The secret of softness, a stiffening truth

An investigation of the molecular and structural properties of the mutable collagenous tissue in a European sea cucumber

Némo LEMAIRE[1], Nathalie SINGH[3], Amy LIN[3], Rudy WATTIEZ[2], Jérôme DELROISSE[1], Matthew J. HARRINGTON[3], Patrick FLAMMANG[1]

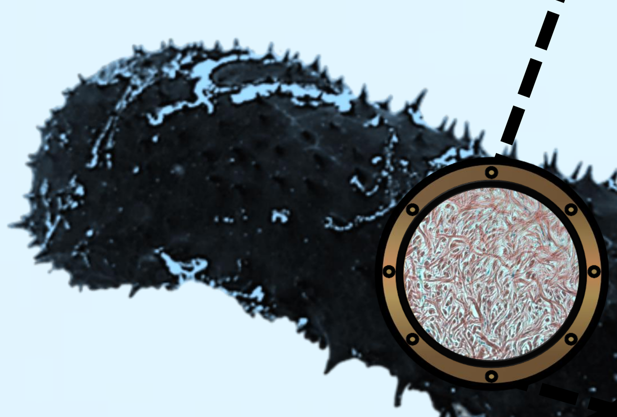
[1] Biology of Marine Organisms and Biomimetics Unit, University of Mons (UMONS), Mons, Belgium
[2] Proteomics and Microbiology Department, University of Mons (UMONS), Mons, Belgium
[3] Department of Chemistry, McGill University, Montreal, Quebec H3A 0B8, Canada

An ever-shifting tissue

In most connective tissue, e.g. tendons and bones, properties are set in time and changes can only occur irreversibly under a handful of conditions. On the contrary, the **mutable collagenous tissue (MCT)** found in echinoderms is a unique connective tissue that can rapidly alter its **mechanical properties** in response to certain stimuli. The tissue can alternate between **three different stiffness states**. Thanks to this, echinoderms can minimize energy expenditure.

The body wall of **sea cucumbers** (Holothuroidea) is a typical MCT capable of reversible modulation and irreversible softening. In this model, mechanical properties are modified by the secretion of molecular factors in the extracellular matrix, resulting in the formation or removal of **transient cross-bridges** between collagen fibrils.

Surprisingly, despite this unique ability and its potential as a **tunable bio-scaffold**, we are currently lacking much information regarding the molecular and structural characteristics of MCT compared to usual collagen-based tissue. A thorough investigation of the principal actors involved, notably collagen, could shed some light on the process.



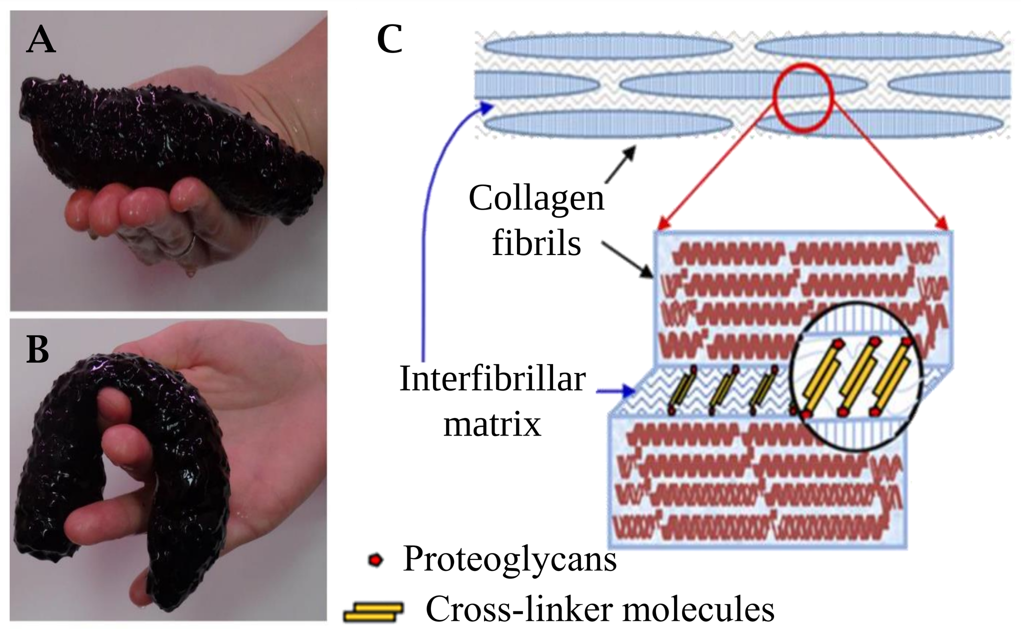


Figure 1: Sea cucumber *Holothuria forskali* in (A) the stiff and (B) the soft state. (C) Simplified diagram of an MCT: spindly-shaped collagen fibrils are assembled into fibers but are not permanently crosslinked together. Under mechanical stimulation, specific neurosecretory cells will release molecules (stiffeners or softeners) capable of interacting with surface proteoglycans.

Objectives

The aim of this project is to analyze the ultrastructure of the MCT in the European black sea cucumber (*Holothuria forskali*). By doing so, we hope to determine if the composition and/or spatial organization of collagen account for the tissue adaptability.

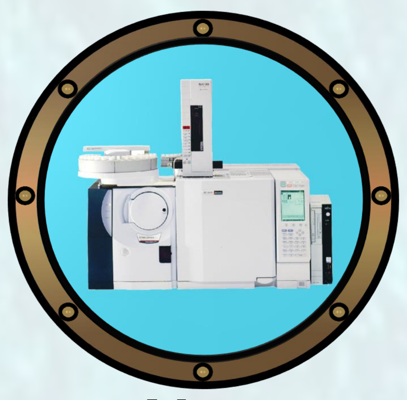
Strategy:

ATR-FTIR

Polarized light microscopy

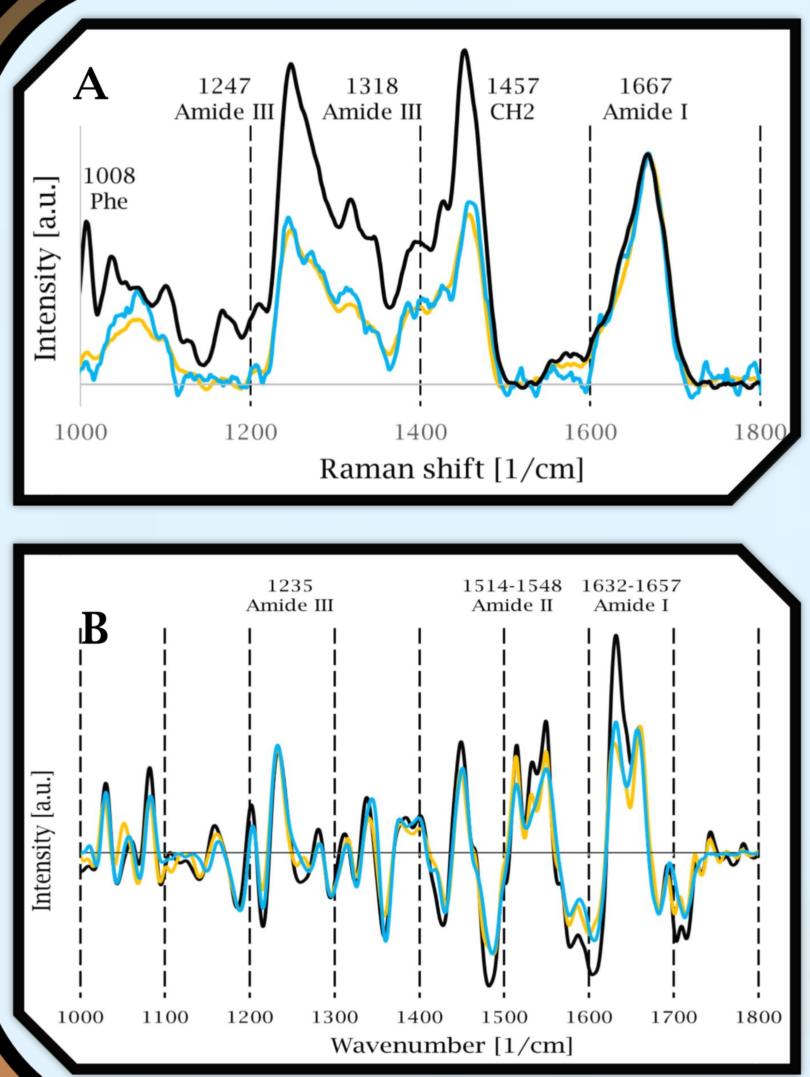
Raman microscopy

X-ray scattering



Mass spectrometry

Molecular composition



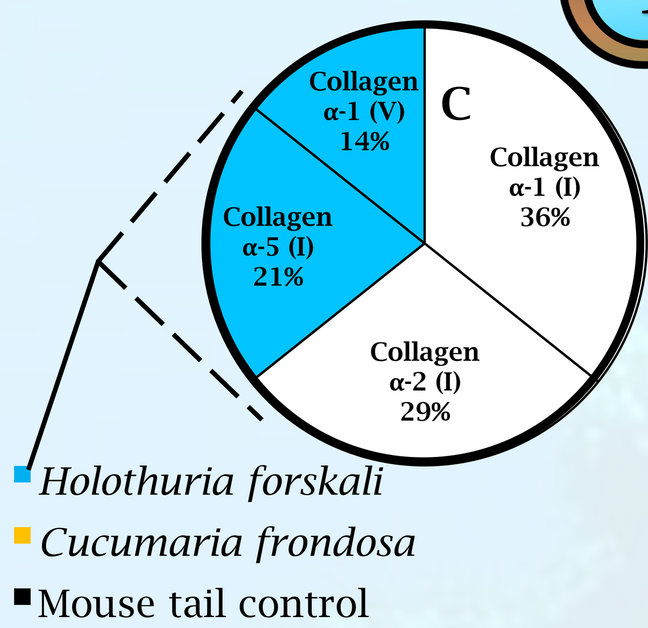


Figure 2: Comparison of *Holothuria forskali* (blue), *Cucumaria frondosa* (yellow), and mouse tail type I collagen (black) molecular signal given by **(A)** Raman microscope and (B) ATR-FTIR for isolated fibrils; the intensity level of amide I was used for normalizing spectra and collagen's related peaks are mentioned. (C) Collagen chains identified during tandem mass spectrometry.

Similarity with:

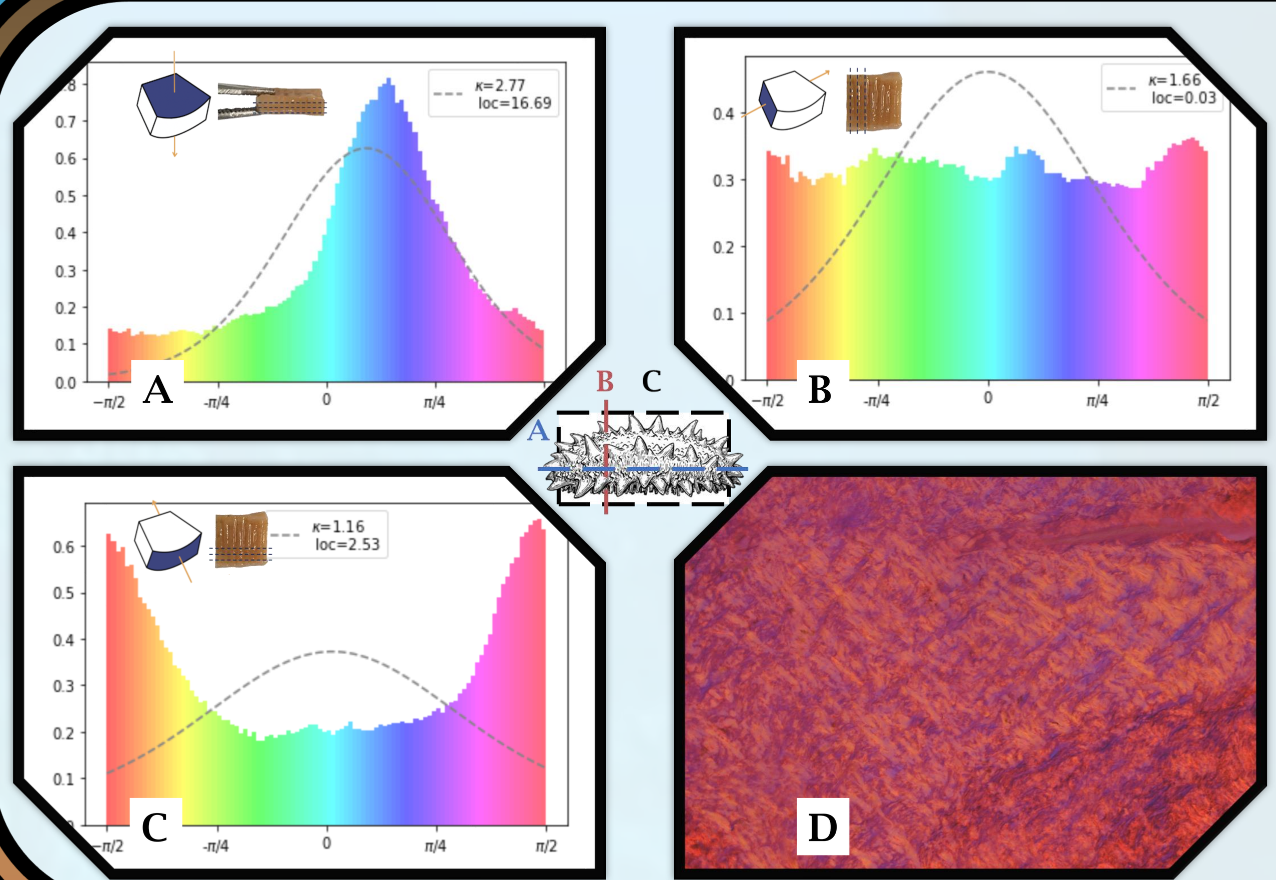
- Mammalian type I collagen
- Distant sea cucumbers

But slight shifts in peaks intensity

Majority of signals:

- Fibrillar collagen (type I, V)
- Usual heterotrimer: $(\alpha\text{-}1)_2\alpha\text{-}2$
- Uncommun heterotrimer: $(\alpha\text{-}1)_2\alpha\text{-}5$
- Scarce type V collagen

Structural organization



Preferential alignment of fibrils:

- Slightly in directions A and C (kappa value)
- Random distribution in direction B
- Potentially related to the lifestyle of the sea cucumber

Figure 3: Preferential alignment of collagen fibrils in three sectioning plans: (A) frontal (anterior to posterior), (B) transversal (dorsal to ventral), and (C) sagittal (side to side). (D) Birefringent samples, collagen fibrils stained with Sirius Red, under a polarized light microscope. Fibrils show a different coloration depending on their orientation to the incident light (blue when parallel and yellow if perpendicular).

Take home message

The mutable collagenous tissue's unique particularity can be envisioned as the result of a **multi-layered association** of common pieces in a **novel pattern**.

Prospects

- **Ultrastructure**: SAXS/WAXS, second harmonic
- **Induced mechanical states:** chemical means
- **Neural control:** related neuropeptides

Reference: **Wilkie, I. C., Sugni, M., Gupta, H. S., Carnevali, M. C., & Elphick, M. R. (2021).** The mutable collagenous tissue of echinoderms: from biology to biomedical applications. **Mo, J., Prévost, S. F., Blowes, L. M., Egertová, M., Terrill, N. J., Wang, W., ... & Gupta, H. S. (2016).** Interfibrillar stiffening of echinoderm mutable collagenous tissue demonstrated at the nanoscale. *Proceedings of the National Academy of Sciences*, *113*(42), E6362-E6371.

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