

# The elastic fibre network of the human lumbar anulus fibrosus: architecture, mechanical function and potential role in the progression of intervertebral disc degeneration

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**Abstract** Elastic fibres are critical constituents of dynamic biological structures that functionally require elasticity and resilience. The network of elastic fibres in the anulus fibrosus of the intervertebral disc is extensive, however until recently, the majority of histological, biochemical and biomechanical studies have focussed on the roles of other extracellular matrix constituents such as collagens and proteoglycans. The resulting lack of detailed descriptions of elastic fibre network architecture and mechanical function has limited understanding of the potentially important contribution made by elastic fibres to healthy disc function and their possible roles in the progression of disc degeneration. In addition, it has made it difficult to postulate what the consequences of elastic fibre related disorders would be for intervertebral disc behaviour, and to develop treatments accordingly. In this paper, we review recent and historical studies which have examined both the structure and the function of the human lumbar anulus fibrosus elastic fibre network, provide a synergistic discussion in an attempt to clarify its potentially critical contribution both to normal intervertebral disc behaviour and the processes relating to its degeneration, and recommend critical areas for future research.

**Keywords** Intervertebral disc · Anulus fibrosus · Elastic fibres · Elastin · Structure–function

## Introduction

Intervertebral disc degeneration is characterised in its late stages by progressive microstructural derangement of the anulus fibrosus extracellular matrix. Comprehensive descriptions of the structural and functional inter-relationships between the constituents of this matrix are, therefore, critical for understanding the degenerative process and developing effective treatments.

The anulus matrix has a complex, hierarchical architecture comprises collagens, proteoglycans and elastic fibres. Elastic fibres, possessing unique mechanical properties that include high linear elasticity and high extensibility, perform essential functional roles in dynamic biological structures, including arteries, lungs and skin and many other connective tissues [30]. Early studies examining the presence of elastic fibres in the intervertebral disc tended to dismiss their potential structural and mechanical contributions as inconsequential, perhaps due to the perceived sparseness of their distribution and low relative percentage of total tissue weight. Recently however, new experimental findings have expanded the field's understanding of the elastic fibre network from both structural and mechanical perspectives, demonstrating that, together with more ubiquitous constituents such as collagens, proteoglycans and water, elastic fibres perform an important functional role.

The objectives of this review were: to summarise the findings of recent and historical studies which have examined both the structure and the function of the human lumbar anulus fibrosus elastic fibres network; to provide a synergistic discussion in an attempt to clarify its potentially

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critical contribution to intervertebral disc behaviour; to discuss its potential role in the onset and progression of intervertebral disc degeneration; and to propose critical areas for future research.

## Background

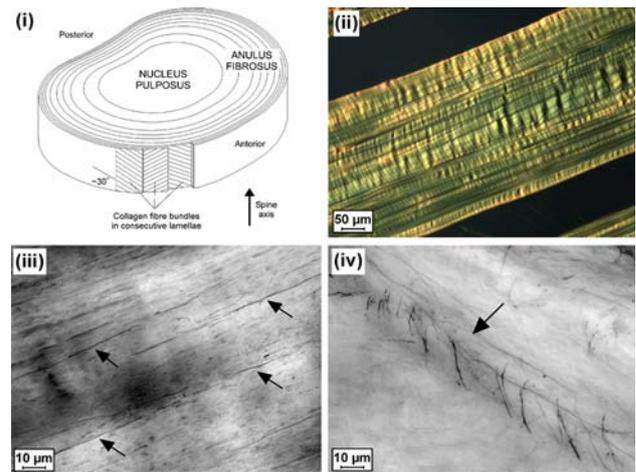
### The annulus fibrosus

Water forms the bulk of the tissue weight of the annulus, 65–75% in inner regions and 55–65% in outer regions [67]. Collagens constitute the bulk of the extracellular matrix in terms of dry weight, comprising 40–60% of the outer annulus and 25–40% of the inner annulus [21, 67]; collagen types I and II are the most prevalent; opposing gradients between these collagen types exist radially from the disc periphery to the nuclear transition zone, with the concentration of collagen I greatest at the periphery [13]. Proteoglycans, principally aggrecan, represent the second greatest constituent of the disc in terms of dry weight after collagen constituting 5–8% of the outer annulus and 11–20% of the inner annulus [67]. An increasing gradient in proteoglycan concentration exists from the annulus periphery to the transition zone [12, 67]. The balance of the extracellular matrix comprises elastic fibres, minor collagens, small proteoglycans, other glycoproteins and lipids [12, 21].

The mechanical function of the annulus is twofold: to contain the radial bulge of the central nucleus pulposus, enabling the uniform distribution and transfer of compressive loads between the vertebral bodies; and to distend and rotate, facilitating joint mobility. These functions are made possible by its unique, microstructural composition, which comprises interacting compressive and tensile load-bearing structural elements. Resistance to compression is provided by inter-fibrillar water, the presence of which is regulated by poly-electrolytic glycosaminoglycans. The fundamental tension-bearing elements are bundles of type I collagen fibrils, which are arranged obliquely to the axial plane of the disc in discontinuous, approximately concentric lamellae around the nucleus [38]. This architecture is illustrated schematically and histologically in Fig. 1 i and ii, respectively. Like its biochemistry, the microstructure of the annulus is functionally graded—the number, angle and crimp morphology of the collagen bundles exhibit heterogeneity with both radial and circumferential position, as do the thickness and continuity of the lamellae [8, 38].

### Elastic fibres

Mature elastic fibres consist of a core of the protein elastin, which is integrated within a scaffold of microfibrillar



**Fig. 1** *i* Schematic illustration of intervertebral disc anatomy showing alternating collagen bundle orientations in consecutive lamellae, *ii* van Gieson staining of a collagen bundle in the annulus fibrosus of healthy, 40-year-old L3–L4 disc, viewed in the plane parallel to the circumferential surface of the disc under cross-polarised light at  $\times 20$  objective magnification, *iii* resorcin-fuchsin staining of an adjacent section in the same orientation, showing intra-lamellar elastic fibres (arrows, viewed under phase-contrast at  $\times 100$  objective magnification) oriented parallel to the direction of the collagen fibres, *iv* resorcin-fuchsin staining of elastic fibres in the annulus fibrosus of a non-degenerate, 28-year-old L3–L4 disc, forming a cross-connecting meshwork at a lamellar interface (arrow). This section is oriented in the plane transverse to the axis of the spine, viewed under phase-contrast at  $\times 100$  objective magnification. Detailed methods used to obtain *ii*, *iii* and *iv* have been published previously [61, 62]

glycoproteins [29, 30]. Previously considered amorphous, the elastin core has more recently been shown to be composed of laterally packed, thin-beaded filaments; the microfibrils form loosely packed parallel bundles [30]. Molecular constituents of elastic fibres can be divided into three categories: those of the elastic fibre core; those co-localising with microfibrils; and those associated with the core–microfibril interface [30]. In the core, the mature, insoluble elastin polymer comprises multiple tropoelastin molecules (65–70 kDa) bound covalently by bi-, tri- or tetra-functional crosslinks, including lysinonorleucine, desmosine and isodesmosine; the fibre core also contains fibulin-1 and the proteoglycan biglycan [11, 29]. The principal structural molecules of elastic microfibrils are the fibrillins 1 and 2, in addition to microfibril associated glycoproteins (MAGPs) and the proteoglycans perlecan, versican and decorin [29]. Molecules associated with the elastin–microfibril interface include latent TGF- $\beta$  binding protein 2 (LTBP-2), fibulin-2, emilin-1, and the proteoglycans versican and decorin [29]. Although commonly referred to somewhat generically in the literature as either ‘elastic fibres’ or ‘elastin fibres’, specific terminologies exist to describe those with differing ultrastructures and biochemical compositions. These include: mature elastic fibres; elaunin fibres, which have a reduced elastin

component relative to the microfibrillar component; and oxytalan fibres, which have no elastin component and are thus composed entirely of microfibrils [44]. As well as existing as fully developed fibres in their own right, during development, elaunin and oxytalan fibres may appear as progenitors of mature elastic fibres [44]. Henceforth, the use of the term ‘elastic fibres’ in this review will encompass all three fibre types. Where the term ‘elastin’ is used, it is to refer specifically to the primary protein constituent of the mature elastic fibre core.

Elastin, in isolation exhibits high linear elasticity, is highly extensible and has an elastic modulus of approximately 0.5 MPa [16]. The mechanical properties of elastin are distinct from those of fibrillin-containing microfibrils, which have an elastic modulus of between 78 and 96 MPa, around two orders of magnitude greater than that of elastin [60]. Mature elastic fibres can, therefore, be considered composites in which mechanical properties are defined according to the combined responses of the elastin core and the surrounding microfibrillar scaffold [60]. In addition, the mechanical properties of pure elastin are highly dependant on hydration. Elastic fibres require water to exhibit their rubber-like elasticity. Without the exposure to an appropriate swelling agent, elastin behaves as a rigid solid similar to glass [17]. As a result, elastin is said to undergo a ‘glass transition’, which is also dependent on temperature [28].

### Elastic fibres in the non-degenerate anulus fibrosus

#### Ultrastructure and distribution

Ultrastructural studies have described mature elastic fibres in the anulus as having both a central elastin core and surrounding microfibrils [2, 7, 41, 54]. Age-related differences in the ultrastructure of these fibres are consistent with those observed in other tissues; those of young specimens have a less developed central core, with these differences appearing to be more pronounced in the anulus fibrosus than the nucleus pulposus [7]. Developing fibres in foetal discs consist almost entirely of microfibrils [20].

Gravimetric methods have estimated the overall elastin content of the human disc to be 1.7% of its dry weight [41], being slightly higher content in the anulus (approximately 2%) than in the nucleus (approximately 1.5%) [48]. Most recently, a commercial dye-binding assay was used to demonstrate that the non-degenerate disc contains approximately 2% elastin (in dry weight terms), but found no significant differences in content between the inner anulus, outer anulus and nucleus [10].

Early microstructural observations of the anulus elastic fibre network included: significant numbers of elastic fibres

in regions of the disc directly associated with adjacent vertebral bodies; elastic fibres within anulus lamellae having circular, longitudinal and oblique orientations; and elastic fibres in the vicinity of the transition zone having a three-dimensional mesh pattern [27]. A close association between location and orientation of elastic fibres and collagen fibres was also observed, and it was noted that like collagen fibres, elastic fibres appear to extend from the disc into the vertebral body at the anulus periphery as Sharpey’s fibres, in an apparent anchoring role. Estimated histologically, the overall area occupied by elastic fibres in the anulus was found to be 10.3% [25], which, when compared with biochemical estimates, may reflect the significant proportion of those fibres consisting of non-elastin components.

More recent microstructural studies have revealed that elastic fibres exist in a more extensive and organised network. Immunohistochemical techniques have demonstrated a complex network of elastic fibres extending throughout all regions of the disc [72, 74]. In bovine tail discs, elastic fibres within the anulus lamellae were found to be predominantly parallel to the collagen fibre bundles, and appeared densely concentrated at the interfaces between consecutive lamellae [74]. Similar findings were subsequently made in the human disc [72]. These distribution patterns appear to be distinct from those present in the nucleus pulposus, where fibres appear to radiate outwards, both horizontally towards the transition zone and vertically towards the end plates [74]. Immunohistochemical techniques have been used to examine the distribution of both elastin and fibrillin-1 in intervertebral disc specimens from adolescent humans and bovine tails [73]. Fibrillin-containing microfibrils were found to be co-distributed with elastin in all regions of the disc. The apparent wide-spread presence of fibrillin relative to elastin may reflect a relatively important role for these stiff, reinforcing microfibrils in the mechanical function of the anulus matrix; this potential role should be considered in the context of the significant contribution made to radial anulus stiffness and extensibility by elastic fibres [61] discussed elsewhere in this review.

Variations in the density of elastic fibres within the anulus lamellae with both radial and circumferential position have been described [62]. Fibre density was found to be significantly higher in lamellae close to the periphery than in those closer to the nucleus, and significantly higher in the posterolateral quadrant of the anulus than the anterolateral. These findings suggest that lamellar elastic fibre density may be commensurate with the magnitude of the tensile deformations experienced by the anulus in bending and torsion. This study also described microarchitectural differences in the arrangement of elastic fibres at distinct levels of the collagen structural hierarchy: in

contrast to fibres within lamellae, which have a single preferential alignment (Fig. 1iii), those at lamellar interfaces form discrete criss-cross meshworks (Fig. 1iv). An additional, qualitative observation included that elastic fibres in the lamellae of the inner annulus appeared to have a looser, less uniform pattern of arrangement than those in the outer lamellae. These architectural observations are in agreement with the earlier findings made with respect to canine cervical discs [26].

The existence of trans-lamellar ‘cross-bridges’ in the annulus, and their chemical composition, has been described [40, 57, 73, 74]. Observed in both the transverse and sagittal planes, these structures appear to form connections between collagen bundles in non-consecutive lamellae. The histology presented in these studies suggests that cross-bridges may traverse lamellae by passing between adjacent bundles in those lamellae, i.e. through inter-bundle spaces [38], enabling them to function without interrupting the intralamellar structure. In addition to containing aggrecan, versican and type VI collagen [40], these cross-bridges contain densely arranged elastic fibres [73, 74].

## Function

The findings of the structural studies which have been outlined have provided an essential framework allowing researchers to hypothesise as to the possible contributions made by elastic fibres to the mechanical behaviour of the annulus fibrosus; nonetheless, direct experimental examination of that contribution, both at the tissue and motion segment levels, is critical. At the motion segment level, such studies are yet to be undertaken, and only one such study has been conducted at the tissue level. Before discussing the findings of this study and their implications, it is constructive to briefly examine such contributions in several other dynamic tissues, for which there is already a substantial body of experimental evidence.

In skin, for example, which comprises just one percent elastin by dry weight, a delicate, scattered network of elastic fibres between the collagen fibres enhances quasi-static mechanical properties in the toe region of the stress–strain response [49]. In the human aorta, where the elastin content peaks at approximately 50% with maturity [56], elastic fibres form dense concentric laminae between the intimal and medial, and between the medial and adventitial layers of the artery, enhancing toe region modulus and extensibility, but contributing little to the response at higher strains [3, 42]. In the aortic valve, where elastin constitutes around 13% of the dry tissue weight, it has been theorised that elastic fibres act as a ‘house keeper’, by restoring the rest state of mobile collagen fibres within the matrix following large deformations [68]. Elastic elements are arranged in a complex network of sheets, tubes and

fibres; the nature and magnitude of their tissue-level mechanical contributions has been demonstrated to be unique in each layer of the valve and with varying orientations [68]. Following the removal of elastic elements, valves have been observed to undergo passive distension, possibly due to an associated relaxation of the collagen matrix, and exhibit reduced toe region modulus under tensile loads [37, 42]. In the elastic wing tendon of the domestic fowl, a tissue morphologically similar to the ligamentum nuchae [46], selective removal of elastin from the tissue matrix has been found to eliminate the toe region of the stress–strain response, presenting as a large decrease in extensibility; it has also been observed that the conformational folding of the collagen matrix in the unloaded tissue relaxes, with histological analyses suggesting that this rest state morphology is maintained by elastic fibres. A reduction in the tensile strength of these tendons was also observed. In the mature human lung, elastin constitutes approximately 30% of the dry tissue weight [9]; elastic elements form a complex, three-dimensional fibrous network, interwoven with collagenous elements, and there is evidence of mechanical connections between the two [65]. In a guinea pig model, the effect of enzymatically removing elastin on the quasi-static mechanical properties of lung parenchymal strips was to cause a large reduction in initial modulus and an increase in extensibility [75].

The results of these studies demonstrate that the nature and magnitude of the contribution made by elastic fibres to the mechanical behaviour of composite, dynamic, biological tissues varies considerably with tissue type. Differences appear to be attributable to a variety of factors, which likely include: the relative orientations of elastic and collagenous constituents with respect to the direction of the applied load; the ability of fibrous elements to straighten and reorient within the tissue towards principal stress axes; the existence and nature of physical connections between co-distributed elastic and collagenous elements; the chemistry and ultrastructure of the elastic fibres; and the total number of elastic fibres present.

To date, only one study has examined the tissue-level contribution made by elastic fibres to the quasi-static, tensile mechanical properties of human lumbar annulus fibrosus, specifically in radially oriented specimens [61]. Using biochemically validated, targeted enzymatic treatments, removal of elastin from the extracellular matrix was found to result in a significant decrease in toe region modulus (from 0.07 to 0.004 MPa) and linear-region modulus (from 0.21 to 0.02 MPa), and a significant increase in extensibility (from 0.16 to 0.93 mm/mm). The magnitude of this contribution is interesting in consideration of the fact that the elastic fibre content as a percentage of dry tissue weight in the annulus is relatively small [10, 48]. To understand how elastic fibres convey this

functionality, it is helpful to examine their potential interactions with other matrix constituents, in particular collagen, with which at the microstructural level they appear to closely associate. Bending and compression of the intervertebral disc within the confines of each motion segment are made possible by the circumferential and axial expansion of the lamellae; this expansion is facilitated by both the direct extension of the collagen fibre bundles and their tilting relative to the transverse plane [18, 31, 32, 51]. In circumferential expansion, direct extension of collagen fibre bundles (fibre strain, measured at the peripheral surface) as a total percentage of tissue deformation is relatively large [59, 64]. In contrast, with respect to the positive axial deformations associated with bending, the percentage contribution of collagen fibre strain to total deformation (increase in disc height) is relatively small, suggesting that collagen fibre reorientation plays a more dominant role [50, 64]. Analytical structure–function modelling has highlighted the relative importance of shear and normal interactions in determining the tensile mechanical response anulus fibrosus specimens, particularly in the axial direction [19], which is consistent with the idea that relative collagen fibre reorientation is the predominating deformation mechanism for this orientation.

The structural mechanism which enables the extension of the collagen fibre bundles along their principle load-bearing axis is the straightening of the collagen fibre planar crimp; extension of the bundles beyond the straightening of this crimp is limited and leads to progressive localised structural failure [51]. The structural mechanism which facilitates tilting is relative collagen fibre reorientation, or inter-fibre ‘sliding’ [6]. In circumferential tension, the tilt angle (the angle of the fibres to the transverse plane) decreases as fibres re-orient towards the loading direction; in axial tension the opposite occurs [5, 18, 31, 32]. Tensile deformation of lamellae could therefore be considered a two-stage process: on the initial application of load, the straightening of crimp occurs first, functioning perhaps more as a shock-absorbing mechanism to prevent sudden impact damage to the collagen fibres, similar to in tendons; larger-scale tensile deformation, particularly that which occurs axially in bending, then follows, facilitated by collagen fibre re-orientation. The question that arises from this two-stage mechanism of deformation is: in which of these two modes is the functional role of intralamellar elastic fibres most important—crimp extension or collagen fibre reorientation?

There are a number of factors which appear to discount the possibility that elastic fibres play a significant role maintaining collagen crimp under zero strain and re-establishing crimp following deformation. First, three-dimensional reconstructions of elastic fibre arrangements in the lamellar plane [62] and immunohistochemical

studies in the transverse plane have demonstrated that intralamellar elastic fibres mimic the crimp pattern of the surrounding collagen [73]. If fibres were to be maintaining crimp, it is reasonable to assume that they would initially be under tension and hence appear straight, but these observations suggest collagen crimp must straighten before elastic fibres become mechanically viable. Secondly, collagen crimp is present in other tissues which contain no documented elastic fibres, such as Achilles tendon, suggesting it is an intrinsic property determined by collagen ultrastructure [14, 55]. Finally, the relatively small strains along the collagen fibre axes attributable to uncrimping [51, 64] would seem to make elastic fibres redundant in consideration of their high extensibility [16].

With respect to the second of these possibilities—that elastic fibres limit and reverse relative fibre reorientation—it would be, by inference, necessary for them to provide cross-collagen fibre mechanical connectivity. Uniaxial strains applied to radially orientated anulus fibrosus specimens, perpendicular to the plane containing the collagen fibres have been found to result in multidimensional rearrangement of intralamellar elastic fibres [63]. Some fibres appeared long and straight, suggesting they were experiencing tension, and others shorter and crumpled, suggesting they were relaxed or experiencing compression. This pattern of arrangement stands in contrast to that observed in unstrained specimens, where arrangement is largely unidirectional [62, 73]. These observations suggest that elastic fibres aligned parallel to collagen fibres maintain discrete points of connection between adjacent collagen fibres. It is possible that these points of connection are located at either the extreme ends of elastic fibres only or at multiple locations along their lengths—the fact that a number of elastic fibres have been observed to undergo sharp changes of transition along their length [63] suggests that the second of these possibilities is the case.

Such cross-collagen fibre connectivity by elastic fibres may also be important in the context of radial deformations. Nuclear migration during bending subjects the anulus to tensile radial strains of up to 10%, depending on disc condition, region and bending modality [66]. Anulus deformation in response to such strains occurs by way of both transverse collagen bundle elongation and separation at lamellar interfaces [52]. Elastic fibres potentially provide important transverse mechanical integrity that limits this deformation and assists its reversal.

Elastic fibres located at the interfaces between consecutive lamellae display greater structural complexity in their arrangement than those within lamellae, perhaps due to the fact that they integrate with collagen fibres with opposite preferential orientations. In bending, compression and torsion, the ‘interlamellar angle’ between collagen fibres in consecutive lamellae varies as those fibres tilt in opposite

directions. This change in interlamellar angle constitutes significant shear at the lamellar interface [6]. The mechanical function of interlamellar elastic fibres may be similar to intralamellar elastic fibres, but conveyed at a higher level of the structural hierarchy—i.e. between lamellae instead of between fibres.

Indeed, at these interfaces elastic fibres have been observed to maintain physical connections between collagen bundles in consecutive lamellae undergoing localised separation [63]. Evidence that fatigue-related damage is commonly manifested as separation of anulus lamellae highlights the potential importance of these connections [23]. Importantly, elastic fibre connections were observed not to be continuous along the entire interface, but instead appeared to form discrete points of adhesion. The length of these adhesions should be considered with reference to the fact that in axial plane of the disc, collagen bundle cross-sections are oblique. It is unclear from these results as to whether interlamellar connections comprises elastic fibres alone, or a combination of elastic fibres and collagen—the inclusion of collagen would provide the connections with additional tensile strength. Elastin and collagen co-distribution at these locations could be investigated in a future study using immunohistochemistry.

The physiological relevance of cross-connecting elastic fibres within and between lamellae is evident when considered in the context of the most common manifestations of matrix damage which have been observed following both fatigue loading and over-pressurisation [22, 23, 53]. For example, in the absence of the elastic fibres which maintain adhesion between lamellae, the shear strains which occur as a result of relative reorientation between those lamellae in bending and torsion may increase the propensity for delaminations and circumferential tears to form. Within lamellae, in the absence of elastic fibres providing cross-collagen fibre connectivity, relative reorientation between adjacent collagen fibres could potentially occur more easily, and the restoration of the homeostatic configuration of those collagen fibres be less effective and less complete. A consequence of such a loss of cohesion may be progressive disorganisation within lamellae, which over time may enable radial fissures, including those that ultimately lead to nuclear prolapse, to propagate more easily.

With respect to trans-lamellar cross-bridges, the presence of elastic fibres in these structures [73, 74] suggests they may be capable of extending to, and recovering from, significant tensile strains, while the presence of collagen VI [40] suggests they also possess some intrinsic tensile strength. Evidence of such a composite mechanical capability may indicate the potential importance of anular cross-bridges in restoring the hierarchical lamellar structure following the complex normal and shear strains

experienced by the anulus during physiological deformation.

### **Roles of elastic fibres in the ageing and degeneration of the anulus fibrosus**

Elastic fibre loss and damage are major contributing factors in the degradation of a number of tissues. Typically, pathogenesis begins with a decrease in the proportion of microfibrils to elastin, followed by degradation of the elastin core [29]. Common degenerative conditions affecting dynamic tissues as a direct or indirect result of elastic fibre degradation include vascular aneurysm formation, pulmonary emphysema and photo-ageing of skin. Hereditary disorders such as Marfan's syndrome and Beal's syndrome directly affect fibrillin-containing microfibrils, leading to severe vascular disease and physical deformity [29].

In the intervertebral disc, there is conflicting evidence as to whether elastic fibres, as a percentage of the total tissue, increase or decrease with ageing and degeneration. Using gravimetric techniques alone, an early study found no apparent change in total elastin content of the intervertebral disc with age [41]. In a subsequent study however, it was found that the elastin content as a percentage of total dry tissue weight increased steadily in both the anulus and the nucleus until reaching a peak at approximately 40 years, then decreased progressively until death [48]. The peak elastin content of the anulus (in terms of dry weight) was found to be approximately two percent, and the peak elastin content of the nucleus was found to be slightly less, at approximately 1.6%. In addition, it was found that the elastin to collagen ratio decreased steadily from birth until death, and that the elastin to glycosaminoglycan ratio increased steadily until approximately the age of 40 before plateauing. Recently, degeneration related variations in the elastin content of human discs were described [10]. In terms of dry weight, the mean elastin content, measured using a commercial dye-binding assay, of non-degenerate discs was found to be approximately 2%. Elastin content was found to be positively correlated with degeneration, with the highest elastin content of 9.3% occurring in the inner anulus of degenerate specimens. In addition, degenerate specimens exhibited significant differences in elastin content between the inner anulus, and the outer anulus and the nucleus.

Any significant change in the elastic fibre content of the disc, be it an increase or decrease, would almost certainly have associated mechanical consequences. Tissue-level changes in anulus fibrosus mechanical properties with ageing and degeneration have been well documented. In the radial orientation, tissue from moderately degenerate discs displays weakened yield strength and ultimate tensile

strength, and increased toe and linear-region modulus compared with non-degenerate discs [15, 61]. In the circumferential orientation, Poisson's ratio increases, and tensile yield strength, strain energy density and fibre reorientation decrease with degeneration [1, 18]. Toe region modulus is positively correlated with age [18]. These changes appear consistent with an increase in elastin content, given the demonstrated role of elastic fibres in reinforcing the stiffness of the extracellular matrix [61], although other matrix changes such as increased glycation and decreased hydration also likely contribute [1, 69].

There is some evidence that elastic fibre network architecture in the disc changes as a result of spine related pathologies. The arrangement of fibres in discs from patients exhibiting neuromuscular and idiopathic scoliosis is sparse and disorganised compared with that observed in discs from non-scoliotic cases [72]. It is unclear, however, whether these changes are causal or consequential with respect to the pathology.

Deposition, degradation and remodelling of the anulus fibrosus elastic fibre network is likely mediated by specific serine proteases, matrix metalloproteinases and their respective tissue inhibitors. Elastin is readily degraded by the serine proteases pancreatic and neutrophil (or leukocyte) elastase, as well as several matrix metalloproteinases including MMP-2, MMP-7 (matrilysin), MMP-9 and MMP-12 (metalloelastase) [24, 29, 39]. In the disc, only limited descriptions of the presence and function of these enzymes currently exist. MMP-7 expression by cells of the nucleus and inner anulus has been demonstrated, more so for degenerate discs and those having suffered prolapse than for non-degenerate discs [35]. MMP-2 has been shown to be present in nucleus material from discs suffering from the early stages of degeneration, and also in the vicinity of nuclear clefts and anular tears in degenerate discs [34, 70]. MMP-9 has been found to be expressed only at very low levels [70]. As of yet no studies examining the presence of MMP-12 appear in the literature. Serine elastase has been identified in the human disc, and while its activity was shown to be greater in the anulus than the nucleus, its function remains undetermined [58]. As discussed previously, elastic fibres contain molecules other than elastin, in particular fibrillins, which are the principal components of elastic microfibrils. Fibrillins are degraded by several members of the matrix metalloproteinase family [4]; in particular, these include MMPs-2, -3, and -13, which demonstrate increased presence in degenerate discs [36].

### Future work

Given the demonstrated importance of elastic fibres for the correct mechanical function of the anulus fibrosus,

clarification of the nature of the changes in elastic fibre distribution and density with age and pathology is critical. Such studies could be either histological or biochemical, but should be conducted in the context of the significant structural heterogeneity in the elastic fibre network which has been described. In the context of these potential changes, irrespective of their nature, biological investigations examining the presence and distribution of elastic fibre degrading proteases and matrix metalloproteinases relative to their respective tissue inhibitors are also potentially very important. An example of such an investigation would be to compare the relative genetic expressions of these enzymes in intervertebral discs at various ages and stages of degeneration.

Future studies should address the contribution of elastic fibres to mechanical behaviour at the motion segment level. The application of the enzymatic degradation techniques used previously for tissue-level studies may be problematic for studying motion segments, as the ability to ensure uniform diffusion of the enzyme over such a large area would be difficult; however, they may be applicable for well-established small animal models, such as rats. Alternatively, elastin or fibrillin knock-down and knock-in animal models may potentially be valuable future tools for such studies.

The mechanical contribution of elastic fibres to anulus behaviour at the tissue level has so far only been investigated for radially oriented specimens. Future studies should expand on these results to include other test orientations and other structure–function associations. For example, it has been suggested in this review that elastic fibre degradation impacts significantly on the mechanisms which underlie collagen fibre reorientation in axial and circumferential expansion—studies could, therefore, investigate potential correlations between elastic fibre density and changes to collagen fibre tilt angle, and examine the effect of targeted enzymatic removal of elastic fibres on that property. Targeted enzymatic degradation techniques could potentially provide a useful tool for experimentally validating current and future structure–function analytical models of anulus fibrosus behaviour.

To date, only one study has examined the distribution of elastic fibre associated microfibrillar proteins in the anulus at the microstructural level [73], and both this and ultrastructural studies have been qualitative in nature. As the mechanical properties of elastic fibres are determined by interactions between the elastin core and surrounding microfibrillar scaffold [60], careful examination of how the ratio between these two ultrastructural components may vary with position in the disc should be a focus of future research.

Tissue engineering is potentially a very important tool for the repair and replacement of degenerate intervertebral discs [47]. Studies to date have focussed on the successful

synthesis of proteoglycans and collagen by anulus fibrosus and nucleus pulposus cells seeded on scaffolds, as those constituents which are required to achieve in vivo structural and functional equivalence [43, 45, 71]. In light of the findings discussed in this review, future studies should be expanded to investigate and promote the production and integration of elastic fibres. It is likely that inclusion of elastic fibres which transversely integrate collagenous elements would enhance the ability of tissue-engineered structures to replicate the mechanical anisotropy of the anulus matrix. Organ culture systems of the kind used in tissue engineering, and those recently developed specifically for the intervertebral disc [33], also present the opportunity to investigate the types of mechanical stimuli which result in the production of elastic fibres and their distribution in the patterns described at each level of the anulus structural hierarchy.

Finally, while this review has focussed on the anulus fibrosus, the nucleus pulposus also contains a network of elastic fibres with its own distinct architecture [73]. Future experimental studies should aim to uncover more about the structure of this network, and its contribution to healthy and degenerate nucleus behaviour.

**Conflict of interest statement** None.

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