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Review

The cellular matrix: a feature of tensile bearing dense soft connective tissues

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Summary. The term connective tissue encompasses a diverse group of tissues that reside in different environments and must support a spectrum of mechanical functions. Although the extracellular matrix of these tissues is well described, the cellular architecture of these tissues and its relationship to tissue function has only recently become the focus of study. It now appears that tensile-bearing dense connective tissues may be a specific class of connective tissues that display a common cellular organization characterized by fusiform cells with cytoplasmic projections and gap junctions. These cells with their cellular projections are organised into a complex 3-dimensional network leading to a physically, chemically and electrically connected cellular matrix. The cellular matrix may play essential roles in extracellular matrix formation, maintenance and remodelling, mechanotransduction and during injury and healing. Thus, it is likely that it is the interaction of both the extracellular matrix and cellular matrix that provides the basis for tissue function. Restoration of both these matrices, as well as their interaction must be the goal of strategies to repair these connective tissues damaged by either injury or disease.

Key words: Tendon, Ligament, Meniscus, Intervertebral disc, Healing, Cytoarchitecture

Introduction

Dense soft connective tissues (i.e. ligament, tendon, meniscus, intervertebral disc) are composed of cells and their surrounding extracellular matrix. While the extracellular matrix of these tissues has been the subject of intense study, their cellular architecture has historically received only casual attention. Much of this information has been conflicting and subject to debate (Ghadially et al., 1978; Ghadially, 1983). However, recent studies using a variety of microscopic and indirect immunofluorescence techniques suggest that these apparently diverse groups of tissues, which are characterized by a spectrum of functions and in vivo mechanical environments, are organized around similar architectural principles.

The purpose of this review is to highlight recent developments in our understanding of the cellular architecture of dense soft connective tissues and to discuss their functional implications. It will become clear that a 3-dimensional cellular arrangement, a "cellular matrix", consisting of fusiform cells with cytoplasmic projections and gap junctions may define a specific class of hypocellular, tensile-bearing, dense connective tissues. This organization may be important in tissue matrix organization, maintenance, and remodelling and may be altered as a result of injury. Further, it is now evident that changes in cell-to-cell relationships as well as the distribution of specific cellular phenotypes may underlie the inability of these tissues to effect repair following tissue injury and disease.

The cellular matrix of tensile bearing dense connective tissues

In contrast to other tissues (liver, spleen, kidney), dense connective tissues perform specific biomechanical functions and are generally characterized as having a large volume of extracellular matrix and a low cell density. This arrangement is typical of restraining structures (ligaments) and force-transmitting (tendon), or force-dissipating (meniscus) structures. The general architecture of each of these structures is specifically fashioned for its role in normal diarthrodial joint function. However, specific aspects of the organization of ligaments, tendons, menisci (peripheral) and outer annulus fibrosus of the intervertebral disc appear to be related because they share a common function and as a consequence they also share common features in their extracellular matrix. For example, each of these tissues is subjected to longitudinal tensile loads and their extracellular composition is characterized by a

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predominance of type I collagen (Amiel et al., 1984). While considerable effort has gone into the characterization of the extracellular matrix, the relationships between the cellular organization and the function of dense connective tissues has only recently been investigated. It now appears that these tissues also share a common cellular architecture.

Ligament

Ligaments are anatomically discrete dense connective tissue structures that connect bones across joints and form a typical tensile bearing dense connective tissue. Ligaments serve a number of important functions including kinematic, biomechanical, and possibly neurosensory roles in both guiding and protecting joint movements (Blacharski et al., 1975; Markolf et al., 1976; Butler et al., 1978, 1980; Woo et al., 1982; Andriacchi et al., 1983; Akeson et al., 1985; Frank et al., 1985; Brand, 1986). During normal day-to-day functions, ligaments are likely subjected to repeated low tensile loads (Holden et al., 1994).

Ligaments are composed of two major components, ligament cells and the extracellular matrix, the majority of which (excluding water) is composed of type I fibrillar collagen (Amiel et al., 1984, 1990). Conceptually, the gross internal organization of ligament is similar to that of tendon, and has been described as a collection of fascicles, composed of longitudinal groups of collagen fibers (Arnoczky et al., 1993). These fascicles, which appear separated by septae in transverse sections, are enveloped in a thin connective tissue sheath, the endoligament. The endoligament itself is connected to the epiligament, a more vascular connective tissue that surrounds the entire ligament (Chowdhury et al., 1991). In standard histological preparations each fascicle appears hypocellular, and the cells are aligned into rows that are inter-dispersed between bundles of collagenous fibers (Amiel et al., 1984, 1990). The general hypocellularity of ligaments has been taken to suggest that ligament cells are functionally isolated and relatively inert.

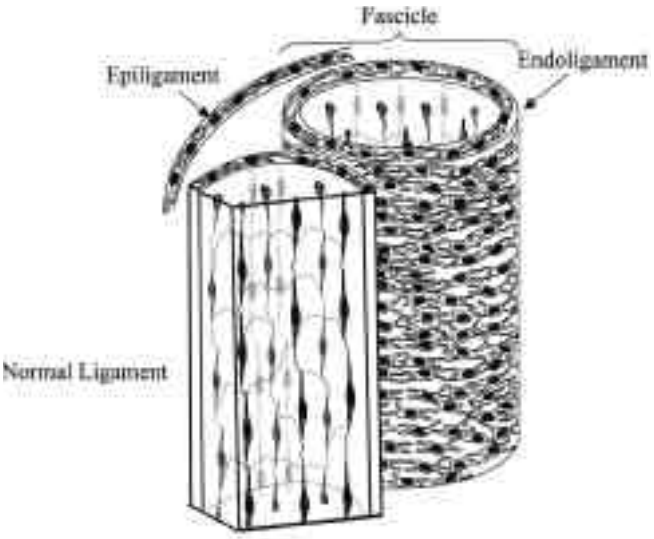

Fig. 1. Schematic representation of the 3-dimensional array of cells in a normal ligament. Cells are organized into parallel sheets in which the cells are arranged in adjacent rows. Within each row, the cells are spindle-shaped and have long cytoplasmic projections connected by gap junctions. Cells between sheets are also connected by long projections and gap junctions. Each fascicle, containing multiple sheets of cells is enveloped by the endoligament. The cellular projections of the epiligament cells are oriented perpendicular to ligament cells. The fascicles are then surrounded by epiligament.

More recently, the cellular morphology and interrelationship between ligament cells has been clarified (Benjamin and Ralphs 1997, 2000; Schwab et al., 1998; Lo et al., 2000, 2001). Although ligament cells may appear functionally and physically isolated in standard haemotoxylin and eosin preparations, evidence obtained combining standard light and confocal microscopy with immunohistochemical techniques has demonstrated that this is not the case. For example, using indirect immunofluorescence and antibodies to cytoskeletal proteins (vimentin and ß-tubulin) the cellular architecture and organization of midsubstance ligament cells has been demonstrated in the ovine and rabbit models (medial collateral ligament and anterior cruciate ligament of the knee) (Lo et al., 2000, 2001). When thick (30 µm) frozen sections of ligament tissue were stained for these components, it was possible to visualize the full extent of the cells along with their spatial relationship to one another within the tissue. In the normal ligament, as described below, the cells were arranged in a complex 3-dimensional network based on parallel rows of cells in the sagittal plane. This organization is summarized in Figure 1. We have termed this network the cellular matrix.

When sectioned along the long axis of the ligament, the nuclei appeared as long thin structures arranged in rows parallel to the long axis of the ligament (Fig. 2a). When the same preparations were stained for vimentin, the cell body could be visualized around the nucleus and extending both longitudinally along the row and peripherally, above, below and to the side of the rows of nuclei via thin cytoplasmic projections (Fig. 2b). Extensions extending perpendicular to the long axis of the cell were observed and functioned to connect cells between rows (Fig. 2c,d). The stellate nature of these projections was confirmed by electron microscopy (Fig. 3).

Although the majority of cells appeared as long thin structures oriented along the length of the ligament, interspersed between these rows of cells were endoligament cells displaying roughly spherical shaped nuclei that were more closely spaced than those of the ligament cells (Fig. 4). The endoligamentous cells and their projections formed an enveloping cellular mesh, which surrounded each fascicle and similar cells (epiligamentous cells) surrounded the entire ligament (as the epiligament) (Fig. 1).

At points of cell-to-cell contact along the ligament cell network, gap junctions were detected by immunohistochemical detection for the gap-junction protein connexin-43 (Fig. 5). Similar staining was also detected in regions of the endoligament and epiligament.

A family of trans-membrane proteins called connexins forms gap junction channels. There are more than 20 members in this multigene family and their

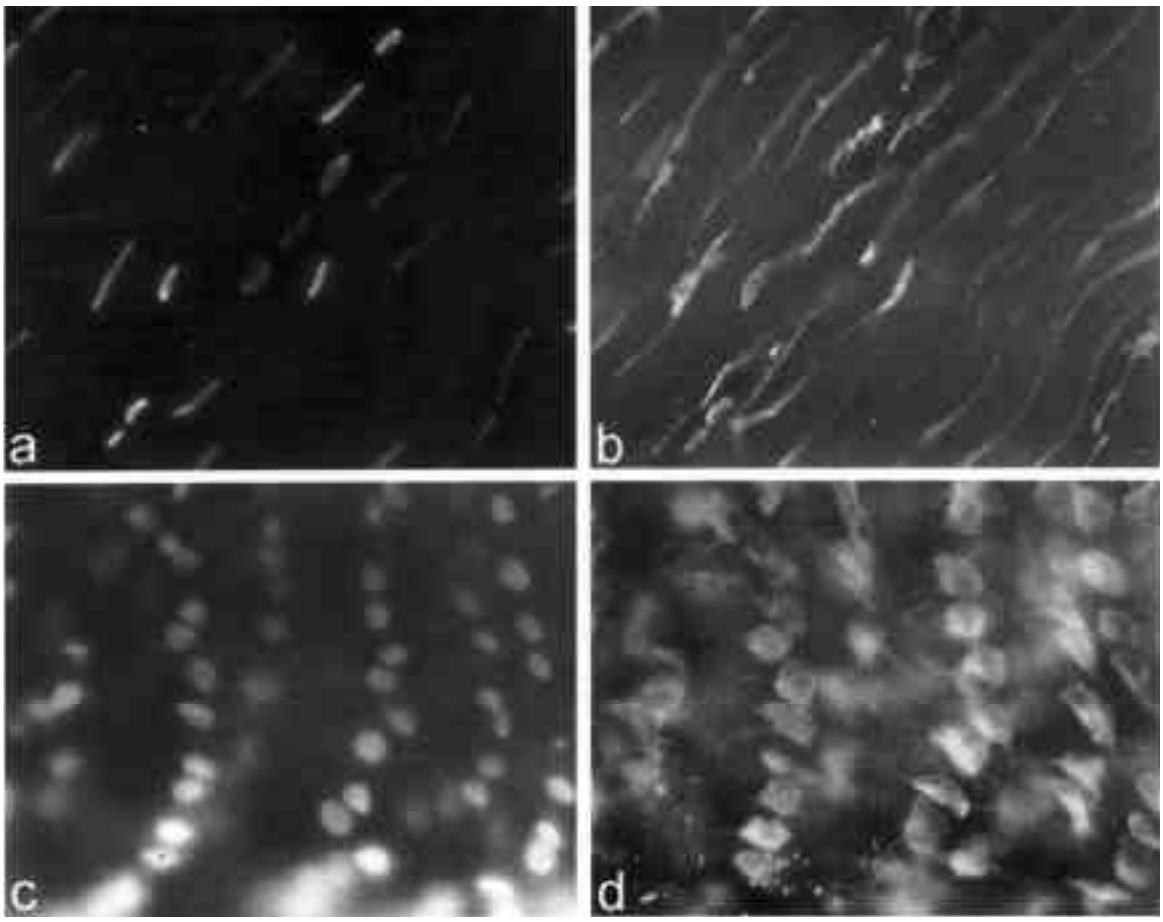

Fig. 2. Histological section of normal ovine medial collateral ligament **(a, b)** and rabbit medial collateral ligament **(c, d)**. When sectioned along the longitudinal length of the ligament the cells demonstrated longitudinal cytoplasmic processes connecting cells within rows **(b)**. When sectioned obliquely, the cells appear in rows with rounder nuclei and long cytoplasmic processes between rows. Histologic sections stained with DAPI **(a,c)** for cell nuclei and immunofluorescence labelling for vimentin **(b)** and ß-tubulin **(d)**. x 1,000

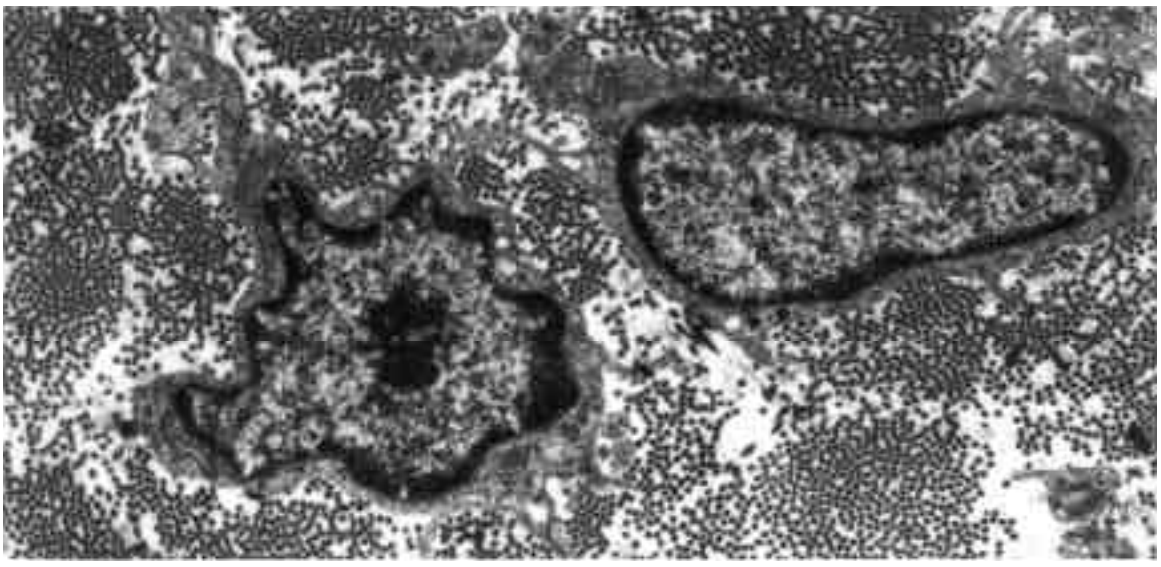

Fig. 3. Electron-micrograph of a transverse section of the ovine MCL demonstrating several long cytoplasmic projections extending into the extracellular matrix and between cells. x 7,000

name is based on the predicted molecular weight of the protein (Kumar and Gilula, 1996). The basic building block of a gap junction is the connexin molecule. A hemichannel or connexon is formed from a hexamer of connexin subunits. Connexons of opposing cells dock in the intercellular gap to form a complete connexin channel or gap junction, directly connecting the cytoplasm of adjacent cells. Gap junction channels thus span the plasma membrane of two cells and directly connect the cytoplasm of neighbouring cells, allowing the passage of a wide variety of small particles up to approximately 1200 Da. This allows the passage of amino acids, nucleotides, ions and secondary messengers including cyclic nucleotides, inositol triphosphate, and Ca^{2+}. The presence of these long cytoplasmic projections and gap junctions potentially connects the entire length of the ligament allowing ligament cells to coordinate their behaviour similar to other tissues (i.e. spread of excitation in the heart) (Evans, 1997). Further, gap junctions are critical in maintaining tissue homeostasis, synchronizing responses to stimuli and controlling growth and development (Citi, 1994; Kanno et al., 1995; Kumar and Gilula, 1996; Peracchia, 2000; Kelsell et al., 2001). Thus, the new finding that there are cell-to-cell communications within ligaments provides a new insight into how ligament function is mediated.

Tendon

Tendons are soft tissue structures that connect muscles to bone and are primarily responsible for the transmission of muscle-generated force to bone. Tendons are normally subjected to tensile loads along their length however these in vivo loads are significantly higher than that of ligament. While several studies have examined the organization of cells within tendon (Merrilees and Flint, 1980; Squier and Magnes, 1983; Squier and Bausch, 1984; Birk and Zycband, 1994; Senga et al., 1995; Tanji et al., 1995), the most extensive studies have been performed by Benjamin and Ralphs (1997, 1998, 2000), McNeilly et al. (1996) and Ralphs (1998). These studies have demonstrated, that tendon cells are arranged in a manner similar to that found in ligament (compare Benjamin and Ralphs, 1997; Lo et al., 2000, 2001) and also form an elaborate 3-dimensional network or a cellular matrix based on fusiform cells, cytoplasmic projections and gap junctions. Tendons also have an epitenon layer which is similar to epiligament (compare McNeilly et al., 1996; Benjamin and Ralphs, 1997 with Lo et al., 2000, 2001). Interestingly, connexin expression was noted to be spatially distributed, that is, connexin-32 was localized to cells within a longitudinal row and connexin-43 was localized to contacts between cells of rows and within a row.

Although the majority of load seen by tendons and ligaments is tensile in nature, in certain instances, these two tissues may be under compressive loads. For example, certain portions of the transverse ligament of the atlantoaxial joint of the cervical spine and the proximal medial collateral ligament of the knee are likely under compressive loads, where fibrocartilaginous changes in ligaments can occur. Tendons can also exhibit compressive loads, such as at the insertion of the Achilles tendon or as the posterior tibial tendon wraps around the medial malleolus. At these points, tendons appear fibrocartilaginous with an increased production of glycosaminoglycans, type II collagen and aggrecan (Merrilees and Flint, 1980; Vogel et al., 1989, 1993, 1994; Vogel and Koob, 1989; Vogel, 1995; Benjamin and Ralphs, 1998; Ralphs et al., 1998). This tissue metaplasia is likely at least in part related to mechanical load since redirecting these tendons during maturation outside its pulley (and thus decreasing compression) prevents the development of fibrocartilaginous changes (Ploetz, 1938; Gillard et al., 1979; Malaviya et al., 1996).

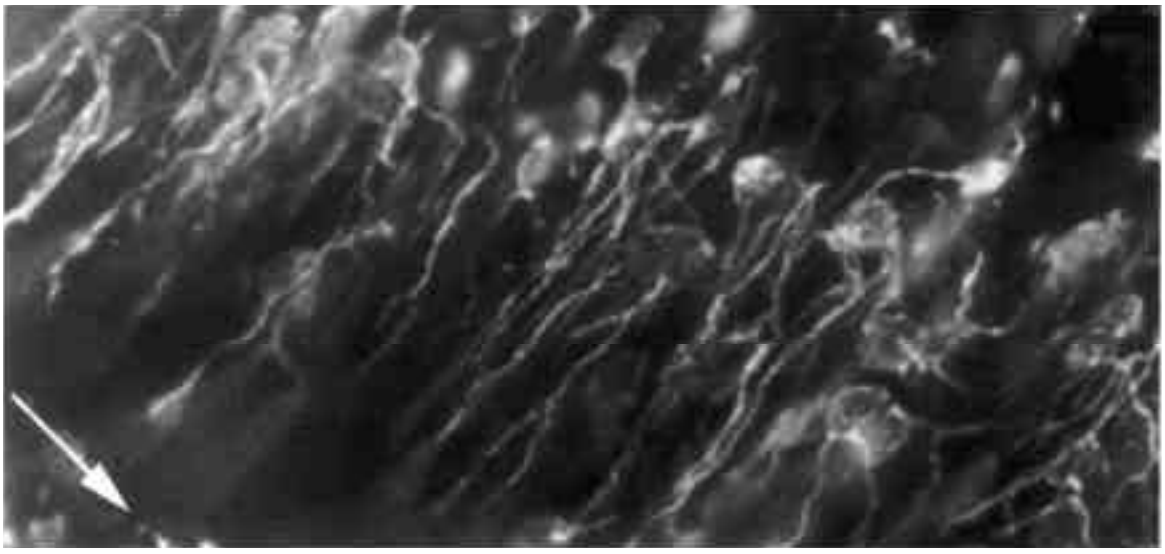

Fig. 4. Histological section of normal medial collateral ligament displaying epiligament cells. Note the spherical nuclei and cytoplasmic extensions generally located perpendicular to the long axis of the ligament (arrow denotes direction of long axis of ligament). x 1,000

Ralphs et al. (1998), have demonstrated that these changes are coincidental with alterations in cell shape and expression of gap junction proteins. Where the tendon is under compression, tendon cells lose their elongated shape, retract their cell processes and appear rounded. Tendon cells in these regions lose contact with their neighbouring cells and gap junction proteins (connexin-43 and connexin-32) are no longer expressed.

Menisci

While tendons are generally subjected to tensile loads and have some regions under compressive loads, tissues such as the menisci and intervertebral disc are typically compressed but have regions subjected to tensile loads. Menisci are intraarticular, fibrocartilaginous structures inserted between the articular surfaces of certain joints (i.e. the knee). Typically, menisci are wedge-shaped, with the wider outer portion of the wedge attached to the peripheral joint capsule and the internal thinner portion of the wedge located centrally. In the knee, the menisci (medial and lateral) may perform several functions including load bearing, shock absorption, neurosensation, joint stabilization and assisting in joint nutrition (King, 1936; Walker and Erkman, 1975; Ghadially, 1983; Arnoczky and McDevitt, 2000).

Menisci are subjected to complex loads including compressive, tensile and shear loads. Shrive et al. (1978) have proposed a model for how the meniscus converts compressive loads into radially directed forces. During normal joint loading, it was hypothesized that the anterior and posterior insertions of the meniscus are tensioned along with the circumferential fibers of the meniscus creating hoop stresses at the periphery of the meniscus. Thus, the compressive loads are resisted by the anterior and posterior attachments of menisci, and the radial forces are balanced by tensile stresses developed in the circumferentially oriented collagen bundles of the matrix (Bullough et al., 1970; Shrive et al., 1978). The mechanical principles outlined in this model would therefore predict the presence of circumferential tensile loads in the periphery of the meniscus. Interestingly, the various cells of the rabbit meniscus are organized in such a manner so that a specific sub-population of cells will occupy each of the predicted mechanical environments found in the meniscus.

In the rabbit model, four morphologically distinct cellular phenotypes have been identified, which are related to the basic architecture and functional domains of the rabbit meniscus (Fig. 6) (Hellio Le Graverand et al., 2001a). These include two classes of cells present within the fibrocartilaginous region of the meniscus. Both these cell types have long cellular projections extending from the cell body; however, the most peripheral cells contain more cellular projections while those more central in position contained fewer and shorter processes. While the projections of the tendons and ligament appear more precise, cellular projections in some meniscal cells with multiple branches can each be associated with gap junctions. A third cell type in the inner hyaline-like region of the meniscus has a rounded morphology and no cellular projections. A fourth cell

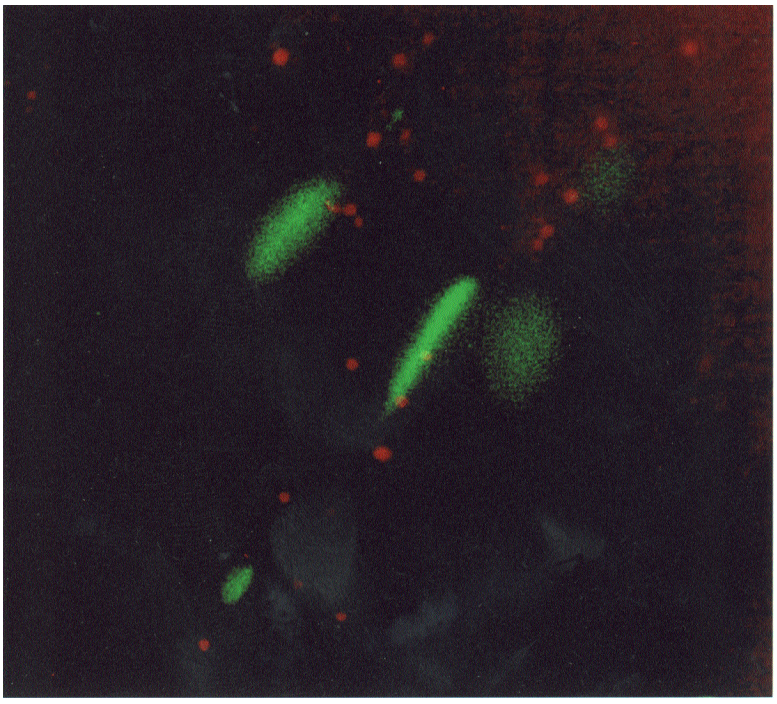

Fig. 5. Gap junctions between adjacent cells within the normal medial collateral ligament demonstrated in sections immunolabelled for connexin-43 (red) and counterstained with DAPI (false coloured green). x 600. (Reproduced from Lo et al. The cellular networks of normal ovine medial collateral and anterior cruciate ligaments are not accurately recapitulated in scar. J. Anatomy, in press).

type with a fusiform shape and no cytoplasmic projections is seen along the superficial regions of the meniscus. The cells in this layer share some morphological similarities with the cells of the superficial layer of articular cartilage (Ghadially et al., 1978; Ghadially, 1983). Thus, there appears to be a relationship between the distribution of morphologically distinct structural cell types in the rabbit meniscus and other dense soft connective tissues. For example cells from regions that experience tensile loads such as the peripheral meniscus, ligament and tendon appear to be morphologically similar. In addition, morphological similarities can also be observed in chondrocytes and cells from the central meniscus, which experience compressive loads. These similarities suggest that specific cellular phenotypes may be associated with specific types of connective tissue function. These cellular phenotypes also correlated with different types of extracellular matrix and are summarized in Table 1 (McNicol and Roughley, 1980; Adams and Muir, 1981;

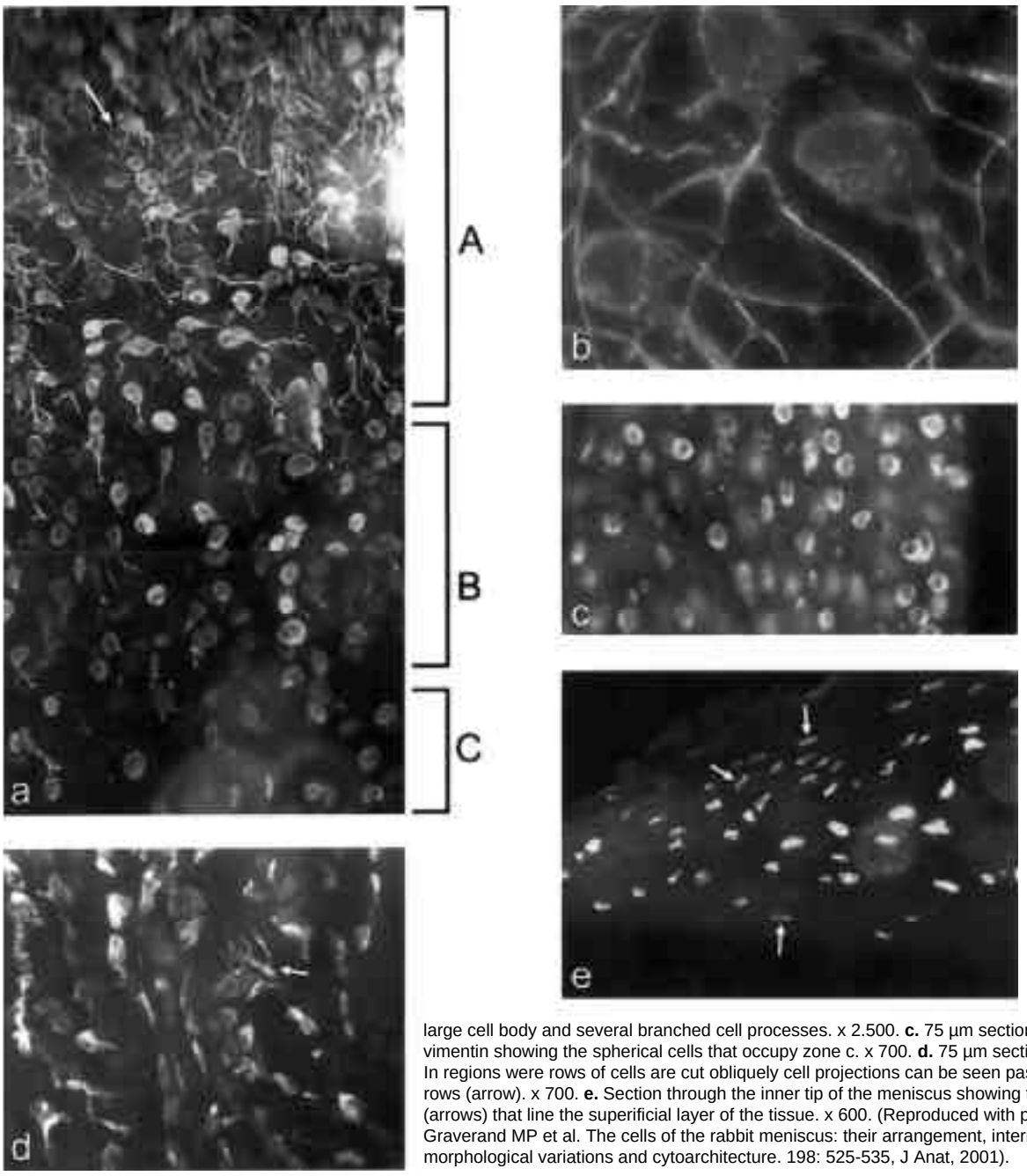

Fig. 6. a. Montage of an oblique 75 µm section of the rabbit meniscus stained with an antibody to vimentin. Cells with distinct morphologies are found in each of the 3 zones. The arrow denotes a row of cells found in zone A. x 600. **b.** High magnification view of cells from zone A as shown in **a**. Each cell has a large cell body and several branched cell processes. x 2.500. **c.** 75 µm section stained for vimentin showing the spherical cells that occupy zone c. x 700. **d.** 75 µm section through zone A. In regions were rows of cells are cut obliquely cell projections can be seen passing between the rows (arrow). x 700. **e.** Section through the inner tip of the meniscus showing the fusiform cells (arrows) that line the superificial layer of the tissue. x 600. (Reproduced with permission: Hellio Le Graverand MP et al. The cells of the rabbit meniscus: their arrangement, interrelationship, morphological variations and cytoarchitecture. 198: 525-535, J Anat, 2001).

Eyre and Wu, 1983; Adams et al., 1986; Adams and Ho, 1987; Cheung, 1987; Nakano et al., 1997; Scott et al., 1997).

Intervertebral disc

Three regions of the intervertebral disc have been described: the nucleus, and the inner and outer annulus fibrosus (Szirmai, 1970). There are marked regional differences in the matrix composition of each of these regions. The nucleus is more cartilaginous (i.e. aggrecan and collagen II) and the outer annulus is more ligament or tendon like (i.e. collagen I) (Eyre and Muir, 1976; Pritzker, 1977; Eyre, 1979; Bayliss et al., 1988; Oegema, 1993; Johnstone and Bayliss, 1995; Urban and Roberts, 1996). The inner annulus appears to be a mixture of both and has been described as fibrocartilaginous containing aggrecan and both type I and type II collagen (Eyre and Muir, 1976; Eyre, 1979; Johnstone and Bayliss, 1995).

The outer annulus is arranged in a distinct pattern of collagenous lamellae (Hickey and Hukins, 1980; Humzah and Soames, 1988). Within each lamella, collagen fibres run parallel to each other at an oblique angle to the long axis of the spine. The collagen fibres of successive lamellae run in opposite angles to each other thus providing the annulus with a cross-ply arrangement. The unique structure of the intervertebral disc allows specific functional roles of each of its regions. For example, the cross-ply arrangement of the collagenous outer annulus fibrosus is uniquely organized to resist the large tensile stresses which develop during complex loading of the spine (i.e. axial compressive loading, torsional loading, sagittal and transverse bending) (Buckwalter et al., 2000).

The cells of these regions of the intervertebral disc also show distinct differences (Postacchini et al., 1984; Errington et al., 1998). Although some processes were demonstrated in all regions of the intervertebral disc, the cells of the outer annulus were most elongated and appeared to extend from and in the direction of the long axis of the cell. Outer annulus fibers have also been shown to have processes that run perpendicular to the long axis of the cell. However, cells from the nucleus and inner annulus were observed to be more round with fewer processes and were separated by extensive extracellular matrix. Although the presence of gap junctions in these regions has not been confirmed, this organization closely resembles that found in the rabbit meniscus and this possibility requires further investigation.

In summary, within ligament, tendon, meniscus, and the intervertebral disc there exist certain regions of tissue where the cellular arrangement forms an elaborate 3-dimensional network or cellular matrix, consisting of fusiform cells, cytoplasmic projections and gap junctions. This arrangement appears to be coincident with the tensile-load bearing property of the tissue and thus may define a specific class of hypocellular, tensile-bearing, dense connective tissues.

Function of the cellular matrix of tensile bearing dense connective tissues

The identification of a cellular matrix in tensile bearing dense connective tissues has led to several investigations on its possible function. Several functions have been suggested including roles in 1) the organization of collagen fibrils; 2) sensing the mechanical environment; 3) injury and healing.

Organization of collagen bundles

Classically the extracellular matrix has been thought of as the scaffold in which cells attach and proliferate. However, the presence of a network of interlacing cells and cytoplasmic projections suggests an alternative hypothesis. That is, the network of interconnected cells itself, may act as a scaffold for the deposition of oriented collagen fibrils and extracellular matrix. These long cellular processes seem to be particularly well suited for matrix deposition since, bundles of collagen fibrils can be seen in cell processes at long distances away from the cell body (Birk and Zycband, 1994). This hypothesis is further supported by observations that during early development the total cell number within tissues such as ligament remains relatively constant while the matrix is deposited in an orderly oriented fashion around each cell (Frank CB, unpublished observations).

Birk and Zycband (1994) have described 3 extracellular compartments in which collagen is formed

Table 1. General organization and composition of ligament, meniscus and cartilage.

	MENISCUS			
	Cartilage	Inner Zone	Outer Zone	Ligament
Cell Shape	Round	Round	Fusiform	Fusiform
Cell Projections	Minimal	Minimal	Long connects cells	Long connects cells
Major Collagen Type	Type II	Type II	Type I	Type I
Proteoglycan	Large PG	Large PG	Less Large PG More Small PG	Small PG

and the matrix is deposited. Initially fibrils are deposited in narrow channels linked to the cell surface originating deep in the cytoplasm. In a second compartment where these channels fuse, bundles of collagen fibrils form at the cell surface. A third compartment where the bundles become laterally associated is defined by the close apposition of two to three fibroblasts. Recently, McNeilly et al. (1996) have also described a fourth compartment defined by successive cells which enclose each fibril bundle with fine sheet-like lateral cell processes. These cellular tunnels or rings (formed of cells and cell projections) form the basis whereby collagen fibrils are deposited in an organized fashion.

Importantly cell orientation often precedes matrix deposition and alignment. This is particularly apparent in the annulus fibrosus of the intervertebral disk where annulus fibrosus cells are first aligned in a parallel fashion within each lamellae of the annulus (Hayes et al., 1999). Subsequently, the collagen fibers are deposited along these oblique cells thus, forming the characteristic cross-ply nature of successive lamellae in the mature annulus fibrosus.

Sensing mechanical environment

The 3-dimensional cellular matrix has the potential to connect the entire length of the tissue (in the case of the ligament or tendon) or significant portions of the tissue (in the case of the meniscus or annulus fibrosus). Thus, characterizing this network is essential to understanding not only cell-to-cell interactions but also cell-to-matrix interactions. Further, intercellular communication may be critical in maintaining and remodelling the extracellular matrix and thus impact tissue biomechanics.

Direct cell-to-cell communication would allow the coordination of cellular behaviour particularly in response to mechanical load within large portions of the tissue. It has long been presumed that cells are able to respond to mechanical load in some fashion and maintain or remodel the extracellular matrix in response to loading (Banes et al., 1995a-c; Guilak et al., 1997; Grodzinsky et al., 1998). Indeed there is a significant body of knowledge with respect to chondrocytes and their response to changes in their local mechanical environment (Benya and Shaffer, 1982; Palmoski and Brandt, 1984; Sah et al., 1989; Korver et al., 1992; Parkkinen et al., 1992; Buschmann et al., 1995, 1996; Knight et al., 1998; Ragan et al., 1999). Several studies have evaluated the response of ligament, tendon, meniscal and annulus fibrosus cells or tissue to mechanical load in vitro (Boitano et al., 1992; Banes et al., 1995a-c, 1999a-c; Sood et al., 1999; Waggett et al., 1999, 2001; Hsieh et al., 2000). However, in many studies cells are isolated and expanded in vitro. This process completely disrupts this 3-dimesional network, inter-cellular communication and cell-matrix interactions and thus may not be representative of the in vivo environment (Banes et al., 1995c, 1999a,c; Boitano et al., 1992; Waggett et al., 1999, 2001; Hsieh et al., 2000).

Evidence for a mechanosensory role of cells within dense connective tissue is growing. As described above, these tissues do demonstrate changes in the extracellular matrix in response to mechanical load. Where tendons or ligaments are under compression or in the inner aspect of the meniscus, these tissues exhibit cartilaginous like changes with increases in type II collagen, and aggrecan (Merrilees and Flint, 1980; Vogel and Koob, 1989; Vogel et al., 1993, 1994; Vogel, 1995; Benjamin and Ralphs, 1998; Ralphs et al., 1998).

In addition, several in vitro studies in tendon cells grown in monolayer have demonstrated that gap junction inhibitors can disruption calcium wave propagation or inhibit type I collagen synthesis when subjected to mechanical load (Sood et al., 1999; Waggett et al., 1999, 2001). Although these cells were taken out of the 3-dimensional network, findings suggest that isolated tendon cells in vitro are capable of responding to mechanical load and that gap junctions at least in part regulate this response.

Perhaps more relevant, ex vivo studies have demonstrated increased DNA and collagen synthesis when whole tendons were subjected to cyclic tensile loads (Banes et al., 1999b). These tensile load-induced changes were further blocked with specific gap junction inhibitors suggesting that gap junctions and inter-cellular communication are important in sensing and responding to changes in the mechanical environment.

Injury

Clinically, dense connective tissues such as tendons, ligaments and menisci are commonly injured and may subsequently undergo degenerative changes. These disease processes have been studied both clinically and experimentally in animal models and have demonstrated that the extracellular matrix undergoes changes in both composition and structure (Daniel et al., 1994; Marshall and Chan, 1996; Birch et al., 1998; Gillquist and Messner, 1999; Hellio Le Graverand et al., 2001b,c). The identification of a cellular matrix raises the question as to whether the cellular matrix is also altered during the onset and progression of these degenerative processes.

Osteoarthritis is a common degenerative disorder that leads to changes of the joint tissues. Although the changes in cartilage have been well characterized other tissues such as the meniscus also undergo pathological changes. Using the ACL transection model of osteoarthritis, alterations including apoptosis, cell cluster formation, matrix remodelling of type I, II, and III collagens, matrix degradation (increased MMP levels, cleavage of type I and II collagens) and tear formation occur in the meniscus (Hellio Le Graverand et al., 2001b,c). More recently, the effects of ACL transection on the cellular networks of the meniscus and a possible model for the formation of cell clusters within menisci have been described (Fig. 7) (Hellio Le Graverand et al., 2001d).

This study demonstrated that following ACL transection in the rabbit model, there is a progressive sequence of events, which leads to meniscal tear formation and is initiated by the disruption of the normal cellular network of the meniscus. The cellular network typically seen in the periphery of the meniscus is first disrupted as cells retract their cellular processes in response to either apoptosis or cellular proliferation. This retraction results in the formation of isolated islands of cells and associated changes in their morphological phenotype. Unlike typical cells found in the periphery of the meniscus, which are characterized by long cytoplasmic projections, cells found in islands or clusters retract their cellular projections and form three types of cell clusters of different morphologies. These morphologies include stellate cells, round cells or stellate and round cells. With time, the clusters of round cells become more prominent and increase in size, presumably due to cellular proliferation. This observed rounding up of meniscal cells not only results in changes to cell-to-cell interactions but also in cell-to-matrix interactions.

In addition to the formation of these clusters, a change in the surrounding extracellular matrix was demonstrated which was characterized by increases in type X collagen, MMP-13 levels, collagen degradation products and calcium deposition. Interestingly, the increased amounts of MMP-13 and type II collagen degradation products were found to be localized to meniscal tears which could potentially be contributing factors to the development of meniscal degeneration (Hellio Le Graverand et al., 2000).

Although this sequence of events needs to be confirmed in other models, this observation suggests that the disruption of the cellular network of the meniscus may be one of the initial processes leading to meniscal cell cluster formation and eventually to meniscal degeneration. Thus, the maintenance of the cellular network may be very important in maintaining tissue integrity. Failure or disruption of the cellular network leads to alterations in the extracellular matrix and eventual tissue failure.

Healing

Ligaments do not heal by the regeneration of normal tissue but by the formation of scar tissue. This tissue is biomechanically inferior and biochemically, histologically, and ultrastructurally different when compared to normal ligament (Loitz and Frank, 1993; Frank et al., 1999a,b; Lo et al., 2000). The detection of a highly organized cytoarchitecture in normal ligament raised the question as to whether this cytoarchitecture exists in scar and how the cytoarchitecture of the scar

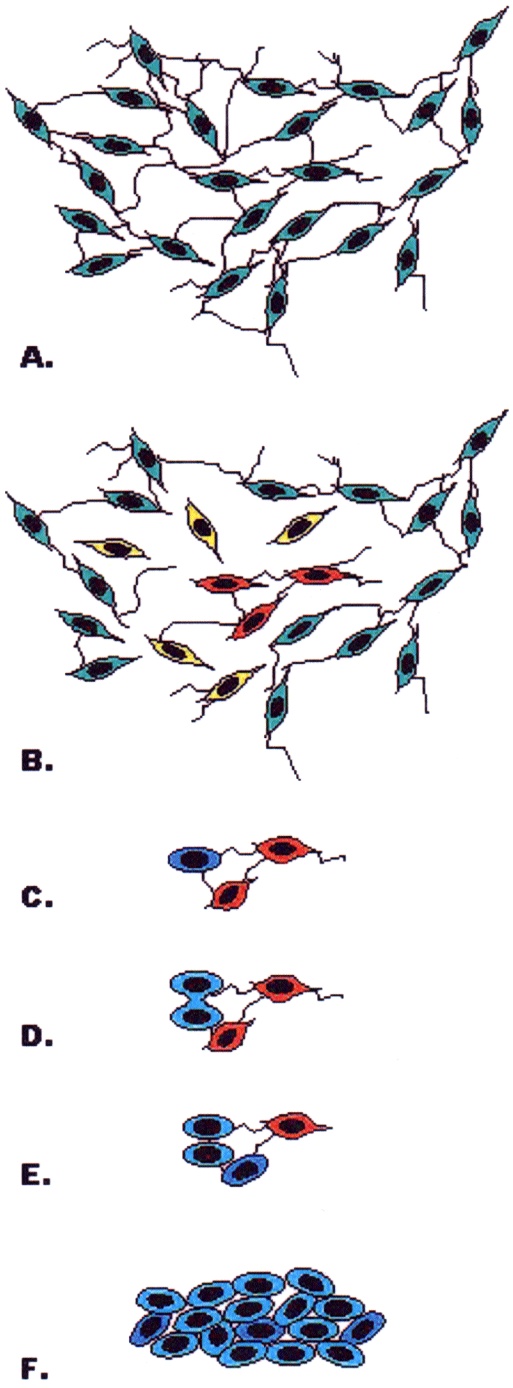

Fig. 7. Diagrammatic representation of the proposed mechanism for cluster formation in the rabbit meniscus. **a.** In a normal meniscus, cytoplasmic projections joins adjacent meniscal cells to form a complex cellular network. **b.** During early meniscal degeneration, numerous meniscal cells undergo apoptosis altering the cellular network and isolating groups of cells. **c.** Islands of meniscal cells alter their morphological phenotype transforming to a round shape and losing cytoplasmic projections. **d and e.** Some cells within islands enter the cell cycle resulting in the increase in the number of cells within a cluster. **f.** As the size of the clusters increase, they form elongated rods. (Reproduced with permission: Hellio Le Graverand M.P., Sciore P., Eggerer J., Rattner J.P., Vignon E., Barclay L., Hart D.A. and Rattner J.B. Cell clusters in osteoarthritic meniscus: their formation and phenotype. Arthritis & Rheumatism, 44: 1808-1818, 2001).

and adjacent uninjured tissue are related. Recent studies have addressed this question in healing ovine medial collateral ligaments (MCL) and posterolateral bands of the anterior cruciate ligament (ACL) (Lo et al., 2001). These two ligaments are of particular interest since the medial collateral ligament (MCL) has been described as

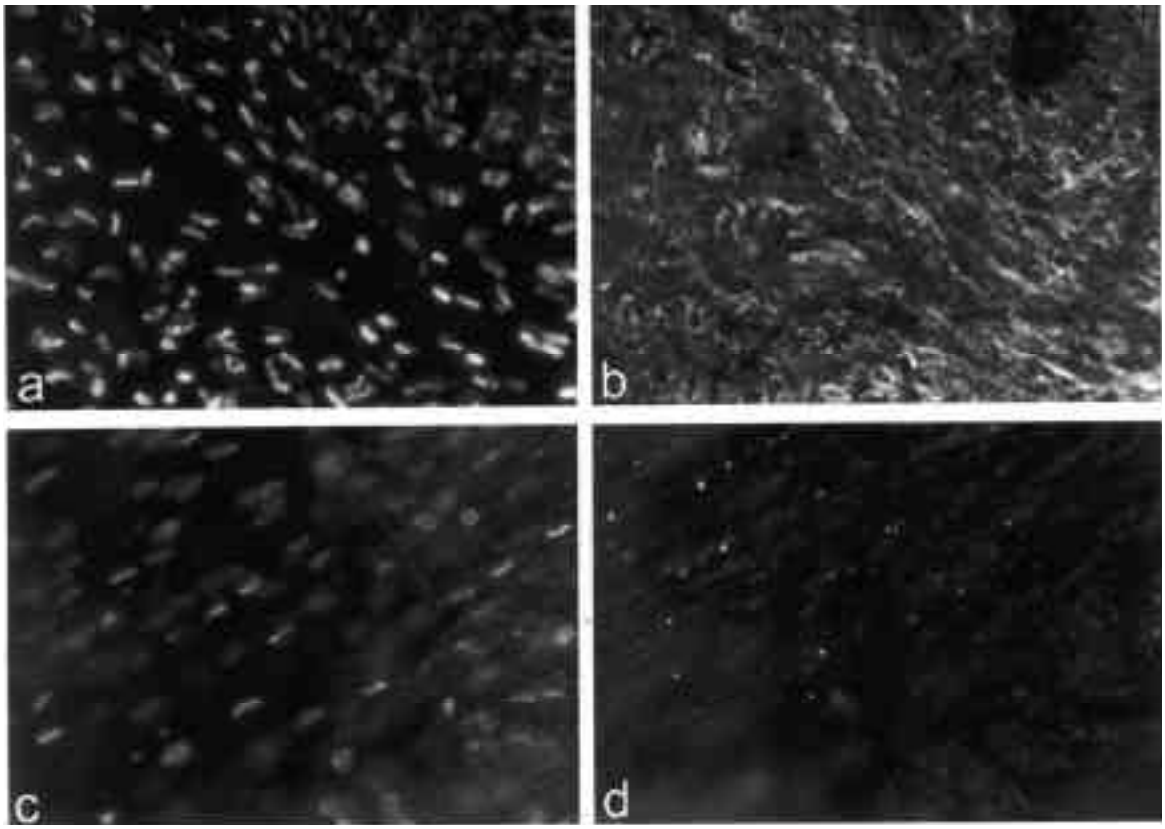

Fig. 8. Histologic section of 6 week healing ovine anterior cruciate ligament demonstrating increased cellularity and disorganization **(a, b)**. The scar tissue is positive for connexin-43 **(c, d)**. Sections stained with DAPI **(a, c)** and immuno-fluorescence labelling for vimentin **(b)** or connexin-43 **(d)**. x 500

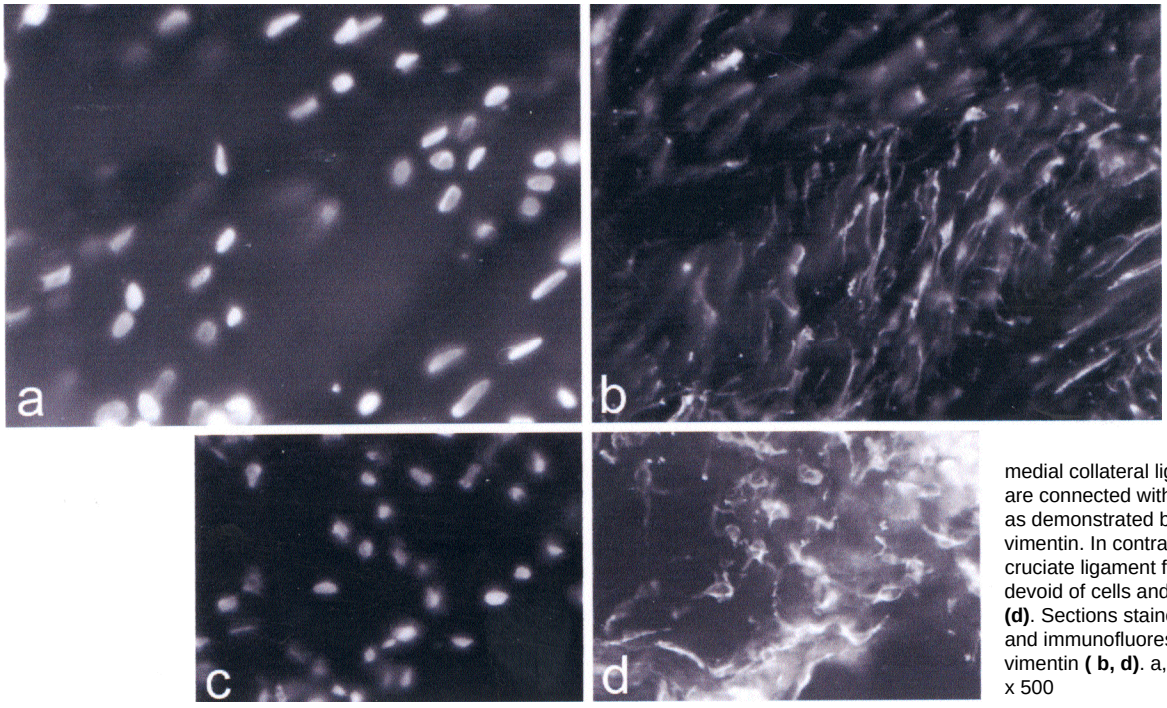

Fig. 9. Histologic section of 3 month healing medial collateral ligament **(a, b)** and posterolateral band of the anterior cruciate ligament **(c, d)** demonstrating cellular flaws as demonstrated by DAPI staining. Note that the medial collateral ligament flaws **(a, b)** are connected with cellular projections as demonstrated by staining for vimentin. In contrast the anterior cruciate ligament flaws **(c)** remain devoid of cells and cellular projections **(d)**. Sections stained with DAPI **(a, c)** and immunofluorescence labelling for vimentin **(b, d)**. a, b, x 1,000; c, d, x 500

an extra-articular ligament with an excellent capacity to heal, while the anterior cruciate ligament (ACL) is an intra-articular ligament and is described as having a poor capacity to heal. Injuries to the ACL commonly do not heal functionally and require surgical intervention (Daniel et al., 1994).

This study demonstrated a number of important findings (Lo et al., 2001). First, the overall cellularity of all scar samples was greater than that found in either normal ligaments or uninjured controls. This increase in cellularity was due in part to active cell proliferation as demonstrated by immunoreactivity for the cell cycling antigen Ki-67. These cycling cells appeared as clusters

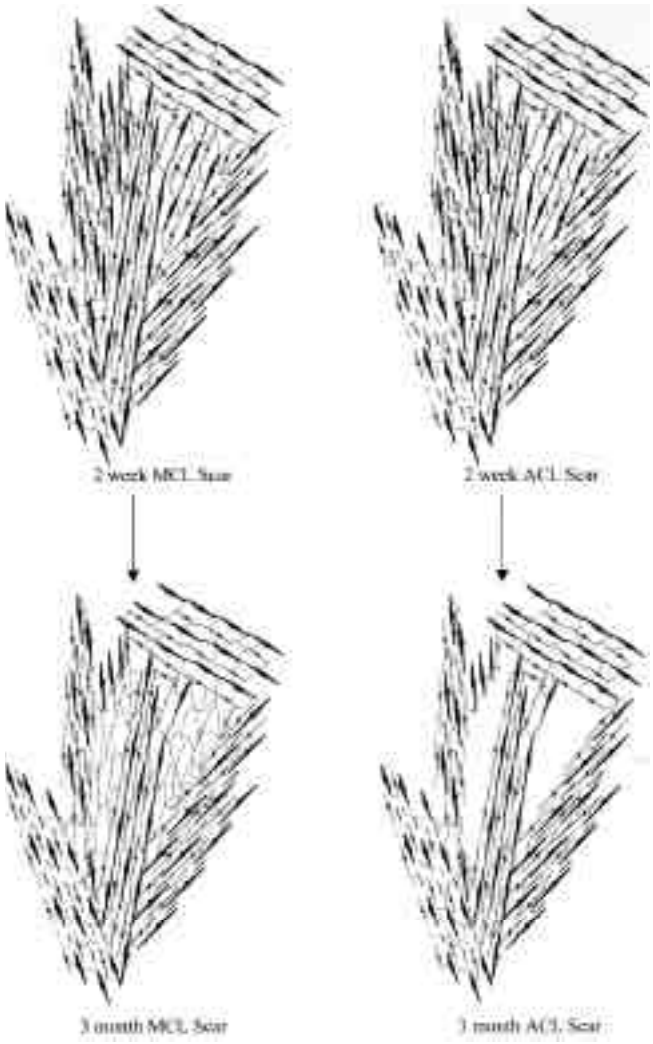

Fig. 10. Schematic representation of healing medial collateral ligament and anterior cruciate ligament. At 2 weeks both the medial collateral ligament and anterior cruciate ligament contained bundles of cells in a disorganized fashion. At 3 months both ligament scars contain flaws, however, in the medial collateral ligament the flaws are filled by long cytoplasmic processes and connected with gap junctions. In the anterior cruciate ligament the flaws remain devoid of cells and cytoplasmic processes.

throughout the tissue and also decreased with time. The overall cellularity of scar in both types of ligaments decreased with time similar to previous reports in other species, but did not return to levels found in uninjured tissue (Loitz and Frank, 1993, Murphy et al. 1993).

The cells within both MCL and ACL scar tissue did not display the extensive longitudinal arrays of cells characteristic of normal tissue (described above). Rather when stained for cytoskeletal proteins, ligament scars appeared to be composed of multiple short bundles of cells. These bundles had a random orientation with respect to the long axis of the ligaments and also to one another so that some bundles were perpendicular to one another (Fig. 8). Within each bundle adjacent sheets of cells were more closely packed than that found in either uninjured ligaments or undamaged tissue adjacent to the scar. Similar to cells in normal ligaments, these cells stained for the gap junction protein, connexin-43 (Fig. 8). These randomly arranged bundles, may themselves contribute to inferior biomechanical properties of tissue but my also result in randomly arranged collagen bundles, further compromising tissue biomechanics.

In general, the organization of the scar tissue in terms of cellular bundles did not change over time. However, both the healing MCLs and ACLs showed prominent discontinuities at 3 months. These discontinuities (Fig. 9) were defined as regions within the tissue that were devoid of nuclei when stained with DAPI. Interestingly, in the MCL scars, the majority of these discontinuities were in fact filled with cellular processes (identified by vimentin staining, figure 9a,b) and were connexin-43 positive. In contrast, in ACL scars, the discontinuities were devoid of cells, cellular processes, and gap junctions as shown by the absence of staining with antibodies to connexin-43 and vimentin (Figs. 9c-f, 10). Future studies will be needed to determine if the type of connexin expression also changes with scar formation.

The presence of discontinuities in the cellular matrix of scars may have a number of implications. Although these discontinuities may be secondary changes due to a failing ACL healing process, because gap junctions connect cells chemically and electrically, the discontinuities detected in the healing ACL likely prevent cells within the scar and perhaps between the scar and the residual uninjured ligament from communicating effectively. These discontinuities may impair metabolic cooperation between cells and further prevent a coordinated response to changes in their biomechanical environment and therefore compromise ligament integrity. Thus, the presence and characteristics of these discontinuities may explain some of the differences between the healing capacity of the MCL and the ACL (Murphy et al., 1993). In addition, differences in the expression of α and ß integrin subunits in the MCL compared to the ACL have been reported (Schreck et al., 1995). Thus, ligament scar discontinuities and the healing capacity of the two ligament types may be the result of complex changes in

the relationship between cells and between their relationship with the extracellular matrix.

Future directions and conclusions

Connective tissues are a diverse group of tissues, which reside in different environments and must provide a spectrum of load-bearing properties. Tensile bearing dense connective tissues may be a subset of connective tissues and demonstrate a specific cellular organization. This organization based upon fusiform cells, with long cytoplasmic projections, and gap junctions, can be detected in ligaments, tendons, meniscus, and the intervertebral disc.

While the identification of the cellular matrix in several tissues is both exciting and intriguing, further research is required to understand its implications. In particular, the specific role of connexins, their subtypes, and distribution within different types of dense connective tissues should be investigated and may provide insight into their possible functional roles. In addition, the developmental organization of the cellular matrix and how this correlates with other cell-to-cell interactions (i.e. adherens junctions) and cell-to-matrix interactions will provide important baseline information. This information will be valuable in interpreting how this organization is altered during pathological conditions (i.e. tendon degeneration, disc disease) and injury responses (i.e. healing).

While the demonstration of connexin expression is indicative of gap junctions, the demonstration of functional gap junctions (i.e. specific dye transfer techniques, intra/inter-cellular Ca^{2+} propagation) in situ and identification of possible secondary messengers that flow through these gap junctions needs to be elucidated. Finally, how the maintenance and disruption of the cellular network affects the biomechanical properties of tissues may be important in understanding disease processes and provide the opportunity for novel therapeutic interventions.

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