Structural Organization of Mutable Collagenous Tissue

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Echinoderms (starfish, sea-urchins and their close relations) possess a unique type of collagenous tissue that is innervated by the motor nervous system and whose mechanical properties, such as tensile strength and elastic stiffness, can be altered in a time frame of seconds. Intensive research on echinoderm 'mutable collagenous tissue' (MCT) began over 50 years ago, and over 20 years ago, MCT first inspired a biomimetic design. MCT, and sea-cucumber dermis in particular, is now a major source of ideas for the development of new mechanically adaptable materials and devices with applications in diverse areas including biomedical science, chemical engineering and robotics.

Keywords: biomimetic nanocomposites ; collagen ; juxtaligamental cell ; mechanically tunable implants ; proteoglycan ; soft actuators ; soft robotics

1. Introduction

Collagenous tissue is an important structural biomaterial in most multicellular animals. The mechanical properties of collagenous tissue are usually relatively stable, with any significant non-pathological changes taking place over timescales of days (during relaxation of the mammalian uterine cervix [1]) to years (during maturation and ageing [2]). However, all five living classes of the phylum Echinodermata (sea lilies and featherstars, sea-urchins, sea-cucumbers, starfish, brittlestars) possess collagenous tissue that can drastically alter its mechanical properties within a timescale of seconds under the direct control of the nervous system. Although such mutable collagenous tissue (MCT) appears to be unique to echinoderms, some specific features underpinning its mechanical adaptability—particularly the absence of permanently stable crosslinks between its constituent collagen fibrils—may be ancestral and may have permitted the emergence of similar phenomena in other phyla. There are, for example, parallels between MCT and the collagenous mesohyl of demosponges whose tensile properties are under non-neural physiological control [3].

MCT demonstrates a micro-architectural diversity comparable to that of vertebrate fibrous connective tissue, occurring as three-dimensional fiber networks in dermal layers, parallel-fibered ligaments linking skeletal components, and crossed-fiber helical arrays in the walls of tubular organs. These anatomical structures perform the same functions as their vertebrate equivalents: they resist, transmit and dissipate mechanical forces, and they store and release elastic strain energy. Their variable tensility, however, adds another dimension to their functional versatility, which is of widespread importance to echinoderm biology and may have contributed to the evolutionary success of the phylum. In echinoderms, prolonged postural fixation depends on passive MCT stiffening rather than active muscle contraction, resulting in a considerable energy saving [4][5]. Another process associated with MCT is irreversible destabilization, which enables defensive self-detachment (autotomy) [6] and asexual reproduction by division of the whole body (fission) in brittlestars, starfish and sea-cucumbers [7][8][9]. Possibly related is the 'autolysis' or 'melting' of the whole dermis exhibited by some sea-cucumbers in adverse conditions, a pathological phenomenon of great commercial importance [10][11][12].

2. Extracellular Components

The MCT of all echinoderms consists predominantly of extracellular materials and includes a relatively small volume fraction of cellular components. With one exception, the extracellular materials comprise mainly transversely banded collagen fibrils aggregated into bundles (fibers) accompanied by loose arrangements of microfibrils and interfibrillar proteoglycans. The exception is the tendon tissue of the intervertebral muscles at the autotomy planes of brittlestar arms, which is an extension of the basement membrane of the muscle cells (see [13] for further information).

The banded collagen fibrils of MCT, like those of vertebrate connective tissue, are parallel arrays of trimeric collagen molecules with a regular stagger between adjacent molecules ranging from 40 to 80 nm, a much wider variability than the 65–67 nm reported for vertebrate fibrils [14][15]. The collagen molecules of most echinoderm fibrils comprise two fibrillar α chains (1α and 2α) which form $(1\alpha)_2 2\alpha$ heterotrimers [16][17][18]. A small proportion of collagen molecules in the fibrils of sea-urchin MCT contain a third fibrillar chain (5α) and have a $(1\alpha)_2 5\alpha$ stoichiometry [16]. The 5α chain is unusual in that its

N-propeptide is not removed prior to fibril assembly, as occurs in the echinoderm 2α chain and in all vertebrate fibrillar procollagens [19], and is located at the surface of the fibrils. The 5α N-propeptide is also notable because it contains 11 SURF ('sea-urchin fibrillar') modules, which are also present in the 2α N-propeptide and in fibrosurfin—an interfibrillar protein of unknown function. Since cleaved 2α N-propeptides have been immunolocalized to the periphery of fibril bundles in a sea-urchin mutable ligament [20], it has been suggested that these SURF-containing molecules play a role in MCT variable tensility [16].

So far, there has been only a very limited application of comparative '-omics' methodologies to the elucidation of MCT molecular organization. Surveys of the *Strongylocentrotus purpuratus* genome and transcriptome analysis of a sea-cucumber body wall revealed no unusual features of the extracellular matrix (ECM) that could explain the mechanical adaptability of MCT. For example, echinoderms have up to four fibrillar collagen genes of the vertebrate I/II/III type and two of the V type, all of which encode molecules occurring in varying combinations in the banded fibrils of vertebrates [9][21][22]. These investigations also demonstrated the presence of fibrillin genes and their transcripts, complementing biochemical and immunological evidence that the beaded microfibrils that are ubiquitous in MCT and non-mutable echinoderm ligaments [15][23][24] consist at least partly of fibrillin-like proteins. These microfibrils may facilitate slippage between adjacent fibril bundles during MCT deformation and contribute to passive elastic recoil after the removal of external forces [25].

Other interfibrillar components include molecules that are responsible for the cohesion between adjacent collagen fibrils and therefore have a major influence on the mechanical properties of MCT. Proteoglycans, which consist of a protein core and glycosaminoglycan (GAG) sidechains, are present both within and on the surface of the collagen fibrils [25]. There is biochemical evidence that surface proteoglycans act as binding sites for other molecules that form interfibrillar crossbridges. Staining with the cationic dyes cuprolinic blue or cupromeronic blue, which label GAG sidechains, reveals both punctate/globular precipitates on the surface of fibrils and linear structures that extend between adjacent fibrils and are attached to specific sites within each fibril D-period [15][26][27]. In featherstar ligaments and sea-cucumber dermis, the GAG components of these surface proteoglycans have been identified as chondroitin sulfate [26][27]. MCT contains several other molecules that contribute to interfibrillar cohesion but whose extracellular disposition is unknown; these are discussed below.

3. Cellular Components

The most abundant and characteristic cellular components of MCT are neurosecretory-like cell processes containing large (diameter > ca. 100 nm) dense-core vesicles (LDCVs). These were first described in the brittlestar intervertebral ligament and named 'juxtaligamental cells' (JLCs), because in brittlestars, the perikarya are always located outside, but usually closely adjacent to, collagenous structures [28]. In almost all investigated mutable collagenous structures, there are two or more populations of LDCV-containing processes distinguished by the size, and sometimes the shape and electron density, of their LDCVs [23][29][30][31][32].

An expanding body of evidence indicates that JLCs are neurons. In brittlestars, they are located in aggregations, known as juxtaligamental nodes, that have a central, neuropil-like region penetrated by the axons of motoneurons and chemical synapses between axonal and juxtaligamental processes [23][29][33][34][35]. Juxtaligamental nodes have an outer capsule of neuroglia-like cells with centrally directed partitions that compartmentalize the juxtaligamental perikarya. This suggests that the nodes could be ganglionic integrating centers that coordinate changes in the tensile properties of MCT with the activities of other effector systems [23]. Less organized cellular aggregations with ganglion-like features are associated with MCT in other echinoderm classes [36].

It is highly likely that JLCs are the effectors that directly alter the tensile properties of MCT, since their processes terminate in MCT, have no possible cellular targets, and link the ECM to the motor nervous system, and since they are also absent from non-mutable echinoderm collagenous structures [25]. Putative effector molecules occur in sea-cucumber JLCs. The stiffening proteins tensilin and stiparin have been immunolocalized to the LDCVs of JLCs in the dermis [37][38] (also Keene and Trotter, unpublished data), and Demeuldre et al. [32] detected tensilin by immunohistochemistry and in situ hybridization in JLCs in the connective tissue of the Cuvierian tubules (extrudable adhesive structures used for defense [39]). In some mutable collagenous structures, alterations in tensile state are accompanied by changes in JLC ultrastructure. These usually include indications that LDCVs or their contents are released into the extracellular compartment. Such changes have been seen mainly in structures undergoing an irreversible alteration in mechanical properties, which can be in the form of either drastic weakening (as occurs during autotomy) or stiffening (as undergone by Cuvierian tubules after expulsion) [15][29][32][40].

Other cellular components of MCT include heterogeneous vacuole-containing cells, which may be phagocytic, and, in a few starfish and sea-urchin structures, myocytes [25][41]. Most echinoderm collagenous tissue, including MCT, appears to lack fibroblasts [23][31][32][40][42]. As echinoderms show indeterminate growth [43][44], they must possess as yet unidentified populations of cells with the capacity to maintain the continuous expansion of connective tissue structures.

References

1. Socha, M.W.; Flis, W.; Pietrus, M.; Wartęga, M.; Stankiewicz, M. Signaling pathways regulating human cervical ripening in preterm and term delivery. Cells 2022, 11, 3690.
2. Svensson, R.B.; Heinemeier, K.M.; Couppé, C.; Kjaer, M.; Magnusson, S.P. Effect of aging and exercise on the tendon. J. Appl. Physiol. 2016, 121, 1237–1246.
3. Sugni, M.; Fassini, D.; Barbaglio, A.; Biressi, A.; Di Benedetto, C.; Tricarico, S.; Bonasoro, F.; Wilkie, I.C.; Candia Carnevali, M.D. Comparing dynamic connective tissue in echinoderms and sponges: Morphological and mechanical aspects and environmental sensitivity. Mar. Environ. Res. 2014, 93, 123–132.
4. Takemae, N.; Nakaya, F.; Motokawa, T. Low Oxygen consumption and high body content of catch connective tissue contribute to low metabolic rate of sea cucumbers. Biol. Bull. 2009, 216, 45–54.
5. Motokawa, T.; Sato, E.; Umeyama, K. Energy expenditure associated with softening and stiffening of echinoderm connective tissue. Biol. Bull. 2012, 222, 150–157.
6. Wilkie, I.C. Autotomy as a prelude to regeneration in echinoderms. Microsc. Res. Tech. 2001, 55, 369–396.
7. Wilkie, I.C.; Emson, R.H.; Mladenov, P.V. Morphological and mechanical aspects of fission in Ophiocomella ophiactoides (Echinodermata, Ophiuroida). Zoomorphology 1984, 104, 310–322.
8. Rubilar, T.; Pastor, C.; Díaz de Vivar, E. Timing of fission in the starfish Allostichaster capensis (Echinodermata: Asteroidea) in laboratory. Rev. Biol. Trop. 2005, 53 (Suppl. 3), 299–303.
9. Dolmatov, I.Y.; Afanasyev, S.V.; Boyko, A.V. Molecular mechanisms of fission in echinoderms: Transcriptome analysis. PLoS ONE 2018, 13, e0195836.
10. Zhu, B.; Zheng, J.; Zhang, Z.; Dong, X.; Zhao, L.; Tada, M. Autophagy plays a potential role in the process of sea cucumber body wall “melting” induced by UV irradiation. Wuhan Univ. J. Nat. Sci. 2008, 13, 232–238.
11. Sun, L.-M.; Wang, T.-T.; Zhu, B.-W.; Niu, H.-L.; Zhang, R.; Hou, H.-M.; Zhang, G.-L.; Murata, Y. Effect of matrix metalloproteinase on autolysis of sea cucumber Stichopus japonicus. Food Sci. Biotechnol. 2013, 22, 1–3.
12. Liu, Z.; Liu, Y.; Zhou, D.; Liu, X.; Dong, X.; Li, D.; Shahidi, F. The role of matrix metalloprotease (MMP) to the autolysis of sea cucumber (Stichopus japonicus). J. Sci. Food Agric. 2019, 99, 5752–5759.
13. Wilkie, I.C.; Sugni, M.; Gupta, H.; Candia Carnevali, M.D.; Elphick, M.R. The mutable collagenous tissue of echinoderms: From biology to biomedical applications. In Soft Matter for Biomedical Applications; Azevedo, H.S., Mano, J.F., Borges, J., Eds.; The Royal Society of Chemistry: Cambridge, UK, 2021; pp. 1–33. ISBN 978-1-78801-757-2.
14. Blowes, L.M.; Egertová, M.; Liu, Y.; Davis, G.R.; Terrill, N.J.; Gupta, H.S.; Elphick, M.R. Body wall structure in the starfish Asterias rubens. J. Anat. 2017, 231, 325–341.
15. Ribeiro, A.R.; Barbaglio, A.; Benedetto, C.D.; Ribeiro, C.C.; Wilkie, I.C.; Candia Carnevali, M.D.; Barbosa, M.A. New insights into mutable collagenous tissue: Correlations between the microstructure and mechanical state of a sea-urchin ligament. PLoS ONE 2011, 6, e24822.
16. Cluzel, C.; Lethias, C.; Garrone, R.; Exposito, J.-Y. Distinct maturations of N-propeptide domains in fibrillar procollagen molecules involved in the formation of heterotypic fibrils in adult sea urchin collagenous tissues. J. Biol. Chem. 2004, 279, 9811–9817.
17. Cui, F.; Xue, C.; Li, Z.; Zhang, Y.; Dong, P.; Fu, X.; Gao, X. Characterization and subunit composition of collagen from the body wall of sea cucumber Stichopus japonicus. Food Chem. 2007, 100, 1120–1125.
18. Tian, M.; Xue, C.; Chang, Y.; Shen, J.; Zhang, Y.; Li, Z.; Wang, Y. Collagen fibrils of sea cucumber (Apostichopus japonicus) are heterotypic. Food Chem. 2020, 316, 126272.
19. Hulmes, D.J.S. Collagen diversity, synthesis and assembly. In Collagen: Structure and Mechanics; Fratzl, P., Ed.; Springer US: Boston, MA, USA, 2008; pp. 15–47. ISBN 978-0-387-73906-9.
20. Cluzel, C.; Lethias, C.; Humbert, F.; Garrone, R.; Exposito, J.Y. Characterization of fibrosurfin, an interfibrillar component of sea urchin catch connective tissues. J. Biol. Chem. 2001, 276, 18108–18114.

21. Sea Urchin Genome Sequencing Consortium; Sodergren, E.; Weinstock, G.M.; Davidson, E.H.; Cameron, R.A.; Gibbs, R.A.; Angerer, R.C.; Angerer, L.M.; Arnone, M.I.; Burgess, D.R.; et al. The genome of the sea urchin Strongylocentrotus purpuratus. Science 2006, 314, 941–952.
22. Whittaker, C.A.; Bergeron, K.-F.; Whittle, J.; Brandhorst, B.P.; Burke, R.D.; Hynes, R.O. The echinoderm adhesome. Dev. Biol. 2006, 300, 252–266.
23. Wilkie, I.C. Functional morphology of the arm spine joint and adjacent structures of the brittlestar Ophiocomina nigra (Echinodermata: Ophiuroidea). PLoS ONE 2016, 11, e0167533.
24. Wilkie, I.C.; McKew, M.; Candia Carnevali, M.D. Functional morphology of the compass-rotular ligament of Echinus esculentus (Echinodermata: Echinoida): A non-mutable collagenous component of Aristotle's lantern. Zoomorphology 2005, 124, 9–26.
25. Wilkie, I.C. Mutable collagenous tissue: Overview and biotechnological perspective. In Echinodermata. Progress in Molecular and Subcellular Biology 39; Matranga, V., Ed.; Springer: Berlin/Heidelberg, Germany, 2005; pp. 221–250. ISBN 978-3-540-24402-8.
26. Erlinger, R.; Welsch, U.; Scott, J.E. Ultrastructural and biochemical observations on proteoglycans and collagen in the mutable connective tissue of the feather star Antedon bifida (Echinodermata, Crinoidea). J. Anat. 1993, 183, 1–11.
27. Wang, J.; Chang, Y.; Wu, F.; Xu, X.; Xue, C. Fucosylated chondroitin sulfate is covalently associated with collagen fibrils in sea cucumber Apostichopus japonicus body wall. Carbohyd. Polym. 2018, 186, 439–444.
28. Wilkie, I.C. The juxtaligamental jells of Ophiocomina nigra (Abildgaard) (Echinodermata: Ophiuroidea) and their possible role in mechano-effector function of collagenous tissue. Cell Tissue Res. 1979, 197, 515–530.
29. Charlina, N.A.; Dolmatov, I.Y.; Wilkie, I.C. Juxtaligamental system of the disc and oral frame of the ophiuroid Amphipholis kochii (Echinodermata: Ophiuroidea) and its role in autotomy. Invertebr. Biol. 2009, 128, 145–156.
30. Hennebert, E.; Haesaerts, D.; Dubois, P.; Flammang, P. Evaluation of the different forces brought into play during tube foot activities in sea stars. J. Exp. Biol. 2010, 213, 1162–1174.
31. Motokawa, T. Mechanical mutability in connective tissue of starfish body wall. Biol. Bull. 2011, 221, 280–289.
32. Demeuldre, M.; Hennebert, E.; Bonneel, M.; Lengerer, B.; Van Dyck, S.; Wattiez, R.; Ladurner, P.; Flammang, P. Mechanical adaptability of sea cucumber Cuvierian tubules involves a mutable collagenous tissue. J. Exp. Biol. 2017, 220, 2108–2119.
33. Mashanov, V.S.; Charlina, N.A.; Dolmatov, I.Y.; Wilkie, I.C. Juxtaligamental cells in the arm of the brittlestar Amphipholis kochii Lütken, 1872 (Echinodermata: Ophiuroidea). Russ. J. Mar. Biol. 2007, 33, 110–117.
34. Charlina, N.A.; Mashanov, V.S.; Dolmatov, I.Y.; Wilkie, I.C. Morphology of the juxtaligamental system in the ophiuroid Amphipholis kochii. In Proceedings of the 12th International Echinoderm Conference; CRC Press: Boca Raton, FL, USA, 2010.
35. Zueva, O.; Khoury, M.; Heinzeller, T.; Mashanova, D.; Mashanov, V. The complex simplicity of the brittle star nervous system. Front. Zool. 2018, 15, 1.
36. Wilkie, I.C.; Candia Carnevali, M.D. The juxtaligamental cells of echinoderms and their role in the mechano-effector function of connective tissue. In Frontiers in Invertebrate Physiology: A Collection of Reviews; Saleuddin, A.S., Leys, S., Roer, R., Wilkie, I.C., Eds.; Apple Academic Press: Cambridge, MA, USA, 2024; Volume 3, pp. 345–430.
37. Trotter, J.A.; Tipper, J.; Lyons-Levy, G.; Chino, K.; Heuer, A.H.; Liu, Z.; Mrksich, M.; Hodneland, C.; Dillmore, W.S.; Koob, T.J.; et al. Towards a fibrous composite with dynamically controlled stiffness: Lessons from echinoderms. Biochem. Soc. Trans. 2000, 28, 357–362.
38. Bonneel, M.; Hennebert, E.; Aranko, A.S.; Hwang, D.S.; Lefevre, M.; Pommier, V.; Wattiez, R.; Delroisse, J.; Flammang, P. Molecular mechanisms mediating stiffening in the mechanically adaptable connective tissues of sea cucumbers. Matrix Biol. 2022, 108, 39–54.
39. Chen, T.; Ren, C.; Wong, N.-K.; Yan, A.; Sun, C.; Fan, D.; Luo, P.; Jiang, X.; Zhang, L.; Ruan, Y.; et al. The Holothuria leucospilota genome elucidates sacrificial organ expulsion and bioadhesive trap enriched with amyloid-patterned proteins. Proc. Natl. Acad. Sci. USA 2023, 120, e2213512120.
40. Bobrovskaya, N.V.; Dolmatov, I.Y. Autotomy of the visceral mass in the feather star Himerometra robustipinna (Crinoidea, Comatulida). Biol. Bull. 2014, 226, 81–91.
41. Wilkie, I.C. Is muscle involved in the mechanical adaptability of echinoderm mutable collagenous tissue? J. Exp. Biol. 2002, 205, 159–165.
42. Santos, R.; Hennebert, E.; Coelho, A.; Flammang, P. The echinoderm tube foot and its role in temporary underwater adhesion. In Functional Surfaces in Biology; Springer: Dordrecht, The Netherlands, 2009; Volume 2, pp. 9–41. ISBN

978-1-4020-6694-8.

43. Bodnar, A.G. Cellular and molecular mechanisms of negligible senescence: Insight from the sea urchin. Invertebr. Reprod. Dev. 2015, 59, 23–27.
44. Mashanov, V.; Whaley, L.; Davis, K.; Heinzeller, T.; Machado, D.J.; Reid, R.W.; Kofsky, J.; Janies, D. A subterminal growth zone at arm tip likely underlies life-long indeterminate growth in brittle stars. Front. Zool. 2022, 19, 15.