為何ECM (GAG)可以保水????????????

GAG (--------)→attaract cations (+)→
more concentrate → osmotic pressure
↑↑↑→ water income → keep in ECM
The extracellular matrix (ECM)

Three types of molecules are abundant in the extracellular matrix of all tissues:

1. proteoglycan: a glycoprotein, high viscosity, it can bind variety of ECMs
2. Collagen fibers: provide mechanical strength and resilience.
3. Soluble multiadhesive matrix proteins: bind to and cross-link cell-surface adhesion receptors and other ECM components
**Fibronectins** connect many cells to fibrous collagens and other matrix components

Fibronectin = example of “adhesive” ECM protein
Is a protein with multiple domains with number of specific binding sites for macromolecules and for receptors on cell surface.

Plasma Fibronectin; soluble, circulates in the blood and other body fluids → enhances blood clotting, wound healing and phagocytosis
Fibronectin Fibrils; insoluble → assemble on surface of cells & deposited in ECM
Fibrils assemble on surface of cells via fibronectin-binding integrins.
Usually aligned with adjacent intracellular actin fibres.

Fibronectin is a dimer, two chains linked via disulfide bonding at the carboxyl terminus.
Fibrin, heparan sulfate proteoglycan, and collagen:
- bind to distinct regions in fibronectin
- integrate fibronectin fibers into the extracellular matrix network

Some cells express integrin receptors that bind to the **Arg-Gly-Asp (RGD)** sequence of fibronectin.

At least 20 different forms of fibronectin have been identified.
- All of them arise from alternative splicing of a single fibronectin gene.

The soluble forms of fibronectin are found in tissue fluids.
The insoluble forms are organized into fibers in the extracellular matrix.
Fibronectin fibers consist of crosslinked polymers of fibronectin homodimers.
Fibronectin proteins contain six structural regions.
- Each has a series of repeating units.
RGD is the letter designation for the amino acids Arginine, Glycine and Aspartic Acid
FIBRONECTINS: attach cells to collagen matrices
Regulates cell shape/cytoskeleton
Dimers of two similar polypeptides linked by disulfide bonds
Forms fibrils when exposed to cells expressing integrins
Circulating fibronectin can bind to fibrin, with the result that platelets associate at the site of blood clots via integrin binding

◆ 纖連蛋白是高分子量糖蛋白（220-250KD）
◆ 纖連蛋白模型
◆ 纖連蛋白的主要功能：
  ⊲ 介導細胞黏著，進而調節細胞的形狀和細胞骨 架的組織，促進細胞鋪展；
  ⊲ 在胚胎發生過程中，纖連蛋白對於許多類型細胞的遷移和分化是必需的；
  ⊲ 在創傷修復中，纖連蛋白促進巨噬細胞和其它免疫細胞遷移到受損部位；
  ⊲ 在血凝塊形成中，纖連蛋白促進血小板附著于血管受損部位。
Specific tripeptide sequence RGD is important role of the cell-bind region of fibronectin is require adhesion of cells

• Arg-Gly-Asp
• Cell binding region
• Type III repeat/binds integrins
Integrins mediate linkage between fibronectin in the extracellular matrix and the cytoskeleton.

Green: integrin  
Red: actin

Stress fibers are long bundles of actin microfilaments that radiate inward from points where the cell contacts a substratum.
Adhesive interactions in motile and nonmotile cell

Integrins relay signals between cells and their 3D environment

Integrins interact with ECM: regulation of integrin-mediated adhesion and signaling controls cell movement

Integrins cluster into adhesive structures with various morphologies in nonepithelial cell

Human fibroblast
Cell grown directly on the flat surface of culture dish (a)
On 3D ECM components (b)
At least 12 distinct $\alpha$-subunits and 9 $\beta$-subunits identified; single subunit can associate with more than one partner

Difficult to crystallize and therefore 3D structures not readily available

Cloning experiments indicate that integrins have short cytoplasmic tail (~ 50 amino acids) and 4 $\text{Ca}^{2+}$ binding sites identified in the $\alpha$ subunit

Numerous disulfide bonds- difficult identify all of them
Muscular dystrophy: connections between the ECM and cytoskeleton are defective

About 1/3300 boys, heat or lung failure

Mutation of dystrophin (a cytosolic protein), bind to dystroglycan

Dystroglycan: $\alpha$-subunit (peripheral protein) plus $\beta$-subunit (transmembrane protein); the $\alpha$-subunit also has O-linked oligosaccharides to bind various basal lamina components

DGC (dystrophin glycoprotein complex) links extracellular matrix to the cytoskeleton and singling pathways enzyme for muscle’s function

Mutations in components of this pathway (e.g. muscular dystrophy) results in mechanical instability of muscle cells.
Duchenne muscular dystrophy (DMD)

裘馨氏肌肉萎縮症

X染色體
P21基因缺損(DMD)。
DMD基因會製造一種重要的肌肉萎縮蛋白
(Dystrophin)，此症患者的細胞內完全缺少肌肉
萎縮蛋白，肌纖維膜變得無力脆弱，經年累月伸
展後終於撕裂，肌細胞易死亡。
患者的肌肉隨著年齡的增長會逐漸退化消失。

Affects cytosolic adapter protein-Dystrophin
Interacts with dystroglycan (glycoprotein)

Affects structure (DGC) involved in linking muscle cell to basal lamina
This structure provides strength to cell membrane
        disruption leads to weakening of mechanical stability of cell
Diversity of ligand-integrin interaction contributes to number of biological processes.
Cell-matrix adhesion is modulated by changes in the binding activity and number of integrins.

Model for integrin activation

X-ray crystal structure (inactive form)

Ligand bind and induced conformational change
Integrin superfamily 的黏著分子主要參與細胞與細胞外基質的黏附，使細胞得以附著而形成 integration。此外，這些分子還參與白血球與血管內皮細胞的黏附。這些分子都是由 α、β 兩條鏈以非共價鍵連接組成的 heterodimer。

α 鏈的分子量為 120～180kD, β 鏈的分子量為 90～110kD, 不同的 α 鏈或 β 鏈胺基酸組成和序列有不同程度的同源性，在架構上有其共同的特點。

α 鏈和 β 鏈均由胞漿區、穿膜區、胞膜外區三部分組成。胞漿區一般較短，可能與細胞骨架相聯。穿膜區富含疏水胺基酸。

α subunits 和 β subunits 組合構成並不是隨機的，多數 α subunits 只能與一種 β subunits 結合成 heterodimer，而大部分 β subunits 則可以結合數種不同 α subunits。目前依 β subunits 的不同將與動脈粥狀硬化相關的 integrin superfamily 分為 3 個不同的組。

Integrin 在與 ligand 結合時所識別的只是 ligand 分子中由數個胺基酸組成的序列，例如 Arg-Gly-Asp (RGD) 序列。不同的 integrin 可以識別相同的序列或同一個 ligand 中不同的序列。

Integrins 在體內分佈很廣泛，多數 integrins 可以表現於多種組織、細胞，如 VLA 組的 integrins 在體內廣泛分佈於各種組織、細胞，而多數細胞可同時表達數種不同的 integrins。
Laminin and fibronectin provide an adhesive substrate for cells.
The Binding of Cytoskeleton to the Extracellular Matrix Through the Integrin Molecule
Many cell-matrix and some cell-cell interactions are mediated by integrins.
Immunoglobulin superfamily

Immunoglobulin Superfamily CAMs (ICAMs)
Ca²⁺ independent cell-cell adhesion in neuronal and other tissue is mediated by CAMs (IgCAM) in the immunoglobulin superfamily.

Movement of leukocytes into tissue depends on a precise sequence of combinatorially diverse set of adhesive interactions.

Movement across an endothelial cell layer of:

• Monocytes (macrophage precursors - cells that ingest foreign particles)
• Neutrophils (release antibacterial)
• T and B Lymphocytes (antigen-specific)

Movement is mediated by selectins, a class of CAMs specific for leukocyte/vascular cell interactions

• Activate integrin binds ICAM-1 and ICAM-2
• Cells move from the blood into infected or inflamed tissue
Immunoglobulin superfamily的家族成員眾多，且均與immunoglobulin有一定的同源性，其分子架構中含有多個90-100個胺基酸的Ig-like domains。廣泛分佈於淋巴細胞、單核細胞、內皮細胞等多種細胞的表面。

重要的成員有intercellular adhesion molecule-1、2、3（ICAM-1、2、3）、vascular cell adhesion molecule-1（VCAM-1）和platelet-endothelial cell adhesion molecule（PECAM）。

該家族的主要功能是參與不同細胞間的識別與黏附，在與免疫、發炎有關的細胞黏附中發揮重要作用。

ICAM-1是最早發現的immunoglobulin superfamily黏附分子之一，以後又相繼發現了ICAM-2和ICAM-3，它們的胺基酸序列具有同源性，且都可以結合LFA-1分子（一種integrin）。不同的ICAM分子在體內的分佈範圍有較大差異，ICAM-1分子分佈廣泛，如白血球、內皮細胞、某些腫瘤細胞、上皮細胞、肝細胞、平滑肌細胞等，IL-1、TNF-α、和LPS可促進ICAM-1分子的表現；ICAM-2則分佈較局限，主要表現在血管內皮細胞；而ICAM-3表現在T細胞、單核細胞。
IgCAMs comprise a diverse group of adhesion receptors, that are defined by the presence of one or several Ig folds; classical examples are:

Neuronal CAM (NCAM) is implicated in neuronal guidance and establishment of new synapses
NCAM forms homotypic contacts
Intercellular CAM (ICAM) interacts heterotypically with integrins
CAMs differ widely in their cytoplasmic binding partners
**Selectins:** mediate transient cell-cell adhesion in the bloodstream

White blood cells (WBCs) utilize the adhesive properties of selectins (and integrins) in order to move: blood ↔ tissue.

- Selectins are “lectins” – carbohydrate binding proteins. (Ca²⁺ dependent)
- TM protein with a highly conserved lectin domain that binds to a specific oligosaccharide.
- Transient, calcium-dependent interactions.
- Selectin types:
  - L-selectin: WBCs
  - P-selectin: platelets and endothelial cells
  - E-selectin: activated endothelial cells
lectins” – carbohydrate binding proteins. (Ca2+ dependent
目前已發現selectin家族中有三個成員：L-selectin、P-selectin和E-selectin，L、P和E分別表leukocyte, platelet和endothelium, 是最初發現相應selectin分子的三種細胞，故得名。

P-selectin，分子量140KD，由單核巨細胞跟內皮細胞形成，貯存於血小板的α顆粒及內皮細胞的Weibel-Palade小體。當受到thrombin跟histamine刺激時，P-selectin會迅速到達血小板或內皮細胞表面，介導白血球與內皮細胞的起始黏附。

E-selectin，分子量115KD，正常的內皮細胞表面並無E-selectin存在，細胞內也沒有儲存。當受到IL-1及TNF-α等細胞因子的刺激時，會活化內皮細胞，刺激E-selectin的合成。E-selectin會幫助白血球與內皮細胞的黏附作用。

L-selectin廣泛存在於各種白血球的表面，參與發炎部位白血球的出血管過程。白血球表面L-selectin分子上的sLeA與活化的內皮細胞表面的P-selectin及E-selectin之間的識別與結合，可召集血液中快速流動的白血球在發炎部位的血管內皮上減速滾動（即透過黏附、分離、再黏附……，如此循環往複），最後穿過血管進入發炎部位。
MACROMOLECULAR ORGANIZATION OF ECM
Movement of leukocytes into tissue depends on a precise sequence of combinatorially diverse set of adhesive interactions.

Bacterial, infection or inflammation $\rightarrow$ tissue dysfunction $\rightarrow$ blood (leukocyte) $\rightarrow$ expressed special adhesion molecules at endothelial surface $\rightarrow$ bind leukocyte $\rightarrow$ induced adhesion molecule activation $\rightarrow$ extravasation $\rightarrow$ extracellular ..................
P-selectin, a lectin (protein that binds carbohydrates) on activated endothelial cells, binds a specific ligand (an oligosaccharide sequence) on T cells. PAF (Platelet Activating Factor - a phospholipid) activates integrin on the leukocyte surface.
CAM directly bind to leukocyte

1. Endothelium activation → selectin or carbohydrate ligand → weak, reversible binding
2. Infection or inflammation signal → chemokines or PAF → expressed special molecules → attached leukocyte
3. Additional activation dependent CAM, integrins → strong adhesion
Multi-step Model of Leukocyte Adhesion and Extravasation

C. Chemokine-activated leukocyte
   - Integrin (high-affinity state)

D. Motile leukocyte

E. Leukocyte migration in tissue
   - Chemokines
   - Macrophage
   - Fibrin and fibronectin (provisional matrix)

Extravasation

Spreading and firm adhesion via integrins
Leukocyte-adhesion deficiency
淋巴球黏力缺失症

Genetic disorder
Leukocyte did not extravasation
Plant tissue

Unlike animals, plants **do not** replace or repair old or damaged cells or tissues; only grow new organs.

Plant only four broad types of cells (form four basic classes of tissue):

1. *dermal tissue*: interact with environment
2. *vasculat tissue*: transport water and dissolved substances
3. *ground tissue*: space filling
4. *sporogenous tissue*: forms the reproductive organs

Contain polysaccharides: cellulose (tensile 张力 拉力 strength), hemicellulose

Allow soluble factor to pass to cell membrane, but less permeable than animal cell matrix.
Functions of cell walls:
For plants:
Mechanical strength--> plant heights
Glue cells together--> dictate the way in which plant develop
Exoskeleton--> control cell shapes
Control balance between cell turgor pressure and cell volume
Diffusion barrier for macro-molecules and pathogens
Food reserves: endosperm cell walls degrade during seed during germination
Signaling
The plant cell wall is a laminate (薄板) of cellulose fibrils in a matrix of glycoprotein.

Primary wall consists of the following basic features:
- Cellulose (strength)
- Hemicellulose
- Pectins (flexibility)
- Structural proteins (rigidity)
- Non-structural proteins

**CELLULOSE AND HEMICELLULOSE ARE PRESENT IN A MATRIX OF PECTIN POLYMERS**
Cellulose micorfibrils: Liner chains of (1->4)-linked beta-D-glucose. Each microfibril may consist of 6 to 30-50 chains. Each chain has 2000 to 25,000 glucose residues. Cellulose has a high tensile strength, equivalent to steel. Insoluble, chemically stable. Excellent “bones” for building a strong cell wall.
Cellulose molecules can be arranged together to form fibrils that have great tensile strength. These fibrils are the main structural element in the cell walls of plants.

a “glucan”: a polymer made up of glucose
…”xylan”.................................xylose
Xyloglucan: a glucan backbone (a linear chain of glucose) with xlylose attached as side chains
Other sugars are hydrogen bonded to cellulose.

- Hemicellulose (red backbone) is similar to cellulose but has branched sugar residues.
- Branches (blue) link microfibrils to pectins (purple).
- Interlinked network binds cells together.
<table>
<thead>
<tr>
<th>Cellulose</th>
<th>Microfibrils of $(1\rightarrow4)\beta$-d-glucan</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Matrix polysaccharides</strong></td>
<td></td>
</tr>
<tr>
<td>Pectins</td>
<td>Homogalacturonan</td>
</tr>
<tr>
<td></td>
<td>Rhamnogalacturonan</td>
</tr>
<tr>
<td></td>
<td>Arabinan</td>
</tr>
<tr>
<td></td>
<td>Galactan</td>
</tr>
<tr>
<td>Hemicelluloses</td>
<td>Xyloglucan</td>
</tr>
<tr>
<td></td>
<td>Xylan</td>
</tr>
<tr>
<td></td>
<td>Glucomannan</td>
</tr>
<tr>
<td></td>
<td>Arabinoxylan</td>
</tr>
<tr>
<td></td>
<td>Callose $(1\rightarrow3)\beta$-d-glucan</td>
</tr>
<tr>
<td></td>
<td>$(1\rightarrow3,1\rightarrow4)\beta$-d-glucan  [grasses only]</td>
</tr>
</tbody>
</table>

[Diagram of xyloglucan structure]
Walls also contain some **proteins**. They may serve structure purposes or function in signaling.

<table>
<thead>
<tr>
<th>Class of cell wall proteins</th>
<th>Percentage carbohydrate</th>
<th>Localization typically in:</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRGP (hydroxyproline-rich glycoprotein)</td>
<td>~55</td>
<td>Phloem, cambium, sclereids</td>
</tr>
<tr>
<td>PRP (proline-rich protein)</td>
<td>~0–20</td>
<td>Xylem, fibers, cortex</td>
</tr>
<tr>
<td>GRP (glycine-rich protein)</td>
<td>0</td>
<td>Xylem</td>
</tr>
</tbody>
</table>

A repeated hydroxyproline-rich motif from a molecule of HRGP from tomato
Loosening of the cell wall permits elongation of plant

Auxin $\rightarrow$ induced weakening of cell wall $\rightarrow$ water into cell $\rightarrow$ expansion of intracellular vacuole $\rightarrow$ elongation of cell
Cell Wall Structure

The middle lamella, primarily pectin, ‘glues’ neighbouring cells together.

**Primary cell wall:**
The first wall laid down during growth.
This is soft and flexible so that the cell can expand during growth.
It contains a mixture of biopolymers, with typically ~20-30% cellulose.

Once growth has stopped, the secondary cell wall is laid down, which provides structural support for the cells.

**Secondary Cell Wall Structure:**
Some cells have very thick secondary cell walls, to provide maximum support.
In the case of flax it can be μm in thickness (compared with ~100nm for the primary wall), and this was why flax was used for this study.

Typical cellulose content is ~50%, though can reach ~100% for e.g. cotton.
Structure is complex, and thought to consist of aligned layers of cellulose microfibrils in a general biopolymer matrix, with systematic misorientations between the layers.
THE SECONDARY CELL WALL CONTAINS A SERIES OF CELLULOSE LAYERS

- THE PRIMARY CELL WALL CONTAINS EXTENSIN, A GLYCOPROTEIN SIMILAR TO COLLAGEN, THAT IS INCORPORATED INTO THE INSOLUBLE POLYSACCHARIDE NETWORK.

- THE INNER SECONDARY WALL LAYER IS LAID DOWN AS CELLS MATURE
PLASMODESMATA INTERCONNECT CYTOPLASMS OF ADJACENT PLANT CELLS

60 nM diameter allows passage of molecules of up to 1000 MW
ER extensions (desmotubule) can pass through, allowing transit of membrane bound molecules
Elevation of cytosolic calcium inhibits transports (similar to gap junction)
<table>
<thead>
<tr>
<th>Kind of Organism</th>
<th>Extracellular Structure</th>
<th>Structural Fiber</th>
<th>Components of Hydrated Matrix</th>
<th>Adhesive Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals</td>
<td>Extracellular matrix</td>
<td>Collagens (ECM)</td>
<td>Proteoglycans and elastins</td>
<td>Fibronectins and laminins</td>
</tr>
<tr>
<td>Plants</td>
<td>Cell wall</td>
<td>Cellulose</td>
<td>Hemicelluloses</td>
<td>Pectins and extensins</td>
</tr>
</tbody>
</table>
The extracellular matrix and the cell wall are the "outside" of the cell

**Extracellular matrix (ECM):** consists of collagen fibers and proteoglycan. Collagen are a group of insoluble glycoproteins that contain large amount of glycine and the hydroxylated forms of lysine and proline. (examples, tendons, cartilage, and bone)

**Cell wall:** consists cellulose microfibrils embedded in a matrix of other polysaccharides and small amounts of proteins (extensins)

**Primary cell wall:** cellulose fibrils and gel like polysaccharides, thus flexible and extensible

**Secondary cell wall:** additional cell wall materials deposited on the inner surface of the primary cell wall, thus thicker and rigid. Second cell wall also contains high concentration of lignin, a major component of wood

**Communication between cells:**

**Plasmadesmata:** cytoplasmic bridges between plant cells

**Animal cells:** gap junctions, right junctions and adhesive junctions

**Prokaryotes:** cell walls consist of peptidoglycans with GlcNAc-MurNAc units