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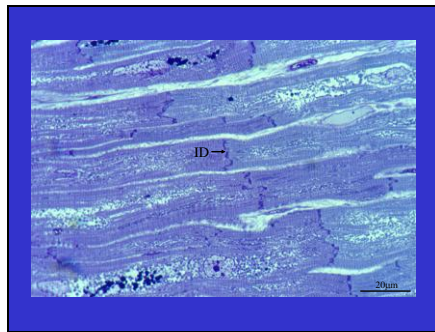
Subcellular structures of the cardiac myocyte

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Aims of Lecture:

1. To illustrate how a range of cellular imaging techniques can be used to build up a comprehensive picture of the structural features of the cardiac muscle cell
2. To relate these structural features to the function of the cardiac muscle cell

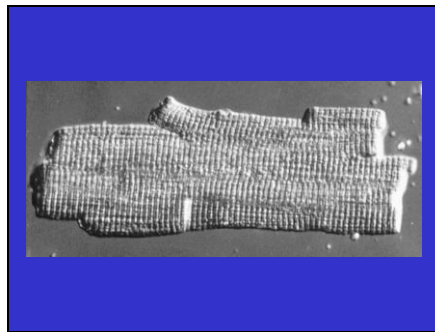
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What do we see by imaging cardiac tissue by light microscopy?

Structure of myofibres, made up from cardiac muscle cells joined by intercalated disks (ID).
Striated appearance

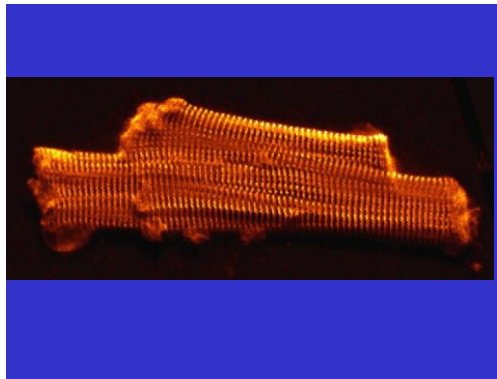
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Light microscopy of isolated ventricular cardiac muscle cells reveals overall features of cell morphology

Elongate, branching appearance
Cells are approximately 120 μm in length, and 25 μm in width
Sites of intercalated disks, at blunted ends of cell
Striations

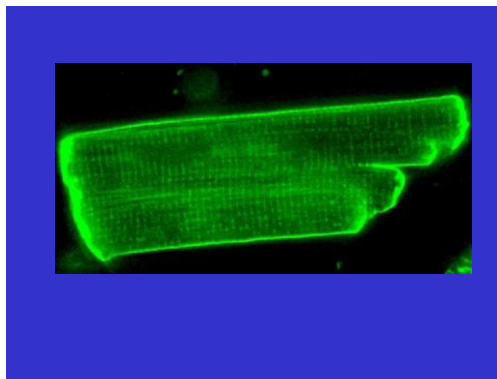
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Fluorescent microscopy (fixed cells)

Protein alpha-actinin is located in the Z-disks and helps hold sarcomeres together

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Fluorescent microscopy (live cells)

ANNEPS staining for T-tubule network

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Electron microscopy

1. TRANSMISSION ELECTRON MICROSCOPY

Electrons that pass through the specimens are used to form the image.

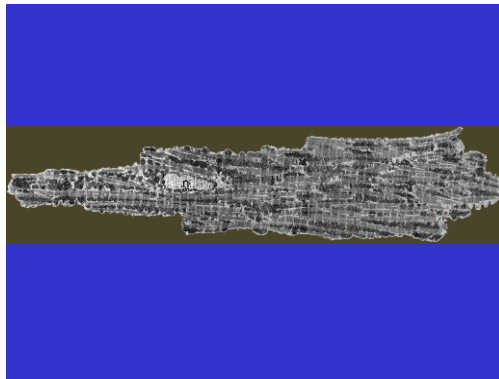
2. SCANNING ELECTRON MICROSCOPY

Electrons scattered or emitted from the surface of the specimen form the image.

Electron microscopy

There are many approaches to cellular imaging by electron microscopy of which the previous example is just one. Electron microscopes are of two main types, transmission electron microscopes (in which electrons that pass through the specimens are used to form the image), and scanning electron microscopes (in which electrons scattered or emitted from the surface of the specimen form the image).

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Low power thin-section electron microscopy of isolated ventricular cardiac muscle cell

Electron microscopy of the isolated cell at a similar magnification to the light microscopical view permits visualisation of further detail of the cell's internal structure

The cell is packed full of striated myofibrils and mitochondria, reflecting its highly specialised function in contraction

Other features resolved – the nucleus (n) and plasma membrane

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TRANSMISSION ELECTRON MICROSCOPY

- Thin-sectioning
- Freeze-fracturing

Different preparation techniques in transmission electron microscopy give different types of image.

Two main techniques in transmission electron microscopy: thin-sectioning, and freeze-fracture

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THIN SECTION EM

- Fixation
(glutaraldehyde to fix proteins; osmium tetroxide to fix lipids)
- Dehydration
- Embedding
(in a liquid plastic resin; polymerising the resin – the sample is now in and part of a block of hard plastic)
- Sectioning
(cutting ultrathin sections using an ultramicrotome)
- Staining
(heavy metal salts – uranyl acetate and lead citrate)

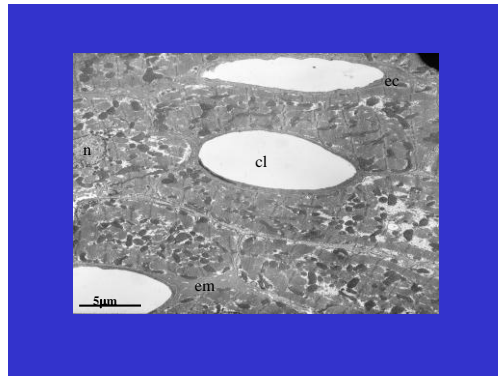
Thin-section electron microscopy

The standard and most widely used approach in electron microscopy

There are many variants; the conventional approach involves:

- 1) Fixation. Glutaraldehyde to fix proteins; osmium tetroxide to fix lipids
- 2) Dehydration
- 3) Infiltrating with and embedding in a liquid plastic resin
- 4) Polymerising the resin – the sample is now in and part of a block of hard plastic
- 5) Cutting ultrathin sections using an ultramicrotome
- 6) Staining the sections with heavy metal salts – uranyl acetate and lead citrate

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Myocardial tissue imaged at low power by thin-section electron microscopy (atrial myocardium)

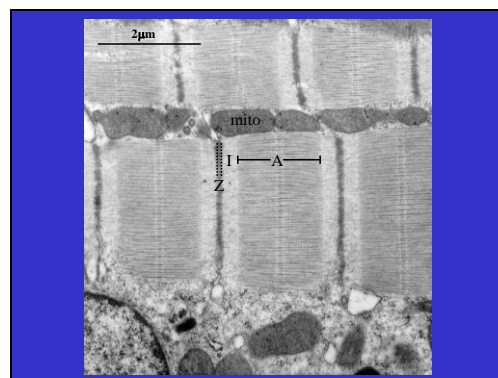
More complex to interpret than images of isolated cells

Portions of multiple cells within the field.

Features identified; extracellular matrix (em), nuclei (n), capillaries (cl), endothelial cells (ec).

As before, the cardiac muscle cells are seen to be packed full of mitochondria and striated myofibrils

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High magnification image of myofibril structure by thin-section electron microscopy

Myofibrils are made up from two types of myofilament; thin-filaments (principally actin) and thick filaments (myosin)

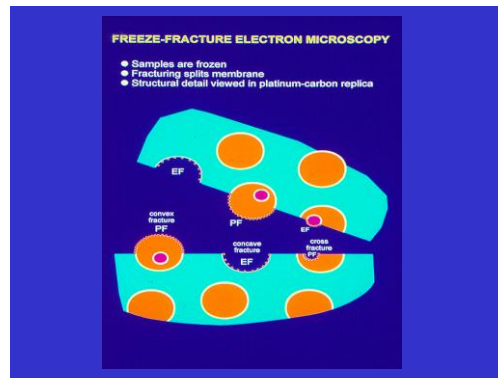
Striated appearance of myofibrils arises from organisation of the myofilaments

Thin filaments extend out on either side from each Z-band, crossing the I-band to penetrate partially into the A-band where they overlap with thick filaments

Thick filaments – within A band only
Contraction brought about by sliding of thin filaments over thick filaments – requires calcium and energy in form of ATP supplied by mitochondria (mito) situated alongside

I bands reduced in width upon contraction – reduced sarcomere length.

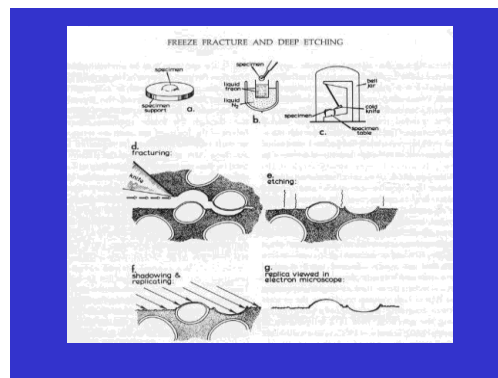
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The main features of structural relief that we see in a freeze-fracture replica result from changes in the fracture plane when it meets membranes.

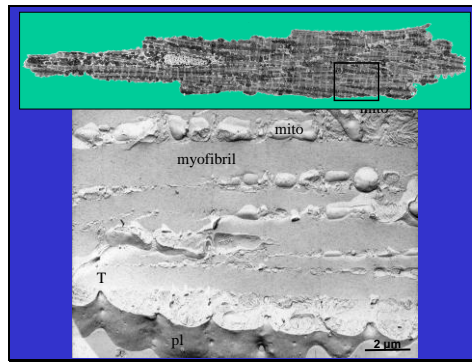
Remind yourself of the images of the cardiac muscle cell corresponding to the three types of fracture depicted in the diagram.

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- Specimen is fixed by glutaraldehyde and cryoprotected with glycerol before mounting step.
- The mounted sample is frozen by Freon 22.
- The frozen specimen is transferred onto the cold stage of a freeze-fracture machine.
- The frozen specimen is fractured by using a liquid nitrogen-cooled microtome.
(temperature -100 C to -120C)
- Etching (vacuum sublimation of ice; temperature is raised between -100C and -95C).
- Replication- deposition of a fine layer of platinum (total replica thickness about 15nm).
- Biological material removed by digestion in chromatic acid. The replica is finally mounted on a grid for examination in the TEM.

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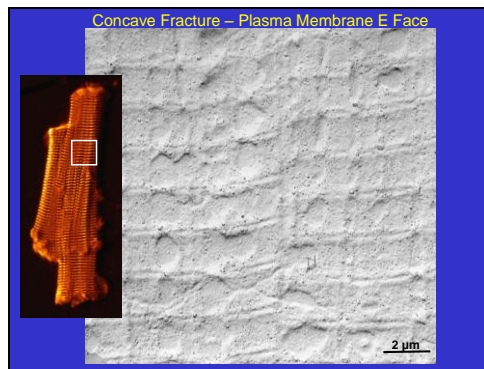
Freeze-fractured myocyte – cross-fracture image

Fracture plane has travelled along the plasma membrane (pl) before breaking into the cell to reveal its internal structure

Main features revealed: myofibrils (myo) separated by rows of mitochondria (mito). Some mitochondria have been cross-fractured to reveal the cristae (mito/cr) Transverse tubules (T) invaginating opposite Z-bands of superficial myofibrils. Sarcoplasmic reticulum (sr) is also revealed

Scale – width of image = 15µm

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Freeze-fractured myocyte in concave fracture

µ

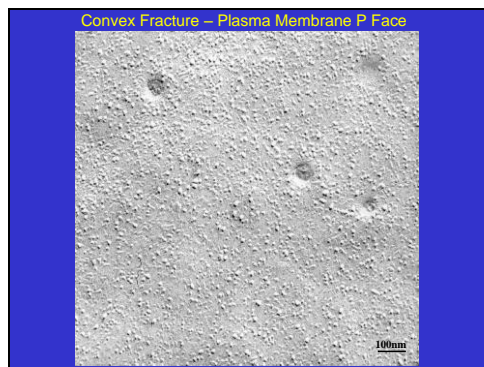
This image shows an example of a concavely fractured cell at low magnification

E-face view of plasma membrane
Three-dimensional topology of the plasma membrane revealed – sculptured appearance

Pairs of folds at regular intervals – sites of costameres

As a guide to scale, the height of this image represent 16 µm

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Freeze-fractured Myocyte – convex fracture

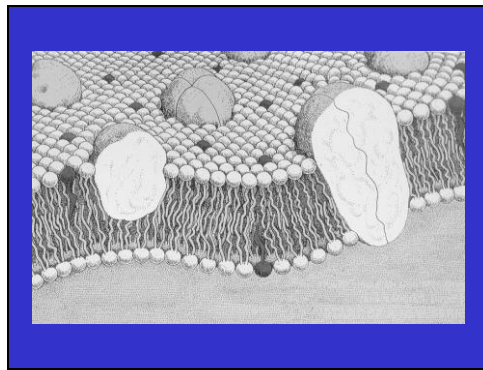
This example illustrates a convex fracture at high magnification – P-face image of plasma membrane

Illustrates how freeze-fracture at high magnification can reveal details of the membrane's internal structure at high resolution

The plasma membrane is seen as a smooth background matrix on which are scattered minute particles, 3-10nm in diameter

The smooth matrix represents the lipid
The particles are termed intramembrane particles – represent the integral proteins of the membrane

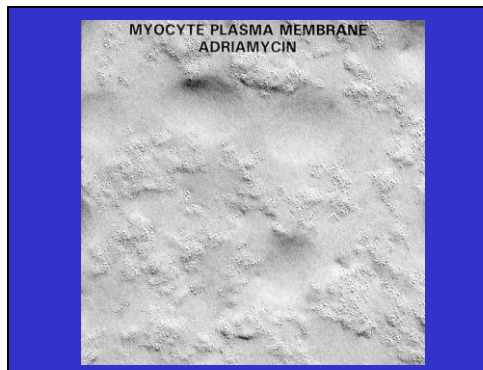
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Fluid mosaic model of membrane structure

The details of membrane structure seen by freeze-fracture formed part of the evidence for the fluid mosaic model of membrane structure. Depicts proteins as globular entities embedded in lipid bilayer. The fracture plane follows a path in the hydrophobic core of the membrane, between the monolayers of lipids.

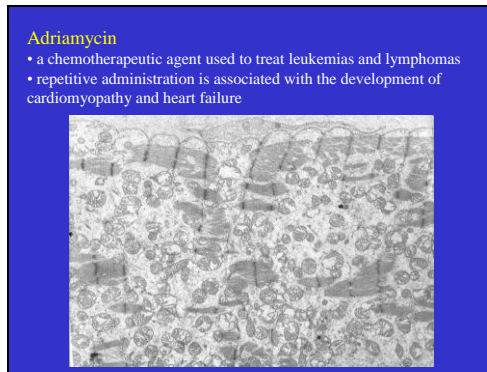
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Freeze-fracture image of membrane morphology from adriamycin-treated cell

This example illustrates dramatic alteration to the plasma membrane structure when cells are exposed to the cardiotoxic drug, adriamycin. The intramembrane particles (representing integral membrane proteins) become aggregated, with extensive lipid islands between. Altered membrane structure - impaired cell function.

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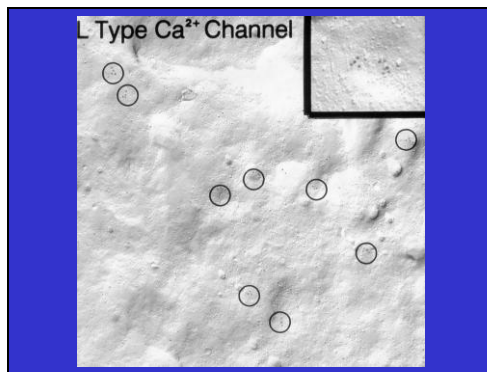
Background information on adriamycin

Adriamycin treatment can result in cardiac problems

This biopsy is from a patient whose lymphoma was successfully treated but later suffered heart failure

Note – sparse myofibrils and disrupted mitochondria – long term effects of adriamycin treatment

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Identification of proteins in freeze-fracture images of the plasma membrane

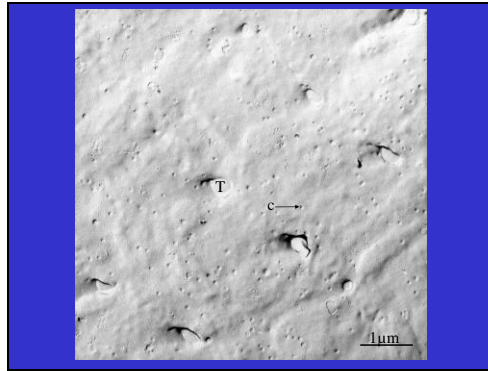
Classical freeze-fracture view of membrane reveals proteins as intramembrane particles but gives no information on their chemical identity
There are now ways we can look at this

This example illustrates the identification of L-type calcium channels by using an antibody against the channel, in combination with minute gold marker particles and freeze-fracture

The gold markers seen as black dots demonstrate that the calcium channels are displayed in clusters in the plasma membrane

These clusters lie opposite the junctional sarcoplasmic reticulum – structural basis for calcium-induced calcium release, basic mechanism of cardiac excitation-contraction coupling

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Other features of the general plasma membrane in freeze-fracture images: transverse tubules and caveolae

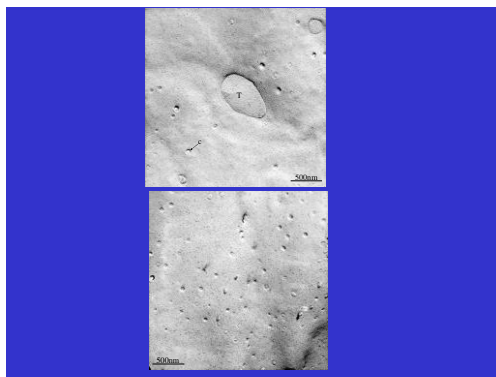
Two further features of the plasma membrane in freeze-fracture images are transverse tubule openings and caveolae

Transverse tubules (T) are finger like extensions of the plasma membrane which penetrate into the cell

The transverse tubule openings appear as large, irregular breaks (100-500+ nm across) Caveolae (c) are smaller structures – vesicle like in-pocketings of the plasma membrane, seen as small breaks where the fracture skips across the neck of the invagination

Can you tell whether this is an E-face or P-face view of the plasma membrane?

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Ventricular myocyte plasma membrane: freeze-fracture image

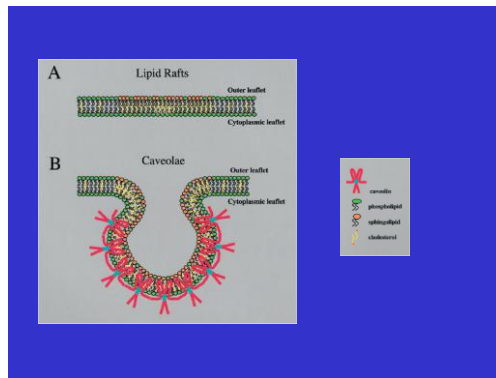
Comparison of freeze-fracture views of the plasma membrane of ventricular and atrial myocytes reveals differences in structure

In the ventricular myocyte, there are numerous transverse tubules (T) and a moderate number of caveolae (c)

Atrial myocyte plasma membrane: freeze-fracture image

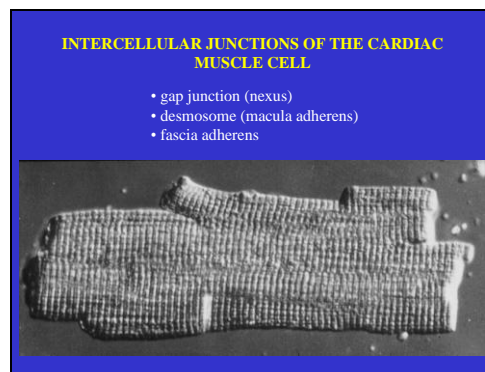
In the atrial myocyte plasma membrane, there is a lack of transverse tubules but more abundant caveolae

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Caveolae are specialised membrane domains, enriched in cholesterol and a protein called caveolin
Sites for the organisation of membrane proteins – water channel proteins, nitric oxide synthase, etc.

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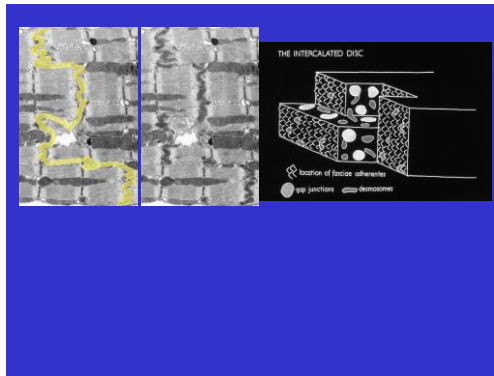
The intercalated disk plasma membrane: gross morphology of the intercalated disk

The features illustrated so far are those of the “general plasma membrane”
In addition, the specialised portions of the plasma membrane at sites of interaction between cells form the intercalated disk

As illustrated at the outset, intercalated disks occur at the blunted ends of the cell – at the ends of the main body of the cell and at side branches

Each myocyte is joined to ~10 other myocytes by intercalated disks to build up the three-dimensional cellular branching structure of the myofibre
Three types of cell junction are present at the intercalated disk: gap junctions, fascia adherens junctions and desmosomes

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The intercalated disk – thin-section electron microscopy

In longitudinal thin-sections of the myocyte, the intercalated disk has a step-like structure

The three types of cell junction have distinct distributions within the intercalated disk:

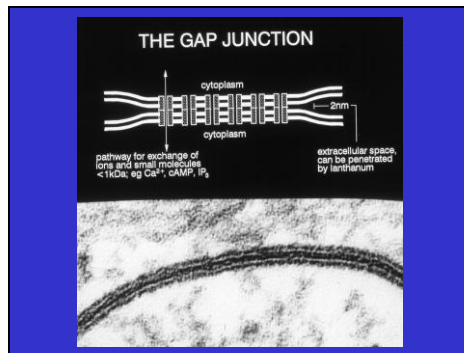
gap junctions – predominantly in longitudinal segments of the membranes

fascia adherens junctions – vertical segments of membranes

desmosmes – both longitudinal and vertical segments of membranes

Study the image of the intercalated disk in the picture on the right. Use the picture on the left to trace the course of the intercalated disk (shown in yellow). Can you identify cell junctions in the disk, and other structures in the image?

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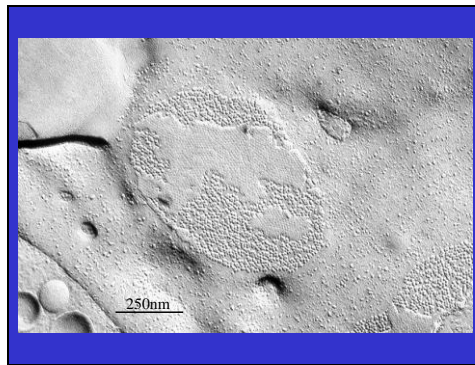
Gap junctions: thin-section electron microscopy image

At the gap junction, the plasma membranes of the adjacent cells come into very close contact

Thin-section electron microscopy reveals the gap junction as a pentalaminar structure formed from two very closely apposed unit membranes

Channels span the pair of membranes, allowing direct communication between the cytoplasmic compartments of the neighbouring cells

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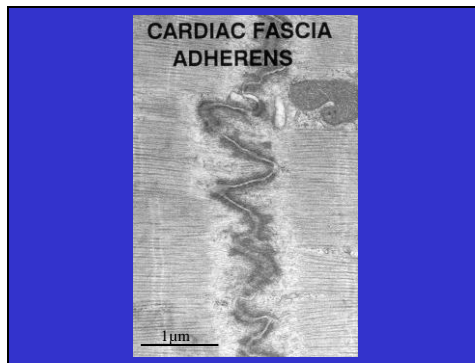


Gap junction viewed by freeze-fracture

When the membranes of the gap junction are split by freeze-fracture, the gap junction is seen as a cluster of particles in the plane of the membrane. Each of these particles represents a gap-junctional channel.

The major function of the gap junction in the heart is to provide the low resistance conduction pathways which allow orderly spread of the wave of electrical activation from cell to cell.

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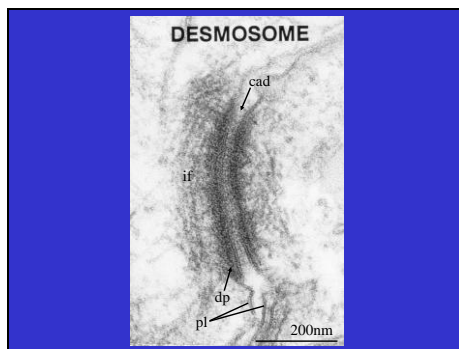
Fascia adherens junctions: thin-section image

Fasciae adherentes anchor the contractile apparatus (myofibrils) of the pair of cells to the plasma membranes: a series of proteins is involved in the attachment, including vinculin and alpha-actinin.

The plasma membranes are bonded together by cadherins – adhesive molecules.

These junctions ensure efficient transmission of the mechanical force of contraction from cell to cell.

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Desmosome: thin-section image

Desmosomes anchor the intermediate filament (desmin) cytoskeleton.

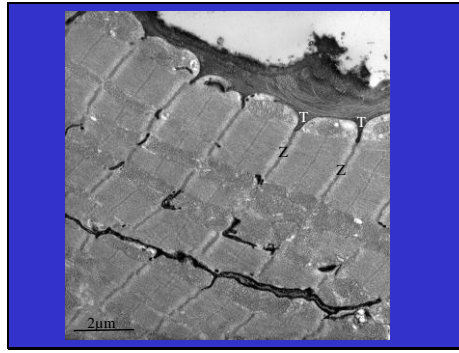
Intermediate filaments (if) composed of desmin attach to the desmosomal plaques (dp).

The desmosomal plaques are composed of desmoplakin.

Desmosomal membrane domains are bonded by cadherins (cad).

(pl = plasma membrane)

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Plasma membrane, transverse tubules and cell interior: thin-section image

The series of images so far completes the survey of the intercalated disk plasma membrane

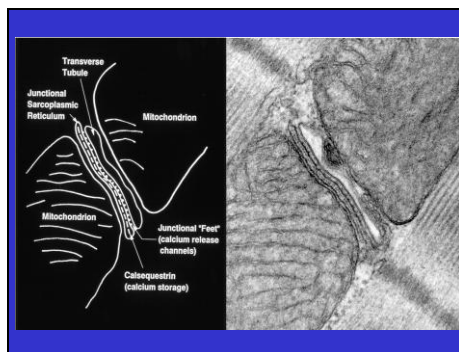
This image picks up the theme of the transverse tubules, so we can now follow them as they penetrate into the cell interior

The transverse tubules (T) penetrate adjacent to the Z-bands (Z) of the superficial myofibrils

An electron dense tracer has been used to mark the extracellular space – this allows us to see how deeply the transverse tubules penetrate into the cell, as they meander in and out of the section plane

Note that transverse tubules are not always transverse – within the cell they may turn and take a longitudinal course

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Transverse tubule and junctional sarcoplasmic reticulum: thin-section image

Making contact with the transverse tubule membranes (and also with portions of the plasma membrane) are elements of junctional sarcoplasmic reticulum

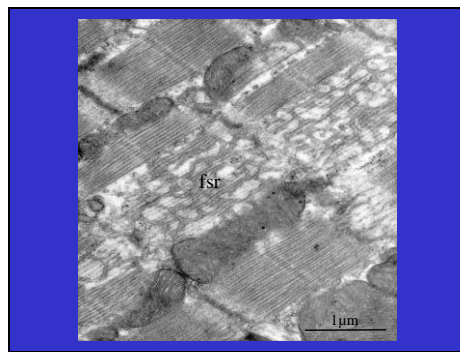
Electron-dense “feet” project from the junctional sarcoplasmic reticulum – these are the calcium release channels
Electron-dense material within the junctional sarcoplasmic reticulum cisternum represents calsequestrin, a protein that binds large quantities of calcium

Depolarisation of the transverse tubule membrane triggers release of the stored calcium in the junctional sarcoplasmic reticulum, through the calcium release channels, into the cytoplasm – stimulates contraction

Check that you can identify the structures in the thin-section electron

micrograph (right) using the diagram (left)

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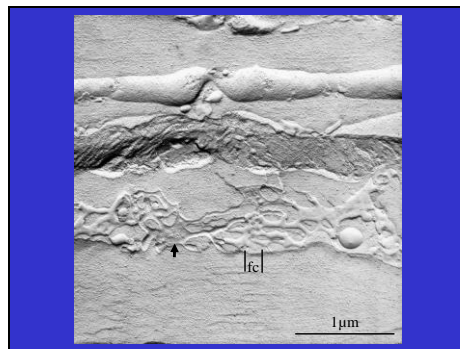


Sarcoplasmic reticulum: thin-section image

The junctional sarcoplasmic reticulum does not exist in isolation – it is intercalated within an extensive sarcoplasmic reticulum network
The non-junctional sarcoplasmic reticulum is termed the “free sarcoplasmic reticulum”

The free sarcoplasmic reticulum (fsr) forms a collar-like network around each myofibril

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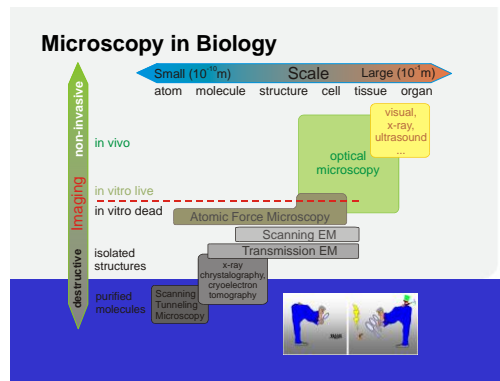


Sarcoplasmic reticulum: freeze-fracture image

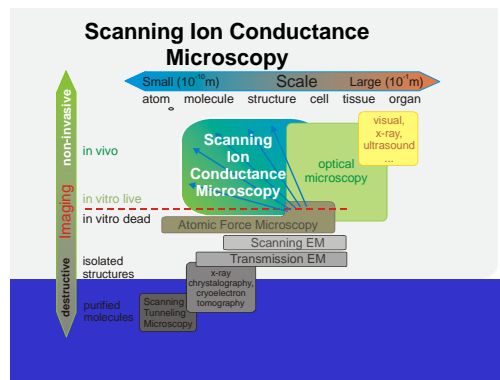
Freeze-fracture reveals that the free sarcoplasmic reticulum is structurally differentiated along the length of the myofibre
Both tubular and cisternal elements are present

In line with the centre of the myofibre, there is a fenestrated cisternum (fc)
This is connected to predominantly longitudinally oriented tubules on either side, which then join to more extensively interconnected tubules and cisternae in the Z-band region
The intramembrane particles seen here on the P-face of the sarcoplasmic reticulum membrane (arrow) represent the calcium ATPase enzyme, which pumps calcium back into the lumen of the sarcoplasmic reticulum after contraction

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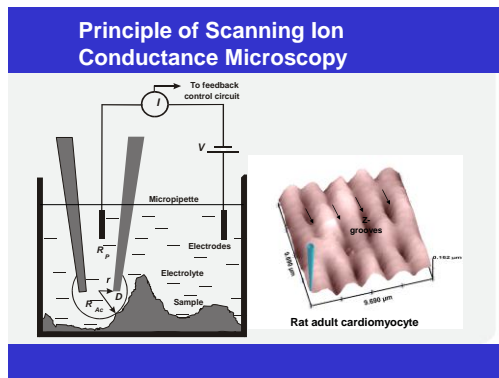


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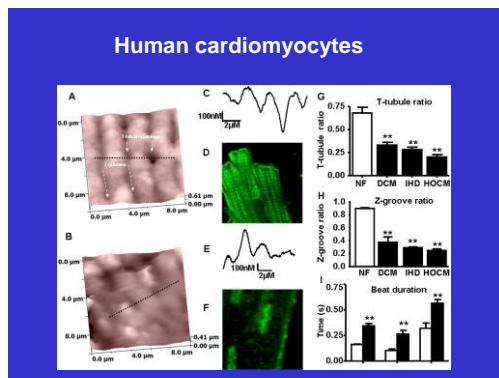
. SICM is a scanning technique therefore it uses a probe that scans over the surface of an object. SICM uses glass micropipette as probe. This is a sharp pipette, its diameter can vary between 20 and 200 nm. The resolution of a SICM image depends on the diameter of the pipette.

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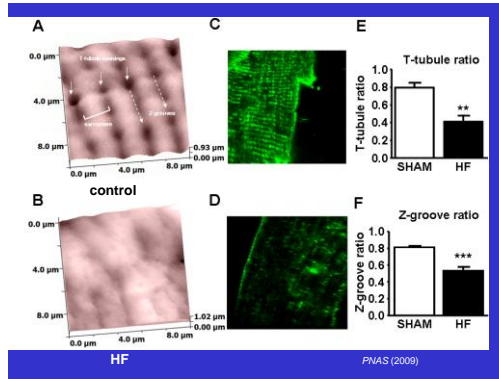
The pipette is filled with electrolyte and attached to a piezo drive, which can move in three dimensions. While scanning the sample in X-Y plane, the pipette moves over the sample surface. SICM feedback control mechanism keeps the pipette at a fixed distance from the surface and in doing so we can collect the Z-displacement of the pipette, and produce an image of the surface.

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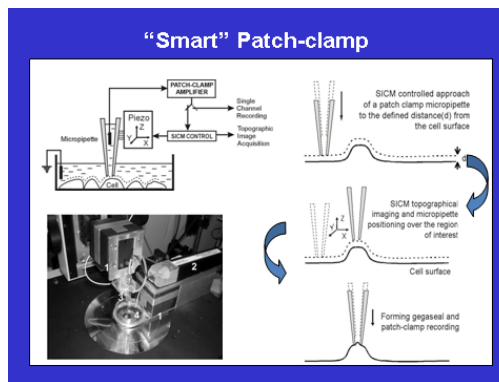
We studied isolated ventricular myocytes from patients with different pathologies. Fluorescent dye that stains T-tubules was used to reveal the structure of these tubules changes with pathology and at the same time SICM scanned the surface to visualise the changes on the cell surface with flattening and loss of Z-groove definition common to all etiologies.

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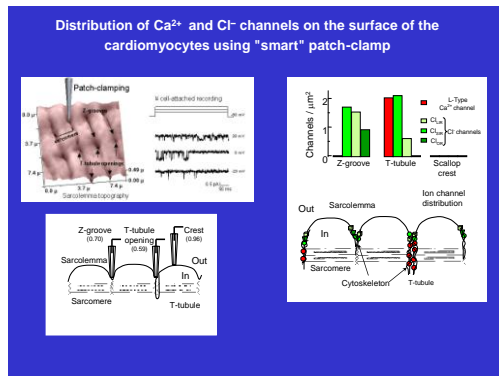
We compared ventricular myocytes from a rat model of chronic heart failure following myocardial infarction. Rat myocytes from the chronic heart failure showed both T-tubule and Z-groove loss.

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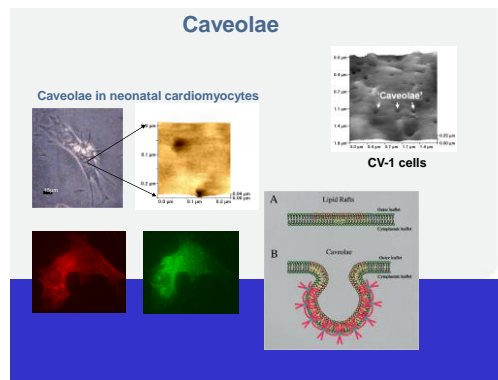
“Smart” patch-clamp technique combines scanning ion conductance microscopy and patch-clamp recording through a single glass nanopipette probe. In this method the nanopipette is first scanned over a cell surface, using current feedback, to obtain a high-resolution topographic image. This same pipette is then used to make the patch-clamp recording. Because image information is obtained via the patch electrode it can be used to position the pipette onto a cell with nanometer precision.

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In cardiac myocytes, as in most excitable cells, action potential propagation depends essentially on the properties of ion channels that are functionally and spatially coupled. We found that the L-type calcium and chloride channels are distributed and colocalized in the region of T-tubule openings, but not in other regions of the myocyte. In addition, chloride channels were found in narrowly defined regions of Z-grooves.

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EXCITATION-CONTRACTION COUPLING IN CARDIAC MUSCLE

- 1) Influx of Ca^{2+} through plasma membrane L-type calcium channels (DHP receptors)
- 2) Ca^{2+} -induced Ca^{2+} release through calcium release channels (ryanodine receptors) of the junctional sarcoplasmic reticulum

