Mutable Collagenous Tissue: Overview and Biotechnological Perspective

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Abstract. The mutable collagenous tissue (MCT) of echinoderms can undergo extreme changes in passive mechanical properties within a timescale of less than 1 s to a few minutes, involving a mechanism that is under direct neural control and coordinated with the activities of muscles. MCT occurs at a variety of anatomical locations in all echinoderm classes, is involved in every investigated echinoderm autotomy mechanism, and provides a mechanism for the energy-sparing maintenance of posture. It is therefore crucially important for the biology of extant echinoderms. This chapter summarises current knowledge of the physiology and organisation of MCT, with particular attention being given to its molecular organisation and the molecular mechanism of mutability. The biotechnological potential of MCT is discussed. It is argued that MCT could be a source of, or inspiration for, (1) new pharmacological agents and strategies designed to manipulate therapeutically connective tissue mechanical properties and (2) new composite materials with biomedical applications.

1 Introduction

1.1 Collagenous Tissue

The collagens are a family of at least 19 proteins characterised by the presence of triple helical regions composed of repeating Gly-X-Y triplets. The majority of collagens occur in the extracellular matrix (ECM) of connective tissue, the most prevalent of these being types I, II and III, which self-assemble into par-

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allel aggregations known as fibrils, and type IV, which forms a network in the lamina densa of all basement membranes. In the ECM, collagen fibrils are accompanied by a variety of other components, which may include other fibrous structures, such as elastin fibres or microfibril aggregations, proteoglycans, glycoproteins and glycosaminoglycans. Some of these other components link together adjacent collagen fibrils and are thereby responsible for interfibrillar cohesion (Kannus 2000; Sarras and Deutzmann 2001).

Connective tissue in which the mechanically dominant component is collagen is the most widespread and evolutionarily ancient structural material in the animal kingdom. With the exception of possibly only the phyla Rotifera (Clément 1993) and Placozoa (Syed and Schierwater 2002), it is present in the bodies of all multicellular animals, including sponges, the internal 'mesohyl' of the latter being histologically and functionally a collagenous connective tissue that, unlike the situation in other animals, is not separated from its investing epithelia by basement membranes (Garronne 1978; Bonasoro et al. 2001). (It is possible that the absence of basement membranes from most sponges is due to secondary loss: see Maldonado 2004.)

1.2 Mechanical Adaptability of Collagenous Tissue

In its role as a structural material, the principal functions of collagenous tissue are to transmit, resist and dissipate mechanical forces and to store and release elastic strain energy. These functions depend on the tissue as a whole possessing certain 'fit for purpose' mechanical properties (tensile strength, stiffness, resilience, etc.), which are the result of a combination of the mechanical properties of its individual structural components, the micro-architectural organisation of these components and the nature of the interactions between them.

The net mechanical properties of most collagenous structures are relatively stable within a physiological timescale (seconds to hours). They may change during maturation and ageing, partly as a result of alterations in collagen fibril diameter and the composition of the interfibrillar phase mediated by adjustments in the synthetic activities of fibroblasts and, in the case of ageing, partly as a result of the non-adaptive biochemical modification of the ECM, e.g. by non-enzymatic glycation (Vogel 1980; Bruel and Oxlund 1996; Reddy et al. 2002; Silver et al. 2003). The mechanical properties of collagenous structures can also be modified in response to a long-term shift in the pattern of force to which they are subjected. For example, exercise increases the tensile strength and stiffness of tendons, again probably through fibroblast-mediated changes in fibril diameter and interfibrillar composition (Buchanan and Marsh 2002; see also Chiquet 1999 for a discussion of the regulatory pathways that might be involved). These age-related and adaptive changes occur over timescales of days to years and are quantitatively undramatic. However,

some collagenous structures undergo more drastic changes in mechanical properties over a shorter timescale, which result in a qualitative modification of the mechanical functioning of the tissue and thus justify it being regarded as a mechano-effector. In *Homo sapiens* and other mammals, this phenomenon is demonstrated by various collagenous structures associated with the female reproductive tract. For example, at the end of pregnancy, the compliance of the uterine cervix increases temporarily by a factor of 12. It switches from being tough and inextensible, in which state it helps to prevent expulsion of the conceptus, to being soft and easily extensible, a condition that permits the dilatation and effacement required for the passage of the fetus through the birth canal. Cervical 'relaxation' depends on two separate processes: (1) changes in the synthetic activity of the stromal cells, which secrete less collagen types I and III and small proteoglycans and secrete more of the large proteoglycan versican; and (2) the enzymatic degradation of the ECM by matrix metalloproteinases (MMPs) including MMP-1, MMP-3 and MMP-8, together with increased expression of the tissue inhibitors of metalloproteinases TIMP-1 and TIMP-2 (these presumably acting as a brake on the degradative process). The resulting change in mechanical properties occurs over hours to days and is controlled by a combination of hormonal events and the direct effect of stretch on the stromal cells (Uldbjerg 1994; Westergren-Thorsson et al. 1998; Yoshida et al. 2002; Sennström et al. 2003). Similar mechanisms and controlling factors are involved in the rupture of the fetal membranes at term (Bryant-Greenwood 1998) and of the ovarian follicle at ovulation (Robker et al. 2000).

The above examples of connective tissue mechanical adaptability have been given in order to highlight the distinctiveness of the subject of this chapter – the mutable collagenous tissue (MCT) of echinoderms. MCT can undergo extreme alterations of mechanical properties within a timescale of less than 1 s to a few minutes, involving a mechanism that is under direct neural control and coordinated with the activities of other mechano-effectors, viz. muscles. The rest of this chapter provides a review of current knowledge of the physiology and organisation of MCT and discusses its biotechnological potential.

2
Mutable Collagenous Tissue: Physiology and Organisation

2.1
Overview

MCT is present at a variety of anatomical locations in all living echinoderm classes, is involved in every investigated autotomy (defensive self-detachment) mechanism and provides a mechanism for the energy-sparing maintenance of posture (Wilkie 1996, 2001, 2002; Wilkie et al. 2004a). MCT is there-

fore crucially important for the biology of extant echinoderms and, in view of its apparent absence from other animals, has been regarded as one of the distinguishing characteristics of the phylum (Byrne 2001).

MCT occurs in the form of dermal connective tissue, interossicular ligaments, tendons linking muscles to skeletal elements and the connective tissue layer in the walls of tubular structures. It performs the same mechanical functions as the collagenous connective tissue at analogous locations in the bodies of vertebrates. Although there have been few attempts to correlate the supramolecular organisation of mutable collagenous structures with their 'conventional' mechanical functions (O'Neill 1989; O'Neill and Withers 1995), there is no reason to doubt that, despite their additional property of variable tensility, all such structures are as exquisitely designed to meet the subtly different functional demands of each anatomical location as are their vertebrate equivalents (see, e.g., Bauer et al. 1989; Munns et al. 1994; Gupte et al. 2002).

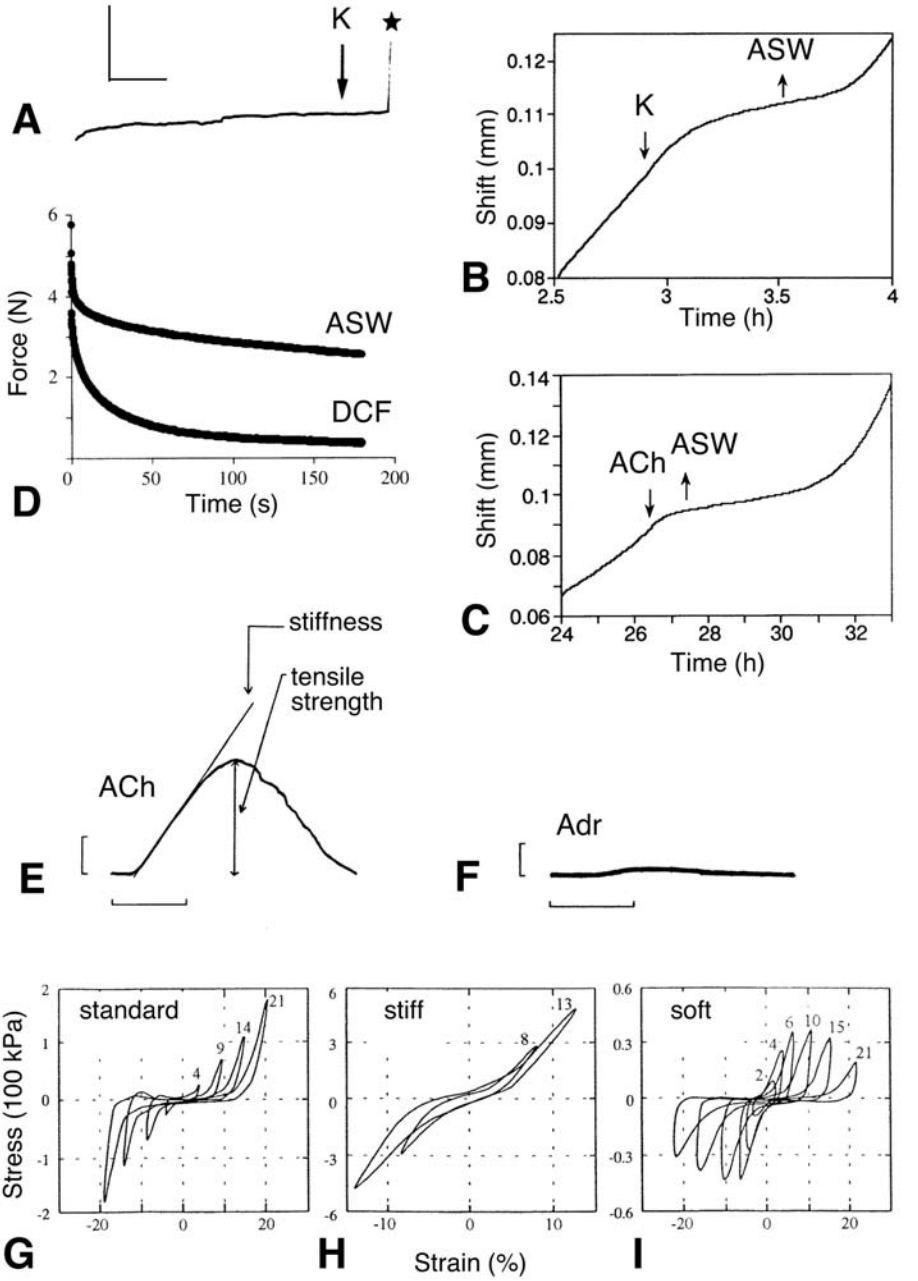
2.2 Mechanical Adaptability of MCT

2.2.1 Passive Mechanical Properties

Each mutable collagenous structure exhibits one of three patterns of change in its passive tensile properties: (1) only *reversible* changes (e.g. in viscosity or stiffness); (2) *irreversible* destabilisation (always associated with autotomy) as well as reversible changes; or (3) only irreversible destabilisation (Wilkie 2002).

The most extreme manifestation of MCT mechanical adaptability is the rapid and irreversible loss of tensile strength undergone by collagenous structures that cross echinoderm autotomy planes. For example, when an arm of the ophiuroid *Ophiocomina nigra* is autotomised, the ultimate tensile

Fig. 1A–I. Passive mechanical behaviour of MCT. **A** Creep test (in which extension of sample under constant load is recorded): in response to 100 mM K^+ (*K*) syzygial ligament of crinoid *Antedon mediterranea* shows sudden decrease in viscosity culminating in rupture (*star*). *Horizontal scale bar* 1 min; *vertical scale bar* 1 mm (adapted from Wilkie et al. 1999). **B**, **C** Creep tests: viscosity of dental ligament of echinoid *Diadema setosum* is increased reversibly by 1 mM acetylcholine (*ACh*) and 100 mM K^+ (*K*). *ASW* Artificial seawater (adapted from Birenheide et al. 1996). **D** Stress relaxation tests (in which samples are subjected to constant deformation and force is recorded): average force relaxation curves of 20 dental ligaments of echinoid *Dendraster excentricus* treated with artificial seawater (*ASW*) and 15 ligaments treated with divalent cation-free seawater (*DCF*) (adapted from Ellers and Telford 1996). **E**, **F** Stress-strain tests (in which force is recorded while samples are stretched at fixed extension rate) conducted on spine ligament of echinoid *Anthocidaris crassispina*: Examples of stress-strain curves produced by samples treated with **E** 0.1 mM acetylcholine



(*ACh*) and **F** 0.1 mM adrenaline (*Adr*). *Horizontal scale bar* Strain of 10 % in both cases; *vertical scale bar* 10 MPa in **E** and 1 MPa in **F** (adapted from Hidaka and Takahashi 1983). **G–I** Dynamic stress-strain tests (in which force is recorded while samples are subjected to oscillating strain): hysteresis loops produced by repetitive testing of dermis of holothurian *Actinopyga mauritiana* in three mechanical states. During these tests, maximum strain (indicated by *number above each curve*) was increased incrementally; note that sample in '*soft*' state showed strain-induced softening. (Adapted from Motokawa and Tsuchi 2003)

strength of the intervertebral ligament at the autotomising joint drops to less than 0.1 % of the normal value in a timescale of 0.4–5.4 s (Wilkie 1988). These drastic changes in mechanical properties can also be demonstrated experimentally using isolated tissue preparations undergoing creep tests (in which their extension under constant load is recorded). Treatment with neuro-active agents such as elevated [K^+] or appropriate neurotransmitter chemicals causes an abrupt decrease in viscosity (stress-strain rate) culminating in tissue rupture (Fig. 1A).

Reversible changes in mechanical properties have been quantified and analysed by means of various testing methods. Reversible changes in viscosity have been demonstrated in creep tests and stress relaxation tests (in which specimens are stretched to a particular length, which is fixed whilst force decay is recorded) (Fig. 1B–D). Reversible changes in tensile strength, tensile stiffness, dynamic shear stiffness, relative damping, etc. have been investigated by means of standard stress-strain tests (Fig. 1E,F) and by dynamic testing methods in which samples are subjected to oscillating strain (Fig. 1G–I). Evidence of mutability is often apparent in the wide variability in the mechanical properties of untreated tissues. Motokawa (1983), for example, found a 200-fold difference in the viscosity of untreated central spine ligaments of *Diadema setosum* taken from different joints of the same animal. In order to overcome this problem and compare tissues in predictable mechanical states, a common strategy has been to subject them to different treatments that induce either maximal or minimal values of stiffness, viscosity, etc. (see Fig. 1G–I). Although biologically relevant stimuli, such as mechanical compression, have been used in these investigations, the artificial nature of some treatments, especially those employed to bring about a compliant state, engender some uncertainty about the physiological relevance of the results thus obtained. Nevertheless, these methods no doubt give an indication of the magnitude of the reversible changes that MCT can accommodate in vivo. Hidaka and Takahashi (1983), for instance, found that at a low strain rate the ultimate tensile strength and elastic modulus of an echinoid spine ligament in the *compliant* condition (induced by 0.1 mM adrenaline) were around 1 % of the values measured in the *stiffened* condition (induced by 0.1 mM acetylcholine), the latter falling within the range reported for mammalian tendon (Redaelli et al. 2003; Fig. 1E,F).

Data from these biomechanical studies have been used to generate models with the ultimate intention of specifying the contribution of different extracellular components to net mechanical properties and determining which of them contribute to variable tensility. Due to the diversity of testing regimes employed, the conclusions from different investigations have been incompatible (see, e.g., Szulgit and Shadwick 2000; Motokawa and Tsuchi 2003) and are difficult to interpret in the light of the increasingly complex picture of the MCT extracellular matrix that is emerging (see Sect. 2.3 below). It seems likely that the full potential of this methodology will not be realised until it can be applied to tissues from which specific components have been eliminated

chemically, enzymatically or, ideally, by genetic knockout (see Bornstein et al. 2000 and Chakravarti 2002 for examples of this last approach applied to mammalian connective tissues).

2.2.2 Active Contractility

Certain mutable collagenous structures can generate tensile force. In the case of the capsular ligament, or 'catch apparatus', of the echinoid spine-test joint, this is attributable to the presence of fine (diameter 0.1–1.0 μm) muscle fibres that are distributed between the bundles of collagen fibrils and constitute 1–3 % of the total cross-sectional area of the ligament. The neurotransmitter acetylcholine increases the tensile strength and stiffness of this ligament (Hidaka and Takahashi 1983; Morales et al. 1989), but also causes isolated preparations of it to shorten and develop mechanical force, which relaxes as soon as the acetylcholine is removed. Both the contraction and relaxation phases of this response can be very fast, the former in some cases reaching 90 % of the maximum in under 1 s (Fig. 2A; Vidal et al. 1993). The functional significance of the muscle fibres is at present unknown. Del Castillo et al. (1995), ignoring incontrovertible evidence that the spine ligament consists of MCT (see, e.g., Hidaka and Takahashi 1983; Szulgit and Shadwick 1994), hypothesised that they are responsible for varying its passive stiffness, which they achieve by adjusting the frictional forces between the ligament fibres and the skeletal ossicles into which they are inserted. Having provoked a frank exchange of views (see Del Castillo and Smith 1996; Wilkie 1996, 2002; Pérez-Acevedo et al. 1998; Elphick and Melarange 2001), this hypothesis was tested and disproved by Takemae and Motokawa (2002). The muscle fibres also do not seem to be involved in straightening out the wrinkles that form transiently in regions of the ligament that are compressed by contraction of the spine muscle (Pérez-Acevedo et al. 1998). This leaves the possibilities that they assist the reshortening of stretched ligament fibres or that they operate synergistically with the spine muscle, perhaps during specific manoeuvres such as re-erection of the spine.

The reputation of echinoderms for being an inexhaustible mine of biological novelty has been enhanced by the discovery that, as well as varying their passive mechanical properties, ligaments in the cirri and arms of crinoids have the capacity for active contractility, though they lack myocytes. Cirri are finger-like appendages supported by a single series of interarticulating ossicles. The only mechanically significant structures connecting adjacent ossicles are myocyte-free collagenous ligaments. Cirri attached to the stalk of sea lilies bend upwards, against gravity, when the stalk or the cirri themselves are stimulated mechanically. In stress relaxation tests, isolated cirri display slow force production in response to the cholinergic agonists muscarine and methacholine at concentrations as low as 0.1 μM (Fig. 2B). Since force produc-

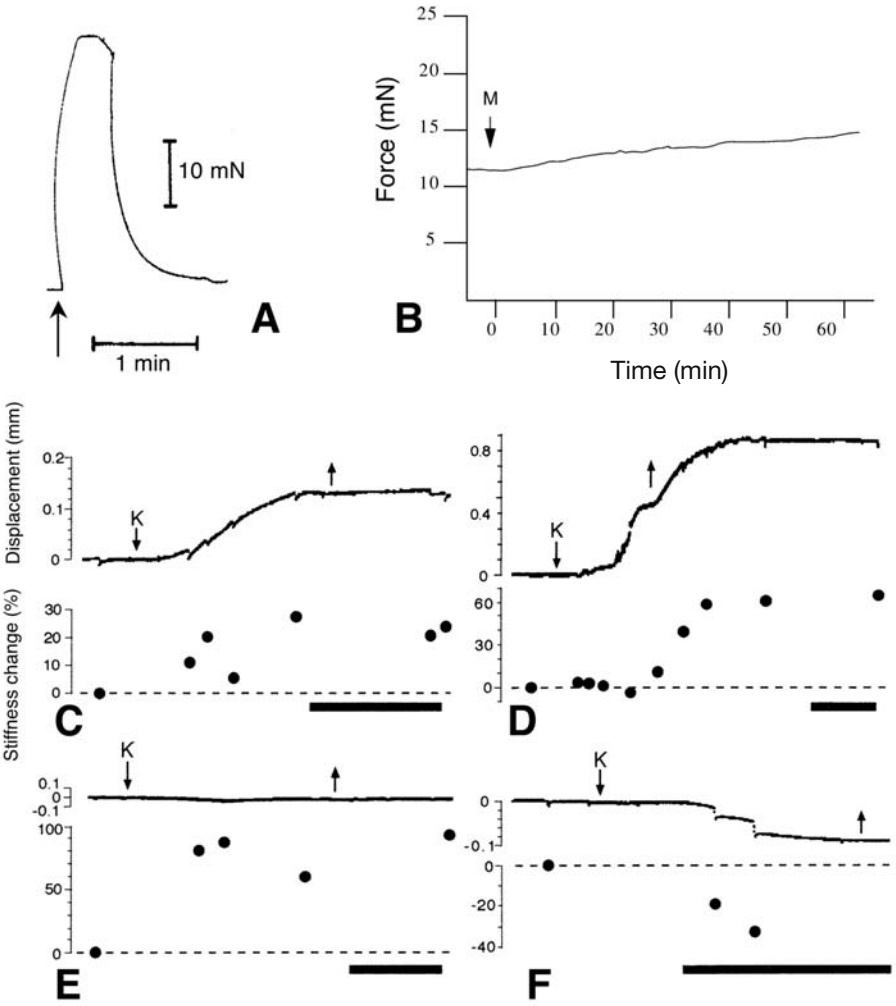


Fig. 2A–F. Force generation by MCT. **A** Contraction of spine ligament of echinoid *Eucidaris tribuloides* induced by 0.1 mM acetylcholine (*arrow*) which was removed as soon as force peaked (adapted from Vidal et al. 1993). **B** Contraction of cirral ligaments of crinoid *Metacrinus rotundus* induced by 0.1 μm methacholine (*M*) (adapted from Birenheide et al. 2000). **C–F** Responses to 100 mM K^+ (*K*) of arm ligaments of *M. rotundus*. In each case, *upper trace* shows upward displacement of arm tip (caused by shortening of ligaments) and *lower trace* shows stiffness changes in ligament. *Horizontal scale bar* 3 min in all cases. **C** Contraction associated with stiffening. **D** Contraction without stiffness change in 100 mM K^+ and contraction with stiffening when excess K^+ was removed. **E** No contraction, but with marked stiffening. **F** No contraction, but with destiffening. (Adapted from Motokawa et al. 2004)

tion can be induced in preparations that have undergone some stress relaxation prior to stimulation, it cannot be explained in terms of the passive recoil of a previously stretched elastic element (Birenheide and Motokawa 1995, 1998; Birenheide et al. 2000).

The mobility of crinoid arms depends on the presence of muscular articulations between adjacent arm ossicles. Below the fulcral ridge of each articulation is a single aboral ligament and above it are paired oral ligaments and paired muscles. Contraction of the muscles bends the arm orally (upwards), yet the power stroke for locomotion by swimming, crawling or climbing is generated by the aboral (downward) flexion of the arm and must be effected by the ligaments. It had long been assumed that this involved the purely passive elastic recoil of stretched aboral ligaments, and perhaps of compressed oral ligaments, these functioning like expanded and compressed springs respectively (see, e.g., Young and Emson 1995). However, Birenheide and Motokawa (1996, 1998) established that the aboral ligament can shorten slowly and generate a tension of up to ca. 5.6 kPa. Because this ligament is a mutable collagenous structure, it is feasible that its apparent contractility results from its becoming stiff whilst it is stretched (by muscle-mediated oral flexion); it would then store strain energy until appropriate stimulation induced its destiffening and thereby allowed it to recoil elastically and reshorten. This 'spring-with-a-lock' hypothesis was shown to be untenable by Motokawa et al. (2004) who, by recording simultaneously stiffness and shortening, demonstrated the independence of passive mechanical properties and contraction: contraction, for example, does not require the ligament to be in a destiffened condition (Fig. 2C–F). The fact that contracting ligaments are usually destiffened, however, indicates that there is coordination of the passive and active mechanical properties, both of which appear to be under cholinergic control.

2.3 Organisation of MCT

2.3.1 Cells

Myocytes, which were discussed in the preceding section, have been found in only a small minority of mutable collagenous structures. All of these structures, however, always contain two other types of cell. The first is characterised by the presence of heterogeneous vacuoles that appear to be lysosomal and may enclose cytoplasmic debris or collagen fibrils. These cells tend to have a roughly fusiform outline and long, sometimes branching, processes (see, e.g., Wilkie 1988; Wilkie et al. 1992). They occur also in echinoderm connective tissue that is not mutable (Wilkie et al. 2004b) and, given the absence of obvious fibroblasts, they are likely to be pluripotential cells that can adopt a fibrogenic phenotype.

The second cellular component that is invariably present in MCT are cell bodies and/or processes containing large, electron-dense, membrane-bounded granules. In ophiuroids, these cellular elements, known as 'juxtaligamental cells', form a complex system of ganglion-like clusters innervated by hyponeural (motor) nerves, with a separate juxtaligamental cluster serving each collagenous structure, including the autotomy tendons of the intervertebral muscles (Wilkie 1979). It is assumed that at least some of the granule-containing cells associated with MCT in the other echinoderm classes are homologous to the juxtaligamental cells, although the cell processes in holothurians differ from those of the other four classes in being separated from the extracellular matrix by a basal lamina (see, e.g., Koob et al. 1999), which presumably represents an ontogenetic rather than a phylogenetic or functional distinction. There is clear morphological evidence for functional contact between motor neurons and juxtaligamental cells in at least ophiuroids, echinoids and crinoids (Cobb 1985; Peters 1985; Welsch et al. 1995), and so, mainly on the grounds that they (1) provide a link between the motor nervous system and the extracellular matrix, (2) terminate in MCT and (3) have no possible cellular targets, it has been hypothesised that they are the effector cells that directly alter the tensile properties of MCT. In all classes it is usual for at least two types of process to co-occur in the same tissue, these being distinguishable by the size and shape of their granules (see, e.g., Welsch et al. 1995; Koob et al. 1999), and it has been proposed that these include separate 'stiffener' and 'plasticiser' cell types. The recent demonstration by immunological methods that juxtaligamental granules of holothurian dermis contain identified chemical factors that influence interfibrillar cohesion provides further evidence that these cells have a role in variable tensility (see below).

2.3.2 Extracellular Matrix

Overview

The extracellular matrix of almost all mutable collagenous structures is dominated by fibres consisting of parallel assemblages of collagen fibrils. These fibres exhibit a range of patterns, including parallel fibre arrays (e.g. echinoid spine ligament: Trotter and Koob 1989), crossed-fibre arrays (e.g. echinoid peristomial membrane: Wilkie et al. 1994) and three-dimensional meshworks (e.g. holothurian dermis: Motokawa 1982), which is comparable in diversity to that shown by vertebrate connective tissue structures. An important exception to this fibrous organisation is provided by the autotomy tendons of ophiuroids. These are extensions of muscle cell basal laminae and consist of non-fibrillar collagen related probably to vertebrate type IV (Wilkie and Emson 1987), although, since their biochemical composition is not known, this is a supposition based only on their histochemistry and ultrastructure. The fol-

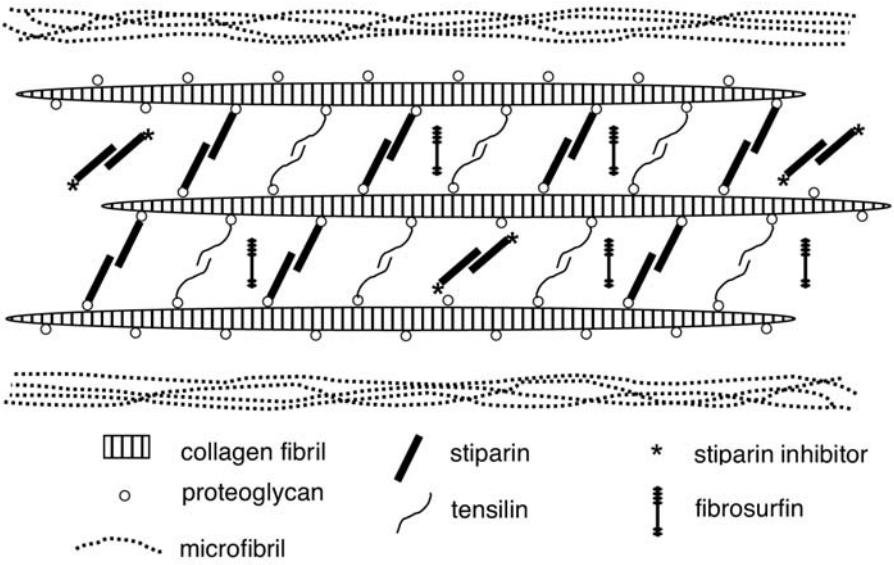


Fig. 3. Model of MCT molecular organisation, which is based on current evidence and assumes that the few mutable structures from which that evidence has been derived are representative. Most MCT consists of parallel aggregates of discontinuous, spindle-shaped collagen fibrils to which are attached PGs and other GAG-containing molecules whose functions include serving as binding sites for molecules responsible for interfibrillar cohesion. Amongst the latter are the proteins stiparin and tensilin, the fibril-aggregating activities of which are modified by a variety of specific inhibitors. The mechanisms by which stiparin and tensilin cause fibril aggregation are incompletely understood. For simplicity, the model assumes that they form dimers that act as interfibrillar crossbridges. Fibril bundles are delimited by loose networks of elastic fibrillin-containing microfibrils that return the tissue to its resting dimensions after it has undergone deformation when in a compliant condition. The functions of fibrosurfin are as yet unknown

lowing sections summarise current knowledge of the molecular constituents of fibrous MCT alone. A generalised model of the molecular organisation of MCT is illustrated in Fig. 3.

Collagen

The fibres of which most MCT is composed are parallel aggregations of cross-banded collagen fibrils that are discontinuous, i.e. shorter than the length of the fibres. The fibrils of two mutable structures with very different microarchitectures – the echinoid spine ligament and holothurian dermis – resemble those of mammalian connective tissue such as rat tail tendon in being composed of molecules with a triple helix length of 300 nm which are assembled in parallel arrays with a regular stagger of 67 nm between adjacent molecules, in having cross-striations in similar positions though varying in stain intensity (an indication of differences in the charge density associated with their

constituent amino acids), and in being stabilised by high levels of trivalent hydroxypyridinium intermolecular crosslinks (Trotter and Koob 1989, 1994; Trotter et al. 1994, 1995). The chain compositions of the echinoid and holothurian collagen molecules were not the same, the former being a heterotrimer of two α1 and one α2 polypeptides, as in mammalian type I and most other echinoderm collagens (see, e.g., Omura et al. 1996; Robinson 1997), and the latter being a homotrimer of three α1 polypeptides. These two collagens also have different solubility characteristics and amino acid compositions (Trotter and Koob 1994; Trotter et al. 1995). The general conclusion from this research is that MCT collagens possess no consistent set of biochemical or structural features that distinguish them from the collagens of other echinoderms or other phyla, or that could be correlated with the mutability of their parent tissues.

Data on gene sequence and gene organisation indicate that at least some echinoderm collagen polypeptides are evolutionarily close to those of vertebrate fibrillar collagens (D'Alessio et al. 1989, 1990; Exposito et al. 1992; Tomita et al. 1994; Cluzel et al. 2000). So far, no primary sequence data on collagen extracted from any MCT have been published. However, an epitope of a fully characterised collagen polypeptide has been detected in two confirmed mutable structures. Cluzel et al. (2001) immunolocalised the amino propeptide of the 2α collagen chain, which, apart from the amino propeptide itself, is closely similar to that of vertebrate fibrillar collagen (D'Alessio et al. 1990), in the mutable peristomial membrane and spine ligament of the echinoid *Paracentrotus lividus*. Since the amino and carboxyl propeptide regions of collagen chains are usually removed by specific proteases during the extracellular maturation process, their retention in the tissues of adult echinoids may have functional significance (Lethias et al. 1997).

Because complete collagen fibrils can be isolated from MCT using mild, non-denaturing extraction methods, more is known about their supramolecular organisation than that of vertebrate fibrils. Fibrils from both echinoid spine ligament and holothurian dermis are spindle-shaped with paraboloidal tips. Despite varying greatly in length, they have a constant aspect (length:diameter) ratio in the order of 2000 and are molecularly bipolar, i.e. in both halves of each fibril the amino termini of the collagen molecules are orientated towards the nearer tip, and near the axial midpoint of each fibril there is a region of symmetrical transition from parallel to antiparallel molecular packing (Trotter and Koob 1989; Thurmond and Trotter 1994; Trotter et al. 1994). As in other fibrillar collagens, this organisation results from the self-assembly of the constituent molecules which occurs automatically after enzymatic removal of their N- and C-propeptides. More recent work by Trotter et al. (1998, 2000a), using digital scanning-transmission microscopy to determine mass per unit length (and therefore the number of molecules) along whole collagen fibrils from both echinoid and holothurian tissues, has provided evidence that the self-assembly mechanism is different from that of vertebrate fibrils.

Although it is not possible at present to isolate whole collagen fibrils from normal adult tissues of animals other than echinoderms, the limited data that are available on vertebrate fibrils indicate that, despite the different mechanism of fibrillogenesis, they are also spindle-shaped with paraboloidal tips (see Trotter et al. 1998). This is the ideal shape for fibrils that reinforce a discontinuous fibre composite, since it allows the full tensile strength and stiffness of the fibril to be exploited along its whole length and avoids shear-stress concentrations near its ends (Trotter and Koob 1989; Trotter et al. 2000b). The fusiform shape of the collagen fibrils in MCT is therefore unrelated to its variable tensility.

Proteoglycans

Proteoglycans (PGs) are present in the fibrous connective tissue of all animals and consist of a protein core to which are attached covalently side chains of polyanionic sulphated glycosaminoglycans (GAGs). The use of the polycationic dyes cuprolinic and cupromeronic blue has revealed that polyanions are localised to specific sites in each D-period on the surface of fibrils in crinoid, echinoid and holothurian MCT, as is the case in vertebrate collagenous tissue (Trotter and Koob 1989; Erlinger et al. 1993; Trotter et al. 1995). Biochemical methods have demonstrated that PGs are attached to the fibrils non-covalently or covalently. Non-covalently bound PGs in the chondroitin/dermatan sulphate class are attached to the collagen fibrils of the echinoid spine ligament (Trotter and Koob 1989). Collagen fibrils in holothurian dermis are associated covalently with three different GAG-containing macromolecules. The most abundant of these includes a fucose-containing GAG that is associated with the fibrils via a non-reducible covalent bond. The structure of the highly sulphated fucose branches of chondroitin sulphate E from *Astichopus japonicus* has been characterised fully by Kariya et al. (1997). The other two covalently bound GAG-containing molecules in holothurian dermis are high molecular weight PGs that are linked to collagen fibrils probably via disulphide bonds and at least one of which acts as a binding site for the glycoprotein stiparin (see below). In addition to these insoluble PGs, holothurian dermis contains at least two soluble PGs that bind stiparin, inhibit stiparin-fibril binding and may be involved in the regulation of stiparin-fibril binding (Trotter et al. 1995 and unpubl.).

Other Non-Collagenous Proteins

Stiparin is the most abundant soluble glycoprotein in the dermis of the holothurian *Cucumaria frondosa* and can be extracted from minced tissue by prolonged treatment with seawater alone (which also results in tissue disaggregation, an indication that the collagen fibrils are normally held together by weak bonds). Trotter et al. (1996) demonstrated that stiparin, which has a molecular weight of about 375 kDa, causes calcium-independent aggregation in vitro of collagen fibrils that have been treated with guanidine-HCl (which

removes non-covalently bound PGs) but has no effect on the mechanical properties of samples of intact dermis (Koob et al. 1999). Whilst it seems likely that stiparin binds to collagen fibrils via a surface-bound PG, the molecular mechanism of stiparin-induced fibril aggregation has still to be determined.

The dermis of *C. frondosa* contains a 62-kDa sulphated glycoprotein that does not bind collagen fibrils but does bind stiparin and thereby inhibits stiparin's fibril-aggregating activity. This molecule has the highest negative charge density of all macromolecules extracted from the dermis, and all of its inhibitory activity is associated with the polygalactose sulphate moiety of the molecule rather than with its protein component. The relative concentration of stiparin inhibitor is 200 times greater in the loose outer dermis of *C. frondosa* than in the dense inner dermis (Trotter et al. 1999).

Tensilin (also known as 'stiffener') is a constituent of the inner dermis of *C. frondosa* that can be isolated only after treatments that cause cell lysis, such as repeated freeze–thaw cycles, indicating that it is present mainly in intracellular locations. Like stiparin, it causes aggregation of isolated collagen fibrils, but, unlike stiparin, it stiffens intact inner dermis, both effects being calcium-independent (Koob et al. 1999). The peptide sequence of tensilin deduced from a full-length cDNA clone (Fig. 4) suggests significant similarity to the tissue inhibitor of metalloproteinase (TIMP) proteins with 21–36 % identity between tensilin and the mammalian TIMPs (Tipper et al. 2003). It seems likely that tensilin interacts with collagen fibrils via surface GAGs. For example, it binds to isolated collagen fibrils that have surface GAGs, but does not bind to GAG-free molecular collagen (Trotter et al. 1995; Tipper et al. 2003). The binding activity of tensilin is unaffected by stiparin inhibitor (Trotter et al. 2000b).

'Plasticiser' is a cell-sequestered <15-kDa protein that is present in only the outer dermis of *C. frondosa* and destiffens samples of intact inner dermis. It appears to act directly on the extracellular matrix, since it is as effective on cell-lysed samples as it is on fresh samples (Koob et al. 1999). Nothing more is known about its mode of action.

Cluzel et al. (2001) characterised a sea-urchin gene that encodes a multidomain interfibrillar protein they called 'fibrosurfin'. This contains 17 epidermal

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1     MEVAFLVLLI GALSLSSADA QCAGCSVKHP QHHFCDATFV MKVTIIDVIL
51    DRQGGDKLIN AEINRSWKKG PSSGDFQFYA PSSFCGATFD SGDTYVVTGT
101   KEETSDGRRY WLHGSCDYMI KWDDMSDQQK AGFKGGYKAR CGECQIAESL
151   TAASVKVEDI AANDYPLATT YWTPTGCYYN PLMTRQFVGR KGSSVVDCED
201   VYGLCKPNEA DKCQWTLTPD YERCLKERDD FVKADSSAFA ITRVEQCDVY
251   TNKRKRKNCR QRFRELQAEM GADEELIFYR
```

Fig. 4. Deduced peptide sequence of tensilin. Leu225 (*underlined*) represents last residue that was found to be in alignment with a known TIMP sequence (*Drosophila* TIMP). Protein database comparison revealed that beyond Leu225 tensilin shows no identity or similarity to any known TIMP sequence or any other sequence. (Tipper et al. 2003)

growth factor (EGF) motifs, 11 of which could potentially bind calcium, and was detected in protein extracts of mainly the mutable spine ligaments and peristomial membrane of *Paracentrotus lividus*. Immunogold labelling indicated that fibrosurfin occurs between, or close to, the collagen fibrils of the spine ligament. Since proteins that contain EGF domains are often involved in protein–protein interactions, it is possible that fibrosurfin contributes to interfibrillar cohesion. Its relevance to mutability is at present unknown.

Microfibrils

Hollow microfibrils 10–14 nm in diameter and sometimes beaded with a periodicity of 30–100 nm are ubiquitous in MCT. They can be aggregated into fibres or sheets (see, e.g., Wilkie et al. 1994, 1998, 2004b), but most often form loose sheaths that surround and separate bundles of collagen fibrils. Thurmond and Trotter (1996) and Thurmond et al. (1997) demonstrated that the microfibrils of *C. frondosa* dermis resemble the fibrillin-containing microfibrils of mammalian connective tissue in their morphology, biochemistry and immunological properties. Isolated microfibrillar networks from *C. frondosa* possess long-range elasticity (Thurmond and Trotter 1996). They thus may confer elasticity on MCT that is in a compliant state and provide it with a predetermined set of dimensions to which it returns when external forces are removed (Trotter et al. 2000b). Microfibrils are also present in echinoderm collagenous structures that are non-mutable (Del Castillo et al. 1995; Wilkie et al. 2004b). There is no evidence that they have a role in the variable tensility of MCT.

2.4
Molecular Mechanism of MCT Mutability

2.4.1
Are Collagen Fibrils Involved?

There is no evidence that the variable tensility of MCT involves changes in the mechanical properties of the collagen fibrils. This would be highly unlikely on a priori grounds, in view of the similarities between the collagen fibrils of MCT and those of vertebrate connective tissue in terms of (1) fibril shape, supramolecular organisation and intermolecular crosslink biochemistry, and (2) the structure of their constituent collagen molecules. Furthermore, numerous ultrastructural investigations have failed to provide evidence that alterations in mechanical properties are accompanied by modification of the shape or organisation of the collagen fibrils. Erlinger et al. (1993), however, interpreted 10–11 nm filaments in a crinoid ligament as being collagen 'protofibrils' produced by a reversible fibril disaggregation mechanism possibly associated with mutability. The ultrastructural observations of Birenheide

and Motokawa (1994) indicate that these filaments are more likely to be homologous to the fibrillin-containing microfibrils that are ubiquitous in echinoderm connective tissue. It is therefore almost certain that mutability depends on changes not in the tensility of the collagen fibrils, but in the cohesive forces holding the fibrils together.

2.4.2
Are Calcium Ions Involved?

The mechanical properties of MCT are sensitive to changes in the extracellular calcium ion concentration. Increasing $[Ca^{2+}]_o$ stiffens and decreasing $[Ca^{2+}]_o$ destiffens almost all mutable structures that have been investigated. These and other findings led to the hypothesis that Ca^{2+} ions contribute directly to interfibrillar cohesion in MCT and that the juxtaligamental cells alter tissue stiffness by controlling the amount of extracellular Ca^{2+} available for such a role (reviewed by Wilkie 1996). This hypothesis was discredited by the demonstration that certain treatments stiffen MCT in the absence of Ca^{2+} ions and that agents that interfere with calcium-dependent cellular processes can change MCT tensility in the presence of a normal $[Ca^{2+}]_o$ (Szulgit and Shadwick 1994; Trotter and Koob 1995; Trotter and Chino 1997). The weight of evidence now favours the view that the influence of $[Ca^{2+}]_o$ manipulation on MCT tensility is due mainly to direct effects on cellular elements rather than on the extracellular matrix itself, and that, although Ca^{2+} ions contribute directly to interfibrillar cohesion in an unknown way (Szulgit and Shadwick 2000), variable tensility does not involve modulation of $[Ca^{2+}]_o$.

2.4.3
Tensilin–Tensilin Protease Hypothesis

The stiffness of MCT is changed dramatically by a range of treatments that cause cell membrane lysis, such as freeze–thawing or exposure to deionised water or detergents (Szulgit and Shadwick 1994, 2000; Trotter and Koob 1995; Trotter and Chino 1997; Wilkie et al. 1999). Extracts prepared from the dermis of *C. frondosa* after it has been subjected to freeze–thawing have the same effects on isolated tissue samples as freeze–thawing itself (Trotter and Koob 1995; Koob et al. 1999; Szulgit and Shadwick 2000), and the analysis of such extracts resulted in the isolation of the active agents tensilin ('stiffener') and 'plasticiser'. The observation that these proteins can be isolated from tissues only after cell lysis indicates that they are present mainly in intracellular reservoirs, and led to the hypothesis that they are regulatory molecules which are secreted from cells and bring about changes in MCT tensility (Koob et al. 1999; Trotter et al. 2000b). The case for tensilin being a secreted effector molecule has been strengthened by its recent immunolocalisation in granules of

juxtaligamental cells in *C. frondosa* dermis (D.R. Keene and J.A. Trotter, unpubl.).

It was noted by Tipper et al. (2003) that tensilin tends to undergo proteolysis in vitro and that the degraded product neither binds collagen fibrils nor induces fibril aggregation. Since analysis of trypsin digests suggested that the C-terminus, which includes a putative fibril-binding site, is susceptible to proteolysis, these authors hypothesised that tensilin-induced stiffening is reversed in vivo by a specific protease. Such a protease could be expressed constitutively, resulting in 'automatic' decay back to the destiffened state, or it could be secreted or activated in response to specific signals (Fig. 5).

At present, the significance for variable tensility of other recently isolated molecules is not clear. Some may have a regulatory and others a constitutive role. Indirect immunofluorescence and immunogold labelling have revealed that stiparin is associated much more abundantly with collagen fibrils than is tensilin (D.R. Keene and J.A. Trotter, unpubl.). This observation and the fact that stiparin, unlike tensilin and 'plasticiser', has no effect on whole tissue samples, suggest it may be a constitutive factor that is not involved in short-term changes in mechanical properties, but functions to hold collagen fibrils in a weak association that facilitates the action of effector molecules such as tensilin (Trotter et al. 2000b). However, the demonstration by immunocytochemistry that stiparin, like tensilin, is present in the juxtaligamental granules

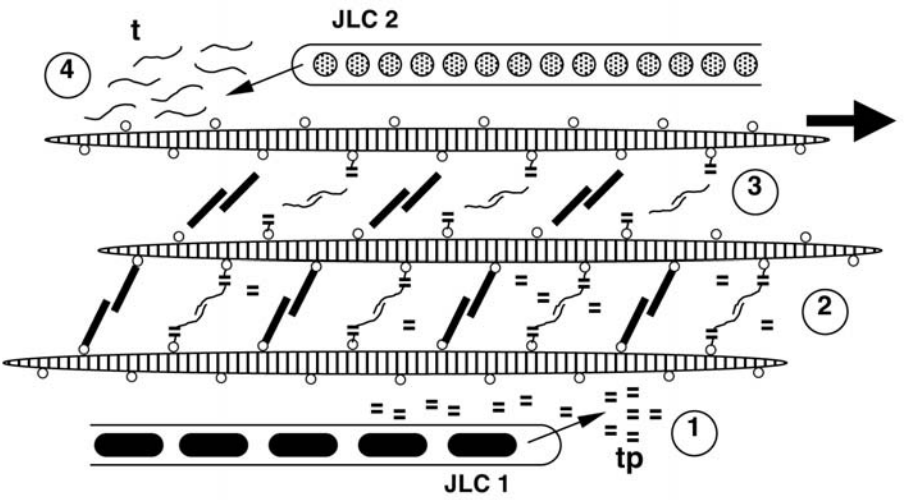

Fig. 5. Model of the tensilin–tensilin protease hypothesis. MCT plasticisation or *destiffening* results from (*1*) the release from, or activation by, a specific type of juxtaligamental cell (*JLC 1*) of tensilin protease (*tp*), which (*2*) cleaves tensilin near its GAG-binding site. This (*3*) allows fibrils to slide past each other, since they are held together only weakly by stiparin. *Restiffening* results from (*4*) the release of fresh tensilin (*t*) from a second type of juxtaligamental cell (*JLC 2*). MCT constituents represented as in Fig. 3

of *C. frondosa* (D.R. Keene and J.A. Trotter, unpubl.) raises the possibility that it also could be a regulatory molecule (or that juxtaligamental cells are a source of both constitutive and regulatory factors).

2.4.4
Active Force Generation

No information is available currently on the mechanism of active force generation in crinoid ligaments.

3
Mutable Collagenous Tissue: Biotechnological Perspective

3.1
Current Commercial Uses of MCT

At the present time, MCT is being exploited commercially for purposes that have nothing to do with its mechanical adaptability, though they may rely on biochemical properties that underpin mutability. The best-known example of this is the use of holothurian body wall as a food item ('trepang' or 'beche-de-mer') throughout SE Asia, China and Japan. The demand for trepang is met by the vigorous fishing of natural populations and by an expanding mariculture sector. It is estimated that in the period 1986–1996 more than 50,000 tonnes of trepang, valued at over US$ 40 million, was imported into Hong Kong, the main world market (Conand 2001; Jiaxin 2003).

For millennia, holothurian body wall has also been revered for its prophylactic and curative properties and it is still a component of many herbal medicines. It has become apparent that its reputed medicinal properties may have a scientific basis. For example, its efficacy as a remedy for joint pain is thought to result from the presence of chondroitin sulphate (which may be an important determinant of the mechanical behaviour of MCT: see above) and, because of its antiviral properties, holothurian chondroitin sulphate has been patented for HIV therapy (Jiaxin 2003). In addition, the body wall of certain holothurians possesses antibacterial activity (Villasin and Pomory 2000) and has a fatty acid profile that suggests it could be of clinical benefit in promoting wound repair (Fredalina et al. 1999).

Echinoids are fished and cultured throughout the world for their edible gonads (Andrew et al. 2002). It has been suggested that discarded echinoid tests, which include sutural ligaments (which may be mutable: Johnson et al. 2002), spine ligaments and peristomial membrane (both of which are mutable: Hidaka and Takahashi 1983; Wilkie et al. 1993), could be used as a source of collagen for the food, cosmetic and biomedical industries (Nagai and Suzuki 2002).

3.2 Biotechnological Potential of MCT

Consideration of the biotechnological potential of MCT must of necessity be highly speculative in view of our currently incomplete knowledge of the molecular organisation of MCT and the molecular mechanism underpinning its variable tensility. Theoretically MCT could be a source of, or an inspiration for, (1) new pharmacological agents or strategies and (2) new composite materials.

3.2.1 Pharmacological Agents or Strategies

It hardly needs to be reiterated that the outstanding property of MCT is its capacity for reversible changes in stiffness. There are certain clinical conditions that would benefit from the therapeutic manipulation of connective tissue mechanical properties, perhaps as an alternative to surgery or other interventions. Most of these conditions would require the temporary or permanent *plasticisation* or weakening of the connective tissue, rather than its strengthening. This applies to problems like joint contractures due to immobilisation, burn scar contractures, breast capsule contractures following enhancement procedures, Dupuytren's contracture and peritendinous lesions following tendon surgery. Previous suggestions for the pharmacological treatment of some of these conditions have focused on the suppression of collagen synthesis and deposition by, for example, the topical application of lathyrogens such as β-aminopropionitrile, which inhibits an enzyme – lysyl oxidase – involved in intermolecular cross-link formation (Chvapil 1988). On the other hand, structures that need to be *strengthened* include ligaments and tendons weakened by immobilisation (Nordin and Frankel 1980) and repair sites in traumatically or surgically transected tendons, which rarely regain full tensile strength (Koob 2002).

Is it possible that MCT contains molecules that could affect the mechanical properties of mammalian connective tissue? As noted above, holothurian chondroitin sulphate relieves joint pain, though there is at the moment no reason to believe that this is due to anything more than the anti-inflammatory or anti-oxidant effect exerted by other chondroitin sulphates and other glycosaminoglycans (GAGs) (Delehedde et al. 2002; Campo et al. 2003). It is intriguing, however, that GAGs contribute significantly to interfibrillar force transfer, and therefore the overall mechanical properties, of mammalian connective tissue (Redaelli et al. 2003), and that stiffness changes in the uterine cervix are accompanied by significant shifts in the expression of certain GAGs (Westergren-Thorsson et al. 1998). Whilst this implies that the best way to treat fibrotic lesions might be to engineer in them a GAG composition mimicking that of the compliant cervix, it is feasible that, since holothurian GAGs

are components of much more mutable connective tissue, they possess features that might facilitate the 'loosening' of fibrotic tissue and that could be incorporated pharmacologically or genetically into the latter. This illustrates the need to determine both the chemical structure and the precise role in MCT mutability of GAGs, the proteoglycans of which they are constituents, and other, as yet incompletely characterised, interfibrillar components. The benefits that would accrue from a therapeutic strategy that treats successfully the fibrotic conditions referred to above, as well as other common pathophysiological processes such as pulmonary fibrosis and connective tissue-related stiffening of the walls of hypertensive blood vessels, cannot be exaggerated.

Regarding a completely separate feature of MCT, Szulgit and Shadwick (1998) discovered that the mutable dermis of the holothurian *Parastichopus parvimensis* has remarkable self-adhesive properties. Dermal autografts or allografts adhered to their implantation site without external pressure or assistance of any sort and shear stresses of 200–500 Pa were required to separate isolated samples after they had been in contact with each other for only 2 h. This property is not due to the entangling of collagen fibres, capillary adhesion or viscous shear forces, but seems to be based on weak chemical bonds. It is independent of the mechanical state of the tissue and is not cell-dependent, and so it seems to be unrelated to mutability, although Szulgit and Shadwick (1998) speculated that a collagen fibril-aggregating factor, such as stiparin, might be involved. This phenomenon merits further investigation, since its elucidation might lead to the identification of chemical factors or mechanisms that could be exploited to promote the adhesion of tissue grafts or artificial skin to wound areas or could be incorporated into MCT-derived artificial tissue (see below).

3.2.2
New Composite Materials

In a recent review, Langer and Tirrell (2004) have drawn attention to the outstanding impact that biomaterials have had on health care, particularly in the context of prosthetic and drug delivery devices, and they commented that the extracellular matrix "provides an important model for biomaterial design". With regard to the development of new structural materials that have medical applications, connective tissue has been employed in three different ways:

1. An entire connective tissue, either living (i.e. with cellular elements left in situ) or with cellular elements removed, may be used as a graft or prosthesis. Examples of this include the use of Achilles tendon allografts for the reconstruction of cruciate ligaments (DeFrate et al. 2004) and skin repair using dermis from different species prepared by various methods (Ramos-e-Silva and Ribeiro de Castro 2002). Obviously, it would be ideal if the mechanical properties of each implant could be adjusted precisely to match the needs of the respective implantation site. For example, skin replace-

ments need to be more extensible and elastic over the extension sides of joints, such as those of the finger, than over the flexion sides. It has to be admitted, however, that there is unlikely to be much scope for using whole MCT in this way, due to the immunological challenge it would present and the low probability that any echinoderm structure would have a microarchitecture more suitable than that of a mammalian alternative. Techniques would also have to be developed to fix the MCT xenograft in the optimal mechanical state. Furthermore, because of the labile nature of the bonds upon which the integrity of MCT depends, many mutable collagenous structures become unmanageably friable in the softened state. It is possible, though, that some of those that demonstrate a limited range of tensile changes and never become compliant to the point of disintegration could be exploited. The echinoid compass depressor ligament and peristomial membrane are examples of such structures (Wilkie et al. 1992, 1993).

2. Components may be isolated from the connective tissue and then reassembled with other biological or synthetic elements to form a novel composite. Examples of biomaterials produced in this way are *Integra* a combination of bovine collagen, shark chondroitin-6-sulphate and a silicone sheet (the last acting as an artificial epidermis), which is used as a skin substitute (Ramos-e-Silva and Ribeiro de Castro 2002), and acellular blood vessel grafts that consist of intestinal submucosa and bovine collagen (Huynh et al. 1999). It is as a source of such components that MCT could make the most direct contribution to biomaterial design, largely by virtue of the extractability of its collagen fibrils. It is notoriously difficult to extract intact collagen fibrils from the post-fetal connective tissue of vertebrates (Trotter et al. 1997). For this reason, the collagen used in existing reassembled biomaterials is in the form of disaggregated molecules. These have the advantageous property of aggregating spontaneously to form fibrils, but, due to the absence of covalent intermolecular bonds, the fibrils have a low tensile strength. Koob (2002) has developed a method for chemically crosslinking such reconstituted fibrils to make a product that could be used to bridge gaps in damaged tendons. In stark contrast to the vertebrate situation, intact collagen fibrils can be isolated easily from MCT by mild non-denaturing techniques. A solution containing 0.5 M NaCl, 0.05 M EDTA, 0.2 M β-mercaptoethanol and 0.1 M TRIS buffer (pH 8.0) disaggregates holothurian dermis, asteroid body wall and echinoid spine ligaments (Matsumura 1973; Matsumura et al. 1973; Trotter and Koob 1989). Even more remarkably, Trotter et al. (1996) discovered that fibrils could be isolated from holothurian dermis using sequential 24-h extractions in artificial seawater alone. This is evidently a consequence of the weak nature of the interactions that maintain interfibrillar cohesion in MCT, and it means that holothurian dermis and other mutable collagenous structures represent a cheap and easily accessible source of intact collagen fibrils that retain their tensile strength and stiffness and could be used for the manufacture of artificial tissues.

However, a more exciting way in which MCT could contribute to the design of new biomaterials, whether these incorporate MCT-derived components or not, is through the mimicking of the mechanisms responsible for its variable tensility and contractility. There is a clinical need for artificial tissues with site-specific micro-architectures and mechanical properties that are either pre-set or continuously adjustable and responsive to changing physiological parameters or to therapeutic manipulation (Langer and Tirrell 2004). Amongst possible applications for such 'smart' materials (i.e. which combine the functions of sensors and actuators) would be vascular implants or cuffs that controlled blood pressure or local blood flow, and whose stiffness and/or contractile state were directly sensitive to blood biochemistry or to precisely targeted pharmacological agents, thereby offering an alternative to systemic antihypertensive drugs and their spectrum of unwanted side effects.

Trotter et al. (2000b) have already examined the possibility of developing a simple 'hybrid' biomaterial assembled from collagen fibrils extracted from holothurian dermis and a synthetic interfibrillar matrix. The interaction between the fibrils and the matrix, and therefore the tensile properties of the whole system, would depend on a pair of synthetic molecules that have been shown to selectively and reversibly associate with each other in physiological conditions. These are a catechol and a phenylboronic acid, which complex to form a boronic ester. This interaction can be reversed by oxidizing the catechol to orthoquinone which does not bind to boronic acid (Fig. 6). The synthetic material would consist of collagen fibrils, to which catechol groups had been attached chemically, linked by a soluble polyacrylamide polymer complexed with phenylboronic acid groups. Trotter et al. envisaged that the association between fibrils and matrix could be repeatedly switched on and off by sequential oxidations and reductions controlled perhaps by optical or electrical signals that change the redox potential of the matrix (Fig. 7). The only MCT-derived elements in this device are collagen fibrils. It is possible that further investigation of MCT will reveal other components that could be built into new controllable biomaterials.

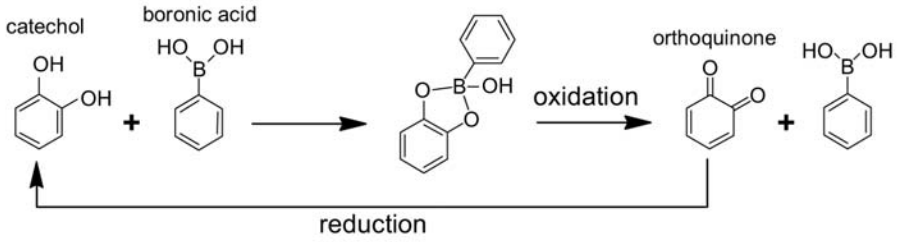

Fig. 6. Interaction between catechol and phenylboronic acid, which provides the reversible cross-links in proposed hybrid biomaterial. (Adapted from Trotter, unpubl.)

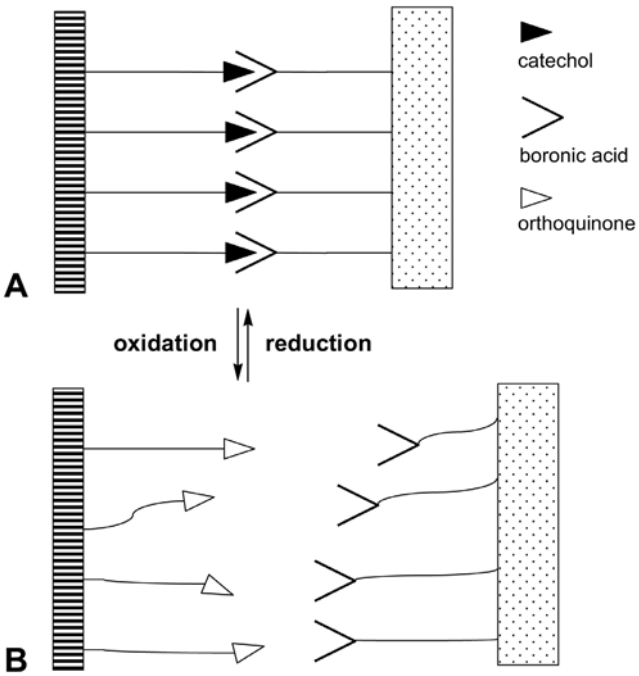

Fig. 7A, B. Model of proposed hybrid biomaterial. Collagen fibrils (*horizontally striated*; only one shown) with attached catechol groups are embedded in polyacrylamide polymer (*stippled*) complexed with phenylboronic acid. **A** Stiff condition, in which cross-links are formed by interaction of catechol and phenylboronic acid. **B** Compliant condition, in which cross-links are reversed by oxidation of catechol to orthoquinone. (Adapted from Trotter, unpubl.)

3. Connective tissue may provide inspiration for entirely synthetic materials. A simple example resulting from this approach is an artificial tendon which is manufactured from poly(ethylene terephthalate) fibres embedded in a swollen hydrogel matrix and can be designed to have mechanical properties that suit specific implantation sites (Kolarik 1995). Trotter (unpubl.) has speculated that MCT could serve as a model system for the construction of dynamically controllable ligaments with adjustable stiffness and adjustable damping. Trotter has also suggested that these might be incorporated into energy-efficient robots, and it is therefore relevant that there has been interest in the design of 'compliant' robots for specialist purposes such as pipeline inspection (Suzumori 1996). As for reassembled biomaterials (see above), both the variable passive mechanical properties and the contractility of MCT may yield design principles applicable to the development of fully synthetic devices.

4 Concluding Remarks

Recent research has done much to elucidate the molecular organisation and functioning of MCT. Whilst a number of interfibrillar molecules have been isolated, only tensilin ('stiffener') has been characterized fully, and the signif-

icance of none of these molecules for either interfibrillar cohesion or variable tensility is fully understood. It would be particularly interesting to find out more about the proteoglycans in view of their critical influence on the supramolecular organisation and mechanical properties of vertebrate collagenous tissue (Redaelli et al. 2003). Further investigation of the biochemistry and molecular biology of MCT could take advantage of the presence in some echinoderms of adjacent structures that differ significantly in their capacity for undergoing tensile changes. Examples include the autotomy and non-autotomy tendons of ophiuroid intervertebral muscles (only the former are mutable: Wilkie and Emson 1987), the distal and proximal oral arm plate ligaments of ophiuroids (the former show both reversible and irreversible changes in mechanical properties and the latter only reversible changes: Wilkie 1992), and the capsular and central ligaments of cidaroid echinoid spines (only the former are mutable: Del Castillo et al. 1995). Qualitative and quantitative comparison of these structures might help to identify the molecular correlates of variable tensility.

A fascinating recent discovery is the homology between tensilin and the tissue inhibitors of metalloproteinases (TIMPs) (Tipper et al. 2003). Matrix metalloproteinases (MMPs) are ubiquitous, connective tissue degrading enzymes and TIMPs are important modulators of MMP activity. One mammalian TIMP (TIMP-3) binds strongly to the extracellular matrix via a GAG-binding site (Yu et al. 2000), as does tensilin. This similarity and the involvement of MMPs and TIMPs in the mechanical changes undergone by female mammalian reproductive tract collagenous tissues suggest that the mechanism underpinning MCT mutability could have evolved from a MMP-TIMP system. There are also surprising similarities between MCT and the collagenous mesohyl of demosponges, which also shows reversible changes in stiffness (Wilkie et al. 2004 c). Mutability may therefore be an ancestral property of the extracellular matrix, which was lost during the evolution of most animals.

It has emerged that MCT is more versatile than previously suspected. As well as showing adaptable passive mechanical properties, some mutable collagenous structures are actively contractile, and another has been found to demonstrate non-cell-mediated self-adhesiveness. This chapter has provided some initial thoughts on the biotechnological potential of these properties, which would seem to be considerable in view of the currently expanding interest in biological systems as a source of molecules and mechanisms that could be developed for biomedical and other applications (Langer and Tirrell 2004). Improved knowledge of the basic biology of MCT should lead to better understanding of how its unique properties could be exploited for such purposes.

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