BIOPHYSICS RESEARCH:-
A FOCUS ON THE EXTRACELLULAR MATRIX

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Introduction:-

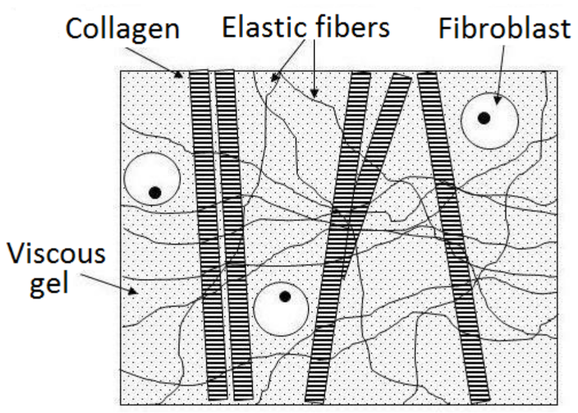
- Biophysics is a multidisciplinary branch of science, applying principles and techniques of physics to biology.
- As Biophysicists, our aim is to gain further insight into understanding biological systems, both in the normal state and when things go wrong.
- One of the major research themes in the Biophysics Group is the extracellular matrix (ECM), where we are trying to answer question such as, "What is the relationships between the physical properties of the macromolecular constituents of the extracellular matrix and their supramolecular assemblies and the physiological functions of the tissue? How is the structure of the ECM changed in disease?".

The Extracellular Matrix (ECM):-

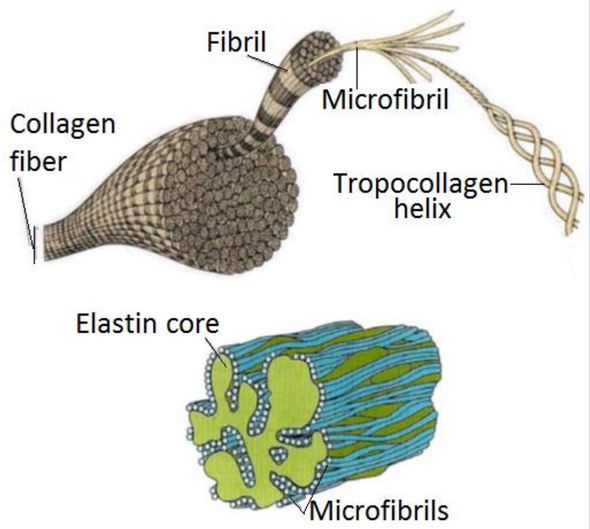
- Tissues and organs are not just made up of cells. The ECM is the non-cellular component of tissues such as cartilage and blood vessels.
- The ECM consists of a complex network of fibrous proteins, elastin and collagen, embedded in a viscoelastic gel rich in proteoglycans[1] (Figure 1).
- ECM composition varies dramatically between tissue types according to their different functional requirements.
- Initially regarded as simply an inert 'filler' material between cells, we now know that the ECM has many other functions including cell support, determining tissue mechanical properties, regulation of cell behaviour and controlling the movement of water, nutrients and other solutes.

Figure 1

(a) Components of the extracellular matrix (ECM)



(b) Collagen and elastin fibers



Matrix Proteins:-

- A combination of collagen and elastin enables tissues to develop a wide range of mechanical properties.

Collagen:-

- The collagens are a family of rigid, triple helical molecules that assemble into essentially inextensible fibers.
- They are the most abundant proteins in mammals.
- The collagens are found in tissues such cartilage, tendons, ligaments and skin where a combination of tensile strength and stiffness are required. In engineering terms collagen may be likened to steel[2].

Elastin:-

- Many vertebrate tissues, such as skin, blood vessels, and lungs have a requirement for long-range elasticity.
- Networks of elastic fibers provides tissues with the required resilience allowing them to recoil to their original shape after being stretched.
- Elastic fibers are composed of the matrix protein, elastin, and a family of microfibrillar glycoproteins[2].
- Elastin is incredibly stretchy being at least five times more extensible than a rubber band.
- Elastin is chemically inert, has to sustain strains of up to 100% and last a lifetime.
- Elastin can be detected either as rod-like fibers, sheets or incorporated into a fine meshwork.

Lamprin:-

- The lamprins are a family of novel elastic proteins found in the agnathans (lamprey and hagfish) and are considered to be evolutionary predecessors of the mammalian protein, elastin.

Multiphoton Microscopy:-

- Multiphoton microscopy is a form of laser microscopy with contrast derived from optical properties of sample[3].
- Confines focal spot to area where photon flux is high.
- Modalities:
  - Two photon fluorescence (TPF)
  - Second-harmonic generation (SHG)
  - Coherent anti-Stokes Raman (CARS)
- Advantages over conventional confocal microscopy:
  - Sub-micron resolution
  - Increased depth penetration (1mm)
  - Label free imaging

Figure 2

Images of multiphoton lab, (a) multiphoton imaging equipment and (b) tensile testing/imaging arrangement.

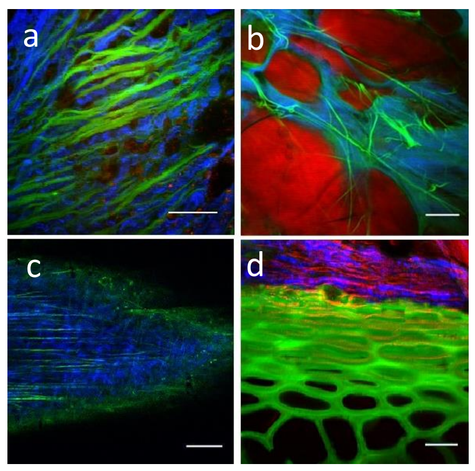


Figure 3

Representative multiphoton images of ECM components in (a) bovine ligamentum nuchae, (b) adipose tissue, (c) Porcine chordae tendineae isolated from heart and (d) lamprey annular cartilage. Scale bar in (a) and (d) is 50 µm and (b) and (c) 25 µm. Collagen is visualised by SHG (blue), elastin by TPF (green) and lipids (red) by CARS.

- Multiphoton microscopy has allowed us to visualise the organisation of collagen and elastin networks in fresh tissues without the need for staining[4,5] (Figure 3).
- This technique has enabled us to detect fine elastin fibers previously overlooked using conventional light microscopy.
- Using the setup illustrated in figure 2b, we have also been able to follow the changes in organisation of collagen and elastin networks under tensile strain.

Mechanical testing:-

- Tool for studying physiological function, behaviour and mechanical properties of biological samples.
- Biological samples generally characterised by relationship between stresses and strains.
- Data interpretation complex because tissues are composite structures.
- Custom-built apparatus permits mechanical strain to be applied under various conditions and under Raman and multiphoton microscope.

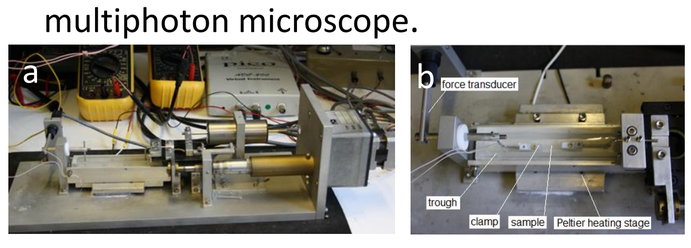


Figure 4

Custom built mechanical testing apparatus in situ, (a) general view and (b) close up of trough arrangement in which sample is contained.

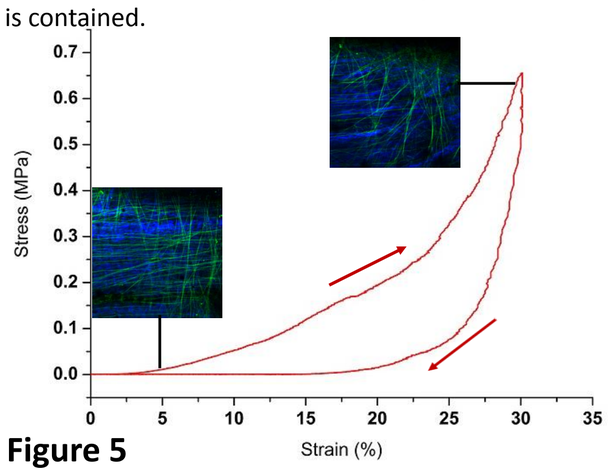


Figure 5

Representative stress-strain curve for anterior strut chordae tendinae isolated from a pig heart mitral valve. Also shown are the corresponding multiphoton images taken at 5% and 20% strain to show the changes in organisation of collagen and elastin networks.

- Nonlinear microscopy enables visualization of ECM components in living tissues, which, in combination with mechanical testing, allows us to relate their mechanical properties to changes in structure

Raman Spectroscopy:-

- Light interacts with matter in different ways (transmission, absorption and scattering).
- Raman spectroscopy looks at the inelastically scattered light to provide us with information on molecular vibrations of the material.
- The Raman effect is very weak (10^{-6} of incident photons). In order to detect the Raman effect we use highly sensitive spectrometers.

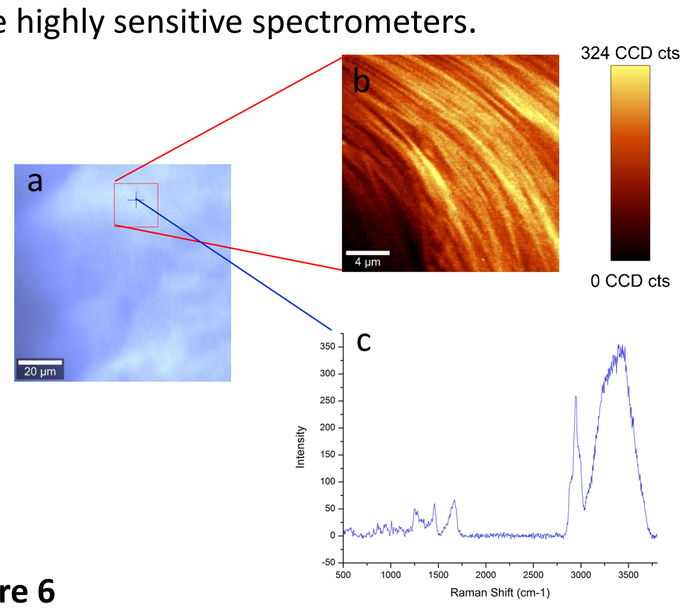


Figure 6

Representative white light image (a) of porcine chordae tendineae, (transverse section) showing area of image scan of 2995 cm-1 CH Raman peak (b) and location of single Raman spectrum (c).

- Raman spectroscopy is a powerful technique for probing the structure of biological molecules under different conditions.
- Using this technique we have characterised the molecular composition of ECM proteins in various biological tissues and investigated the conformational changes that occur under strain, different bathing media and temperature[6,7].

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