Matrix synthesis and degradation in human intervertebral disc degeneration

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Abstract
Degeneration of the intervertebral disc has been implicated in chronic low back pain. Type II collagen and proteoglycan (predominantly aggrecan) content is crucial to proper disc function, particularly in the nucleus pulposus. In degeneration, synthesis of matrix molecules changes, leading to an increase in the synthesis of collagens type I and III and a decreased production of aggrecan. Linked to this is an increased expression of matrix-degrading molecules including MMPs (matrix metalloproteinases) and the aggrecanases, ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) 1, 4, 5, 9 and 15, all of which are produced by native disc cells. Importantly, we have found that there is a net increase in these molecules, over their natural inhibitors [TIMP-1 (tissue inhibitor of metalloproteinases-1), 2 and 3], suggesting a deregulation of the normal homoeostatic mechanism. Growth factors and cytokines [particularly TNFα (tumour necrosis factor α) and IL-1 (interleukin 1)] have been implicated in the regulation of this catabolic process. Our work has shown that in degenerate discs there is an increase in IL-1, but no corresponding increase in the inhibitor IL-1 receptor antagonist. Furthermore, treatment of human disc cells with IL-1 leads to a decrease in matrix gene expression and increased MMP and ADAMTS expression. Inhibition of IL-1 would therefore be an important therapeutic target for preventing/reversing disc degeneration.

Introduction
The human IVD (intervertebral disc) is an important component of the spinal column, forming the ‘shock absorbers’ between each vertebra allowing bending, flexion and torsion of the spine. The normal IVD is composed of three distinct components: the CEP (cartilaginous end plates), the AF (annulus fibrosus) and the central gelatinous NP (nucleus pulposus).

The major components of the IVD are water, collagens, PGs (proteoglycans), non-collagenous proteins and elastins. These occur in unique tissue-specific patterns and contribute to the structure/mechanical function of the disc (Table 1). The turnover of the ECM (extracellular matrix) proteins within the disc is very slow, with turnover rate expressed in years rather than months with lowest rates found within the NP.

The collagen fibres of the IVD provide a strong durable framework which supports the cells and confines the highly hydrated PG aggregates. Approximately 90% of the collagen in the IVD is made up of fibrillar collagen types I and II [1]. The fibrils in the inner annulus are almost pure type II collagen, but the tissue becomes progressively enriched in type I collagen towards the outer annulus [1,2].

The IVD, particularly the NP, has a very high PG content. The high negative charge density of the PG complexes produces a large swelling pressure that is important for the ability of the disc to withstand compressive forces [3]. The major PG of the disc is aggrecan, a large PG that aggregates with HA (hyaluronic acid) via link proteins and contains both CS (chondroitin sulfate) and KS (keratin sulfate) [4]. Additionally the IVD also contains a small amount of versican, a second aggregating PG, which is distributed primarily in the AF [5,6], and a number of leucine-rich, small PGs, including decorin, biglycan, fibromodulin and lumican [6–9]. The function of these PGs is to modulate collagen fibrillogenesis and therefore influence the organization and mechanical properties of the tissue.

The concentration of aggrecan increases towards the inner annulus with corresponding increases in osmotic pressure, decreased hydraulic permeability and decreased diffusion [10,11]. The hydrated PGs of the NP confer viscoelasticity on the disc and provide resistance to compression on axial loading [12].

The NP is a gelatinous structure composed primarily of aggrecan and collagen type II together with small amounts of collagen types VI, IX and XI. The outer AF contains large amounts of collagen type I together with collagen types III, V and VI, and the inner annulus is transitional in its composition between the NP and the outer AF. The normal human IVD contains chondrocyte-like cells within the NP.

Key words: a disintegrin and metalloprotease with thrombospondin motifs (ADAMTS), degeneration, degradation, interleukin 1 (IL-1), intervertebral disc, matrix metalloproteinase (MMP).

Abbreviations used: ADAMTS, a disintegrin and metalloprotease with thrombospondin motifs, AF, annulus fibrosus; CS, chondroitin sulfate; ECM, extracellular matrix; IVD, intervertebral disc; KS, keratin sulfate; IL-1, interleukin 1; IL-1Ra, IL-1 receptor antagonist; LBP, low back pain; MMP, matrix metalloproteinase; NP, nucleus pulposus; PG, proteoglycan; TIMP, tissue inhibitor of metalloproteinases; TNFα, tumour necrosis factor α.

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Table 1 | Components of the IVD

<table>
<thead>
<tr>
<th>Component</th>
<th>AF</th>
<th>NP</th>
</tr>
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<tbody>
<tr>
<td>Water</td>
<td>60–70% (no change with age)</td>
<td>90% at birth</td>
</tr>
<tr>
<td>(no change with age)</td>
<td>80% at age 20</td>
<td>70% at older age</td>
</tr>
<tr>
<td>Collagens</td>
<td>50–60% (dry weight) (little change with age)</td>
<td>15–20% (dry weight) (little change with age)</td>
</tr>
<tr>
<td>PGs</td>
<td>15–20% (dry weight) (decreases with age)</td>
<td>65% (dry weight)  (decreases with age)</td>
</tr>
<tr>
<td>Non-collagenous proteins and elastin</td>
<td>5–25% (dry weight) (increases with age)</td>
<td>20–45% (dry weight) (increases with age)</td>
</tr>
<tr>
<td>Extracellular enzymes, age pigment, cells</td>
<td>Minor - remainder</td>
<td>Minor - remainder</td>
</tr>
</tbody>
</table>

and inner AF but fibroblast-like cells in the outer AF [13]. These cells are responsible for the synthesis and regulation of the matrix in the disc, controlling the homoeostasis between matrix synthesis and matrix degradation.

The matrix composition of the IVD is key to its function and if it degrades then this can result in reduced disc height (due to loss of matrix) and blood vessel and nerve in growth into the normally avascular and aneural IVD [14]. Such changes are associated with LBP (low back pain) [15]. LBP is a major cause of disability within the community, with approx. 11 million people within the U.K. experiencing LBP for at least 1 week in each month and leads to considerable loss of working days (108 million in 2000) with an estimated 11 billion pounds sterling in lost production. LBP also impacts significantly on the NHS (National Health Service) (e.g. over 6 million consultations per annum) and social services [16]. However current therapies, which predominately relieve symptoms, are often unsuccessful, and none to date targets the cause of LBP, which in most of the cases is caused by IVD degeneration.

Matrix synthesis and degradation during disc degeneration

The pathogenesis of IVD degeneration is a complex process and has a number of poorly understood biological and mechanical determinants [17]. However, understanding its pathogenesis is paramount to the development of future therapies. During disc degeneration a number of changes are seen within the ECM of the IVD. Many of these are simply due to breakdown of the normal matrix components (for example, smaller PG aggregates are found, and an overall decline in PG content is observed).

In addition to the overall degradation of the ECM during disc degeneration, patterns of synthesis and the composition of collagens within the disc alter. In the early stages of degeneration, collagen synthesis, in general, is increased, with a clear increase in collagen type II synthesis within the NP, possibly signifying an attempted repair mechanism [18]. With more advanced degeneration, synthesis patterns are altered, with more type II collagen appearing in the outer annulus, and type I, which forms stronger collagen fibrils, within the inner annulus and NP. In addition, type X collagen has been localized in degenerate discs, associated with chondrocyte clusters and cleft formation, signifying abnormal cellular activity [19].

The cross-linking of the collagen fibres within the IVD also alters during disc degeneration. Within the normal disc, especially within the NP, high concentrations of pyridinoline cross-links are found, but with degeneration these cross-links decrease and are replaced with the pentosidine cross-links [20], which make the tissue more prone to mechanical failure, and increase susceptibility to annular tears.

The synthesis of PGs also changes, with decreased aggrecan [13], and increased versican, biglycan and decorin production [8,21]. In addition, a change in the relative proportions of GAGs (glycosaminoglycans), from CS to KS, occurs, with a resultant loss in hydration [8,22]. Fibronectin content also increases with increasing grade of degeneration [23].

As well as changed synthesis patterns there is an increase in matrix degradation. The major degradation process is mediated by the MMPs (matrix metalloproteinases) and the ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs). The MMPs are a large family of extracellular zinc-dependent proteinases, divided into four main sub-families: collagenases, stromelysins, gelatinases and MT-MMPs (membrane-type MMPs) [24]. Throughout disc turnover, development and degeneration, the cells within the IVD produce a wide range of MMPs that degrade components of the ECM within the IVD. However, during disc degeneration the expression and activity of a number of MMPs are increased, including MMPs 1, 3, 7, 9 and 13, which degrade many of the main matrix components [25–28]. Our work has shown that of these MMPs, MMPs 7 and 13 (whose predominant targets are type II collagen and aggrecan) are expressed most highly within degenerate discs particularly in the NP (Figure 1) [25,26].

MMPs are normally secreted in a latent form, requiring activation for proteolytic activity and are inhibited by specific tissue inhibitors [TIMPs (tissue inhibitors of metalloproteinases)] of which four types are now known [29,30]. TIMP-1 and TIMP-2 form irreversible non-covalent complexes to active MMPs in a 1:1 stoichiometric fashion, inhibiting their enzyme activity. Each member of the TIMP family appears to show a degree of specificity to certain MMPs [29,31]. Our studies have shown that production of TIMP-1 and -2 is also up-regulated in the degenerate disc [25], which suggests that those MMPs (e.g. MMP 7) which are more resistant to TIMP-1 and -2 may play a greater role in the pathogenesis of disc degeneration [26].

Aggrecan has two major cleavage sites, the first is between Asn341 and Phe342, and several MMPs have been shown to cleave at this site [32]. However, the second cleavage site between Glu370 and Ala374 is not produced by the major MMPs, with the exception of MT1-MMP [33]. Members of the ADAMTS group of enzymes, namely 1, 4, 5, 8, 9 and 15 are aggrecanases cleaving at this site and have been shown to
play a key role in the PG breakdown in articular cartilage [34–36]. Recently, they have also been implicated in aggrecan degradation in the IVD and we have demonstrated that ADAMTS 1, 4, 5, 9 and 15 are produced by the cells of the IVD and that gene and protein expression are increased during disc degeneration [25,37]. ADAMTS are inhibited specifically by TIMP-3, which unlike the inhibitors of the MMPs is not up-regulated during disc degeneration [25,37], resulting in an excess of aggrecanase activity over the inhibitor, and dysregulation of the homoeostatic mechanism, leading to aggrecan breakdown. These changes within the disc ECM have a number of consequences: the disorganization and diminished levels of PGs and collagens result in loss of structural integrity, decreased hydration and hence a decreased ability to withstand load, resulting in many of the characteristic features of disc degeneration. The degradation of this matrix also results in the release of a number of soluble factors, and cytokines, which are stored within the matrix [38], thus affecting local cellular activities and matrix homoeostasis. Furthermore, the dehydration of the IVD and alterations within the ECM in proximity to the disc cells, also influence the availability of nutrients and growth factors to the cells [38,39], resulting in further impairment of normal disc cell function.

**Future therapeutic targets for degeneration of the IVD**

Conventional therapies for LBP simply address the symptoms and involve treatments such as pain modulators and surgery (e.g. discectomy and spinal fusion). However these therapies do not target the cause of LBP, i.e. the underlying altered cell biology in the IVD. As such developing therapies to interfere with the process of degeneration would be of potential benefit for the treatment of LBP. However, the targeting of therapies that promote matrix regeneration and repair would only be of benefit if this treatment were used in conjunction with therapies that would inhibit disc degeneration, thus removing the underlying defect. Therefore for a future therapy to be successful both the inhibition of disc degeneration and stimulation of regeneration by increased matrix synthesis must be addressed.

As such future therapies would be 2-fold, namely factors to: (i) inhibit the processes of disc degeneration and (ii) stimulate further matrix production. Our studies have suggested that IL-1 is an important cytokine in the pathogenesis of disc degeneration [40] and that its inhibition is possible using an *ex vivo* gene therapy approach to deliver the IL-1Ra
The use of IL-1Ra can then inhibit the stimulation of degradative enzymes and prevent the inhibition of matrix production brought about by IL-1 [40,50,51]. Stimulation of matrix production is a necessity in regeneration and could be achieved by the application of growth factors to the IVD. Our results have suggested that the BMP (bone morphogenetic protein) family of growth factors would be most suited to treat the degenerate disc [52]. Alternatively, stimulation of matrix synthesis could be achieved by the supplementation of the IVD with adult mesenchymal stem cells. Work from our laboratory has shown that such cells differentiate to a disc cell-like phenotype when appropriately stimulated [53,54].

Conclusion
During disc degeneration the careful balance between anabolic and catabolic factors is altered, which results in a loss of matrix and disc function, which can ultimately result in LBP.

References
50 Le Mare, C.L., Freeman, A.J. and Hoyland, J.A. (2006) J. Pathol. 210 (Suppl. 1), 56

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