IHP) on intervertebral disc cells

Objectives	Species/model	Pressure magnitude/frequency/ duration	Findings
Static HP at magnitude and duration response [46]	Bovine caudal disc tissue and pathological human disc tissue	Magnitude: 1, 2.5, 7.5, 10 MPa Time: 20 s, 2 h	20 s: ↑ Matrix synthesis 1 MPa in NP; 1, 2.5, 7.5 MPa in IA ↓ At 10 MPa in NP, IA 2 h: ↑ Matrix synthesis 5 MPa in IA ↓ At 10 MPa in NP, IA
Static HP at various magnitude [36]	Human pathological disc tissue	Magnitude: 0.1, 0.3, 3 MPa; 2 h	GAG synthesis: ↑ 0.1, 0.3 MPa in NP and IA ↓ At 3 MPa in IA *MMP-3* and *TIMP-1* production: ↑ At 0.3 MPa in IA ↑ At 3 MPa in NP
Static HP at various magnitude [42, 43]	Expanded hound dogs, NP and AF cells in alginate beads	0.1, 0.35, 1 MPa; 9 days	1 MPa: ↑ Collagen and PG synthesis in NP but vice versa in AF ↑ *COL1A1*, *COL2A1* and *ACAN* gene expression in NP and AF 0.35 MPa: ↓ Proteoglycan synthesis in NP and AF ↑ Collagen synthesis in NP but vice versa in AF Gene expression: AF: ↓ *COL1A1*, *COL2A1* and *ACAN* NP: ↑ *COL1A1* and *ACAN*, ↓ *COL2A1*
Magnitude response and NO inhibitor [65]	Human lumbar (herniated)disc tissue	0.1, 0.3, 3 MPa	0.3 MPa: ↑ PG content, decrease NO production 3 MPa: ↓ PG production, ↑ NO production
Magnitude and frequency response of IHP [49]	Rabbit AFC in monolayer or NPC in alginate beads	AFC monolayer: 0.3 MPa, 1 Hz, 1.7–2.5 MPa, 20 Hz NPC 3D: 0.75, 1.5, 3 MPa; *f*: 1, 10, 20 Hz; 30 min/day, 9 days	AFC in monolayer: High magnitude and frequency stimulate collagen synthesis NPC in 3D: High magnitude and frequency ↑ ratio of collagen synthesis to collagen degradation
Magnitude and frequency response of cells to IHP [31]	Porcine NPC in agarose	0.4, 3.4, 6.0 MPa; *f*: 3/17 min; Low: 10/10 s; High: 12 h/ day; 7 days	Low frequency: ↑ GAG and OH-proline at 0.4, 3.4 MPa ↓ GAG and OH-proline content at 6 MPa High frequency: 3.4 MPa ↑ OH-proline but ↓GAG 6 MPa largest decrease in GAG and OH-proline

Table 3 continued

Objectives	Species/model	Pressure magnitude/frequency/duration	Findings
Magnitude response to dynamic IHP [124]	Porcine AFC in alginate disc	1, 3 MPa; 0.5 Hz; 3 h	1 MPa: ↑ *COL1A1*, *COL2A1*, and *ACAN* gene expression 3 MPa: ↓ *COL1A1*, *MMP-1* and *TGFβ-1* gene expression
Cyclic strain and IHP [85]	Human pathological disc cells in collagen type I gel	Cyclic strain: 2%; 1 Hz; 24 h, 4 weeks IHP: 0.25 MPa; 0.1 Hz; 30 min/day, 4 weeks	IHP: ↑ *COL1A1* in AF ↓ *MMP-2,-3* in NP 2% cyclic strain: ↑ *ACAN* gene expression in AF ↓ *MMP-3* gene expression in AF
Frequency response of IHP [50]	Porcine IVD cells in alginate	1 MPa; 1, 3, 5, 8, 10 Hz; 30 min/day, 3 days	Frequency of >5 Hz ↓ Collagen synthesis, ↑ Collagen degradation
Magnitude response of IHP [86]	Human lumbar pathological IVD cells and bovine NPC in collagen type I gel	0.25, 2.5 MPa; 0.1 Hz; 30 min (further incubated for 24 h)	0.25 MPa: ↑ **COL1A1**, **ACAN** protein 2.5 MPa: ↓ *COL1A1A1*, *COL2A1A1*, *ACAN*; ↑ *MMP-1,-3,-13* gene expression
Effect of IHP and cyclic strain under various osmolarity [129]	Human lumbar (herniated)disc cells and bovine caudal disc cells in type I collagen gel	Osmolarity: 300, 400 500 mOsm IHP: 0.25 MPa; 0.1 Hz; 30 min Cyclic strain: 4% strain; 1 Hz; 24 h	IHP: ↑ *ACAN*, *COL2A1* gene expression in hypo-osmosis condition ↓ *ACAN*, *COL2A1* gene expression in hyper-osmosis condition
IVD cells-PLLA construct under IHP [98]	Bovine OA and IA cells in PGA-PLLA scaffold	5 MPa; 0.5 Hz; 4 h/day; 11 days	HP improved AF cells infiltration HP resulted in higher COL2A1 protein synthesis by OA cells
Response of healthy and degenerating IVD cells to IHP [63]	Human IVD cells in alginate beads	0.8–1.7 MPa; 0.5 Hz; 2 h	HP ↑ *c-fos*, *ACAN*, *SOX-9* and *COL2A1* gene expression in non-degenerate NPC but not degenerate NPC
Loading and the release of endothelial cells attractants by IVD cells [84]	Human pathological disc cells in collagen gel and HMEC-1	Cyclic strain for AFC: 4%; 1 Hz; 24 h IHP for NPC: 0.25, 2.5 MPa; 0.1 Hz; 30 min	↑ *PTN* gene expression 1 h after cyclic strain and IHP ↑ *ACAN* in *AFC*, ↓ *ACAN* in NPC 24 h after loading 2.5 MPa IHP loaded NPC conditioned medium increased HMEC-1 migration
Glucose level and IHP [100]	Bovine and human NPC in alginate beads	Glucose: 0, 0.5, 5 mM; 2.5 MPa, 0.1 Hz, 30 min;	IHP ↑ *COL1A1*, *COL2A1* gene expression under normal glucose (5 mM) level ↓ *MMP* gene expression at reduced (0.5 mM) glucose

CS cyclic strain, *NPC* nucleus pulposus cells, *AF* annulus fibrosis cells, *IA* inner annulus, *OA* outer annulus, *NO* nitric oxide, *PGA-PLLA* poly(glycolic acid)-poly($_L$-lactic acid), *OH-proline* hydroxyproline, *GAG* glycosaminoglycan, *COL1A1* collagen 1, *COL2A1* collagen 2, *ACAN* aggrecan, *ADAMTS-4* a disintegrin and metalloproteinase with thrombospondin motifs-4, *MMP* matrix metalloproteinase, *PG* proteoglycan, *GAG* glycosaminoglycan, *HMEC-1* human microvascular endothelial cell line, *PTN* pleiotrophin, *TIMP* tissue inhibitor of metalloproteinases, *NO* nitric oxide

cells [31] and AF cells [124], bovine NP cells and human IVD cells [86] using a hydrostatic pressure loading system. Overloading of disc cells even increased the chemokine synthesis, which caused an enhanced migration of endothelial cells [84]. Exceptionally, Kasra et al. [49] found an increased ratio of collagen synthesis to degradation for isolated rabbit IVD cells when hydrostatic pressure of magnitude 3 MPa was applied at 20 Hz. These results

suggest that dynamic hydrostatic pressure of magnitude lower than 2.5 MPa might be beneficial to disc cells but there may be variation across different species and a frequency-dependent response. However, when considering studies using isolated cells, the digestion of the extracellular matrix during cell isolation disrupts the in situ environment; therefore, such systems completely lack the signal transduction from the matrix to the cells.

Response to frequency modulation

The IVD experiences different frequencies of loading during, e.g. walking, running or sitting in a car, tractor or helicopter. Exposure to whole body high frequency loading/vibration was found to be associated with low back pain and disc degeneration [10, 92]. Loading rate influences the extent of disc deformation, possibly also the rate of fluid flow in and out of the disc [88, 95], and affects the biosynthesis of the IVD cell. A lower loading frequency of 0.5 Hz was found to be the best of the tested conditions (0.5, 1.5 and 2.5 Hz) in preserving proteoglycan content of the rat tail model [19]. With the same model, MacLean et al. [70] demonstrated that 0.2 Hz was the best among the tested frequencies (0.01, 0.2 and 1 Hz) in preserving normal disc metabolism. The authors suggested that a "maintenance" stimulus of 0.2 Hz and 0.2 MPa sustained the steady state of structural mRNA transcription and that alterations above or below this loading level would result in remodelling, repair or degeneration [70]. For example, hypo-physiological loading frequency of 0.01 Hz in a rat tail model [122] or ultra-high frequency (10 Hz) in an ovine explant model [45] resulted in increased number of apoptotic cells. Frequency response is also relevant for stimulation by hydrostatic pressure. It has been found that a frequency of >5 Hz of 1 MPa intermittent hydrostatic pressure (IHP) impaired collagen synthesis [50].

Time response

The total time of exposure to mechanical loading is another important factor, which affects the biological response of the disc. Wuertz et al. [127] suggested that the disc responded anabolically to dynamic compression at a high daily exposure, such as 8 h/day instead of 1.5 h/day. For instance, a 2-week dynamic compression of 8 h/day at 1 MPa and 1 Hz caused up-regulation of anabolic gene expression in the NP of the rat tail. However, extended period of loading of the same magnitude for 8 weeks caused accumulation of mild degeneration. It was suggested that cyclic compression at a moderate level simulates physical activities and that this physiological loading might be able to promote repair or postpone disc degeneration. The duration response of IHP on the IVD cells has not been specifically reported in the literature, but an increased anabolic gene expression by human disc cells was demonstrated either after a single 30-min loading [86] or 30-min daily for 4 weeks [85].

Region-specific response

It has been shown that the expression of anabolic and catabolic genes by the IVD cells resulting from mechanical stimulation is region specific [70]. The difference in frequency response in different regions of the disc can be explained from the mechanical point of view. A greater peak-to-peak displacement of the IVD was found at low frequency (0.01 Hz) than high frequency (1 Hz) loading [70], in which the tissue modulus increases with frequency and so the strain decreases. This indicates a lesser deformation of the IVD at high frequency than low frequency at the same magnitude of load. Thus, higher hydrostatic pressures and smaller solid matrix strains were developed under high frequency loading. Therefore, it is likely that the AF response is dominated by deformation-related stimulus, which is more related to the magnitude of the loading where NP response is dominated by hydrostatic pressurisation, which is more related to frequency of the loading [5]. In a study of IHP, Kasra et al. [50] also reported a magnitude-dependent and frequency-dependent response of collagen synthesis in porcine AF and NP cells, respectively. Furthermore, a long duration of loading (8 h/day) was beneficial for the NP, but was degenerative in the AF [127]. It is unclear why the duration of loading resulted in a different response in the NP and the AF; it could be due to the fundamental difference in the characteristic of the cell types or the matrix environment of the two compartments.

Nutrition

Impaired endplate permeability caused by endplates calcification or sclerosis hinders the nutrition supply to the disc [9, 82, 109, 120]. When the IVD cells are in a reduced glucose environment, mechanical loading does more harm than good. Under limited glucose supply, axial compression applied on an ovine caudal disc organ culture model [45] or IHP applied on NP cells in alginate beads [100], resulted in elevated MMPs gene expression and/or protein expression. These negative responses were more prominent under hyper-physiological (10 Hz) loading frequency [45].

Other factors

Condition of the disc cells also influences the response of the cells to loading. When NP cells were cultured in

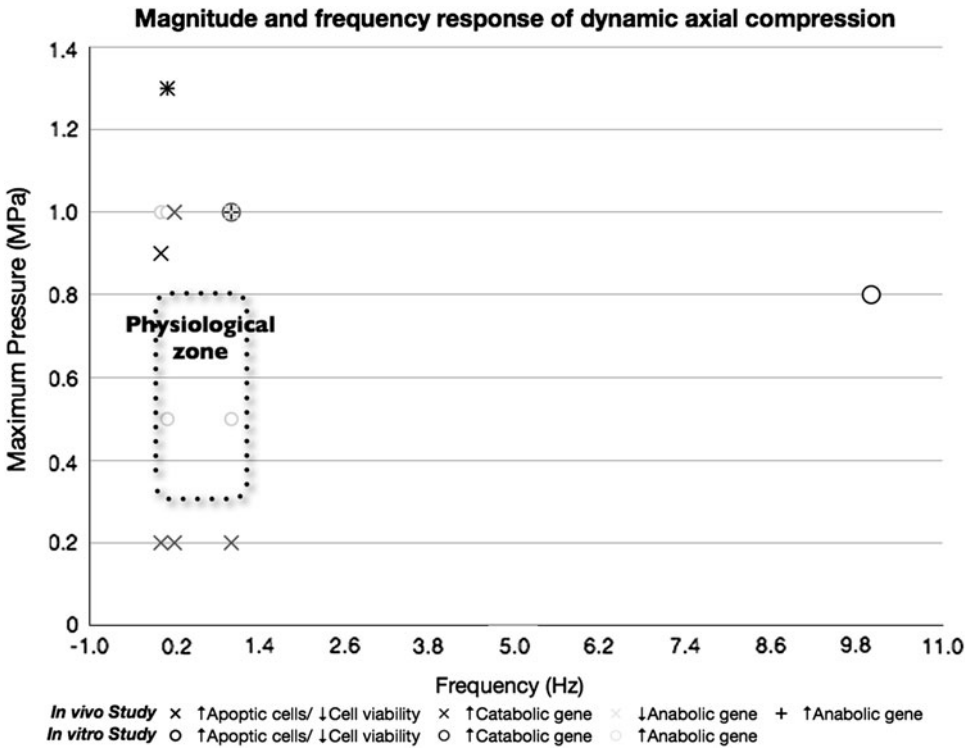

Fig. 1 Influences of magnitude and frequency of the dynamic compression acting on the IVD. *Square in dotted line* indicates possible physiological loading magnitude and frequency for the intervertebral disc. *Plus symbol*, *lighter circle* Increase in anabolic gene (collagen 1, collagen 2, or aggrecan); *lighter times symbol* decrease in anabolic gene; *thin times symbol*, *thin circle* increase in catabolic gene (Adamts-4: a disintegrin and metalloproteinase with thrombospondin motifs-4, MMP-3 or MMP-13 MMP: matrix metalloproteinase); *thick times symbol*, *thick circle* increase in apoptotic cells or decrease in cell viability [45, 58, 70, 72, 122, 123]

various osmotic conditions and exposed to the same IHP, hypo- and hyper-osmotic condition resulted in an anabolic and catabolic response, respectively [129]. This may imply that the initial volume of the cells affects their response to mechanical stimulus. The response of disc cells to loading is also different between 'healthy' and degenerated disc cells, and between young and mature animal disc cells. When isolated 'healthy' or degenerated human disc cells were exposed to IHP at physiological range, 'healthy' disc cells increased the anabolic gene expression while no response was detected in the degenerated disc cells [63]. This may be explained by the altered mechanical transduction mechanism between the degenerated disc cells and the healthy disc cells [64]. Young bovine caudal disc cells (6-month old) respond to axial compression with decreased anabolic gene expression but a higher GAG/DNA ratio than mature cells (18- to 24-month old) [56].

In summary, it is suggested that dynamic axial compression of magnitude 0.2–0.8 MPa, frequency of 0.1–1 Hz (Fig. 1) and a duration of 8 h/day is within the physiological loading level in which structural mRNA level and metabolism are maintained, and may not induce remodelling, repair or degeneration of the disc. For hydrostatic pressure, a magnitude of 0.1 to 2.5 MPa, frequency of <5 Hz and 30 min–4 h of loading per day are beneficial to isolated cells in 3D culture, but the duration of loading may vary with different in vitro systems and/or animal models used. The summarised "physiological" loading parameter space offers a potential beneficial loading regime for disc regeneration or for testing tissue engineering approaches for IVD repair.

IVD tissue engineering

Tissue engineering is an emerging field of study for the biological therapy of IVD degeneration. Due to the difficulty in acquiring healthy disc cells, multipotent stem cells, e.g. mesenchymal stem cells (MSCs), has been an option for IVD regeneration and tissue engineering, with their potential for self-renewal and differentiation into various cell lineages. MSCs can differentiate into various cell lineages, including adipocyte, osteoblast, chondrocyte, myoblast, and tenocyte [15, 91]; it may thus be likely that MSCs can be differentiated into IVD-like cells for tissue engineering applications. MSC differentiation can be directed by different factors, such as growth factors, or more importantly by mechanical factors, including shear, tension, compressive strain and hydrostatic pressure, all depending on the local environment such as matrix stiffness and structural arrangement, and the resulting cell shape and morphology differences, pH, oxygen and nutrient supply [52, 93].

MSCs in the IVD environment

The complex mechanical role of the IVD and the lack of vasculature make the IVD a "harsh" environment for cells. It is presented as low glucose and oxygen levels, high osmolarity, is acidic and experiences fluctuations in load and the resulting hydrostatic pressure. These conditions could jeopardise MSC survival in the disc environment. A very low initial MSC survival inside the IVD has been reported [17, 20]. On the other hand, in vivo studies have shown that MSCs inside the IVD can differentiate into matrix producing cells [59, 99]. The response of the MSC in each specific mechanical condition of the IVD has been studied. Cyclic compression [40, 48, 66, 90] and hydrostatic pressure [21, 53, 79] can promote chondrogenic differentiation of the MSC (Tables 4, 5). Furthermore, the high volumetric stress environment of the IVD could direct MSC differentiation towards a chondrogenic phenotype [16, 94]. Hypoxia has been shown to impair adipogenesis and osteogenesis of human MSCs [38], and as a potent chondrogenesis promoter than dynamic compression or hydrostatic pressure in the presence of transforming growth factor β (TGF-β) [7, 74, 77, 105]. Nevertheless, low oxygen tension and hydrostatic pressure alone without TGF-β could not initiate chondrogenesis of MSC [105]. A low glucose condition stimulates anabolic gene expression of MSCs [128]. An acidic environment and high osmolarity, as in the IVD, decrease MSC proliferation and viability, alter MSC morphology and inhibit anabolic gene expression [126, 128]. Overall, the disc environment certainly contains not only factors that favour MSC differentiation towards matrix producing cells but also factors that may hinder MSC survival.

MSC and dynamic loading

The basic function of the IVD is to provide mechanical support to the spine and therefore the mechanical loading regime applied to the cells used for IVD tissue engineering should be carefully evaluated. The effect of short-term to mid-term cyclic compression (Table 4) and hydrostatic pressure (Table 5) on MSCs has been studied. In the absence of TGF-β, dynamic compression together with dexamethasone could initiate chondrogenesis of rabbit MSCs [40, 48] and human MSCs [66, 90]. However, in some studies, dynamic compression inhibited chondrogenesis of porcine MSC [113] and human embryoid body-derived cells (hEBd) [112]. It has been suggested that dynamic compression applied before the initiation of chondrogenesis, e.g. exposure to growth factors, would inhibit later chondrogenesis [113]. Therefore, first conditioning MSCs towards a chondrocyte-like phenotype may be helpful for IVD tissue engineering, so as to enhance MSC chondrogenesis or 'discogenesis' when exposed to dynamic loading [29].

MSC differentiation influenced by frequency and duration of the loading

Compressive loading

Chondrogenesis of MSCs induced by dynamic compressive loading and hydrostatic pressure is frequency and duration dependent. A compressive loading frequency of 0.15–1 Hz was shown to enhance MSC chondrogenesis, whereas frequencies of 0.03 or 0.1 Hz did not [22, 90]. The duration of the dynamic compression is also important. Loading on alternating days resulted in negligible chondrogenesis, compared with a daily loading regime [54]. A duration of at least 54 min/day compression increased chondrogenesis but not if the duration was only 12 min/day [22]. As for the magnitude, all the studies were performed within the range of 5–20% compressive strain, and the effect of strain magnitude was not compared (Table 4).

Hydrostatic pressure

The effect of different magnitudes of IHP (0.1, 1, 10 MPa) was analysed and it was found that GAG production by ovine MSCs was increased after 10 days of loading of 1 or 10 MPa as compared to unloaded controls [79]. An IHP exposure duration of 2 h/day instead of 30 min/day enhanced GAG and hydroxyproline production of murine embryonic fibroblasts [21]. Angele et al. [3] also suggested that the effect of IHP is cumulative, 1 day or multiple days of loading give different differentiation results with human MSC. Loading with 10 MPa IHP increased hydroxyproline accumulation after 3–14 days but GAG was increased only at 7 and 14 days [79].

In summary, the combination of growth factors and dynamic loading provides a synergistic effect on MSCs differentiating into matrix producing cells. Consecutive compressive strain of 5–20% at frequencies of 0.15–1 Hz for 1–12 h/day or hydrostatic pressure of 0.1–10 MPa at frequencies 0.25–1 Hz increases the production of GAG and/or hydroxyproline by MSCs and may induce MSC chondrogenesis.

Possibilities and limitations

It seems obvious that well-designed dynamic compression and/or hydrostatic pressure loading protocols can induce differentiation of MSCs towards matrix producing cells. The loading parameters summarised in the first and the

Table 4 Effect of cyclic compressive loading on stem cells

Objective	Species/model	Type of load/force/duration	Findings
CD under static or dynamic compression [23]	Chick (23/24 embryos) limb bud MSC in agarose	Static: 4.5 kPa; 2 h × 3 days Dynamic: 0.25–9.0 kPa; 0.33 Hz; 2 h/day × 3 days	Enhanced CD by dynamic compression but not static loading
Frequency and duration response of MSC to cyclic loading [22]	Chick (23/24 embryos) limb bud MSC in agarose	0.25–9.25 kPa; 0.03, 0.15 or 0.33 Hz; 12, 54 or 120 min × 3 days	Enhanced CD at frequency > 0.15 Hz At 0.33 Hz, CD is enhanced by loading for 54 or 120 min
CD under dynamic compression vs. TGF-β [40]	Rabbit (3 m) bone marrow MSC in agarose with TGF-β and dexamethasone	10% strain; 1 Hz; 4 h × 3, 7, 14 days	Cyclic compression alone induces MSC CD as effectively as TGF-β Loading ↑ *TGF-β1* gene expression
CD under dynamic compression [41]	Rabbit (3 m) bone marrow MSC in agarose	15% strain; 1 Hz; 4 h/day × 2 days	↑ gene expression and production of proteins of **Sox9**, **c-jun**, and **TGF-β** receptors
CD under static or dynamic compression in the presence or absence of TGF-β [14]	Human bone marrow MSC in alginate or pellet culture with TGF-β	15% strain; 1 Hz; 10 days	Dynamic loading alone induced chondrogenesis ↑ Chondrogenic marker expression in dynamically loaded and TFG-β presence sample +TGF-β: ↑ *COLXA1*, ↓ *ACAN* −TGF-β: ↑ *COLXA1*
Duration response of dynamic compression, in the presence or absence of TGF-β [112]	Goat (3-year-old) bone marrow MSC; hEBd in PEGDA with or without TGF-β	10% strain; 1 Hz; 1, 2, 2.5, 4 h/day; 1, 2, and 3 weeks	In the absence of TGF-β1: Dynamic loading induces MSC CD Dynamic loading inhibited hEBd CD Dynamic loading enhanced CD when hEBd was pre-conditioned with TGF-β1
Duration of MSC pre-conditioning with CD medium and dynamic loading [83]	Calf bone marrow MSC in agarose with or without TGF-β and dexamethasone	10% strain; 1 Hz; 3 h; 16 days	↑ Chondrogenic gene expression and matrix synthesis by dynamic loading with TGF-β and dexamethasone
Effect of cyclic loading vs. free swelling on CD of MSC [113]	Porcine (4 m) bone marrow MSC in alginate with TGF-β	10% strain; 0.5 Hz; 1 h/day; 42 days	GAG accumulation: dynamically loaded sample < free swelling sample
CD of MSC under TGF -β1 or dynamic loading stimulation [54]	Equine (2- to 5-year-old) bone marrow MSC in agarose with or without TGF-β and dexamethasone	10% strain; 0.3 Hz; 3 h/day every alternate day or 12 h/day; 21 days	↑ Chondrogenic marker gene expression in the presence of TGF-β1 or 12 h daily dynamic loading
Frequency response of MSC towards CD [90]	Human bone marrow MSC in fibrin gel (40, 60, 80 mg/mL)	10% strain; 0.1, 0.5 or 1 Hz; 4 h × 3 days	CD is enhanced at frequency > 0.5 Hz
CD under dynamic compression and TGF-β [66]	Human bone marrow MSC in fibrin polyurethane scaffold with or without TGF-β and dexamethasone	10–20% strain; 1 Hz; 1 h × 7 days	Cyclic compression alone induces MSC CD ↑ Gene expression and the protein synthesis of TGF-β1 and TGF-β3 by MSC
CD under static or dynamic compression [48]	Rabbit (250–400 g) bone marrow MSC in PLCL with TGF-β	5% strain; 0.1 Hz, 10 days	↑ GAG content ↑ Protein and gene expression of *COL2A1* and *ACAN* in dynamically loaded scaffold

CD chondrogenic differentiation, *PLCL* poly(lactide-co-caprolactone), *MSC* mesenchymal stem cell, *PEGDA* poly (ethylene glycol)-diacrylate hydrogels, *hEBd* human embryoid body-derived cells, *GAG* glycosaminoglycan, *COLXA1* collagen 10, *ACAN* aggrecan, *COL2A1* collagen 2, *TGF* transforming growth factor

second part of this article can be useful for designing a mechanical loading protocol to test the performance of the IVD tissue engineering construct and to direct differentiation of stem cells to matrix producing cells. Nevertheless, there is no study available at present that evaluates the differentiation of MSC towards IVD-like

Table 5 Hydrostatic pressure and stem cell (SC) chondrogenic differentiation (CD)

Objectives	Species/model	Loading magnitude/ frequency/duration/others	Findings
Duration of IHP and CD of MSC [3]	Human bone marrow MSC in pellet with TGF-β and dexamethasone	IHP 0.55–5 MPa; 4 h/day; 1 or 7 days, total culture time 14 or 28 days	7 days loading ↑ GAG and collagen content but not single loading event
Influence of oxygen tension, IHP, and TGF-β on MSC CD [105]	Bovine bone marrow MSC in collagen membrane with TGF-β and dexamethasone	IHP: 0.2 MPa; 30/2 min; 24 h; 2 non-consecutive days Oxygen tension: 5, 21%	Low oxygen tension and IHP is not sufficient to induce CD Low oxygen tension enhance CD in TGF-β
Duration of IHP and CD [21]	Murine embryonic fibroblast (C3H/10T1/2) in monolayer	IHP: 0.3–5 MPa; 1 Hz; 30 min/day or 2 h/day; 3 days	2 h/day IHP ↑ GAG, OH-proline synthesis
IHP, TGF-β and CD [80]	Human MSC in pellet with TGF-β	IHP: 10 MPa; 1 Hz; 4 h/day; 3, 7, 14 days	↑ CD marker gene (*SOX-9*, *COL2A1*, *ACAN*) expression IHP < TGF-β < IHP + TGF-β
Magnitude and duration response [79]	Human MSC in pellet	IHP: 0.1, 1,10 MPa; 1 Hz; 4 h/day; 3, 7, 14 days	Magnitude: ↑ *SOX9*, *ACAN* in all magnitude ↑ *COL2A1*, OH-proline in 10 MPa ↑ GAG at 1 and 10 MPa Duration: ↑ GAG content at 7, 14 days ↑ OH-proline content at 3, 7, 14 days
Effect of PHP on pre-differentiated MSC [69]	Ovine bone marrow MSC differentiated in TGF-β3 for 4 weeks in polyester scaffolds	PHP: 0.1 MPa; 0.25 Hz; 30 min/day; 4, 7, 10 days	↑ DNA, GAG, collagen content with PHP than unloaded control at day 10
Difference between steady or ramped IHP [25]	Human MSC in 2% agarose	Steady: 7.5 MPa Ramped: 1–7.5 MPa (0.5 MPa increase/d); 1 Hz; 4 h/day; 4, 9, 14 days	↑ *COL1A1* at steady high HP at d4 ↑ *COL1A1* for both loading group at d14
MSC differentiation under IHP and multiple differentiation factors [121]	Human MSC seeded in type I collagen sponges in TGF-β1 and β-glycerol-phosphate	1 MPa; 1 Hz, 4 h/day; 10 days	↑ *COL1A1*, *COL2A1*, *ACAN*, *SOX9* gene expression with IHP, no change in RUNX2 and TGF-β1
Effect of IHP, TGF-β, BMP-2 on stem cells CD [131]	Bovine bone marrow MSC aggregates in TGF-β, BMP-2 and dexamethasone	0.5–3 MPa; 1 Hz; 1 h/day; 14 days	TGF-β ↑ CD But not enhanced with IHP or BMP-2
IHP on CD of adipose-derived SC [87]	Human adipose-derived SC in collagen scaffold with TGF-β, dexamethansone	0-0.5 MPa; 0.5 Hz; 1 week; further unloaded culture of 1, 2, 3 weeks	↑ Cell number ↑ *COL2A1*, *COLXA1*, *ACAN*, *SOX9* gene expression in IHP > unloaded control after 4 weeks
IHP on NP co-cultured MSC [53]	Rabbit MSC and NPC in alginate beads	0.2–0.5 MPa; 2/15 min; 4 h/day; 3 days	Co-culture MSC and NP, and IHP synergistically increased *ACAN*, *SOX-9* gene expression

IHP intermittent hydrostatic pressure, *PHP* pulsatile hydrostatic pressure, *MSC* mesenchymal stem cell, *OH-proline* hydroxyproline, *GAG* glycosaminoglycan, *COL1A1* collagen 1, *COL2A1* collagen 2, *COLXA1* collagen 10, *ACAN* aggrecan, *TGF* transforming growth factor, *BMP* bone morphological protein

phenotype in the presence of dynamic loading. Due to the lack of specific markers for IVD cells, earlier studies indicated differentiation of MSCs towards IVD-like or chondrocyte-like cells by the expression of the most prominent chondrocytic markers for the extracellular matrix, such as aggrecan and collagen 2 [101]. Recent studies have shown that there are significant differences between chondrocytes, AF and NP cells [29, 30, 32, 78, 102, 103], and the effect of cyclic loading on MSC differentiation towards an IVD cell lineage is worth investigating with these possible IVD markers. On the other hand, although many studies have shown that dynamic loading promotes MSC chondrogenesis, most of the results were based on temporary loading on MSCs from a few minutes up to 12 h/day. The fate of stem cells during continuous diurnal dynamic loading, as in the IVD, is not known. Moreover, the physiological range of deformation of chondrocytes and their organelles may be different than that of IVD cells; therefore, the strain threshold may be different for chondrogenesis or IVD-like differentiation. It

is also possible that, other than TGF-β, other growth factors such as growth and differentiation factor 5 (GDF-5), insulin-like growth factor (IGF) and platelet-rich-plasma (PRP) could direct MSCs to a more IVD-like phenotype under dynamic loading.

Conclusions

Dynamic compression is one of the important factors that determines the biosynthetic activity of IVD and MSCs and affects the fate of cells for tissue engineering applications. Magnitude, frequency and duration of dynamic loading together determine the fate of disc cells as well as cells for IVD tissue engineering. It is essential to establish a beneficial loading regime, which enhances the anabolic response of disc cells and the IVD-like differentiation of stem cells. Particularly when more in vitro disc culture systems are made available for testing the performance of tissue engineering constructs for disc degeneration therapy. Therefore, this review summarises information from the literature and suggests possible beneficial loading regimes for IVD cells and MSCs. Nevertheless, the loading applied on the IVD is more complex than only compression and hydrostatic pressure, other physical factors and different modes of mechanical loading also affect disc cell behaviour. With the recent findings on the difference in transcriptional markers of the IVD cells and articular chondrocytes, the fate of the stem cells within the IVD environment could be investigated in depth. Moreover, although the response of the IVD to loading has been well studied, the specific mechanism that controls the IVD cells' response to applied loads is still not known. The investigation of loading on the IVD will ultimately improve our understanding of the possible prognoses of disc degeneration and provide insight into the prevention of disc diseases and the rehabilitation of the disc after biological therapy. Furthermore, information on the influence of continuous dynamic loading on the possible cell source for tissue engineering, e.g. stem cells and the differentiation of the stem cells, towards an IVD cells phenotype could advance IVD tissue engineering.

Conflict of interest None of the authors has any potential conflict of interest.

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