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Degenerative physiochemical events in the pathological intervertebral disc

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Summary. Low back pain is one of the commonest musculoskeletal complaints that affects individuals of all ages and is a leading contributor towards work loss worldwide. The range of current treatment modalities involving surgeries, injectable agents, and medications is promising but cannot address the reasons behind the occurrence of pain in patients with degenerative disc pathologies. One possible factor for the limited success is the lack of evidence behind the identification of early, intermediate, and late stages of painful changes methodologically in a vast group of populations and the manifestation of the diseases in terms of increased physical activity, hereditary patterns, and various risk factors. However, despite these challenges, steady progress has been achieved in understanding the parameters in abnormally loaded progressively degenerating discs and these features have been elucidated at a physical, biochemical, and cellular level. These recent findings can likely lead to the development of therapeutic interventions that will identify and retard tissue damage, decrease pain, and improve the quality of life in these patients. Therefore, the main aim of this review is to integrate recent updates in intervertebral disc degeneration research for the development of evidence-based screening protocols and more targeted interventions in the management of low back pain.

Key words: Intervertebral disc, Degeneration, Pain, Blood vessels, Nerves

Introduction, epidemiology and pain perception

Back pain is a significant health problem in the world, affecting over two-thirds of the population at some stage in their lives, and with more than 25% of the people reporting it in the last 3 months and 12% suffering for 30 days or more in the last year. It is the second leading cause of disability, and the most common ailment of working adults and is estimated to cost $50 billion annually across the world (Strine and Hootman, 2007; Leboeuf-Yde et al., 2009). Back pain is associated with greater depression, anxiety, and insomnia (Strine and Hootman, 2007), recent evidence indicates that it persists into old age (Bendix et al., 2008; Leboeuf-Yde et al., 2009), and although its impact is masked by retirement and immobility, it continues to have significant economic consequences on the delivery of health care. It is clear, therefore, that this is a musculoskeletal disorder affecting enormous numbers and is one of the leading causes of severe pain and disability in the aging population. Recent studies provide strong evidence that intervertebral disc (IVD) degeneration is associated with the most severe chronic back pain (Cheung et al., 2009; de Schepper et al., 2010; Livshits et al., 2011). IVD degeneration when defined in terms of specific structural changes (Adams et al., 2006 (3rd edition in 2012)), then the relationship with back pain is clear. However, where IVD is defined in terms of age-related loss of water and proteoglycans or in terms of general radial bulging, then the relationship to pain is more complex (Boden et al., 1990; Jensen et al., 1994; Videman et al., 2003), however closely associated with pain are radial fissures in the annulus fibrosus (Videman and Nurminen, 2004, Peng et al., 2006), disc herniation (Hartvigsen et al., 2004; Ghahreman and Bogduk, 2011), and endplate damage (Peng et al., 2009; Wang et al., 2012) that facilitate processes such as proteoglycan loss readily. The ultimate collapse in annulus height is also associated with back pain (Boos et al., 1995; Videman et al., 2003) and increased neo-innervations and vascularisation. Evidence from pain-provocation studies suggests that IVDs account for approximately 50% of severe and chronic low back pain cases (Weber et al., 2015).

Intervertebral discs structure and functions

Located between the consecutive vertebral bodies

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intervertebral discs are white fibrocartilages separating the vertebrae. An adult intervertebral disc is approximately 7-10 mm thick and 40 mm in diameter along the mid-sagittal plane (Roberts et al., 1989; Urban and Roberts, 2003a,b). They are thinnest in the thoracic and thickest in the lumbar regions and provide flexibility allowing bending (flexion, extension) and torsion. They also act as minor shock absorbers and help in the even distribution of compressive forces on the vertebral bodies (Adams et al., 1996). Each intervertebral discs comprise of three integrated parts: the gelatinous nucleus pulposus, the laminated annulus fibrosus, and the cartilage endplate. The nucleus pulposus and annulus fibrosus are sandwiched superiorly and inferiorly by the cartilage endplates (Humzah and Soames, 1988), as shown in Fig. 1.

Annulus fibrosus forms the outer-most part of the discs, with concentrically arranged 15-25 collagen lamellae (Fig. 1A,B) (Urban and Roberts, 2003a,b). Each lamella consists of oblique and regularly arranged type-I collagen fiber bundles orientated at 30 degrees to the horizontal plane with the direction of fibers alternating between adjacent lamellae. The outer annulus contains relatively dense population of cells that tend to be fibroblast-like, are elongated and aligned parallel to the collagen fibers (Errington et al., 1998; Lama et al., 2013). Towards the inner annulus region, the lamella present mainly resists compressive forces and demonstrates a higher amount of proteoglycans molecules thus are more deformable, consequently the cells of the inner annulus are rounded and not necessarily aligned parallel to the collagen fibers (Bruehlmann et al., 2002). Cells may appear in pairs or clusters in between the lamellae. Collagen types II, III, and VI are present around the pericellular matrix of clustered and non-clustered inner annular cells (Roberts et al., 1991).

Nucleus pulposus represents the inner soft core of the intervertebral disc (Fig. 1A-C). It originates from the notochord, and in the fetus and infants, it contains actively dividing notochordal cells, which disappear at approximately eight years of age (Urban and Roberts, 2003a,b). In the adult disc, the nucleus contains type II collagen fibers which are randomly organized. Proteoglycans account for 50% of the dry weight of the adult nucleus (Roughley et al., 2002), and contain type III, V, VI, IX, and type XI collagens, which are pericellular in location (Roberts et al., 1991). Aggrecan is the main component of proteoglycans and has a protein core to which up to 100 sulphated cationic glycosaminoglycans (GAG) chains are covalently attached (Urban et al., 2000). The charge borne on the surface by each GAG possesses a high affinity to attract extracellular ions and water, thus the water content of the nucleus is approximately 80% (McMillan et al., 1996). The nucleus, therefore, behaves as a viscous fluid and resists compressive forces by generating a hydrostatic pressure of approximately 0.05MPa in a cadaveric disc (Adams et al., 2012a,b). Under high pressure, nucleus pulposus can insinuate between collagen fibers of the inner annulus causing micro-disruptions within and between the lamellae (MacLean et al., 2008). In the nucleus, chondrocytes-like nucleus pulposus cells (Urban, 1994) may be present singly, in pairs, or may frequently form small and large clusters.

The cartilage endplate is the third morphological distinct region of the discs (Fig. 1C). It is usually present in the form of a horizontal hyaline cartilage layer with less than 1 mm thickness and is loosely bonded to the bony vertebral endplate of perforated cortical bone with abundant blood vessels and capillaries. The most important function of the endplate is to act as a physical

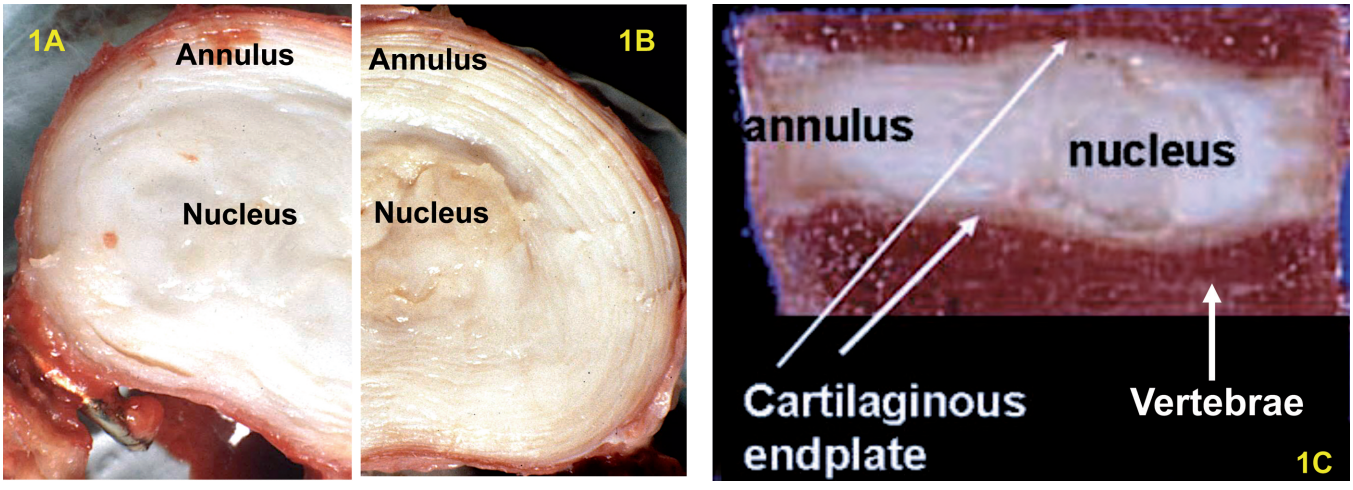

Fig. 1. Normal Intervertebral disc showing the annulus lamellae, nucleus pulposus and cartilaginous endplate regions. **A.** Young disc with a clear white nucleus pulpous region. **B.** Mature disc 'yellowed in appearances' due to overall loss of hydration. **C.** Sagittal section of a spine showing the cartilaginous endplate located between the vertebrae and the intervertebral discs. (Fig. A,B are from the Biomechanics Lab of Michael A Adams and Fig. C from Urban and Roberts, Arthritis Research & Therapy, 2006).

and chemical barrier, thereby preventing the nucleus from pushing into the spongiosa of the vertebral bodies (Harris and Macnab, 1954; Roberts et al., 1989). The endplate also functions as a pressure distributor during loading (Veres et al., 2010). The cartilage endplate maintains the internal pressurization of the discs by hindering the expulsion of water and proteoglycan from the disc into the vertebrae while facilitating the passage of nutrients into the discs through the process of diffusion (Rajasekaran et al., 2004). The elemental chemical composition of the endplate is proteoglycans, type II collagen, and chondrocytes in pairs or clusters (Roberts et al., 1989; Lama et al., 2014).

Blood supply and Nerve Supply of the disc

The human annulus fibrosus is supplied by blood vessels for only three years after birth, and these vessels disappear by late childhood apart from some small capillaries and lymph vessels confined to the outer 1 to 2 mm of the annulus (Urban and Roberts, 2003a,b) (Fig. 2A,B), and this makes the intervertebral disc one of the largest avascular tissue in the adult human body. Essential nutrients such as oxygen, glucose, and substrates for matrix molecules reach IVD's by the process of diffusion and fluid flow through the blood vessels present at the margins of the outer annulus and endplate (Ferguson et al., 2004) diffusion varies according to the duration of axial compression (Adams and Hutton, 1986; Arun et al., 2009), the loaded/unloaded state of the discs, and pumping action generated by the loading or unloading cycles (O'Hara et al., 1990; Ferguson et al., 2004). The cellular viability depends upon oxygen, pH, and glucose concentration, and since the oxygen levels in the center of the nucleus regions are very low approximately 6-8 mm away from the nearest blood supply, metabolism is by glycolysis and accumulation of lactic acid may make the pH of the discs slightly acidic.

Nerve supply to the intervertebral discs is by the branches arising from the sympathetic trunk, the spinal nerves, and the sinuvertebral nerves. The anterior and lateral aspect of the annulus is supplied through a plexus primarily derived from the sympathetic trunk (Bogduk, 1988). Posteriorly the annulus receive branches formed by the sinuvertebral nerves, and the sensory spinal nerves (Bogduk, 1994) (Fig. 2C). The nerves fibers are abundant in the outer ligamentous portion of the annulus, are fewer in the middle third and absent from the inner third and in the nucleus pulposus region (Coppes et al., 1990; Edgar, 2007; Adams et al., 2010).

Intervertebral disc degeneration and pain

Adverse mechanical loading is strongly implicated in the initiation of disc degeneration (Videman et al., 1990; Newell et al., 2017), with many of the structural features of disc degeneration associated with pain being reproducible in cadaveric discs by severe or repetitive loading (Adams and Roughley, 2006; Stefanakis et al., 2014). However, loading alone may not inevitably lead to end-stage disc degeneration (Hutton et al., 2000). Biological factors are equally important in the etiology of disc degeneration and pain and may integrate as genetic influences, inheritance, alongside age-related changes in the composition of the matrix which predisposes the discs to physical disruption during normal activity. Hence, the heritability of disc degeneration and pain is approximately 70% in middle-aged women (Sambrook et al., 1999; MacGregor et al., 2004) but only 30-50% in younger men with greater levels of physical activity (Battie et al., 2008). Another crucial biological factor in disc degeneration is considered to be poor metabolite transport to cells in the

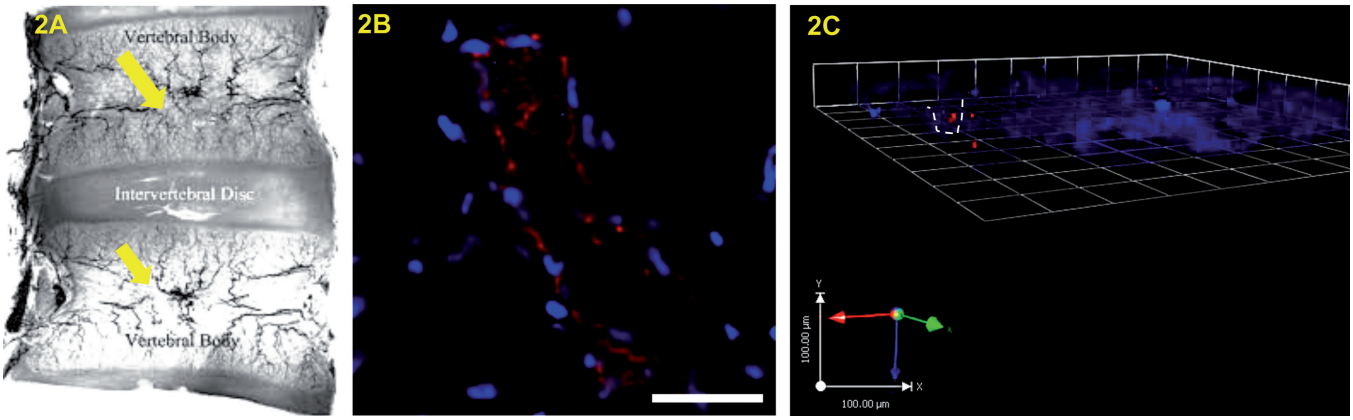

Fig. 2. Blood vessels and nerve terminals of the adult intervertebral disc. **A.** Abundant blood vessels are present at the vertebral bodies (arrow) with relatively avascular and a neural disc. **B.** CD-31 immunopositive endothelial blood vessels located at the peripheries in the inner annulus region of a painful disc. **C.** 3-D Confocal Z stacked section of a tiny, punctuate and arborizing P.G.P 9.5 immunoreactive nerve terminal, seen in red, within 30 μm thick sections of a painful disc. (Fig. 2A is from Johnson and Roberts, 2007 and Fig. 2 B,C are from the Molecular matrix Lab of Polly Lama). Scale bars: B, 50 μm; C, 100 μm.

nucleus from where the degenerative events could commence (Urban et al., 2004). Disc structural failure leading to progressive pathogenic etiology and accelerated degenerative changes stems from both structural and molecular dissonance, the center-stage factors in disc degeneration research (Urban et al., 2004). Neo-innervation and vascularisation of discs have been associated with disc degeneration in general (Freemont et al., 1997), and with annulus radial fissures in particular (Peng et al., 2005), with direct associations between nerve in-growth and back pain (Freemont et al., 1997). Recent evidence thus suggests that discogenic back pain has much to do with nerve in-growth and nerve sensitization phenomena and appear to be associated with mechanical disruption of the annulus fibrosus and alteration of cellular phenotypes.

Cellular changes in relation to nerve in-growth and annular fissures

Recent research exploring cell-mediated events leading to discogenic pain has indicated that proteoglycans (PG) such as aggrecan, inhibit nerve and blood vessel growth (Johnson et al., 2002, 2005), and proteoglycan loss is particularly associated with fissures or disrupted regions of the tissue, (Antoniou et al., 1996; Stefanakis et al., 2012) and may promote in-growth of vessels and nerve in degenerated discs (Fig. 3A) (Melrose et al., 2002). In-growths is induced by neurotrophic factors, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), secreted by blood capillaries (Freemont et al., 2002), nucleus pulposus (NP), and inner annulus fibrosus cells of degenerate discs triggering release of catabolic cytokines (Fig. 3B). Nociceptive nerves become 'sensitized' by various inflammatory-like reactions that may involve an increase in the production of factors such as TNFα secreted by NP cells (Olmarker et al., 2003; Olmarker, 2008) which contributes towards the central mechanisms of neurogenic pain. Cell clustering is also associated with painful discs (Johnson et al., 2001), as is cell senescence (Roberts et al., 2006a,b; Le Maitre et al., 2007) which increases expression of matrix-degrading enzymes (Roberts et al., 2006a,b) all of which are regulated, at least in part, by the increased production of catabolic cytokines particularly IL-1[21, 40]. There have been attempts to block TNFα signaling to cure clinical sciatica following disc herniations (Karppinen et al., 2003; Korhonen et al., 2006), and to cure discogenic back pain by injecting neurotoxic agents such as methylene blue into painful discs (Peng et al., 2010). Each had mixed success, suggesting that the approach is promising but needs to be properly targeted.

Annulus fissures drive focal changes associated with painful discs

Degeneration in painful discs is often focal, with loss of PGs, in-growing nerves, and blood vessels, as well as matrix-degrading enzymes located predominantly around annulus fissures (Weiler et al., 2002; Peng et al., 2005; Lama et al., 2018). Preliminary

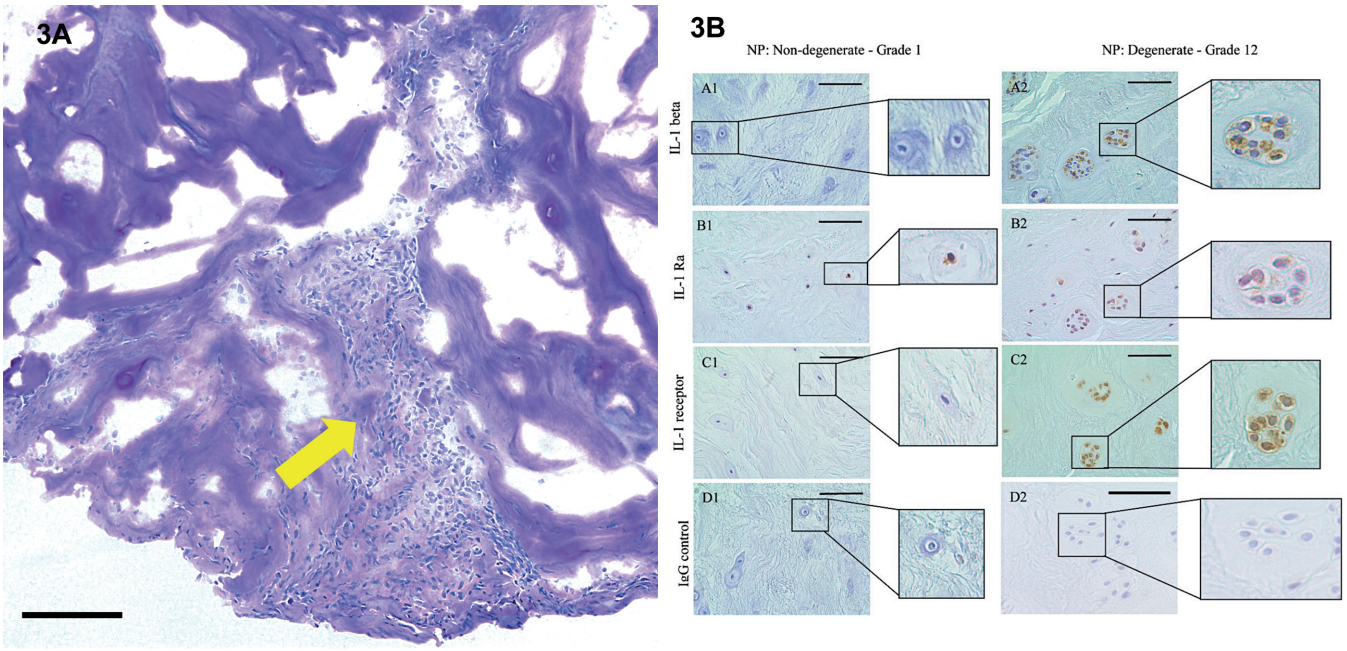

Fig. 3. Inflammatory events in the disc tissue. **A.** Toluidine blue stained herniated disc annulus region with invading inflammatory cells (arrow) in fissured and proteoglycan depleted region. **B.** Expression of cytokine IL-1in degenerated nucleus pulpous cells. (Fig. 3A is from Molecular matrix Lab of Polly Lama and Fig. 3B is from Biomolecular Research Lab of Christine Le Maitre, Le Maitre et al., 2005). Scale bar: 100 μm.

observations from recent studies show that the expression of catabolic factors is highest within micro-fissures in the nucleus pulposus region, and the data emphasize that painful degenerative changes emanate from small regions of disrupted tissue (Lama et al., 2018). Past studies have shown annulus fissures present a micro-environment in which physical stresses are remarkably low and proteoglycans have been lost (Stefanakis et al., 2012), both changes being conducive to nerve and blood vessel ingrowth (Adams et al., 2012b). This is in agreement with evidence showing decreased aggrecan synthesis in disc regions exposed to reduced pressure (Hutton et al., 1999; Neidlinger-Wilke et al., 2009). Thus, there are close associations between annulus fissures, localized swelling, depleted PG, and nerve in-growth (Adams et al., 2012b). Increases in collagenolytic MMP expression localized to fissures can further disrupt the collagen fiber network, reducing its ability to restrain the swelling tendency of proteoglycan molecules. In our past work on surgically retrieved samples of painful disc tissue, a close spatial association between tissue disruption, proteoglycan loss, cell clustering, integrin expression (Lama et al., 2019), and blood vessels and nerve ingrowths (Fig. 4A-G) was observed, and cells from degenerated disc tissue exhibit abnormal mechanotransduction pathways, with integrins unable to convey mechanical stimuli even though they are still expressed by these cells (Le Maitre et al., 2005 and 2009). Thus, uncoupling of the link between the cell and extracellular matrix interactions via the integrins receptors could explain these abnormalities in cellular phenotypes as degenerative events progress. Weakened cell-matrix interaction may encourage disc cells to re-enter the cell cycle, creating cell clusters that typify subsequent senescence in degenerated tissue. These cell clusters accelerate the synthesis of catabolic factors and degrading enzymes produced during severe degeneration. Therefore, focal swelling around fissures may be the trigger for cellular changes identified in painful regions of intervertebral discs.

Neo vascularisation and innervation in pathological painful patient's disc

It has been suggested that the neuropathological basis of low back pain is the sensitization of nerve endings and nociceptors (Kuslich et al., 1991) that are present within the outer three or four lamellae of the relatively avascular and aneural intervertebral discs (Ashton et al., 1994). These nociceptive nerve fibers act as sensors that detect damage or potentially damaging stimuli that signal pain (Roberts et al., 1995; Dimitroulias et al., 2010), respond to changes in intradiscal pressure (Adams et al., 1993), and become hypersensitive during annular injury/tear, as high tensile stresses are especially concentrated near radial fissures (Pezowicz et al., 2006). Stimulation of these nociceptors releases sensory neuropeptides such as Substance P (sub-P) and calcitonin gene-related peptides (CGRP) capable of amplifying the pain response (Fig. 4B) (Brain and Moore, 1999). These nociceptors and tiny nerve fibers fail to fire during normal movements within normal

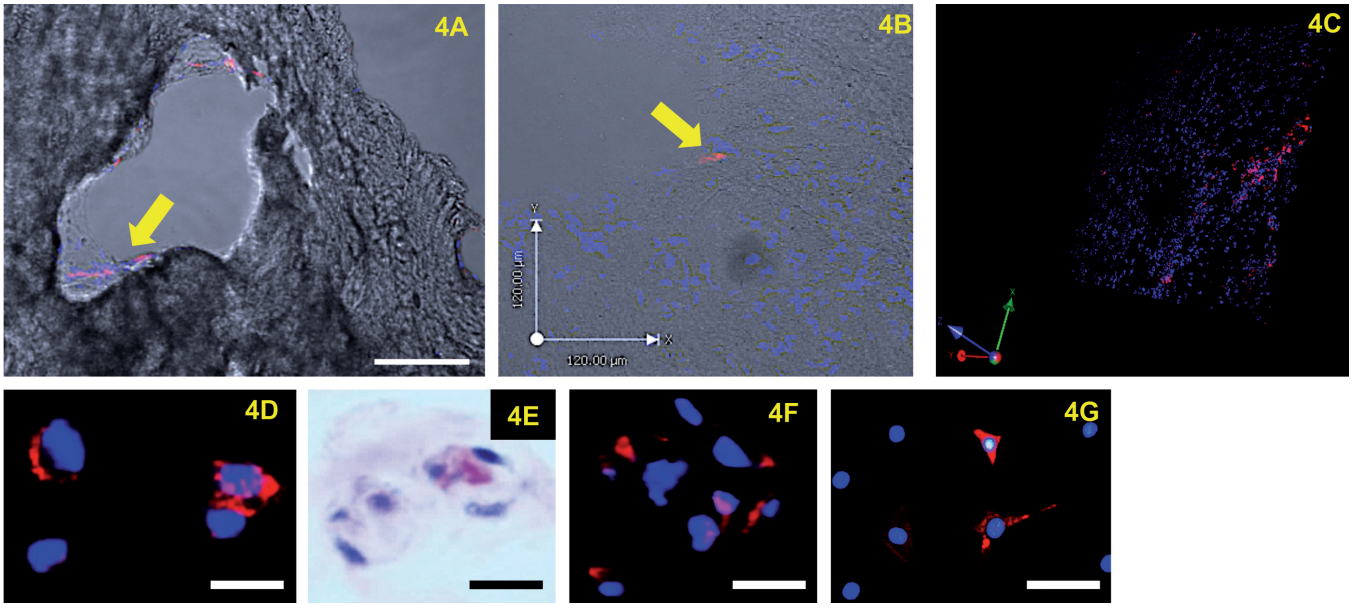

Fig. 4. Immunohistochemical expression of various markers and receptors in painful disc pathologies. **A.** CD-31 positive vessel seen along a fissure/defect (arrow) with a phase contrast background. Nuclei stained blue with DAPI and the vessels red. **B.** Substance-P positive nerve terminal (red), cell nuclei (blue) with phase contrast. **C.** 3-D confocal Z stack of 30 μm thick section with NGF stained vessels in red, cell nuclei (blue). **D.** Matrix degrading enzymes, MMP-1 **(E)** MMP-3 **(F)** Integrin alpha 5 beta 1 **(G)** TLR-2 receptors in cell stimulated with Interleukin-1. All Figs. are from Molecular matrix lab of Polly Lama. Scale bars: A, 50 μm; B, C, 100 μm D-F 50 μm.

ranges of motion but, in response to tissue damage, they become involved in the process of neurogenic inflammation and secrete chemical irritants that complicate the reparative events initiated by the disc cells. In degenerated intervertebral discs, such nerve ingrowths have even been reported to occur within the nucleus pulposus (Peng et al., 2005), following a fissure (Stefanakis et al., 2012). Nerves are accompanied by capillaries that express high-affinity nerve growth factor (trk-A) receptors. Ingrowth of such vessels and nerves into the nucleus pulposus has also been suggested to occur through the discal endplates (Freemont et al., 1997 and 2002) in degenerated discs. Growth factor synthesis and its receptor activation have also been suggested to be a response following disc herniation (Tolonen et al., 1995). Angiogenic receptors are expressed frequently along with Substance P in both herniated and degenerated discs, indicating that it may contribute to pain pathogenesis. Radial annular fissures are strongly associated with disc degeneration, herniation, and discogenic low back pain (Videman and Nurminen, 2004; Peng et al., 2006). The severity of the fissure may determine the degree of herniation, as a well-developed tear extending from the nucleus to the annulus, rupturing the outermost annular layer and the surrounding ligaments, readily allow the disc to herniate and sensitize the peripherally located blood vessels and nerves leading to the compression of the dorsal root ganglion (Rothman et al., 2011). Such fissures may also provide a route for blood vessels and nerves to grow deeper into the matrix (Stefanakis et al., 2012), which could make discs as a painful tissue, and further, the fissure extends into the annulus, the greater the risk of pain (Videman and Nurminen, 2004). Fissures may also act as an easy route for disc tissue to lose its proteoglycan concentration (Melrose et al., 2002) and it is well known that proteoglycan inhibits blood vessels and nerve growths (Johnson et al., 2006). A fully herniated disc may influence other degenerative changes, as it is known that disc tissue on losing its pressurized confines becomes free to swell, and proteoglycans imbibe fluids from the surrounding environment. Continuous swelling leads to break down and eventually leaching of proteoglycan aggregates from the disc tissue (Urban and Maroudas, 1981; Dolan et al., 1987), leaving behind a scaffold of collagen fibers, and such changes may perhaps trigger complex altered events at cellular and biochemical level that may initiate processes leading to the synthesis of catabolic factors that are capable of further degrading the matrix.

Focal swelling and tissue disruptions in degenerated and herniated intervertebral discs

At equilibrium, when there is no fluid loss or gain in the discs, the internal swelling pressure is equal to the magnitude of the external stress (Urban and McMullin, 1988). The lamellar collagen network of the annulus fibrosus and the endplate located around the nucleus pulposus regions thus maintains the internal pressure and prevents glycosaminoglycans loss. The total ion and glycosaminoglycans (GAG) concentration is higher in the nucleus pulposus than the surrounding annulus fibrosus (Jay Lipson and Muir, 1981). Abnormal stress imbalances perturb the GAG's and ion concentrations within the discs tissue and are expressed as fluid lost from the discs (Urban and Maroudas, 1981). The fixed charged density (FCD) of the GAG molecules, the proteoglycans and the resulting osmotic pressure rises with loss of fluids until an equilibrium can be reached. The osmotic pressure tries to balance the applied stress (McMillan et al., 1996), and during this process any damage to the collagen fibers increases the swelling potential of the disc tissue, facilitating more proteoglycans leaching (Urban and McMullin, 1988). Thus, fissured, degenerated tissue that escapes from such pressurized confines swell and undergoes extensive biochemical changes that start as rapid proteoglycans loss, changes in hydration, structural orientation, nutrition, cellular functions, morphology, and micro-mechanics. These changes could typically occur through a progressive and stepwise cascade of events resulting in gradual alterations of the cellular and extracellular matrix (Pfirrmann et al., 2001), with affects collagen synthesis, increases fragmentation of small leucine-rich proteoglycans (SLRP's) and fibronectin (Oegema et al., 2000; Greg Anderson et al., 2003), increases the synthesis of matrix-degrading enzymes, cytokines, increases cell clustering and initiates cell senescence or death (Tschoeke et al., 2008). As a result, swelling alters the physiochemical environment of the tissue to stimulate the increased production of catabolic factors that are commonly seen in fissured and painful discs.

Conclusion for the physiochemical etiology of painful disc degeneration

Ageing, loading history, and an unfavorable genetic inheritance leave some intervertebral discs vulnerable to physical damage, particularly to the vertebral endplate and peripheral annulus. Metabolite transport problems ensure that the disc has a low cell density so that effective repair is possible only in certain peripheral regions. Elsewhere, the disrupted collagen network allows tissue swelling, fissuring, and loss of proteoglycans that makes IVD's conducive to inflammatory cell invasion, and to the ingrowth of blood vessels and nerves. Focal swelling disturbs disc cell binding to their matrix, either directly or following MMP up-regulation, so that the mechanotransduction events are also disturbed. Affected disc cells then form clusters, in an attempted repair process that is frustrated by inadequate metabolite transport and results only in cell death and unregulated production of matrix-degrading enzymes. Inflammatory changes are amplified by repeated minor injuries to a structure that cannot easily be protected from mechanical loading, and in-growing disc nerves become sensitized to minor stimuli.

In this way, physio-chemical disruption to the disc matrix could play a significant role in altering the behavior of disc cells and ensuring that focal disruption is followed by chronic degeneration and pain rather than by effective healing.

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Conflict of Interests. Authors declare no conflict of interests.

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