Elastic tissues of the intervertebral disc

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Abstract

Elastic fibres have been generally considered to play no significant role in the mechanical function of the intervertebral disc since earlier studies reported that the elastic fibre network was sparse and irregular. However, a recent study has reported that the network is highly organized and that the distribution and orientation of elastic fibres varies from region to region. In the annulus, elastic fibres appear densely distributed in the nuclei where long straight fibres are radially organized in the lamellae. They are also organized in the bridges across the lamellae. To whom correspondence should be addressed (e-mail jing.yu@physiol.ox.ac.uk).

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oriented and anchor perpendicularly or obliquely into the cartilaginous endplate. Immunohistochemistry using specific antibodies indicates that elastin is present in the network, as is fibrillin. Biochemical studies show, however, that the amino acid composition of the residue remaining after alkaline (NaOH) extraction or CNBr digestion contains a higher concentration of polar amino acids than ligamentum nuchal elastin. The composition of the residue suggests that disc elastin may cross-link strongly with some other matrix components. With such coupling, it is thought that elastic fibres could play a significant mechanical role even though overall elastin is less than 5 % of the total dry weight of the disc.

Introduction
Elastic fibres in the intervertebral disc (IVD) have been thought to be sparsely and irregularly distributed and to play no significant roles in disc mechanical functions [1-5]. These thoughts have perhaps been the key reason for the fact that the elastic fibres of the IVD have not been extensively studied when compared with the numbers of studies on the other macromolecular components of the disc, such as collagen and proteoglycan. However, a very recent study has found that an abundant elastic fibre network, revealed immunohistochemically, is distributed over all the regions of the disc and has also described a higher degree of organization than seen previously [6]. This recent finding has provided new insight into the functions of the elastic fibres of the IVD.

Overview of elastin and elastic fibres
In general, elastic fibres are found in tissues that need resilience and elasticity for their function, with variation in the structural appearance of the elastic fibres depending on functional requirements. Rod-like fibres have been found in nuchal and other elastic ligaments, concentric elastic lamellae have been found in blood vessels and a three-dimensional meshwork of elastic fibres has been found in auricular cartilage [7]. The function of elastic fibres in tissues such as skin, lung and aorta thus depend not only on the chemical composition of the fibres but also on their organization or architecture.

Elastic fibres in general are composed of two morphologically distinct components: centrally located amorphous elastin is surrounded peripherally by microfibrils [8]. Elastin and microfibrils are different in their morphology and chemical composition [7]. Amorphous elastin is the most hydrophobic and highly insoluble extracellular matrix protein and is formed from its soluble precursor, tropoelastin. Both tropoelastin and insoluble elastin are rich in hydrophobic amino acids such as glycine, alanine, proline and valine, which are considered to contribute to the elastic properties of the molecule, whereas insoluble elastin contains the unique amino acids desmosine and isodesmosine [9]. Microfibrils primarily consist of fibrillins and other structural glycoproteins such as fibulin-5 and emilin [7]. These proteins have a high content of polar amino acids, such as aspartate, glutamate and cysteine.

Elastin and microfibrils are closely related during the process of elastogenesis [10]. During the formation of elastic fibres, tropoelastin is secreted from cells into the extracellular space and subsequently cross-linked by the enzyme lysyl oxidase in the presence of Cu²⁺ into an insoluble elastin. The assembly of tropoelastin requires the microfibrils to act as a scaffold for its deposition and orientation. Elastic fibres contain a high fraction of microfibrils during their early development; as the fibre matures, the proportion of microfibrils gradually falls until the central amorphous elastin forms more than 90 % of the mature elastic fibre, while the microfibrillar components are displaced to the periphery of the growing fibres [11]. Various studies have reported that emilin [12] and fibulin-5 [13,14] are essential components for the formation of new elastic fibre. Emilin seems to be associated with elastic fibres at the interface between amorphous elastin and microfibrils [15] and is thought to be important for the normal alignment and deposition of tropoelastin on to the microfibrils. While fibulin-5 appears able to link elastic fibres to cells and it has been suggested that it may regulate the rate of elastin deposition on to microfibrils by promoting the initiation or termination of fibre formation over a specific cell surface area [16]. Mutations in the genes of elastic fibre components such as elastin, fibrillin or fibulin-5 have been shown to generate abnormal elastic fibre formation and consequently cause disorders [17].

Elastin and elastic fibres in IVDs
Elastin of IVDs
The elastin content of the IVD and its amino acid composition have not been widely studied [5]. Mikawa et al. [5] reported that the elastin content of human annulus fibrosis (AF) was no different to that of nucleus pulposus (NP), and also that there
was no change in elastin content with aging or between sexes. In contrast, Olczyk [18] reported that human AF contained more elastin than that of the NP and also that there were significant age changes in elastin content. Olczyk [18] found that elastin content of both the AF and NP increased during the first four decades of life and then decreased in later life. In addition, with aging, the ratio of elastin to collagen was found to decrease slightly in the AF but sharply in NP whereas the ratio of elastin to glycosaminoglycan (GAG) increased greatly in the first decade and reached a stable level in later life in both AF and NP. The conflicting findings between the studies of Olczyk [18] and Mikawa et al. [5] could result from the differences in the elastin-purification methods used and also variation in elastin content in different levels of the spine since Mikawa et al. examined a broad level of IVD and Olczyk mostly studied lumbar IVD.

Mikawa et al. [5] have also reported the amino acid composition of the IVD residue after 0.1 M NaOH extraction at 98 °C. The residue, presumed to be elastin, was enriched in polar amino acids such as aspartate, glutamate, arginine and particularly histidine and lysine, but depleted in valine, alanine and, to a lesser extent, glycine, compared with ligamentum nuchal elastin. The amino acid composition of the elastic tissue found in bovine IVD (P. Winlove and J. P. Urban, unpublished work) corresponds to the finding by Mikawa et al. [5], and was thus very similar to that found in certain cartilage and pathological tissue [20]. Since IVD disc sections show positive elastin immunostaining [6] and also disc cells express elastin (J. P. Urban, unpublished work), it appears unlikely that disc cells synthesize a different type of elastin, as suggested by Keith et al. [19]. Rather, IVD elastin may cross-link strongly with structural glycoproteins, residues of which may remain after alkaline extraction. In fact, fibrillin has been identified immunohistochemically in human IVD (S. Roberts, unpublished work). In addition, AF cells of bovine IVD have been found to express fibrillin RNA (J. P. Urban, unpublished work). It was thought that such coupling could influence the mechanical properties and physiological functions of the fibres [6].

**Organization of the elastic network of the IVD**

Elastic fibres have been found throughout the AF and NP of human [1,5], dog [3], rat [21] and bovine [6] IVD. Through the use of electron microscopy (EM), elastic fibres were found consisting mainly of microfibrils in the AF of human fetal [22] and infant [1] discs, a typical characteristic of an immature elastic fibre. In contrast, elastic fibres of the NP appear to contain largely amorphous elastin in human infant disc. Since elastogenesis involves an essential deposition of amorphous elastin on to the bundles of microfibrils and then the progressive enlargement of amorphous elastin and attenuation of microfibrils, it was suggested that the development of elastic fibres might differ in AF and NP of the human IVD [1]. Also by using EM, elastic fibres were found around the cells of rat disc [21] and running parallel to the collagen fibres in the AF of human and rat IVD. Using light microscopy, Johnson et al. [2] found that elastic fibres, observed histochemically by orcein or haematoxylin and eosin staining, distribute mainly at the interface between human cervical discs and the vertebral epiphysis but not throughout the human cervical discs [2]. However, by using the same technique, elastic fibres were found throughout the AF and NP of dog cervical discs [3] and the AF of human lumbar discs [4] with the number of elastic fibres decreasing with age. These results suggest the distribution of elastic fibres may vary depending on the level of IVD, aging and also the species. Nevertheless, Johnson et al. [3] found that the elastic fibres of the AF appeared in a loose threedimensional network in the innermost lamellae, radially arranged in mid-zone lamellae and vertically and obliquely arranged in the outermost lamellae. Elastic fibres were also observed in the interlamellar regions.

Although these studies showed that elastic fibres were found throughout AF and NP of IVD, they were reported to be sparsely and irregularly dispersed among the collagen fibres; thus it is not surprising that elastic fibres have not been thought to contribute significantly to the mechanical behaviour of the IVD. However, a very recent study that examined elastin organization by immunolocalization revealed an abundant and wellorganized network of elastic fibres distributed in the all the regions of bovine IVD [6]. Details of elastic fibre organization not described previously were possibly masked by the dense GAG and collagen matrix. Using hyaluronidase and collagenase to remove most of the GAG and collagen matrix, an extensive elastic fibre network was found throughout AF and NP of the bovine IVD with the organization differing between disc regions and changing with age. In the NP region (Fig-
ure 1), elastic fibres appeared long (> 200 μm), straight and mostly oriented radially. Vertically or obliquely oriented elastic fibres were also observed in this region, which suggested that elastic fibres possibly run through the NP from endplate to endplate. At the transitional region between NP and AF, elastic fibres changed orientation gradually into a so-called criss-cross pattern, which possibly corresponds to the report by Johnson et al. [3] that a three-dimensional elastic fibre network was observed in innermost lamellae of the dog cervical disc. In the AF region (Figure 2), elastic fibres were most densely clustered and randomly oriented in the interlamellar space, while within the lamellae they were orientated predominantly parallel to the collagen fibres, which corresponds to the finding by Buckwalter et al. [1] that elastic fibres observed by EM appeared in parallel to the collagen fibrils in the AF of human IVD. In the region of the disc connected into the endplate, long and straight elastic fibres appeared to penetrate into the plate vertically and obliquely. In the adult disc, dense and frequent cross-bridges composed of elastic fibres appeared to run across the lamellae of the outer AF (Figure 2). The orientation of these elastic fibres varied, with some long and straight, and some forming a mesh network.

### Possible functional roles of the elastic fibre network of the IVD

The major functional role of the IVD is to bear loads. It carries compressive load and also provides flexibility to the spinal column by allowing it to bend, flex and twist. With every application of load, the disc deforms and rapidly recovers its size and shape after the load is removed (provided that load application has been brief and fluid expression thus minimal). The disc thus behaves elastically and the elastin network may play an important role in restoration of shape after deformation.

The role of the elastic fibre network may vary with disc region. The NP of IVD is rich in aggregan, and is therefore highly hydrated [23]. Under compressive load, the disc loses height by the deformation of the NP that expands radially with a consequent lateral expansion or bulge of AF. It has been suggested that the long, straight and radially oriented elastic fibres seen in the NP may serve to resist tensile force radially when the load is transmitted to the AF from NP and then contribute to the recovery of the original network conformation [6].

The elastic fibre network of the annulus may have a different role. The AF of the IVD contains less aggregan than the NP. Its main structural features are the concentric, cylindrical lamellae made from bundles of collagen fibres oriented obliquely to the spinal axis within each lamella and with the angle of orientation alternating by around 120° between adjacent lamellae [22,24]. When the spine is bent or flexed, one side of the disc is placed under tension and increases in height while the opposite side is placed under compression and loses height. The increase in disc height on extension far exceeds the maximum extensibility of collagen fibrils [25] and is thought to depend on sliding between the adjacent collagen lamellae [24,26]. The dense and randomly distributed elastic fibre network lying between the lamellae...
may make this sliding possible in any direction and reversible and hence contribute greatly to the recovery of annular lamellae after the deformation. In addition, the elastic fibre network between the fibrils appears linked to the network of elastic fibres parallel to collagen fibres within the lamellae and the network thus may serve to maintain annulus integrity. Vertically and obliquely oriented elastic fibres penetrating the adjacent vertebral bodies are likely also to assist the sliding of lamellae as well as to help to realign original network organization after flexing or extending in any direction.

In addition to its mechanical functions, elastic fibres possibly contribute to regulate disc cell metabolism. The disc tissue is avascular. Disc cell metabolism is dependent on the diffusion of nutrients, metabolites and hormones between the blood supply surrounding disc tissue and the disc cells [27]. The transport of the macromolecules that regulate cellular metabolism can be accelerated by the movement of tissue fluid [28] that results from deformation of the disc under the loads and its recoil when the loads are removed. The degree of the deformation of disc tissue, in part governed by the elastic fibre network, hence may influence disc cell metabolism and hence ultimately the formation and remodeling of the elastic fibres themselves.

Conclusions

Although elastic fibres of IVD have been studied since 1940, their organization and function in human or animal IVD have not been clearly understood. The new approach by using enzyme pre-digestion before histologically examining elastic fibre organization could provide a powerful and rapid technique to reveal details of the organization and hence possible functions of elastic fibres in the IVD. Further studies on the changes of elastic fibres in aging or degeneration could contribute greatly to the understanding pathological conditions of IVD.

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References


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