TISSUE ENGINEERING OF FIBROCARTILAGINOUS TISSUES: THE INTERVERTEBRAL DISC AND THE MENISCUS.

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Synopsis/Abstract
The intervertebral disc in the spine and the meniscus in the knee are two fibrocartilaginous tissues which commonly are injured or become degenerate, causing significant clinical problems. The principals of tissue engineering, which are applicable elsewhere in the body, hold true for the disc and meniscus. Whilst there are some similarities with articular cartilage in terms of the molecules present, these fibrocartilages have their own peculiarities, some of which can be quite challenging. Following a description of the structure and anatomy of the disc and meniscus and the current clinical treatments, the different strategies for biological repair are described focusing particularly on cell therapy. The types of cells and scaffolds being investigated and how these can be modified are discussed.

1. Introduction

Regenerative medicine and tissue engineering have created a vast amount of interest in the recent decade (Fisher *et al.,* 2013; Ringe *et al.,* 2010). In the musculoskeletal field it has been directed in the main to articular cartilage, a specialised form of hyaline cartilage. There are, however, other cartilages which can be damaged or become degenerate leading to clinical symptoms. For example, the intervertebral disc and the meniscus of the knee are both fibrocartilages and are responsible for billions of dollars per annum of healthcare and loss of production in the western world. It is estimated that 850,000 patients are treated each year in USA for meniscal injury whilst 200,000 people there undergo surgery to fuse their spines for degeneration of the intervertebral disc and associated back pain.

Tissue engineering of these fibrocartilages has very different demands and requirements in comparison to articular cartilage. Whilst the main molecular components in all cartilages are generally similar (mostly water, collagen and proteoglycans), the individual types of molecules and their organisation can differ considerably. For example, articular cartilage is predominantly type II collagen, whilst the meniscus and outer part of the intervertebral disc (the annulus fibrosus) are mostly type I collagen. The cells of the cartilages also differ; hyaline cartilage is generally considered to be populated by only one cell type, chondrocytes, whilst the meniscus and disc have cells resembling fibroblasts as well as chondrocytes.
In this review the structure, composition and functioning of the meniscus and disc will be described, in addition to their most common pathologies, before discussing strategy and progress towards their tissue engineering.

Connective tissues characteristically are made up of a fibrous component, typically a member of the collagen family, interspersed between glycoproteins, a large part of which are proteoglycans; these in turn are responsible for retaining a great volume of water within the resulting matrix. In addition to this basic composition (usually making up more than 90% of the matrix) there are many other molecules such as proteins and other glycoproteins (for example, elastin, microfibrillar protein, COMP, CMP, amyloid, CILP and matrilins), growth factors, cytokines, proteases and inhibitors. Whilst individually they are very minor constituents in terms of mass-balance, they may influence the final structure significantly. The physical and mechanical properties result largely from water content; this is a product of the swelling pressure caused by the osmotic pressure of the charged matrix components and is resisted by the tension in the fibrillar molecules. The strength of the fibrillar network will depend not only on the

inherent strength of the fibres themselves, but also on interconnecting molecules and interactions between them including, for example, collagen crosslinks. The mechanical properties of individual tissue matrices vary depending on the composition of that particular tissue. The fibrocartilages, the intervertebral disc and meniscus, are two connective tissues for which the mechanical properties are very important to their role in the body, being key structures in the axial skeleton and lower limb, respectively. Even within one structure, for example, the intervertebral disc, the matrix organisation and mechanical properties vary greatly within it, from being very weak in compression and very strong in tension in the outer annulus fibrosus (AF), to vice versa in the central nucleus pulposus (NP).

2. Anatomy, Structure and Function.

The intervertebral disc and meniscus both facilitate flexion, extension and rotation of joints. They differ in that the meniscus is part of a diarthrodial joint whereas the intervertebral disc forms an amphiarthrosis, without a central synovial cavity (**Figure 1**) <figure 1 near here>.

(i) Meniscus

The meniscus is an integral part of the diarthrodial knee joint with other tissues including articular cartilage, the coronary, posterior, anterior, cruciate, collateral, lateral and transverse ligaments, the synovium and the fibrous capsule. Each knee joint contains two menisci, the lateral and medial menisci, which are both wedge shaped crescents of fibrocartilage sitting between the lateral and medial femoral condyles and tibial plateaux respectively and acting to protect the adjacent articular cartilage (**Figure 1**). The shape of the meniscus is adapted to aid distribution of loads within the knee, with the superior surface in contact with the femoral condyles being concave in shape, in comparison to the flatter inferior surface which contacts the tibial plateau (Dudhia *et al.,* 2004). At various points the outer meniscus interfaces directly with different ligaments which are attached to the bony components of the knee (Gray, 1999).

The shape of the meniscus is determined during foetal development when it is a very cellular and highly vascularised tissue (Gray, 1999). After birth and during skeletal maturation the vascularisation decreases, particularly centrally, resulting in only the outer third of the meniscus having blood vessels present in adulthood (**Figure 2**) <figure 2 near here>. These vessels originate from the peripheral capsule and synovium and provide the outer region of the meniscus with oxygen and nutrients and clearance of waste metabolites. Similarly it is only the outer region of the meniscus which is innervated with free nerve endings and mechanoreceptors (types I, II and III being reported particularly in the posterior horn outer region). The central meniscus is therefore aneural, avascular and alymphatic in adulthood.

The cells of the meniscus are referred to by some as fibrochondrocytes (Almarza *et al.,* 2004), although there is evidence that there are at least 2 distinct populations present: chondrocyte-like cells are found when explants of the inner meniscus are cultured whilst elongated fibroblast cells are observed from explant cultures of the outer region. The morphology of the cells within the meniscus also varies with location *in situ*, being oval and fusiform in the superficial zone, to more rounded and polygonal in the deeper zone (reviewed in (Almarza *et al.,* 2004) and (Dudhia *et al.,* 2004)). They commonly sit in a pericellular capsule and may have cell processes, particularly those in the deeper zone. Mitochondria are not frequently seen, suggesting that energy production is

predominantly via glycolysis, as for other cartilaginous cells such as in articular cartilage. The differential responses seen by articular cartilage cells to cytokines according to depth (i.e. the surface zone cells being more responsive than the deeper cells) has not been studied in the meniscus but different growth and metabolic characteristics *in vitro* have been demonstrated (e.g. the deeper fibroblast cells proliferate more than those from the surface).

(ii) The Intervertebral Disc

The intervertebral discs sit between the centrum of the spinal vertebrae, interfaced superiorly and inferiorly by hyaline cartilage endplates. At birth these constitute more than 50% of the intervertebral space but, during development, this reduces and the layer of hyaline cartilage becomes progressively thinner until in adulthood it is less than 1 mm thick. It also reduces in diameter so that in the skeletally mature individual it does not extend out to the vertebral rim. It integrates completely with the intervertebral disc, with collagen fibers continuing from it into both the annulus fibrosus (AF) and the nucleus pulposus (NP). Its attachment with the bone resembles more of an interlocking 3 dimensional 'jigsaw' arrangement between the 2 calcified tissues with there being few, if any, obvious fibres crossing between them. The adult disc is similar to the meniscus in that the nerves and blood vessels are restricted to approximately its outer 30%. The outer AF is supplied by branches of the sinuvertebral nerve; in the healthy adult there is a limited innervation with even more restricted presence of mechanoreceptors which presumably aid proprioception. Vascularisation in the newborn human is present in the AF and there are large vascular channels throughout the cartilage endplate. Soon after birth and during skeletal development, both of these diminish leaving the cells of the NP dependent on diffusion of nutrients from within the vertebral bone or vasculature within adjacent tissues such as the longitudinal ligaments. Clearance of metabolites is also via a similar route. Since cells within some of the adult intervertebral discs may be up to 8mm from the nearest blood vessels any interruption of the nutrient supply (e.g. reduced vascular flow or calcification of the endplate) risks reducing the normal functioning of the cells or even their viability (Bibby *et al.,* 2004).

The centre of the disc, the nucleus pulposus, in young humans has a gelatinous appearance and is clearly distinct from the surrounding and very organised annulus fibrosus which has the appearance of concentric but interlocking rings. During skeletal development the nucleus becomes more solid and the boundary between these 2 regions much less obvious. This continues with increasing age, during which time the disc changes from being white to pale yellow in colour, attributed to alteration in the chemical cross linking occurring in the matrix components. The cells within the centre of the intervertebral disc, like the meniscus, have been shown to be more rounded or oval than the more fibroblast-like, thin and elongated, bipolar cells of the annulus fibrosus. However, when released from the matrix both the annulus fibrosus and nucleus pulposus cells are a similar size and shape (Roberts *et al.,* 1991). Features which are more commonly seen for cells of the NP and inner AF *in vivo* are the presence of a pericellular capsule and also cell processes. The exact function, however, of each of these is not completely understood with sensing or protection of mechanical load suggested as a possible factor. Glycolysis is the common respiratory pathway for disc cells as for those of the meniscus, and the two populations (from NP and AF) have also been shown to have different synthetic capabilities and respond differently, for example, to mechanical loading or other environmental factors such as osmolality (Ohshima *et al.,* 1989).

During development the human disc has a third and very distinct cell population centrally, that of notochordal cells. Soon after birth the number of these cells in the human diminishes rapidly, perhaps due to the very different and greater energy requirements compared to NP cells (Guehring *et al.*, 2009). In many species, including all rodents, notochordal cells persist in adulthood. This is an important species difference which should be borne in mind when translating results from studies in such species to humans.

3. Composition of the Extracellular Matrix and Its Organisation

Both the meniscus and the intervertebral disc consist primarily of extracellular matrix (ECM) which is produced and maintained by relatively few cells in comparison to most other tissues (Vonk *et al.*, 2010). For example, cells constitute only about 1% of the volume of the disc (compared to 80% of the liver). Few they may be but they remain essential to how the matrix is produced. Due to their interactions with the ECM, their shape and ultimately their metabolism is dependent on the composition and organisation of the matrix and the load on it. There is a common structure for the matrix of the two tissues which is represented in (**Figure 3**) <figure 3 near here>. Structurally the matrix has three main components: collagens, proteoglycans (PGs) and non-collagenous proteins, in addition to water, which constitutes very approximately 70% of the weight of the fibrocartilages. Each of these components will be described in general terms and then more specifically with regards to the two tissues.

(i) Collagens

Collagen is a large family of proteins with at least 28 members. It is the most common structural protein in humans, each molecule being composed, at least in part, of a super triple helix of three amino acid chains. This arises due to every third amino acid being a glycine. The structure of collagen is very specific and well understood but outside the scope of this paper. It is reviewed in (Eyre *et al.*, 2006). In both disc (AF) and meniscus, collagen can make up 70% of its dry weight. In both these locations the type of collagen is mostly type I, whereas in the NP it is predominantly type II collagen (**Figure 4**) <figure 4 near here>. A small amount of type II collagen is also reported in the meniscus, particularly in osteoarthritis.

Besides the fibrillar types I and II collagens, which constitute approximately 80% of the total collagen present, there are many other collagen types present in much smaller quantities. These include collagen types III, V, VI, IX, X, XI, XII and XIV. They may be present only in small amounts (around 1-2%), but can influence the tissue considerably. For example, type IX collagen sits on the surface of type II collagen fibrils and controls its diameter which in turn will affect its mechanical properties. Likewise types III and V collagens may sit on the type I collagen fibrils within the meniscus, controlling their diameter possibly resulting in a more compliant tissue (Dudhia *et al.*, 2004). The structure of the collagen molecules and network is complex, with heterotypic fibrils of types II, IX and XI also being identified (Eyre *et al.*, 2006) in cartilage and the disc. Many collagen fibrils align to form fibres, bundles of which are orientated, both in the meniscus and AF of the disc (Schollum *et al.*, 2008), in a highly organized way, believed to optimize it for the incident loads on the tissues. In the meniscus the majority of the fibres lie circumferentially so that the meniscus is stiffest in the radial plane. There are a small number of collagen fibres running radially, which

it is thought might act to resist lateral spread of the tissue and longitudinal splitting such as might lead to 'bucket handle tears' of the meniscus.

In the AF of the disc the collagen bundles are also arranged circumferentially where they form concentric layers or lamellae (**Figure 4c**). Within each lamella the collagen fibres are parallel and at 60˚ to the vertical axis, but alternating to the right and left of it in adjacent layers. Above and below the disc the collagen bundles insert into the rim of the vertebrae, thereby locking it in place. In the central NP the collagen fibres, which make up less of the matrix, are randomly organised.

(ii) Proteoglycans

Proteoglycans (PGs) are complex and can be exceptionally large molecules with a central protein core to which is attached one or more glycosaminoglycan (GAG) chains. They are a very diverse and ubiquitous family of molecules which owe their main function in the ECM matrix largely to these covalently bound GAG side chains. They are polyanionic chains of repeating disaccharides containing hexosamine and uronic acid, the particular mix of which forms hyaluronan (HA), chondroitin sulphate (CS), keratan sulphate (KS), dermatan sulphate (DS) or heparan sulphate. The fixed negative charge on these GAG chains attracts counter cations, causing a high osmotic and hence swelling pressure. It is this property which controls the hydration of the ECM drawing water in from the surrounding area to create a water filled compartment (Roughley *et al.,* 2006). The N-terminus of the protein core has a globular region, the G1 domain, which interacts with HA, the reaction being stabilised by another protein, the link protein. Since HA forms long chains of up to millions in molecular weight, it can lead to huge conglomerate molecules or 'aggregates' of proteoglycan monomers. Aggrecan is the most common PG in many load bearing tissues such as articular cartilage, the intervertebral disc and also the meniscus. The GAG chains attached are KS nearest the G1 domain and CS towards the C terminal region. There are considerable regional differences with more KS associated with the PGs in the NP than the AF. The degree and location of the sulphate groups (e.g. whether C-4-S or C-6-S) varies with location and also the age and health status of the individual.

Another aggregating PG is versican, small amounts of which have been reported in the disc. There are also other types of proteoglycans called small leucine-rich proteoglycans (SLRPs). In contrast to the large aggregating PGs which have a core protein with molecular weight of >2000KDa, for SLRPs it is ~ 40-50KDa. In addition they only have 1 or 2 GAG chains per molecule, whereas aggrecan can have 100-150 GAG chains. SLRPs include decorin, biglycan, fibromodulin, keratocan and lumican, all of which have been reported in the meniscus and disc. The SLRPs in the human adult meniscus commonly have DS chains which may be highly sulphated, with decorin being predominant. The small PGs can have many physiological properties ranging from binding growth factors within the matrix to controlling collagen fibrillogenesis. As with the aggregating PGs their distribution varies with location. For example, in the meniscus biglycan and fibromodulin are most common in the inner region, whereas there was more decorin in the outer zone.

(iii) Other glycoproteins and molecules

In both intervertebral disc and meniscus several other matrix molecules have been identified including, amyloid, cartilage oligomeric matrix protein (COMP), cartilage intermediate layer protein 1 (CILP), elastin and microfibrillar protein. The function of

some of these is not clearly delineated, whereas for others the function seems more apparent. For example, elastin in the disc appears to have an important structural role forming a complete network within and between the annular lamellae. No doubt there still remain other molecules to be identified and characterised. In articular cartilage many of these structural molecules interact with each other forming a complex series of interactions. It is therefore clear that the matrices of the intervertebral disc and meniscus have a hierarchical structure which is well adapted to the functions it must perform. This should be borne in mind when considering its repair or tissue engineering.

4. Pathologies And Current Treatments Of The Fibrocartilages

The connective tissues all undergo changes as part of the normal aging process, for example, general dehydration and pentosidine cross link formation of collagen molecules. These related changes are the same or similar to those seen in degenerative joint diseases but perhaps the rate is different. Whatever the cause of this degeneration, it commonly results in diminished function and mobility and increased pain, creating a large socio-economic burden on society. The most common disorders are described below together with a very brief outline of current therapies employed.

(i) Meniscus

The menisci can be the subject of traumatic, metabolic, inflammatory or degenerative disease. However, one of the most common pathologies presenting clinically is that of a meniscal tear. These can be classified as horizontal, radial (vertical), longitudinal (vertical), bucket handle or a flap. Whilst there is some inherent ability to repair itself, particularly in the vascularised region where the fibrin clots can act as a scaffold, the quality of repair is poor and often leads to continued clinical problems and early degenerative arthritis. Surgical repair in the form of excision of very damaged tissue may be undertaken but the quantity excised is kept to a minimum to reduce the development of osteoarthritis. Suturing and/or gluing is commonly performed. Meniscal repair was first described in 1883 but has been undertaken in large numbers since the 1980's onwards (reviewed in Jarit *et al.*, 2010). The surgical approach (whether removing or repairing) now depends on the demands of the patient (removal has a quicker recovery time, e.g. for a professional sports person), their age (removal is more likely to lead to long term arthritic changes), the region and extent of the tear, and the beliefs and practises of the surgeon.

(ii) Intervertebral disc

In addition to the spinal deformities (scoliosis and kyphosis, which will not be addressed here) the main pathologies involving the intervertebral disc are degenerative disc disease (DDD) and disc herniation. It is believed that DDD is associated with back pain although the exact pathway involved is not well defined. It is characterised by loss of proteoglycan and water particularly in the NP, resulting in loss of disc height and the formation of vertebral osteophytes. Herniation or prolapse of the disc can occur to varying degrees, ranging from simply a bulge (protrusion) with an intact AF, extrusion where the fibres are ruptured, to sequestration where some disc tissue has detached. It is usually only posterior lateral herniations which are troublesome clinically, when the exact symptoms depend on the spinal level and hence which nerve roots the prolapse contacts. The lower lumbar discs (L4-5, L5-S1) are usually involved so that leg/buttock pain often results. Treatments range from conservative, with analgesia or physiotherapy, or sometimes surgery. For herniations, micro-discectomy is usually

successful, although 5 – 10% re-herniate. For disc degeneration the surgical options are not so obvious and may include spinal fusion, when the adjacent vertebrae are fused immobilising that disc level, or more recently many prosthetic intervertebral discs have been developed. These appear to be more successful in the cervical spine than in the lumbar region.

5. Tissue Engineering

The attraction of a tissue engineering solution to a clinical problem is that it would be hoped that the successful biological approach could elicit a permanent repair, in comparison to, for example, an inert implant which inevitably has a finite and limited lifespan. The concept involves utilising a cellular component and a scaffold, each of which will be discussed in turn.

(i) Cells

The cells which will affect the repair may be the individual's own (autologous), another individual's of the same species (allogeneic) or of a different species altogether (xenogeneic). They may be culture-expanded in the laboratory to increase the population, or have undergone some other transformation in the laboratory, e.g., de-differentiated or differentiated in a specific manner. They may be cells which are committed down the pathway to be the same sort as the tissue being repaired or they may be stem cells. The cells could also have been genetically modified. For example, genes have been transferred, both *in vivo* and *in vitro* to meniscus and disc cells. If these genes encode for modifying species of RNA or proteins such as a growth factors, transcription factors or receptors, they could result in genetically augmented tissue engineering (Evans *et al.,* 1999). For example, both meniscal and disc cells have been transfected by a TGF-β cDNA which resulted in increased proteoglycan production in the rabbit disc. Transfection with other substances such as Lim-Mineralisation Protein-1 (LMP-1) can result in increased proteoglycan production as well as upregulating other growth factors such as BMPs 2 and 7 (Yoon *et al.*, 2004). This gives a multiple response for one alteration in genetic material. Hence in theory such technology could aid the efficiency of the repair and regeneration of the disc and meniscus, though it is being used little, if any, in current practice.

Stem cells are often considered to be the ideal cells for tissue engineering since they have unlimited capacity for self-renewal and the capability to form more than one tissue. Whether they are multipotent or totipotent depends on their source, in general being from either adults or embryonic, respectively. There are actually advantages associated with both. Embryonic stem cells have greater proliferative capacity, and the ability to transform to any cell type is advantageous. However, some individuals have great ethical concern in their use which they would not have with, for example, mesenchymal stromal or stem cells (MSC). In addition, MSCs from bone marrow may have other advantageous properties, such as having an anti-inflammatory effect, as well as a reported capacity 'to home' to a site of damage. For example, Shen et al (2014) reported that human MSCs injected intra-articularly into a rat meniscal injury model led to enhanced meniscal regeneration and protected the articular cartilage from undergoing osteoarthritic changes (Shen *et al.,* 2014). They attribute this to homing of the progenitor cells to the site of injury which was mediated by stromal cell derived factor 1 and its receptor, CXCR4. An alternative approach is to activate those MSCs already in the region of injury by applying growth factors or other stimulatory molecules (Murray *et al.*, 2014).

In a study of tissue engineering of the meniscus in rabbit, the use of mesenchymal cells also appeared to enhance integration of the repair tissue with the host, in comparison to an acellular treatment or those treated with complete bone marrow or platelet rich plasma (PRP) (Zellner *et al.,* 2010). MSCs in disc tissue engineering studies are attributed with not only being able to differentiate to disc-like cells producing the appropriate matrix molecules, but they may also influence the endogenous cell population to increase PG production (Yang *et al.,* 2009). This, together with any anti-inflammatory activity they may have, can present MSCs as attractive cells for use in tissue engineering of the disc, the meniscus and also other tissues.

In addition to cells applied externally to the damaged tissue, it may of course be possible to 'activate' a population of resident cells close to or within the tissue to be repaired. Addition of growth factors or a chemo-attractant to draw stem cells or other cells to the location may be such techniques which could be considered [11].

(ii) Scaffolds

There are many scaffolds which can be chosen for the tissue engineering of fibrocartilage, ranging from a natural product which might be a decellularised allograft tissue to something artificial; these may be degraded and replaced by cells *in vivo*, or the scaffold may be resistant to degradation in which case it will remain permanently in the patient. An excellent example of an allograft was the use of a cadaveric trachea, which was decellularised and used as a scaffold to seed with the patient's own cells (MSCs and bronchial epithelial cells) before implanting for the successful regeneration of a bronchus (Macchiarini *et al.,* 2008). The trachea was decellularised over 6 weeks by incubating in sodium deoxycholate and deoxyribonuclease ensuring total removal of immunogenic (MHC positive) cellular components. Allograft menisci and intervertebral discs have both been used in humans, menisci in several countries worldwide for many years, whereas discs only more recently. Various procedures are undertaken prior to transplant, but not usually as extensive as described for the trachea. Menisci have been used after maintaining in culture whilst some safety checks are performed (Lubowitz *et al.,* 2007) or more generally following cryopreservation via various protocols. Depending on how this is done, it may kill some of the resident cells prior to implantation. It appears that the implanted frozen cadaveric tissue becomes populated with the host cells, though the source of the cells has not been identified (**Figure 5**) <figure 5 near here>. Whilst some success with allograft menisci has been reported they do not provide consistently good results, often appearing to resorb with time post-implantation. Cadaveric intervertebral discs have also been used as replacement motion segments in a series of patients (Luk *et al.*, 2008). At the five-year follow-up, four of the these 5 patients treated in the cervical spine had signs of mild disc degeneration but good motion; as with menisci, apparent resorption and/or loss of disc height appears to occur with time.

Artificial scaffolds have been developed and investigated in the laboratory and cell behaviour (of various populations) has been examined, both for meniscal and disc replacement. They can either be designed to be resorbed and replaced by the implanted cells or as non-resorbable synthetic polymers. Some of these which have been investigated include a Teflon fibre net, Dacron coated with polyurethane, PVA hydrogel and a polyurethane elastomer (Rongen *et al.,* 2014). In the clinic, however, the main use of these scaffolds is as an acellular implant. There are 2 main products

currently in use for replacing the meniscus: one is a bovine collagen product (with some hyaluronan and chondroitin sulphate incorporated to form the Collagen Meniscal Implant (CMI®, now distributed by Ivy Sports Medicine LLC) and the other a polycaprolactone/polyurethane scaffold, marketed as Actifit®. These are not the answer to everything however. The CMI® requires an intact rim for correct placement and is only suitable for replacing a medial meniscus. That notwithstanding, some reports are encouraging, with histology of biopsies from the treated region suggesting that there was tissue 12 months post-treatment resembling native tissue (reviewed in (van Tienen *et al.*, 2009)). However, remnants of the CMI® were also visible and 9% of patients had some synovitis present. The polyurethane scaffold used in the Actifit® meniscal replacement has the advantage over the CMI® that it is easier to suture in place (to the remaining tissue at the meniscal rim). Early clinical results appeared encouraging with histology of biopsies taken from the graft 12 months post-implantation showing vessel sprouts and neovascularisation of the outer region of the implant and population with chondroblast-like cells (Rongen *et al.*, 2014).

Implants which have been developed and are in the clinic for the intervertebral disc mostly use inert materials, without the intention of acting as a scaffold for cellular infiltration. In the laboratory, however, there are many studies investigating numerous scaffolds including chitosan, silk, collagen, alginate, polylactic acid, polygylcolic acid and polycaprolactone (PCL) (reviewed for the disc in Hudson *et al.*, 2013; Kandel *et al.*, 2008) and meniscus (Makris *et al.*, 2011; Rongen *et al.*, 2014; van Tienen *et al.*, 2009). For both meniscus and intervertebral disc applications the addition of growth factors is also being studied. Minehara *et al.*, (2010) have shown that the incorporation of recombinant human bone morphogenetic protein (rhBMP-2) in the decellularised meniscal allograft provides a chemokinetic activity. There is increased migration of chondrocytes to the scaffold, in a dose dependent manner. TGF-β infused into PCL and hydroxyapatite scaffolds has been shown to induce endogenous cells to produce a surface covering on a damaged rabbit humeral head. Most importantly this treatment led to a regenerated thicker and more dense cartilage than non-TGF-β impregnated scaffolds and this cartilage had similar mechanical properties to native cartilage (Lee *et al.*, 2010). Growth factors may not be the solution in all cases however. Pabbruwe *et al.*, (2010) reported that whilst the addition of TGF-β to a stem cell/collagen scaffold encouraged the appropriate differentiation of stem cells, it rendered the construct less well integrated to the adjacent tissue. The introduction of chemokines may be a further way of controlling/attracting an appropriate and effective population of cells to the location (Shen *et al.*, 2014).

(iii) Clinical applications

Many laboratory studies into tissue engineering (of a range of tissues) often have the target of creating or generating a piece of tissue with histological, chemical or mechanical properties mimicking that of the native tissue. Achieving this is only part of the solution and often little thought is given to how it would be applied to and integrate with the host tissue. The application might involve major and /open surgery. If one is creating an avascular tissue such as articular cartilage or the inner region of the meniscus or the intervertebral disc, then integration is more likely to be a problem than for a well vascularised tissue such as bone (**Figure 6**) <figure 6 near here>.

The use of bioreactors has not been described in this paper, as the authors feel that they do not have a role in a clinical service. It would be better if it is possible to create the

correct environment *in vivo* and use the patient as their own bioreactor. This has been achieved by the recreation of a mandible with autologous bone marrow, a bone mineral carrier and BMP7 inside a computer aided design (CAD) scaffold which was implanted in the patient's latissimus dorsi for 7 weeks before locating in the correct position (Warnke *et al.,* 2005). Perhaps one useful way forward for disc and meniscus tissue engineering is to understand better the natural repair processes that exist, however limited.

It would be advantageous if the necessary compounds of the tissue to be engineered can be applied via a needle to the region to be treated (**Figure 7**) <figure 7 near here>. This might be achieved by applying a scaffold of low enough viscosity to be injected which might subsequently polymerise or be rendered more viscous *in situ*. It could have either the correct signalling molecules to attract an active endogenous population, or incorporate an appropriate cell population. The degree of 'maturity' of the cells may well be inversely related to the degree of integration with the native/host tissue, with more mature, apparently better developed cells/tissue in the laboratory, actually being less able to integrate completely into the surrounding host tissue.

Notwithstanding the challenges to tissue engineering, several clinical trials have been undertaken and are on-going utilizing cell therapy approaches, both in the meniscus (Longo *et al.,* 2013) and intervertebral disc (Kregar-Velikonja *et al.,* 2013). These are mostly small, phase I trials though a few companies are progressing to larger Phase III trials.

Conclusion

The concept of a biological repair, such as tissue engineering, is just as appealing for the fibrocartilaginous tissues, the intervertebral disc and the meniscus, as it is for hyaline articular cartilage, but much less developmental, preclinical and clinical work has been done in these areas. It is often thought that because they are cartilages, that what has been developed or found out for articular cartilage will be equally applicable to these other 2 tissues. This is not necessarily the case for tissue engineering however, with both the disc and meniscus requiring a specialised approach and often being more challenging. The properties of both tissues are very variable with location, much more so than for articular cartilage. The *in vivo* loading is more complex and the application and fixation more difficult than for articular cartilage. Nonetheless, the quest continues and developments are being made, which will inevitably be driven further by the large clinical demand in this area.

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Figure legends

Figure 1: Different joints are found throughout the body, depending on the amount of motion required. (a) Synarthroses in the skull (arrowed) permit virtually no movement, whereas the cartilages within amphiarthroses, such as the intervertebral disc (IVD) (b), or articular cartilage and menisci in the diarthrodial joint (c) facilitate greater movement.

Figure 2: The human newborn menisci are vascularised throughout (a), whereas this diminishes during skeletal maturity (b) and further again over the age of 50 years of age (c). (Adapted from Makris *et al.*, 2011).

Figure 3: The extracellular matrix of both meniscus and the intervertebral disc is composed primarily of collagen fibres (shown here in pink) being kept in tension by the water-retaining proteoglycans (some of which link via hyaluronan (shown in yellow) to form large aggregates).

Figure 4: Immunohistochemical staining of the intervertebral disc shows type I collagen (a) predominates in the annulus fibrosus (AF), the lamellae of which can be clearly seen when viewed through polarised light (c). In contrast type II collagen (b) occurs more centrally in the nucleus pulposus (NP) and cartilage endplates (CEP) (which make up a large part of the intervertebral space in this 2 year old human).

Figure 5: Haematoxylin and eosin stained section of a meniscal allograft 12 months post-implantation showing apparently viable cells (with nuclei stained blue) infiltrated throughout the allograft.

Figure 6: Tissue engineering of the intervertebral disc faces particular challenges, some of which are delineated in this schematic. (Reproduced from Kandel *et al.*, 2008).

Figure 7: An injectable tissue engineering solution is attractive in terms of being minimally invasive, for example into a disc (a) X-ray showing cells in a radio-opaque carrier being injected into the intervertebral disc. (b) Fluorescently labelled cells which were injected into the nucleus pulposus of a bovine model system of intervertebral disc degeneration remained viable for at least 28 days in culture.

Article History

Updated items:

Authors
Tissue engineering – cells
Tissue engineering – scaffolds
Clinical applications
Acknowledgements
Figure 1
Figure 2
Figure 4