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TISSUE ENGINEERING:

Function follows form

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When the intervertebral disc degenerates, the disc height shrinks and the spine loses mechanical stability. This mechanism is at work in many cases of back pain. Designing a living-tissue substitute that repairs or replaces the mechanical and physiological function of damaged intervertebral disc structures could provide treatment options beyond pain relief alone[1]. The group of Elliott and Mauck, reporting on page 986 of this issue[2], takes an important step towards engineering a biomimetic analogue of an important part of the intervertebral disc — the annulus fibrosus — by creating a laminated structure on which stem cells can be cultured, and which approaches the strength of the equivalent native tissues. Their results also point to a shearing mechanism between the lamellae that acts to reinforce our discs under stress in undamaged, native annulus fibrosus *in vivo*[2].

If the intervertebral disc structure is vaguely reminiscent of a jelly doughnut, then the jelly is the centrally located nucleus pulposus, a hydrophilic gel that helps to distribute stresses that act on the spine (Fig. 1). The nucleus is surrounded by the cake parts of the doughnut, which include the strong annulus fibrosus forming a thick band around the nucleus pulposus and the cartilage endplates on the top and bottom surfaces. The annulus fibrosus is a collagen-fibre-reinforced laminated tissue, which serves the dual mechanical roles of maintaining the pressurization of the nucleus pulposus and transmitting loads across adjacent vertebrae. Together, the complex intervertebral disc structure can transmit loads across the vertebrae while allowing flexibility of the spine. Intervertebral discs are subjected to compressive forces approaching ten times body weight during certain activities because of the large loads from spinal musculature. Small and large injuries of the annulus fibrosus can lead to substantial remodeling and deterioration of the whole of the disc that are characteristic of degeneration[3], and often associated with pain.

Treatment for intervertebral disc disorders can involve a range of surgical interventions, such as fusion of two or more vertebrae. More recent treatments preserve intervertebral motion through the use of artificial disc replacements such as polyethylene and metal disc implants; the next-generation treatment involves intervertebral disc transplantation[4]. Tissue engineering a live disc replacement is a treatment that it is hoped could be used in the future to reduce the complication risks from more traditional permanent implants, such as polyethylene wear debris[5]. Tissue-engineered implants would also be seeded with living cells that should allow the tissue to remodel with use over time. Tissue engineering of the annulus fibrosus for repairing intervertebral discs is likely to become one of several

At present, when intervertebral discs fail, the only existing treatments are fusion of neighbouring vertebrae or polymer and metal implants. A tissue-engineering approach using an electrospun-fibre laminated scaffold could provide functional equivalence with native tissues in both construction form and strength.

treatment options chosen based on patient age, extent of degeneration, and severity of symptoms[6].

Nerurkar *et al*.[2] created tissue-engineered annulus fibrosus constructs using electrospun nanofibrous scaffolds that were seeded with mesenchymal stem cells. Bilayered laminated constructs were then formed and maintained in culture for up to ten weeks to allow the cells to grow. The cells synthesize and accumulate proteins in the same way they do in the native disc and this was combined with an increase in mechanical stiffness, showing strong potential for future developments and refinements in engineering of annulus fibrosus.

This study is an important advance for tissue engineering of the annulus fibrosus because it demonstrates the possibility of producing and maintaining a biomimetic annulus fibrosus that is as close to native tissue as we have seen so far, both in terms of the structural hierarchy of fibrous lamellae and the resulting strength of the engineered tissue. The work demonstrates an appreciation for the structure and properties of the native tissue on several length scales, including fabrication using nanofibres to emulate the fibrous nature of the tissue that provides an appropriate microenvironment for cells of ~10 μm diameter, and mechanical interactions at the interface of discrete annulus lamellae on the millimetre scale.

The importance of interfaces in laminated tissues was emphasized when the researchers engineered an annulus-mimetic tissue with two layers. A laminated tissue constructed with layers with the constituent fibres at a 30° angle to each other develops shear stresses at the interface that are not found in laminated constructs with parallel fibre orientations[2]. These shear stresses enhance the constructs' stiffness.

Using confocal imaging of natural annular lamellae under shearing stresses, it has been clearly demonstrated that sliding does not occur between annular layers and that interlamellar connections involve a variety of molecular interactions[7]. The natural annulus fibrosus structure is more intricate than the engineered tissue reported by Nerurkar and colleagues, because of the proteins arranged *in vivo* during development and refined during growth and aging as the mechanical and physiological environments remodel and mature (Fig. 2). In humans, annular connectivity between layers becomes more complex in aged and degenerated tissues. Future enhancements of engineered annulus constructs are likely to involve more biomaterials at interfaces between lamellae in the scaffolds, as well as mechanical conditioning during the *in vitro* culture period to refine tissue properties using the known responses of intervertebral cells to mechanical loading[8]. As the number and types of cell in the disc tissue also change with aging and degeneration, refined tissue-engineering approaches will also require improved characterization of native disc cells[9].

One of the biggest challenges in tissue engineering of the intervertebral disc, and tissue engineering more generally, involves implantation and integration of the engineered construct with native tissues, particularly where mechanical strength is required. Integration may be particularly challenging for a tissue such as the intervertebral disc with few blood vessels, where there are few cells and their metabolic rate is lower than in tissues that have more blood supply. Many interfaces exist in the composite disc involving gradual and abrupt transitions between the annulus fibrosus, nucleus pulposus, cartilage and vertebral endplates, and other surrounding soft tissues. Interfaces in the disc warrant particular attention as abrupt discontinuities between annulus and vertebrae are often the sites of injury[3], and even more gradual transitions between annulus and nucleus tissues may also be an initiation site for degenerative changes[10]. Consequently, some researchers strive to engineer entire intervertebral disc–vertebrae constructs, as this ambitious strategy may offer the best opportunity to promote tissue integration and successful tissue implantation[5].

Next-generation tissue-engineered constructs offer promise for repair techniques for restoring biomechanical function, allow tissue integration, and eventually promote clinical success. Future tissue-engineered structures are likely to use biomimicry in a feedback method, much like the path this work suggests. The form of the biomimetically engineered tissue construct emulates that of the natural structure, and following *in vitro* culture or implantation in the body it undergoes further remodelling in response to its mechanical stress environment, so that function of the matured tissue is refined following its biomimetic form.

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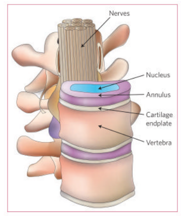

Figure 1.
Spine anatomy. The intervertebral disc supports large loading on the spine while allowing mobility, but is a common source of pain, particularly when the gelatinous nucleus pulposus (shown in blue) is forced through a weakened part of the annulus fibrosus (shown in purple). The ubiquitous problem of lower back pain provides motivation for improved repair techniques including engineering of annulus fibrosus replacements.

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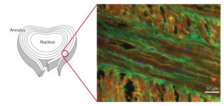

Figure 2.
Intervertebral disc structure. The annulus fibrosus is a hierarchical structure with many levels of complexity within and between annular layers in the native tissue. Successful tissue engineering of the annulus fibrosus requires improved understanding of the interactions between fibres and cells, and the interfaces between annular layers. Blue: DNA in cells; orange: collagen type I; green: collagen type VI. The immunohistology image was obtained in collaboration with Dr Casey Korecki and Dr Marc Levenston.