Oct. 29, 2021

Bio-integrated Materials Science (Online Lectures)

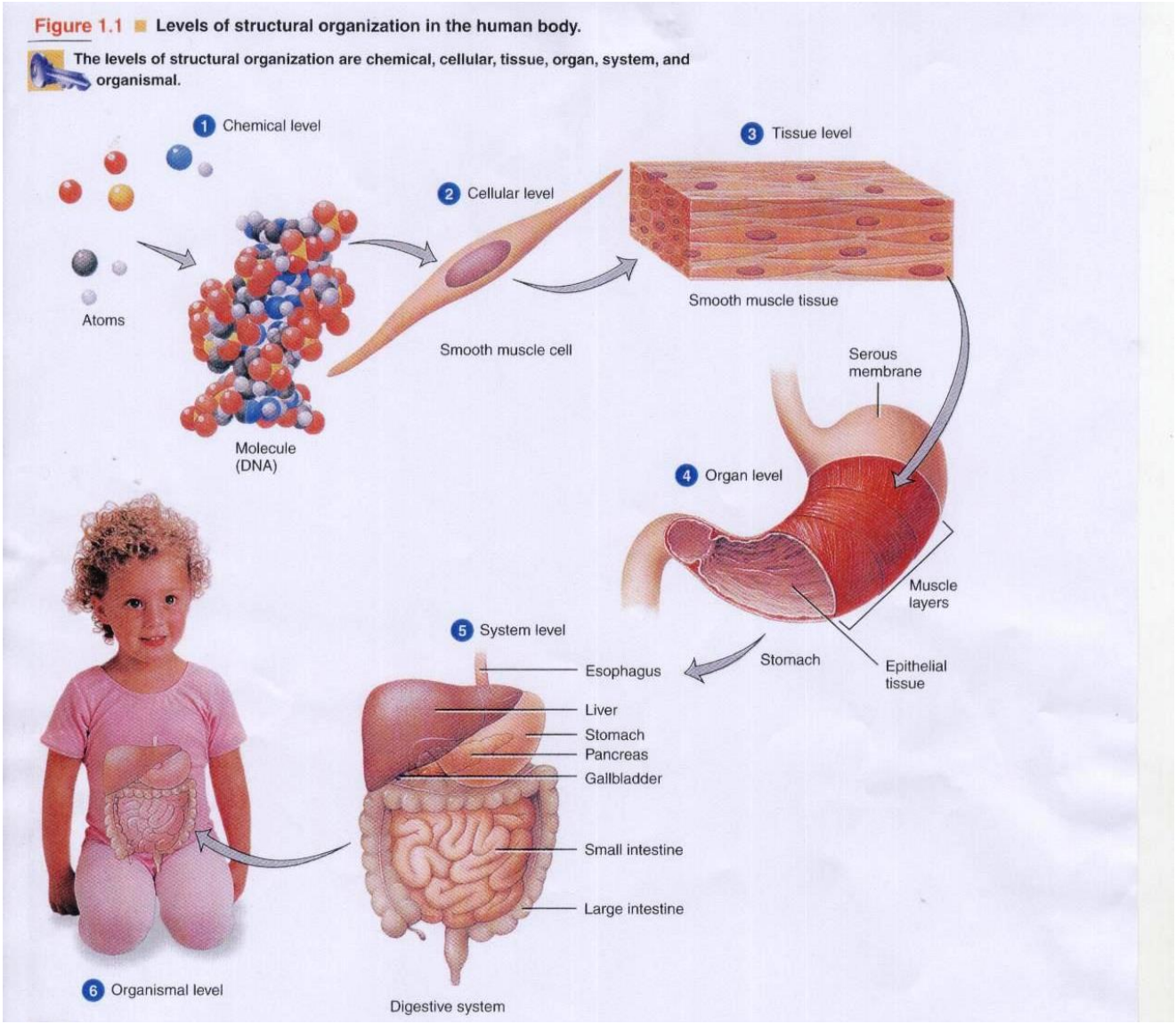
Materials for Tissue Engineering

Lecture 10

Prof. Jung Heon Lee

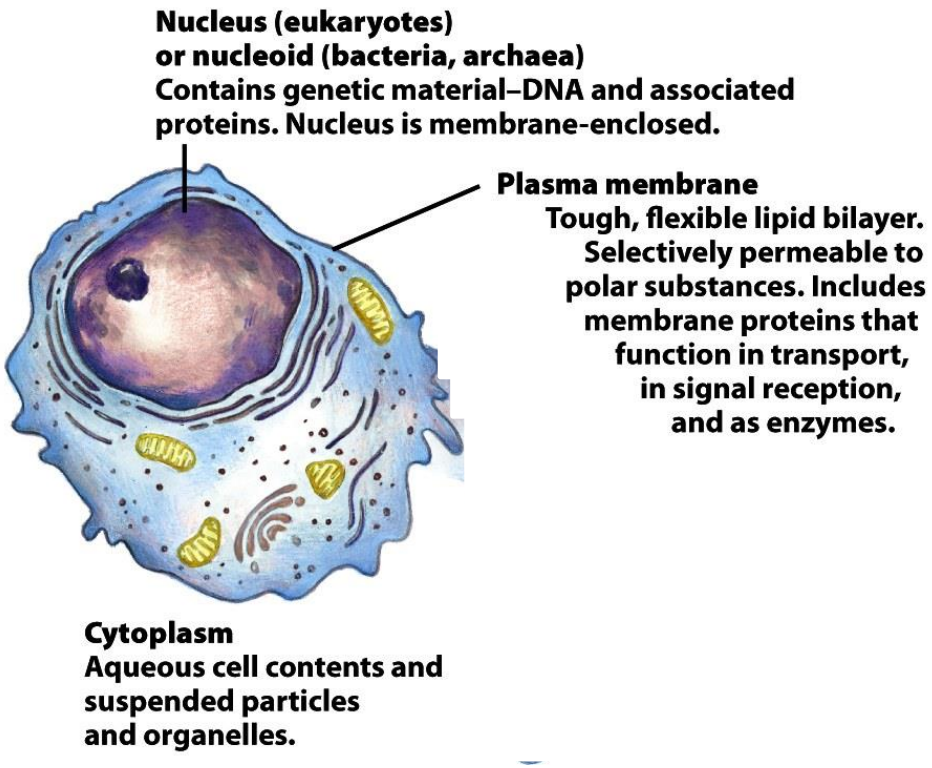
Cells

The structural and functional [ ] of all living organisms



Similar properties of all cells

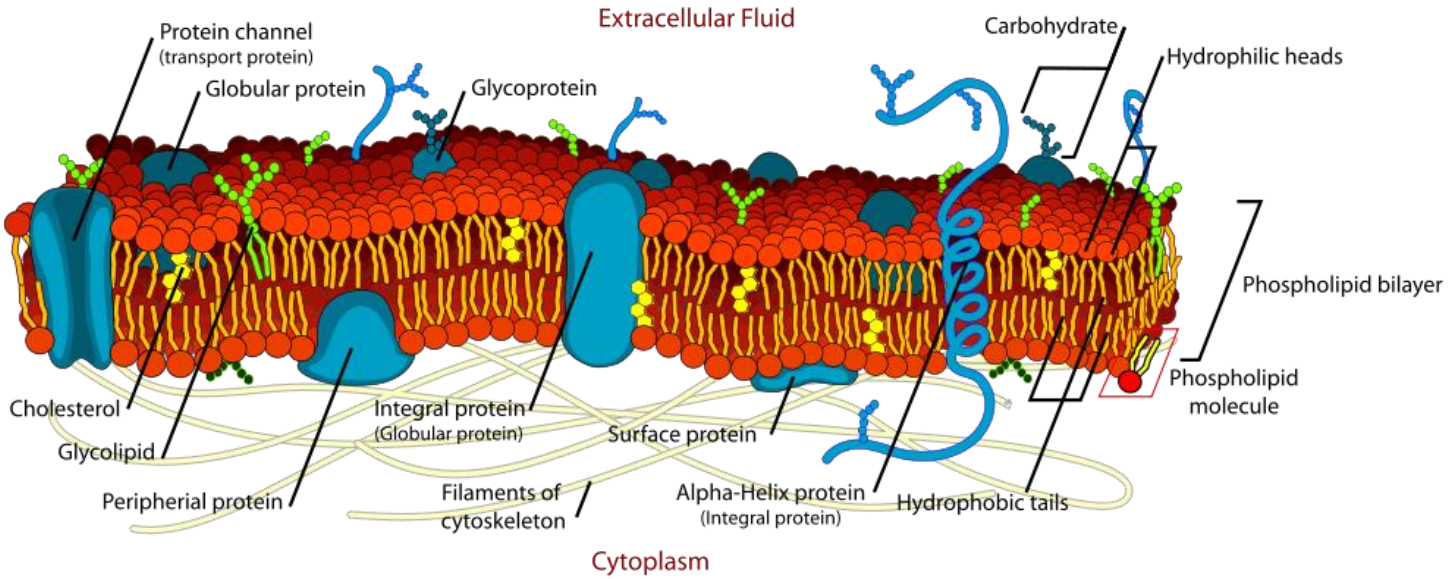
- All cells have a boundary that separates them from their environment, which is called the [ ] membrane (or cytoplasmic membrane)
- The area inside all cells is called the cytoplasm
- All cells contain deoxyribonucleic acid (DNA) as the genetic material
- All cells make proteins to help them function
- All cells have ribosomes that builds proteins



Plasma membrane

Main components of plasma membrane

- Phospholipids: Structural integrity and barrier function
- Proteins: Specialized membrane function
  - Receptor proteins: detect signals from the environment of the cell
  - Transport proteins: helps specific molecules get across the membrane
- Sterols (e.g. cholesterol)
- Carbohydrates



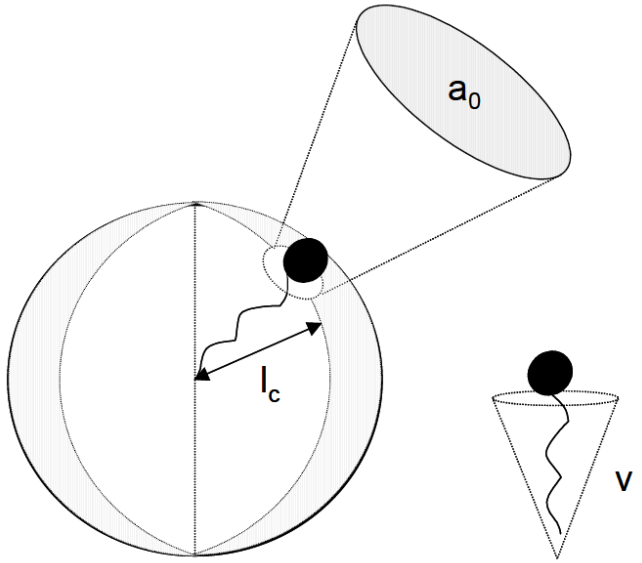
Critical packing parameter

Packing geometry is determined by

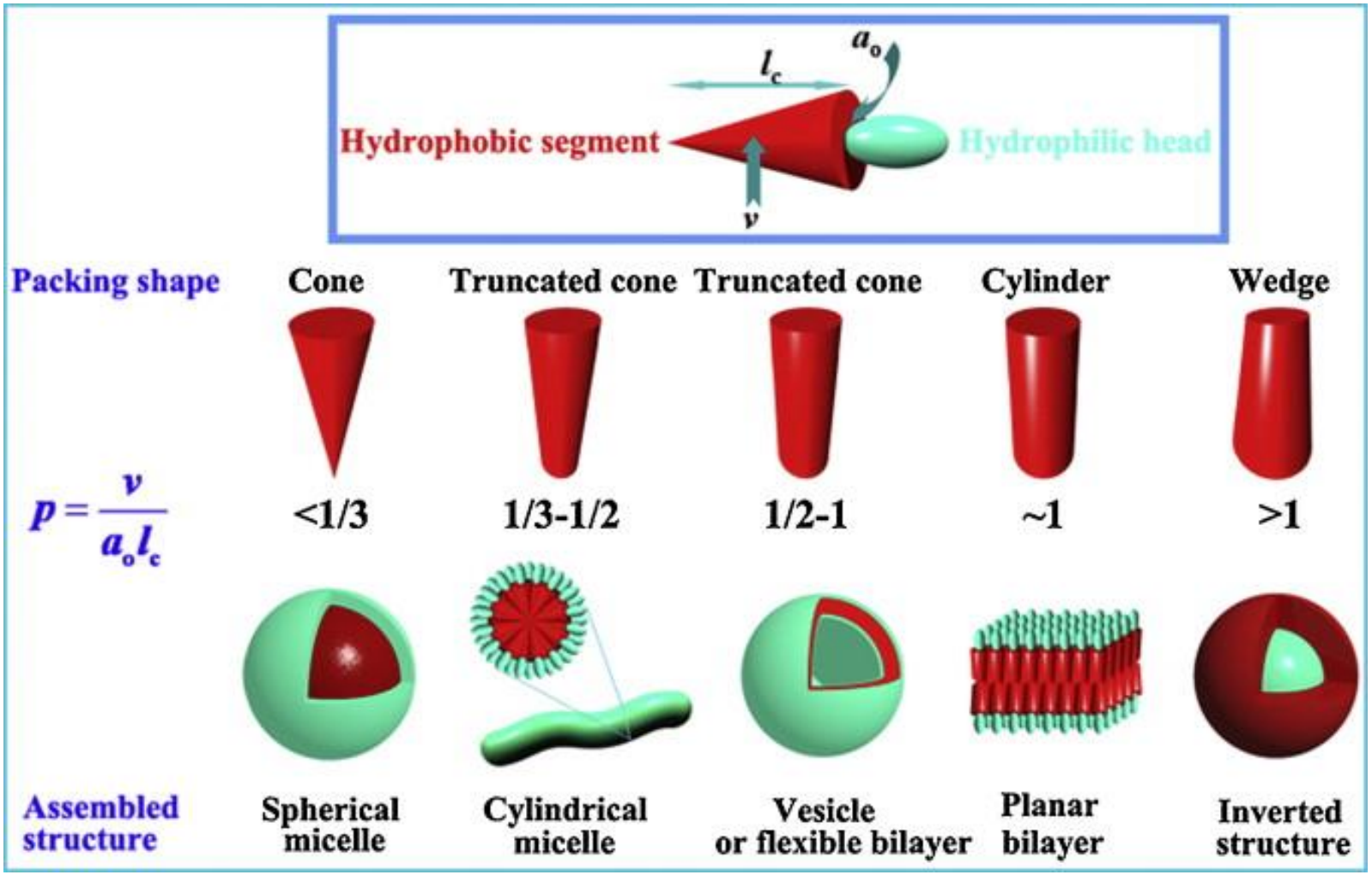
1) a_o: minimum interfacial area occupied by the head group
2) v: volume of hydrophobic tail(s)
3) l_c: the maximum extended chain length of the tail in the micelle core

$$P = \frac{v}{l_c \cdot a_o} \longrightarrow$$

Critical packing parameter: dimensionless number determining the geometry of self assembled aggregate



Packing geometry



Progress in Polymer Science, 37, 8, 2012, 1130–1176

Biological lipids

Why do biological lipids have 2 tails?

- Lipids with 2 chains are likely to form bilayers

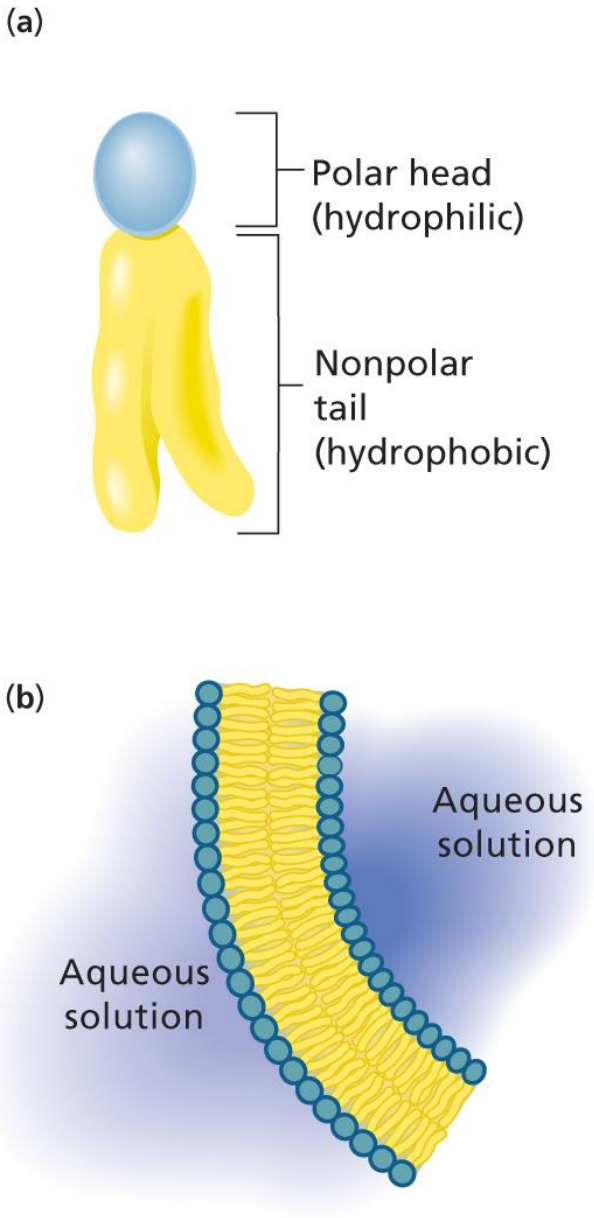
$$\mathcal{P} = \frac{v}{l_c \cdot a_o} \sim 1$$

- Increased hydrophobicity → [ ]
  - CMC of micelle lipids: $10^{-2} \sim 10^{-5}$ M
  - CMC of bilayer lipids: $10^{-6} \sim 10^{-10}$ M

- Increase of the lifetime (τ) of molecules within aggregate

  Molecules are in state of constant exchange between monomer state and aggregated state
  - $\tau_{\mathcal{R}}$(micelles) = 10^{-4} sec
  - $\tau_{\mathcal{R}}$(bilayers) = 10^{4} sec

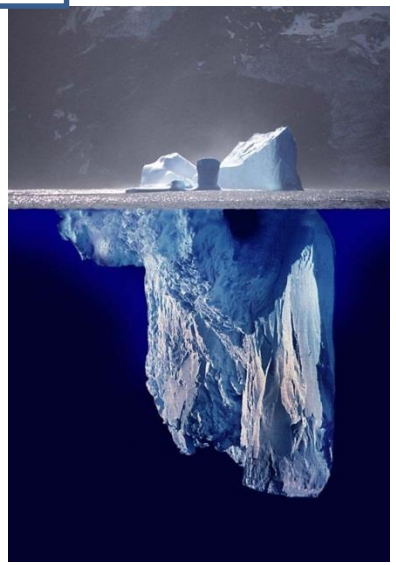
Membrane lipid and bilayer



- Lipid bilayers are the main structural component of all biological membranes.
- The noncovalent interactions of lipid molecules in bilayers make membranes [ ] and self-seal.
- Hydrophobic triacylglycerol and cholesterol cannot form bilayers.
- A lipid bilayer is typically about 5-6 nm thick and consists of two sheets of monolayers.
- The spontaneous formation of lipid bilayers is driven by hydrophobic interactions.

The fluid mosaic model of biological membranes

- A typical biological [ ] contains about 25-50% lipid and 50-75% protein by mass.
- Lipids are complex mixture of phospholipids, glycosphingolipids, and cholesterol.
- Cells will change saturated/unsaturated lipid ratio to maintain bilayer fluidity.
- Cholesterol helps to increase membrane [ ]
- A biological membrane is thicker than a lipid bilayer (6-10 nm)
- Fluid mosaic model: The membrane is a dynamic structure in which both proteins and lipids can rapidly and randomly diffuse laterally or rotate within the bilayer

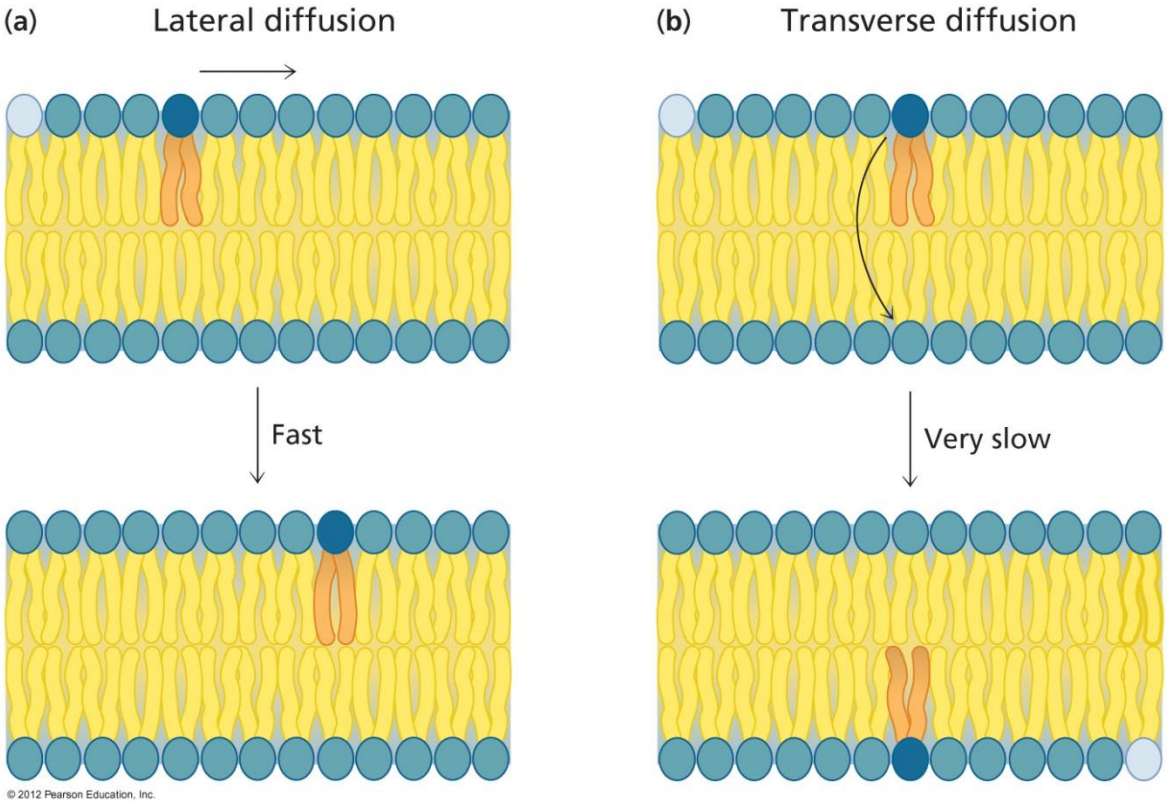


Iceberg: similar to membrane proteins in a highly fluid lipid bilayer sea

Dynamic membrane structures

A lipid bilayer: a two-dimensional solution

Changes in membrane fluidity affect the membrane transport and catalytic functions of membrane proteins



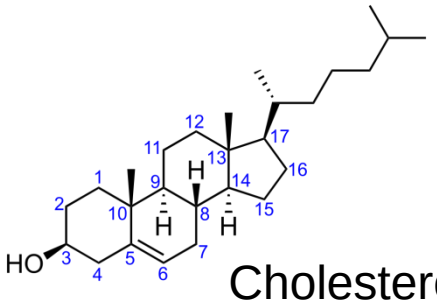
Move 2 μm in 1 sec at 37°C

1 billion (10^9) times slower than lateral diffusion: helps to keep inner & outer lipid compositions separately

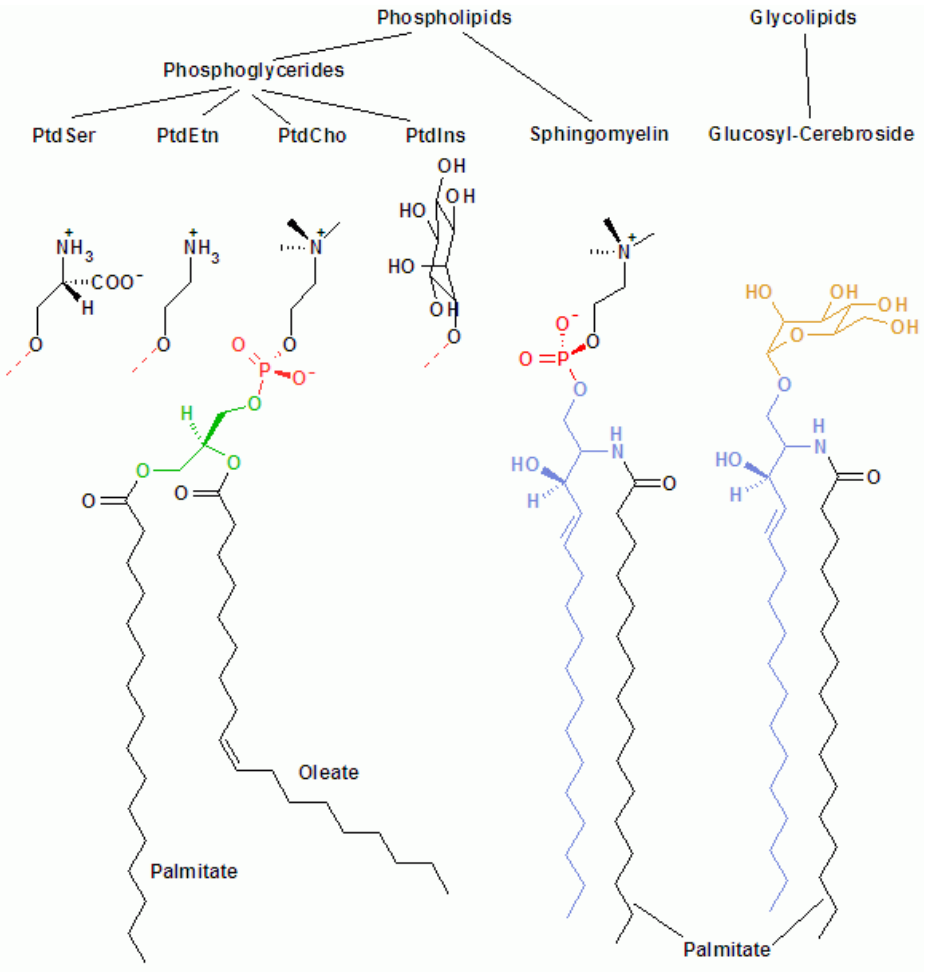
Role of plasma membrane

- Interface between extracellular environment and inner cellular domains
- Facilitate uptake of ions and metabolites
- Communication between (to) cells

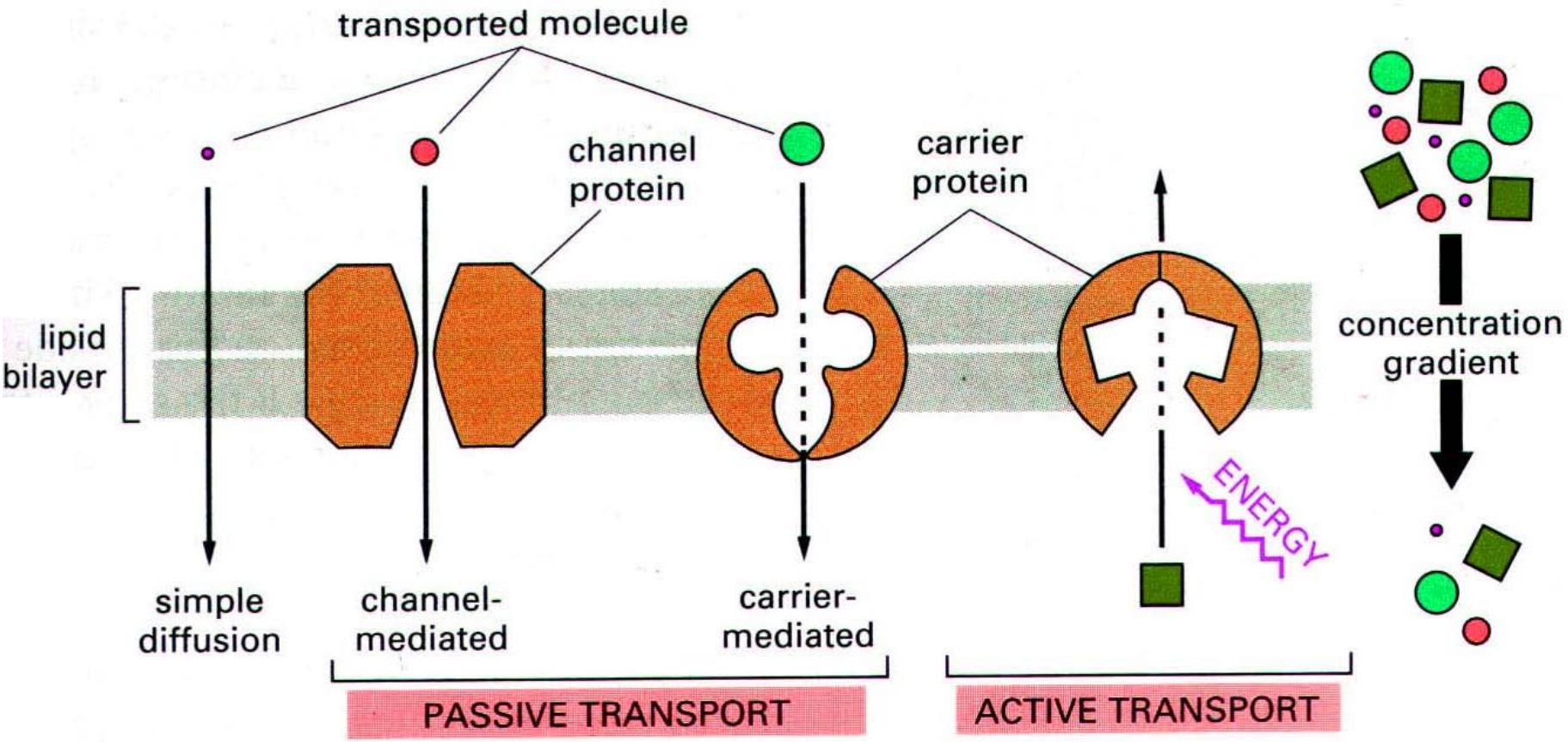
The relative amounts of various phospholipids, cholesterol, and glycolipids (lipids with a carbohydrate) will alter the physico-chemical properties of the membrane



Cholesterol



Molecule transportation through cell membrane



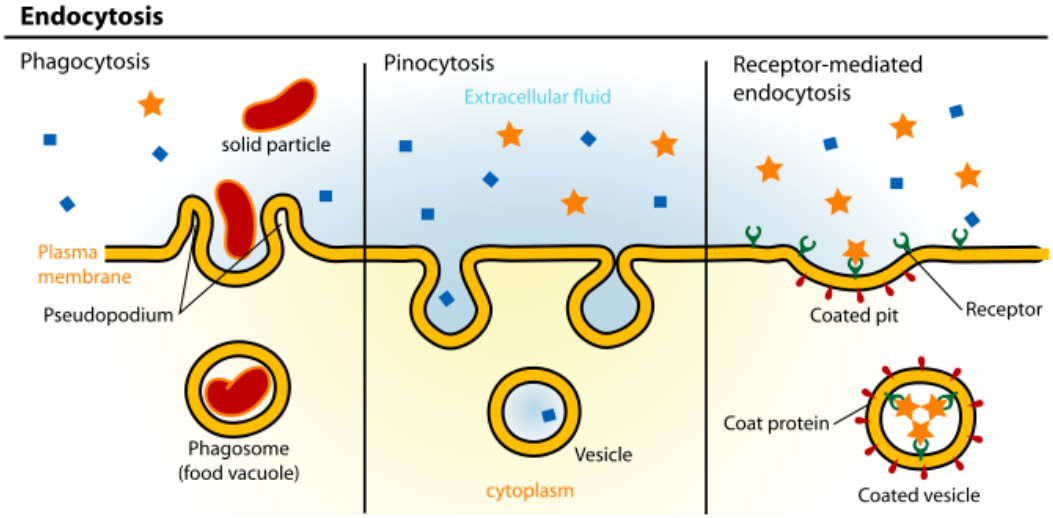
Transport across membranes

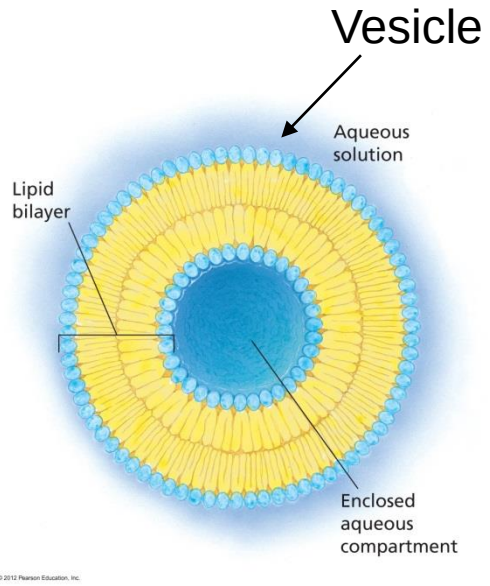
- Simple [ ] though lipid bilayers:
  - Small nonpolar molecules (O_2, CO_2)
  - Large hydrophobic molecules (steroid hormones)
  - Small polar molecules (water (18 Da), ethanol (46 Da), urea (60 Da))
  - However, ions and larger polar molecules (glucose (180 Da)) are excluded

- [ ] proteins: for transport of small MW (<1,000 Da) molecules (ions, glucose, nucleotide, amino acid)
  - Channel proteins: create hydrophilic pores → fast
  - Carrier proteins: bind specific ligand and undergo a series of conformational changes → relatively slow transport
    a. Passive transport: along the concentration gradient
    b. Active transport: against a concentration gradient, need energy

Endocytosis and exocytosis

- Transportation of large molecules, which are beyond the size of channels or transport proteins
- : introduction of macromolecules by plasma membrane inside a lipid vesicle (or cells take in materials).
  - Phagocytosis (cell eating): capture and destroy pathogens and particulate antigens
  - Pinocytosis (cell drinking): extracellular fluid and small molecules
  - mediated endocytosis: absorption of extracellular fluid and substances
- Exocytosis: a process opposite of endocytosis





https://www.youtube.com/watch?v=nXrU6AaPp88

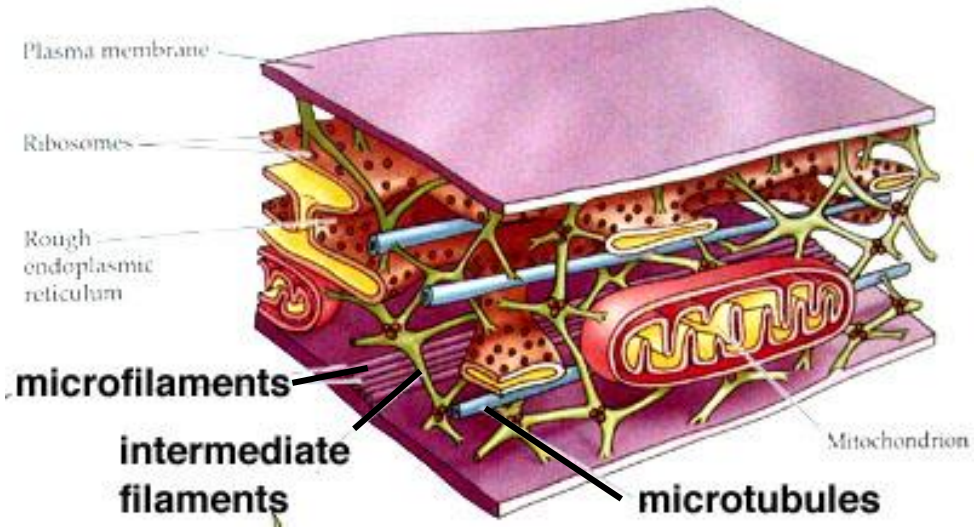
Cytoskeletons

Cytoskeleton (also CSK):

- The cytoskeleton is a network of [ ] extending throughout the cytoplasm.
- The cytoskeleton is responsible for holding the [ ] and structure of the cell and protecting it, and it also helps with movement and stability.

Three main components

- Microfilaments (actin): stained red
- Intermediate filaments
- Microtubules: stained green



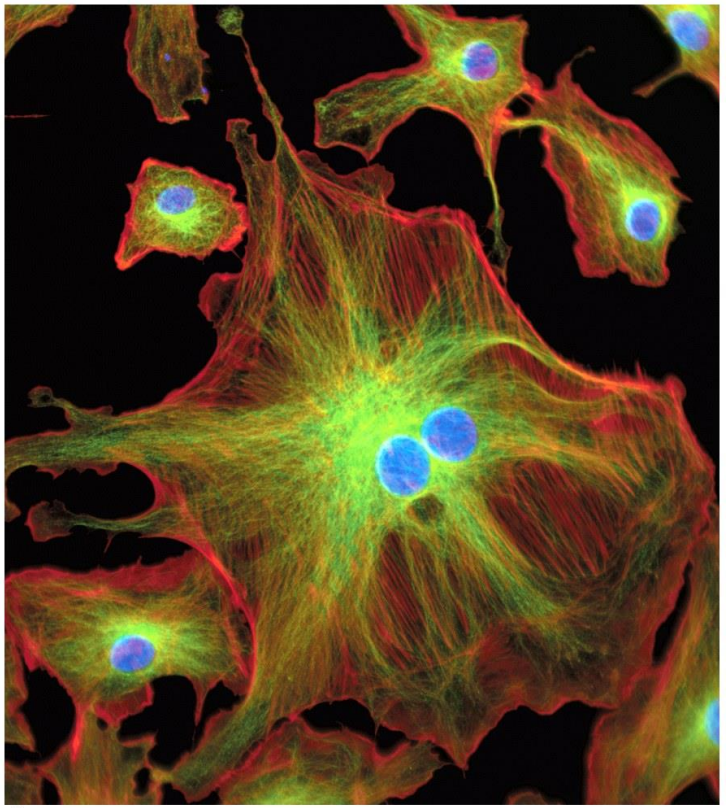


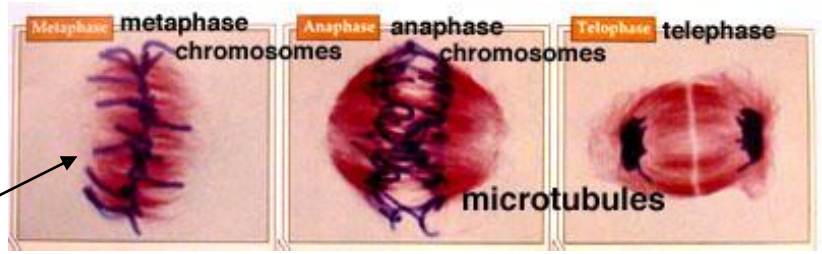
Figure 1-9a
Lehninger Principles of Biochemistry, Fifth Edition

Cytoskeletons

[ ] are made of the protein actin. Microfilaments are the proteins that make muscle cells contract, help pinch animal cells in two during cell division, allow cells like amoebae to crawl, and act as railroad tracks for organelles in some types of cells

Microtubules are made of the protein tubulin. Microtubules are the proteins inside of cilia (섬모) and flagella (편모). They move chromosomes during cell division and act as railroad tracks for the movement of vesicles and some organelles.

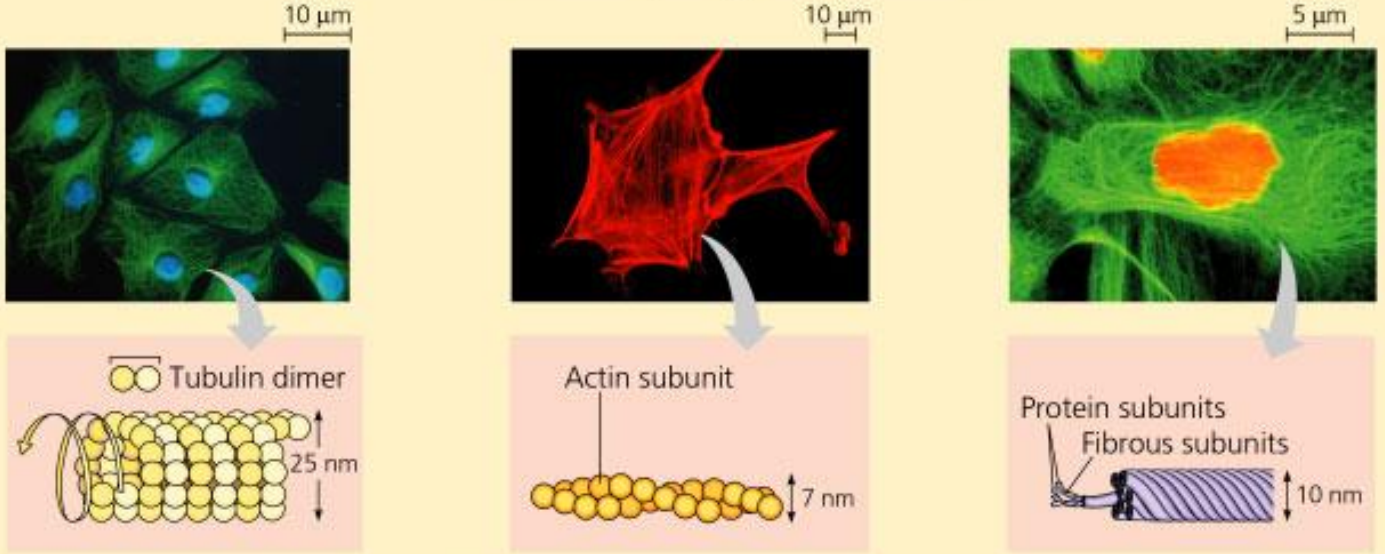
Chromosome: purple
microtubules: red



Intermediate filaments are made of various proteins. They often act as reinforcing proteins. For example, the protein lamin that strengthens nuclear membrane is an intermediate filament. Likewise, the keratin that strengthens your skin cells and make them resistant to damage is an intermediate filament

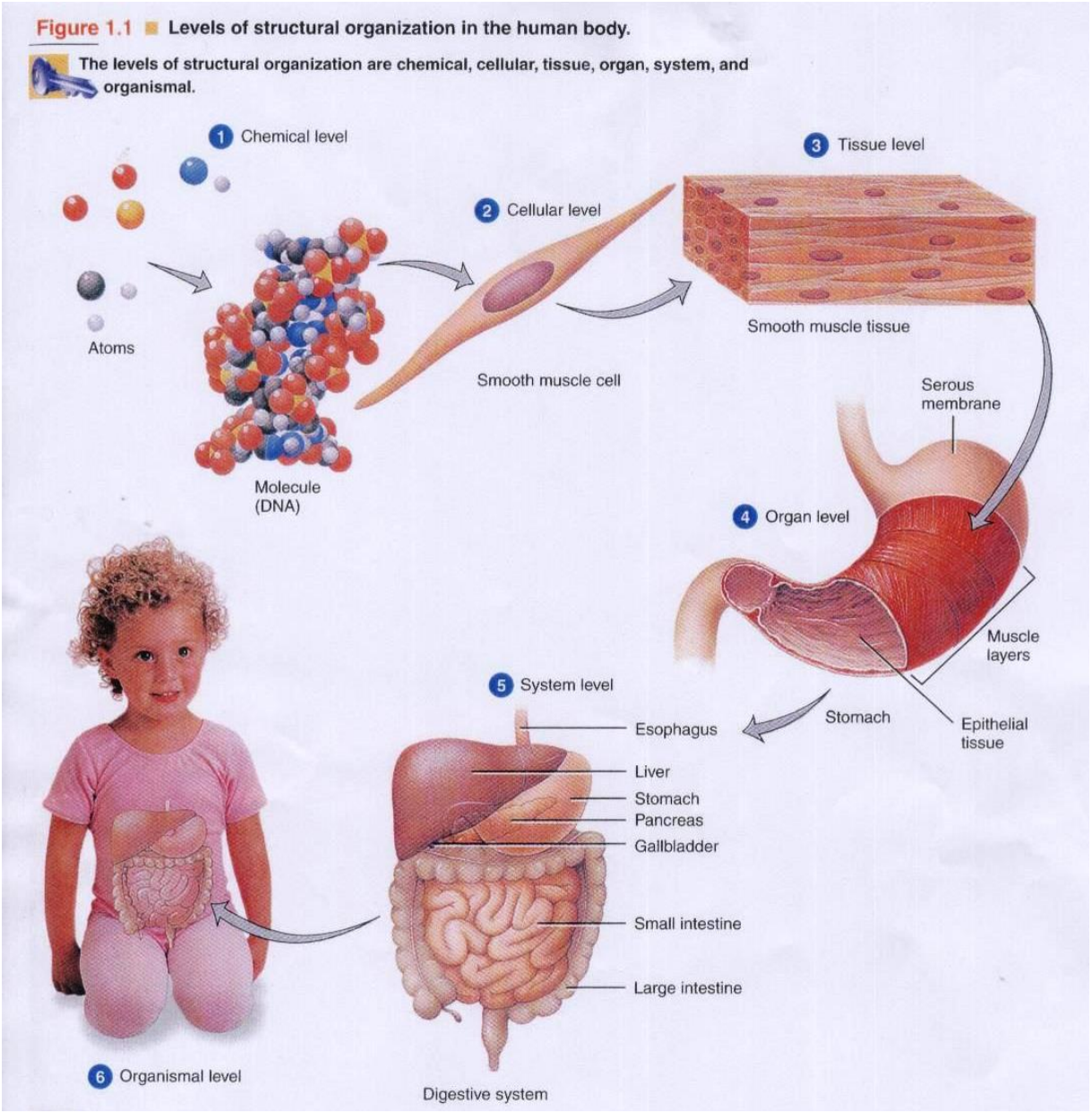
Table 7.2 The Structure and Function of the Cytoskeleton

Property	Microtubules	Microfilaments (Actin Filaments)	Intermediate Filaments
Structure	Hollow tubes; wall consists of 13 columns of tubulin molecules	Two intertwined strands of actin	Fibrous proteins supercoiled into thicker cables
Diameter	25 nm with 15-nm lumen	7 nm	8–12 nm
Protein subunits	Tubulin, consisting of α-tubulin and β-tubulin	Actin	One of several different proteins of the keratin family, depending on cell type
Main functions	Maintenance of cell shape (compression-resisting "girders") Cell motility (as in cilia or flagella) Chromosome movements in cell division Organelle movements	Maintenance of cell shape (tension-bearing elements) Changes in cell shape Muscle contraction Cytoplasmic streaming Cell motility (as in pseudopodia) Cell division (cleavage furrow formation)	Maintenance of cell shape (tension-bearing elements) Anchorage of nucleus and certain other organelles Formation of nuclear lamina



SOURCE: Adapted from W. M. Becker, L. J. Kleinsmith, and J. Hardin, *The World of the Cell*, 4th ed. (San Francisco, CA: Benjamin Cummings, 2000), p. 753.

Levels of organization



Atom

↓

Molecule or compound

↓

Organelle

↓

Cell

↓

Tissue

↓

Organ

↓

Organ system

↓

Organism

Tissue

Tissue: a group of [ ] cells specialized to carry on a particular function

In all tissues, cells are assembled into coherent groupings through specific cell-cell and cell-[ ] interactions

Each type of tissue has a distinctive and genetically determined pattern of structural organization adapted to its particular function

Each pattern is strongly influenced by both metabolic and/or mechanical factors

Extracellular matrix

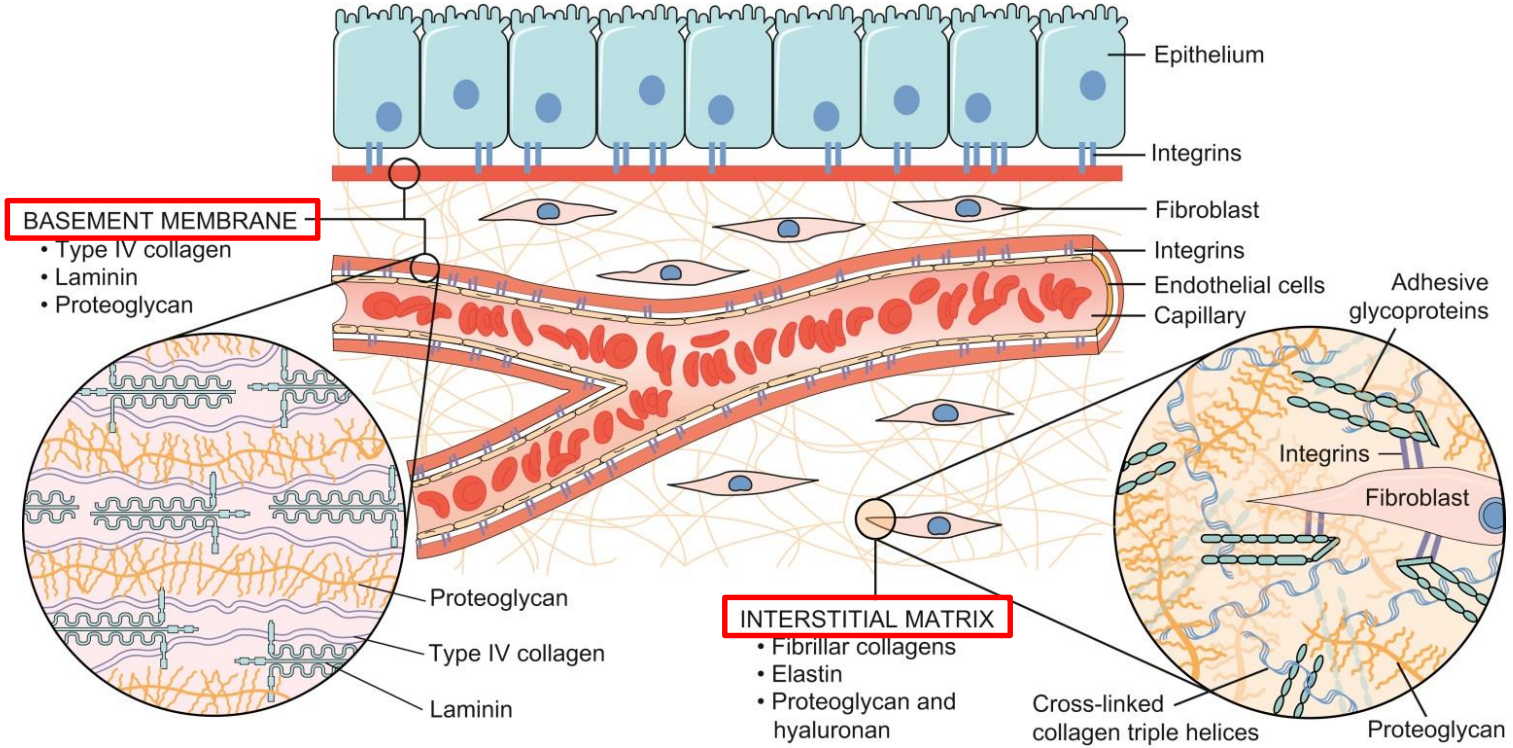
Tissue = Cells + Extracellular matrix (ECM)

Extracellular matrix (ECM): extracellular part of animal tissue that usually provides structural support to the animal cells in addition to performing various other important functions

Extracellular matrix: interstitial matrix + basement membrane

- [ ] matrix is present between various animal cells (i.e., in the intercellular spaces). Gels of polysaccharides and fibrous proteins fill the interstitial space and act as a compression buffer against the stress placed on the ECM.
- Basement membranes are sheet-like fibers on which various epithelial cells rest

Extracellular matrix



Molecules in ECM:

- [ ] (Collagen, Elastin, Fibronectin, Laminin)
- Glycosaminoglycan
- Proteoglycan

Proteins

- Collagen: provides [ ] to ECM
  - a group of naturally occurring long, fibrous structural proteins
  - most abundant protein in the human body
  - found in various forms in almost every tissue
  - different types of collagen have different chemical and physical properties
  - type 1 form strong fibers and type IV form meshlike network

Type	Description
Collagen I	skin, tendon, vascular ligature, organs, bone (main component of the organic part of bone)
Collagen II	cartilage (main component of cartilage)
Collagen III	reticulate (main component of reticular fibers) commonly found alongside type I
Collagen IV	forms basal lamina, the epithelium-secreted layer of the basement membrane
Collagen V	cell surfaces, hair and placenta

Proteins

- Elastin:
  - proteins in connective tissue that is [ ] and allows many tissues in the body to resume their shape after stretching or contracting
  - particularly abundant in certain tissues in which stretch and recovery are important in their functions

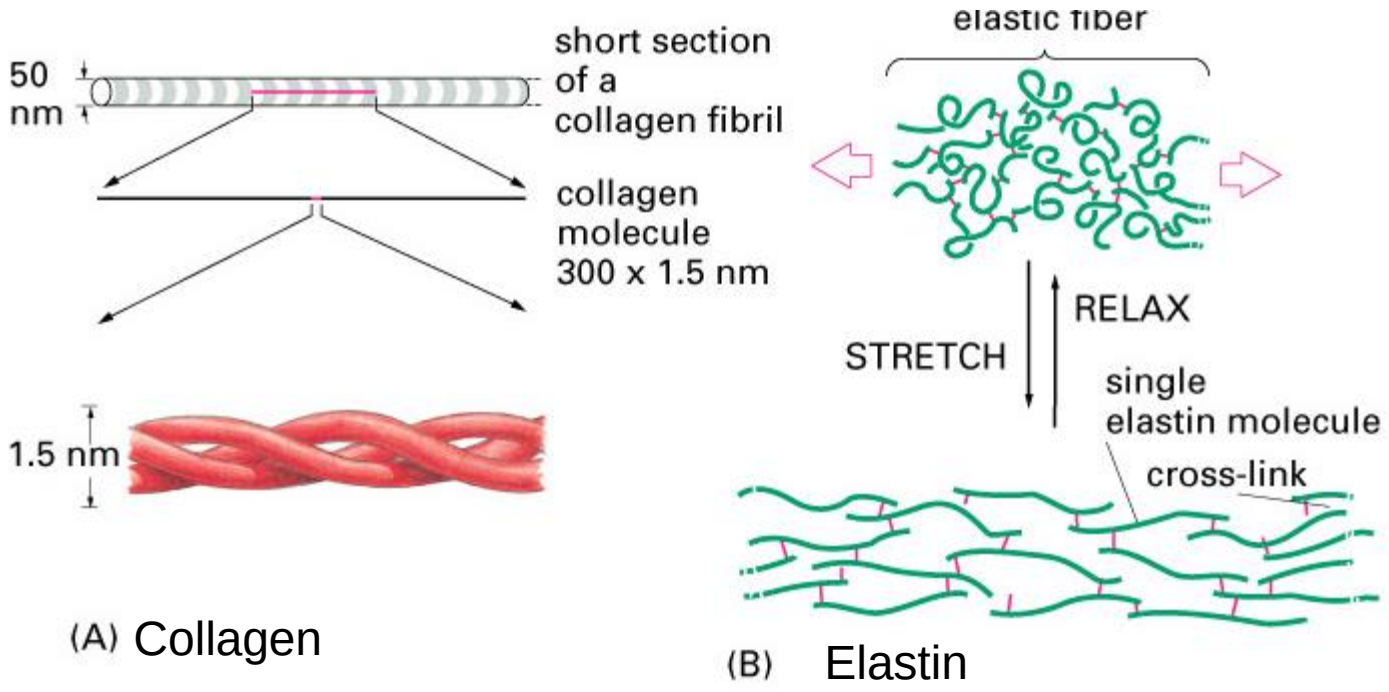


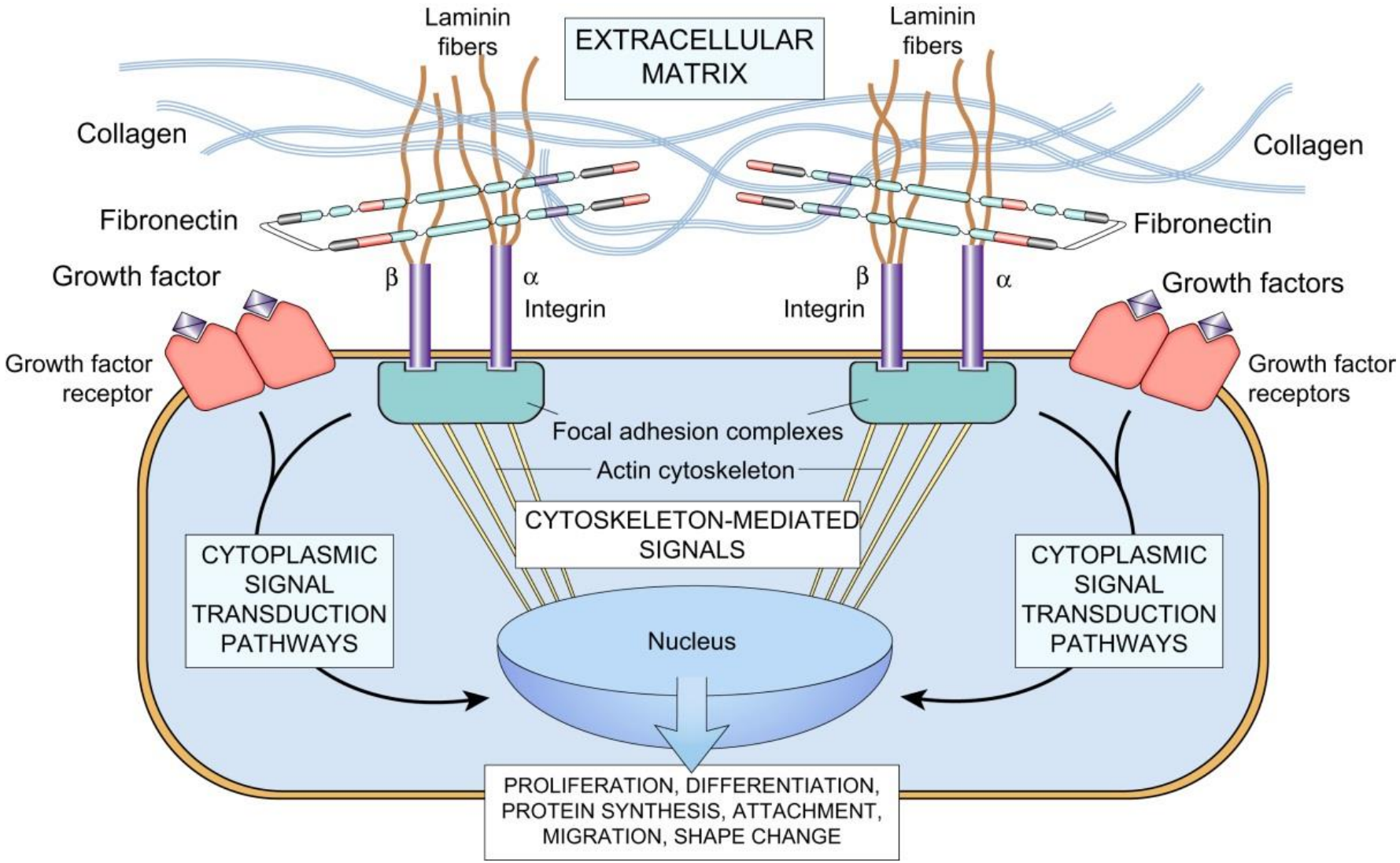
Figure 4-28 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Proteins

- Fibronectin:
  - help to bind to the other matrix component together
  - is a glycoprotein found mainly in connective tissue
  - serves as crosslinker or intermediate in the ECM by binding to both collagen and GAG
  - contains binding sites for adhesion receptors found on cell surface
  - facilitate attachment of cells to the ECM

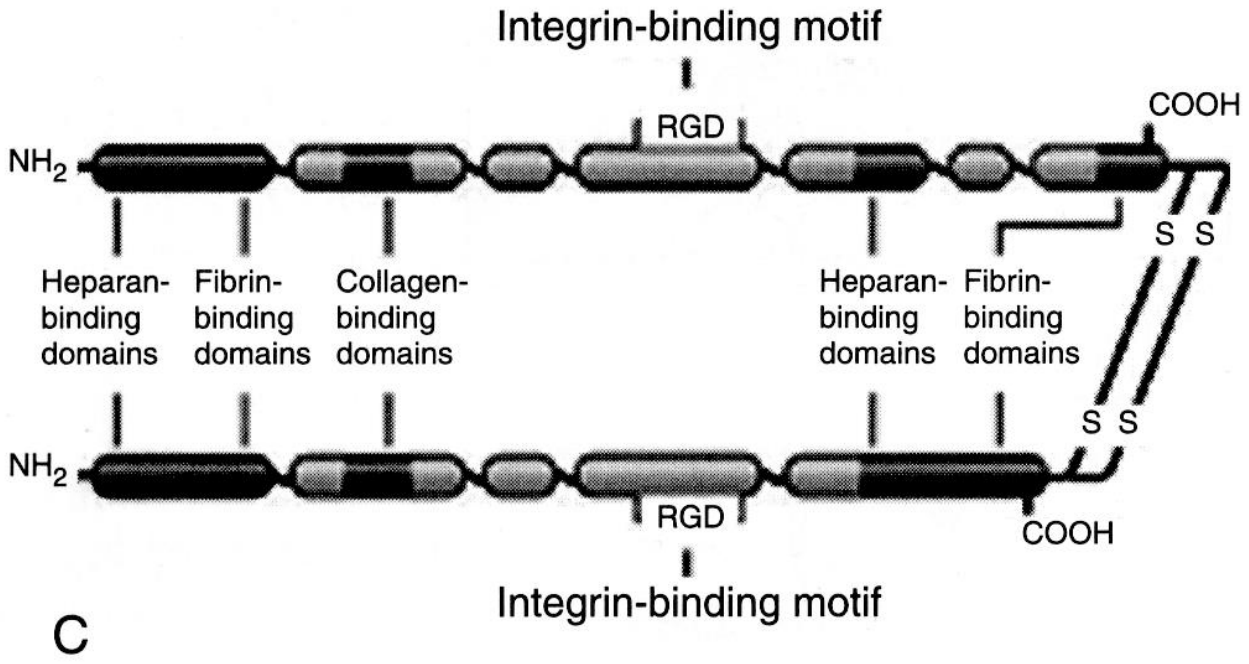
- Laminin:
  - major proteins in the [ ] membrane
  - critically contribute to cell attachment and differentiation, cell shape and movement, maintenance of tissue phenotype
  - contains binding sites that promote neurite outgrowth

Integrin-ECM interaction

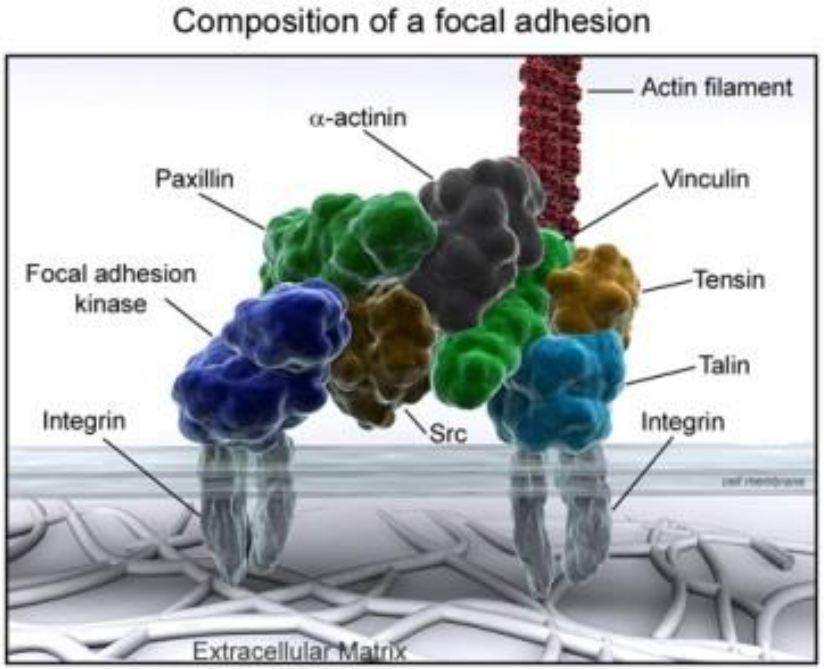


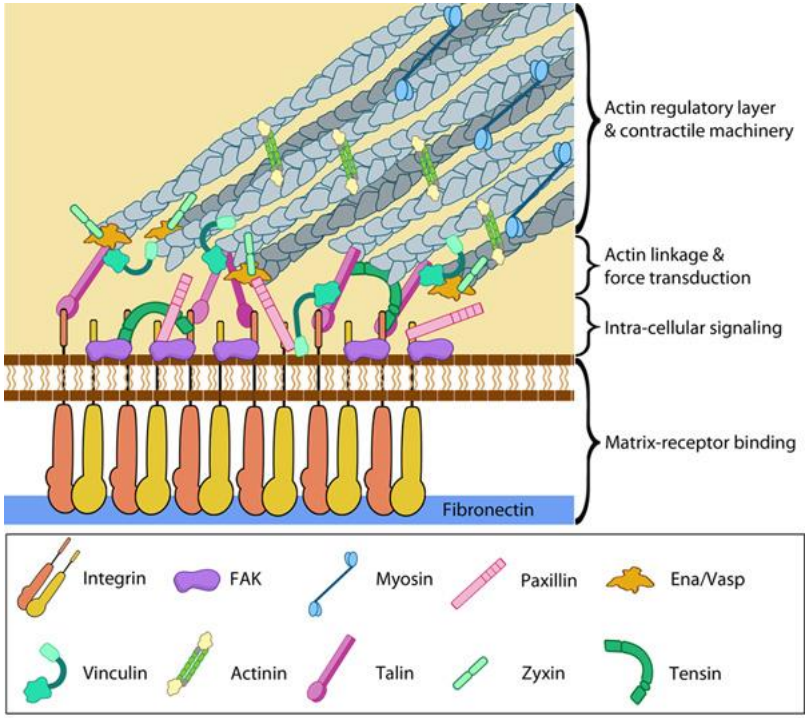
RGD

The prototypical binding site present in the adhesive proteins fibronectin and vitronectin is the three amino acid sequence arginine-glycine-aspartic acid which binds to a specific type of integrin receptors on the cell surface



ECM proteins

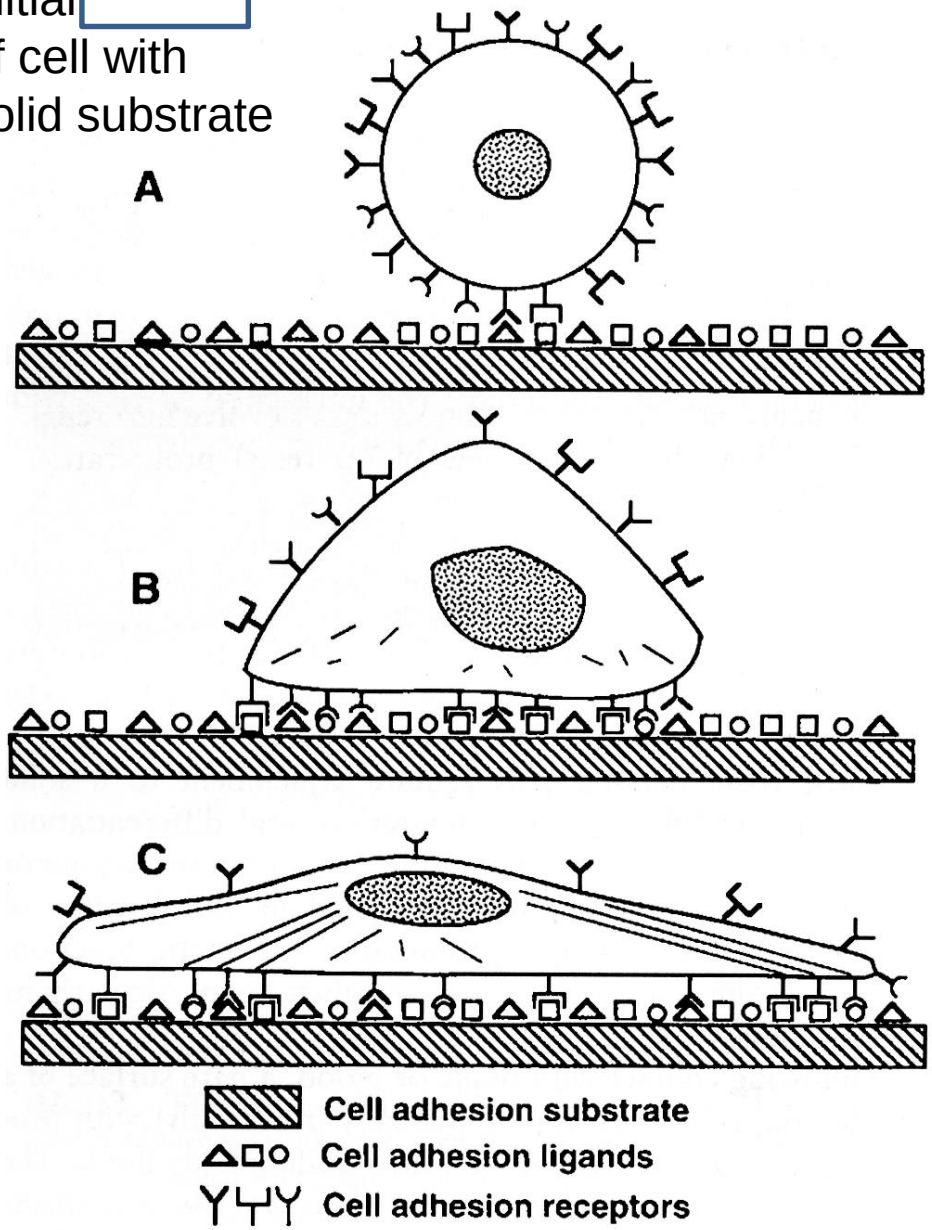


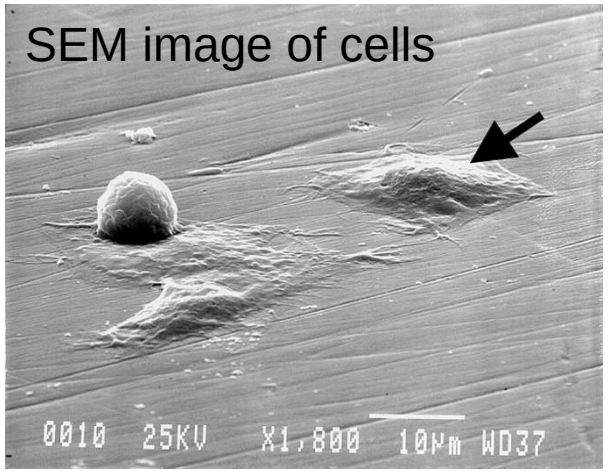


Peptide sequence	ECM protein	Conjugate receptor
RGD	Fibronectin, Laminin α-chain, Collagen, Vitronectin	Multiple integrins
YIGSR	Laminin β1-chain	β_1 integrins
IKVAV	Laminin α-chain	LBP110
REDV	Fibronectin	$\alpha_4\beta_1$ integrin
DGEA	Collagen type 1	$\alpha_2\beta_1$ integrin
KQAGDV	Fibronectin γ-chain	β_3 integrin
VAPG	Elastin	Elastase receptor, $\alpha_5\beta_3$ integrin

Process of cell attachment

Initial [ ] of cell with solid substrate





Formation of bonds between cell surface receptors and cell adhesion ligands.

[ ] reorganization with progressive spreading of the cell on the substrate for increased attachment strength

Process of cell attachment

- Proteins attach on material surface as a monolayer
- Cells make contact with, and anchor to, the absorbed proteins at discrete peptide regions – [ ] points
- Focal adhesions are considered to represent the strongest of such interactions. They comprise a complex assembly of intra-and extracellular proteins, coupled to each other through transmembrane integrins.
- Cell-surface integrin receptors promote cell attachment to substrates, and especially those covered with the [ ] proteins fibronectin and vibronectin.
- These receptors transduce biochemical signals to the nucleus by activating the same intracellular signaling pathways that are used by growth factor receptors.

Cell / tissue – biomaterial interaction

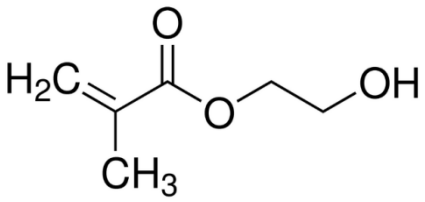
- Most tissue-derived cells require attachment to a solid surface for viability, growth, migration, and differentiation.
- Following contact with tissue or blood, a bare surface of a biomaterial is covered rapidly (usually in seconds) with proteins that are adsorbed from the surrounding body fluids
- Cell [ ] triggers multiple functional biochemical signaling pathways within the cell.

Adhesion proteins on cell-surface interaction

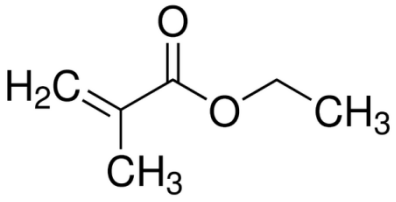
Cell adhesion to HEMA-EMA copolymers

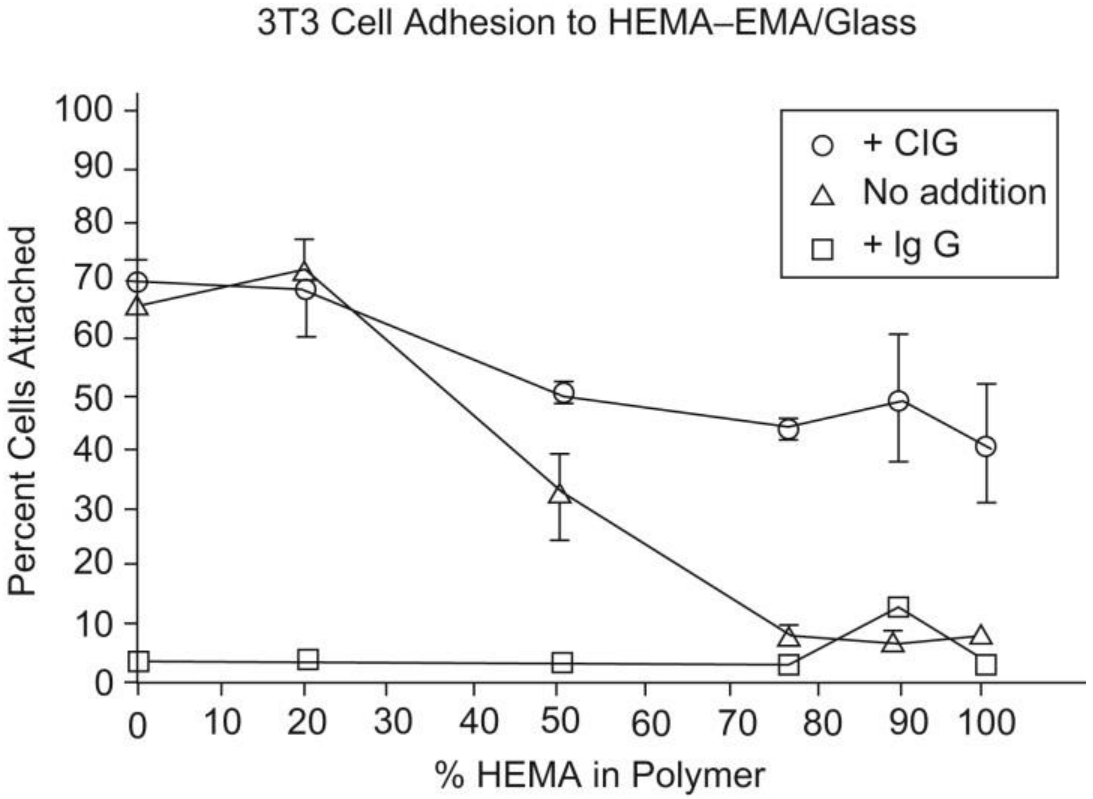
- 3T3 cells bind to the surface with [ ] (CIS)
- 3T3 cells do not bind to surface with [ ]
- [ ] binding of 3T3 cells to surface varies as the property of the surface changes

HEMA: 2-hydroxyethyl methacrylate: hydrophilic



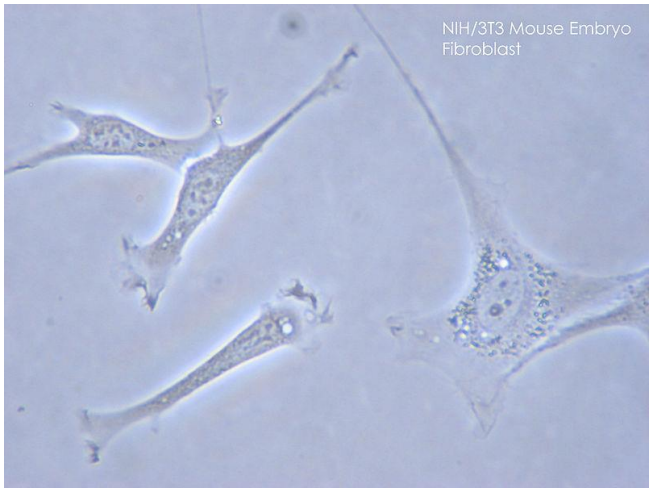
EMA: ethyl methacrylate, hydrophobic





3T3 cells

- Cell line: a permanently established cell culture that will proliferate indefinitely given appropriate fresh medium and space
- 3T3 cell:
  - a cell line established in 1962 by two scientists then at the Department of Pathology in the New York University
  - 3T3 cell line has become the standard fibroblast cell line
- Fibroblast:
  - A type of cell that synthesizes the extracellular matrix and collagen, the structural framework (stroma) for animal tissues, and plays a critical role in wound healing
  - Fibroblasts are the most common cells of connective tissue in animals



Micro-contact printing

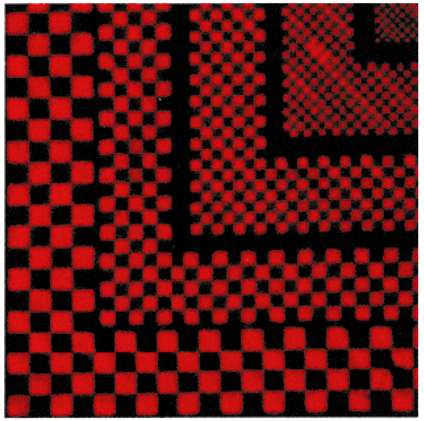
printing (or μCP): a form of soft lithography that uses the relief patterns on a master polydimethylsiloxane (PDMS) stamp to form patterns of self-assembled monolayers (SAMs) of ink on the surface of a substrate through conformal contact

Advantages:

- The simplicity and ease of creating patterns with micro-scale features
- Can be done in a traditional laboratory without the constant use of a cleanroom (cleanroom is needed only to create the master).
- Multiple stamps can be created from a single master
- Individual stamps can be used several times with minimal degradation of performance
- A cheaper technique for fabrication that uses less energy than conventional techniques

Disadvantages

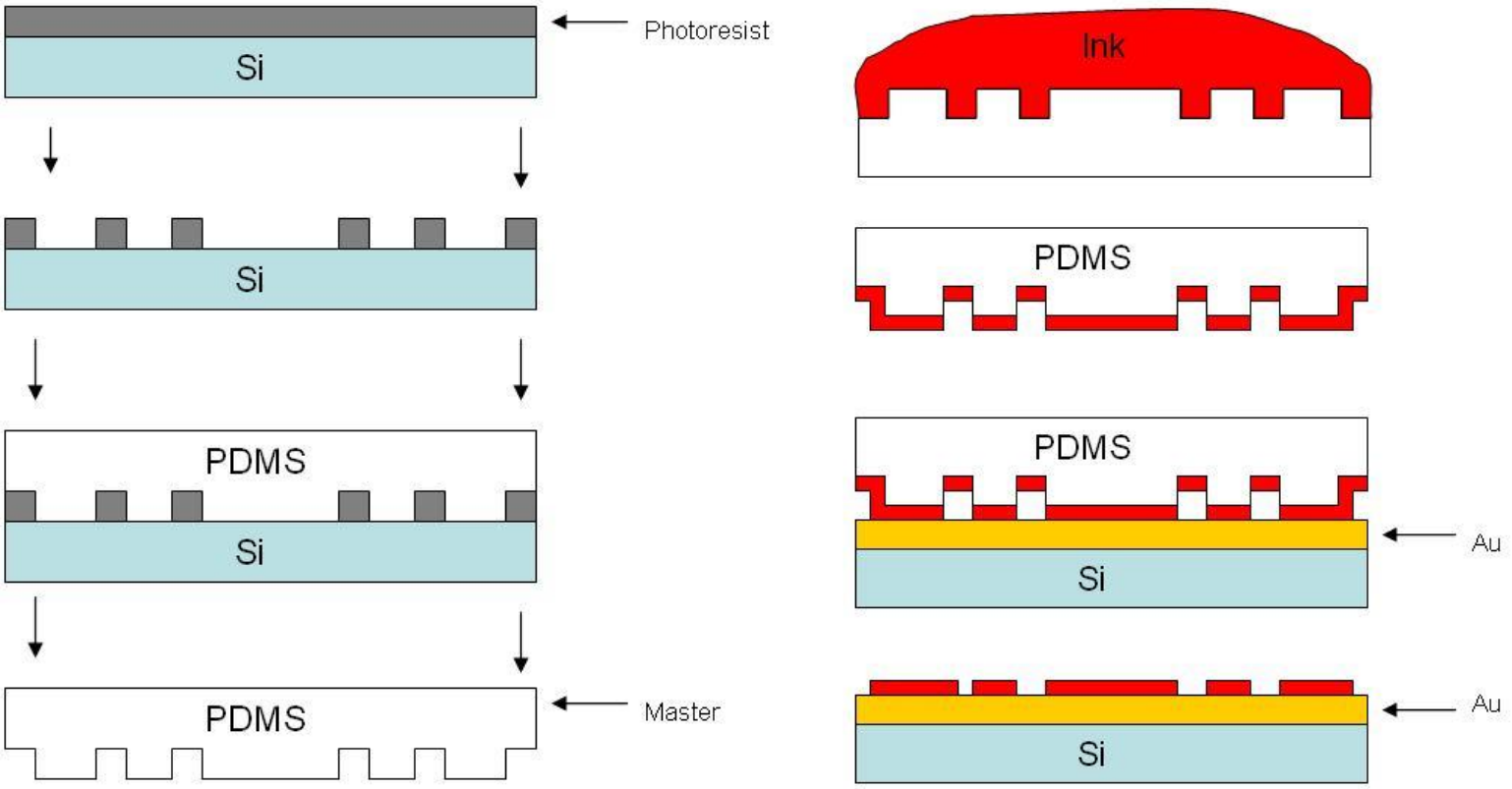
- Difficult for nanoscale assemblies
- Not desirable for patterning different inks



Patterning of proteins

Micro-contact printing: Method

Transfer of SAM precursor with elastomeric stamp onto substrate

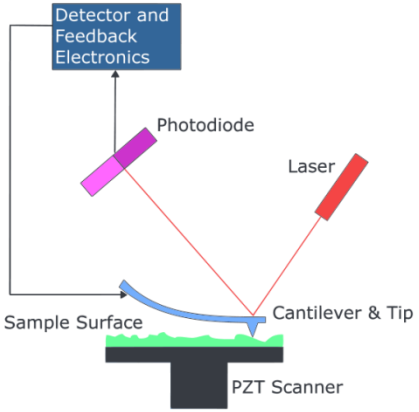


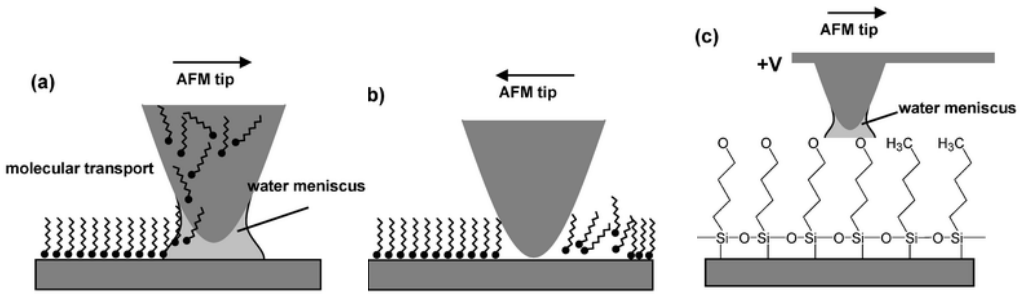
- Master generation by photolithography and similar techniques
- Stamp is obtained by casting of elastomer (PDMS) over master

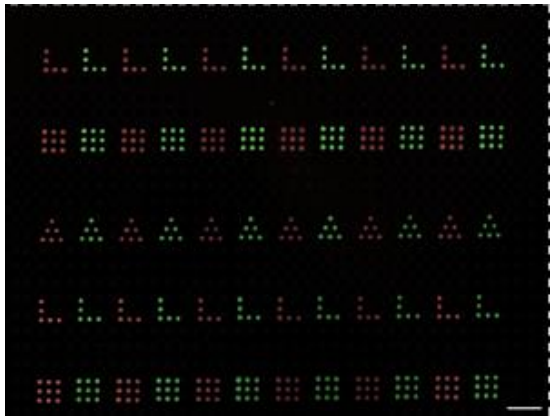
- Pattern generation by stamping of SAM precursor onto substrate

Dip pen nanolithography

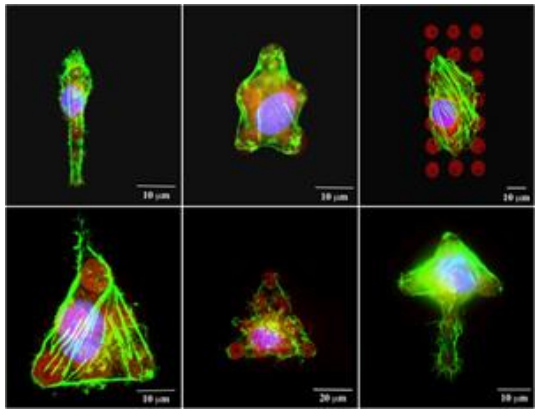
Utilization of Atomic force microscopy (AFM) for nanoscale patterning







Fluorescent image of a multiplexed pattern of various shapes of laminin (green) and fibronectin (red)



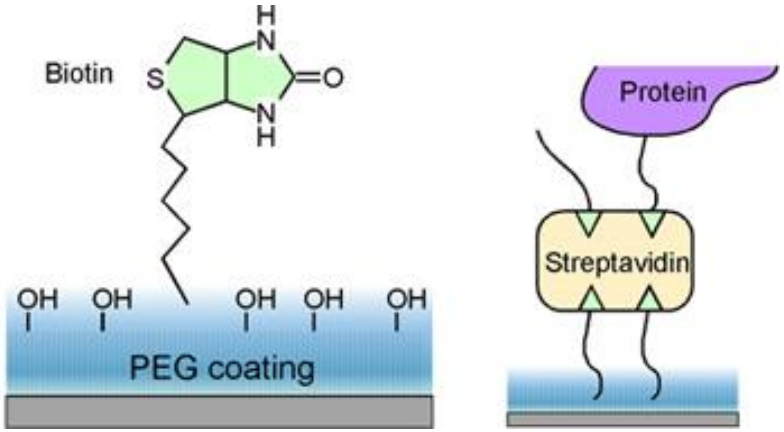
20x fluorescent images of fibroblasts attached to fibronectin (red) patterned on epoxy-functionalized glass slides, showing actin (green) and nuclei (blue)

Functionalization of biomolecules on surfaces

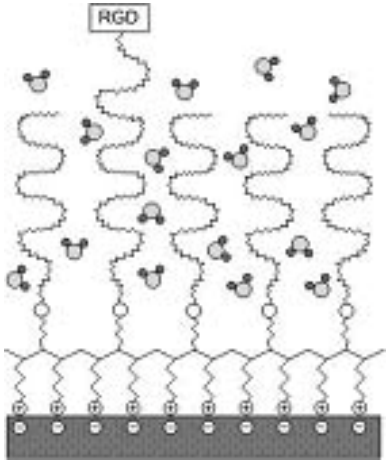
Use the preceding techniques to add functional groups to the surface.

Examples are:

- Avidination / Biotinylation
- Epitopes (e.g. RGD for promoting cell adhesion)
- Plasma treatment (promotes protein adhesion)
- Adsorption of whole bioactive molecules (patterns)



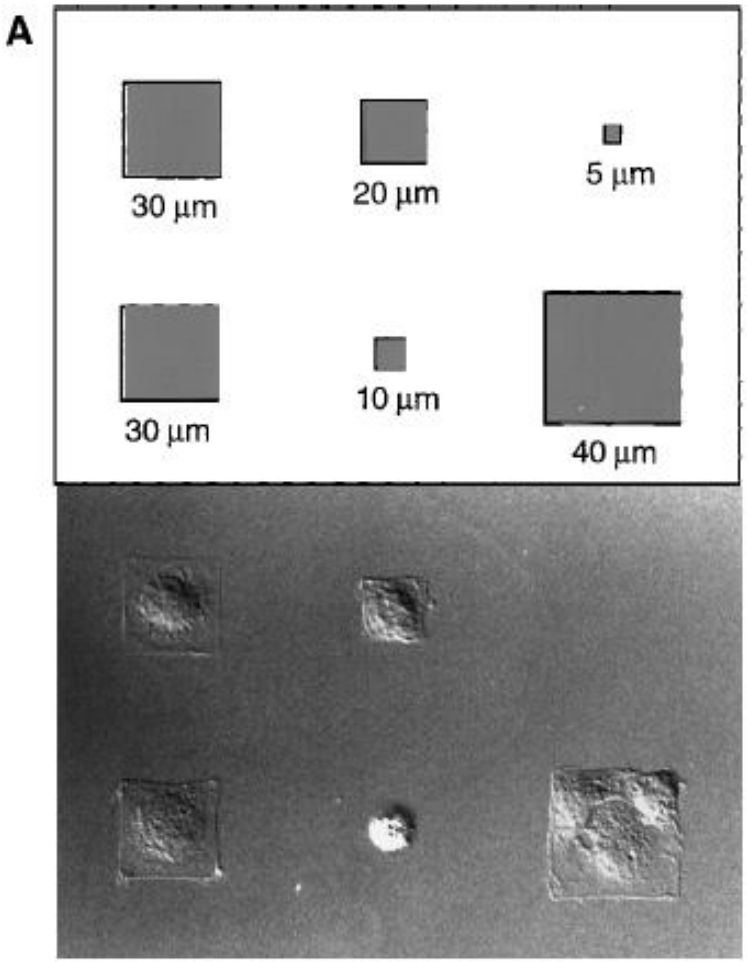
Biotin-Streptavidin: The strongest among non-covalent binding



Model view of a titanium oxide surface covered by RGD-modified PLL-g-PEG polymer.

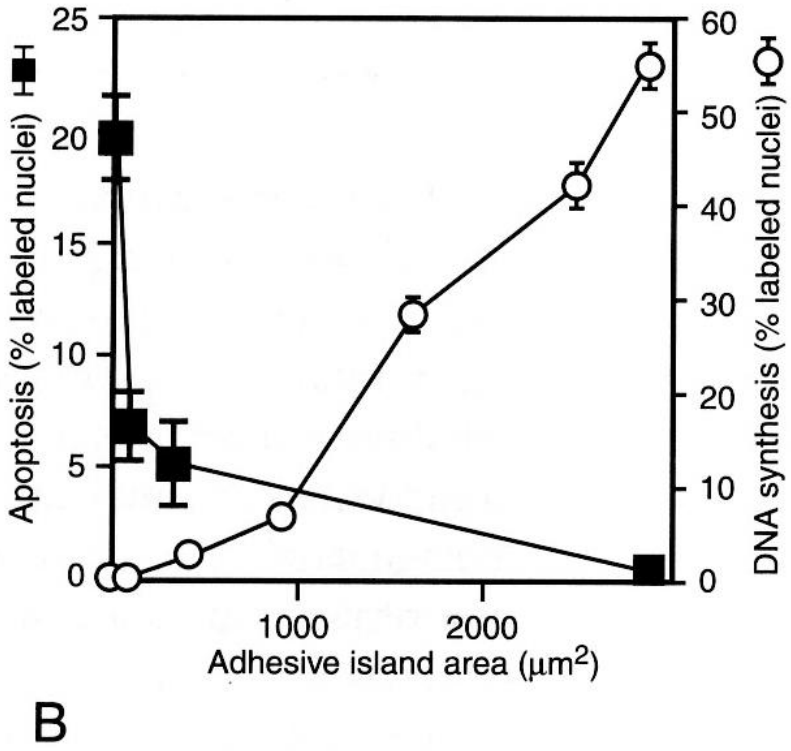
Proteins that contain the Arg-Gly-Asp (RGD) attachment site constitute a major recognition system for cell adhesion

Cell growth on patterned surface



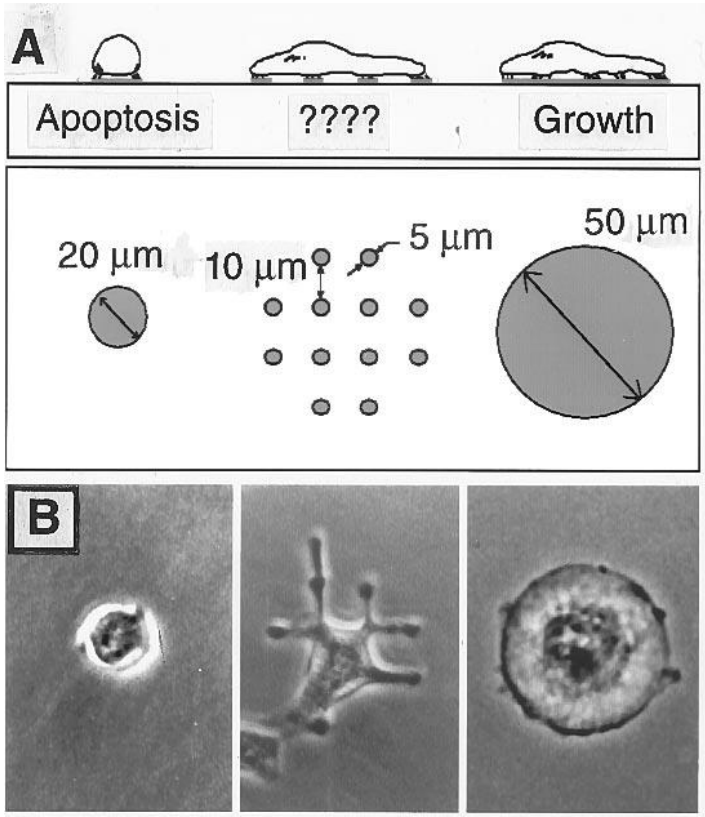
When cells were plated on square-shaped islands coated with fibronectin, square-shaped cells were produced that closely matched the size and shape of the adhesive island

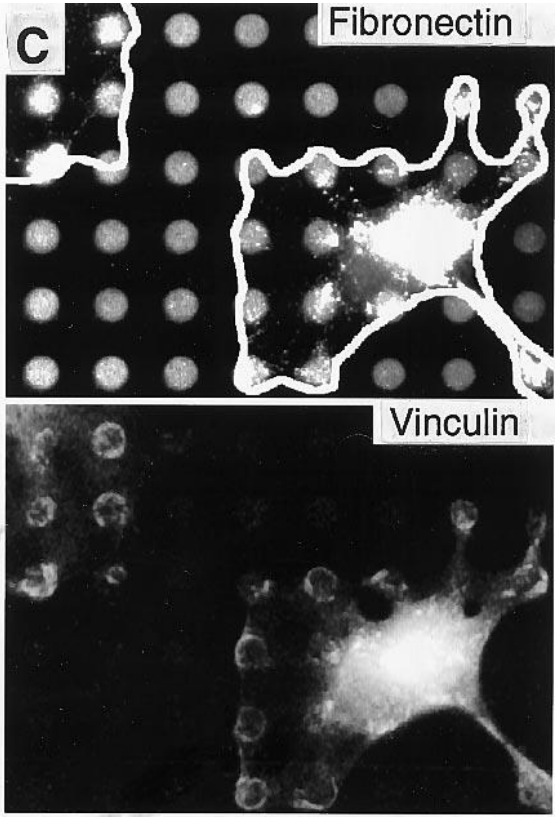
Chen *et al*., Science, 276, 1425

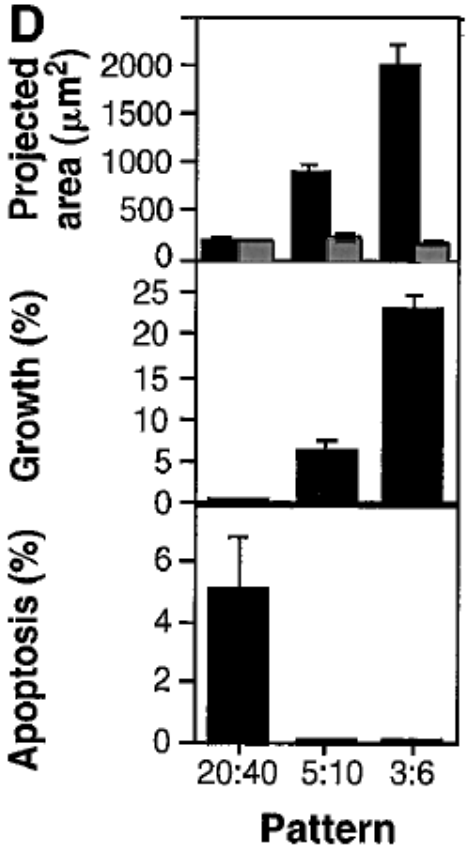


Apoptotic index and DNA synthesis index after 24 hours, plotted as a function of the projected cell area.

Cell growth on patterned surface







- The more cells spread, the higher their rate of
- Not dependent on the of fibronectin

Cell distortion on proliferation

- The ability of a cell to proliferate depend directly on the degree to which the cells were allowed to [ ] physically, and not on the actual surface area of substrate binding.
  → Thus, cell distortion is a critical determinant of cell behavior.

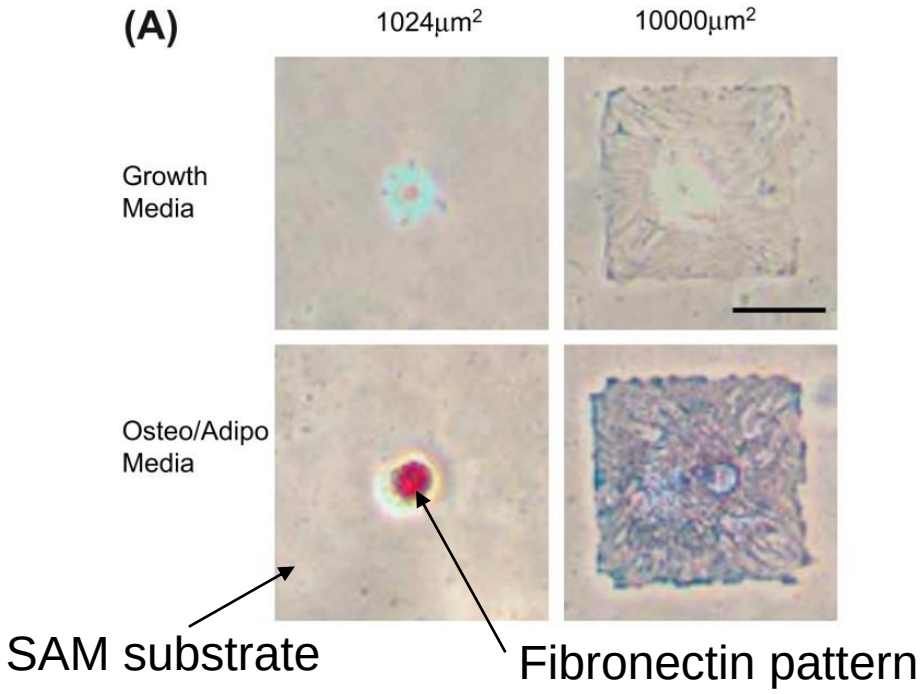
- Interactions of cells with ECM differ from those with soluble regulatory factors owing to the reciprocal interactions between the ECM and the cell's actin cytoskeleton

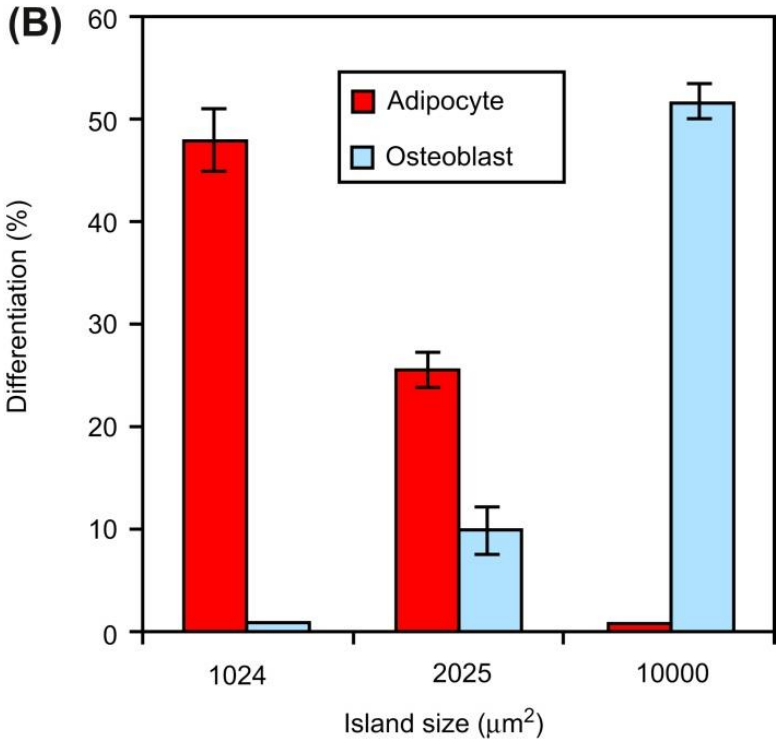
Properties of the substrate on cell

- Thus, the properties and configuration of the surface-bound ECM on a substrate and the properties of the substrate itself can [ ] cell-biomaterials interactions.

- The key concept is that a biomaterial surface can contain specific chemical and structural information that controls tissue formation, in a manner analogous to cell-cell communication and patterning during embryological development.

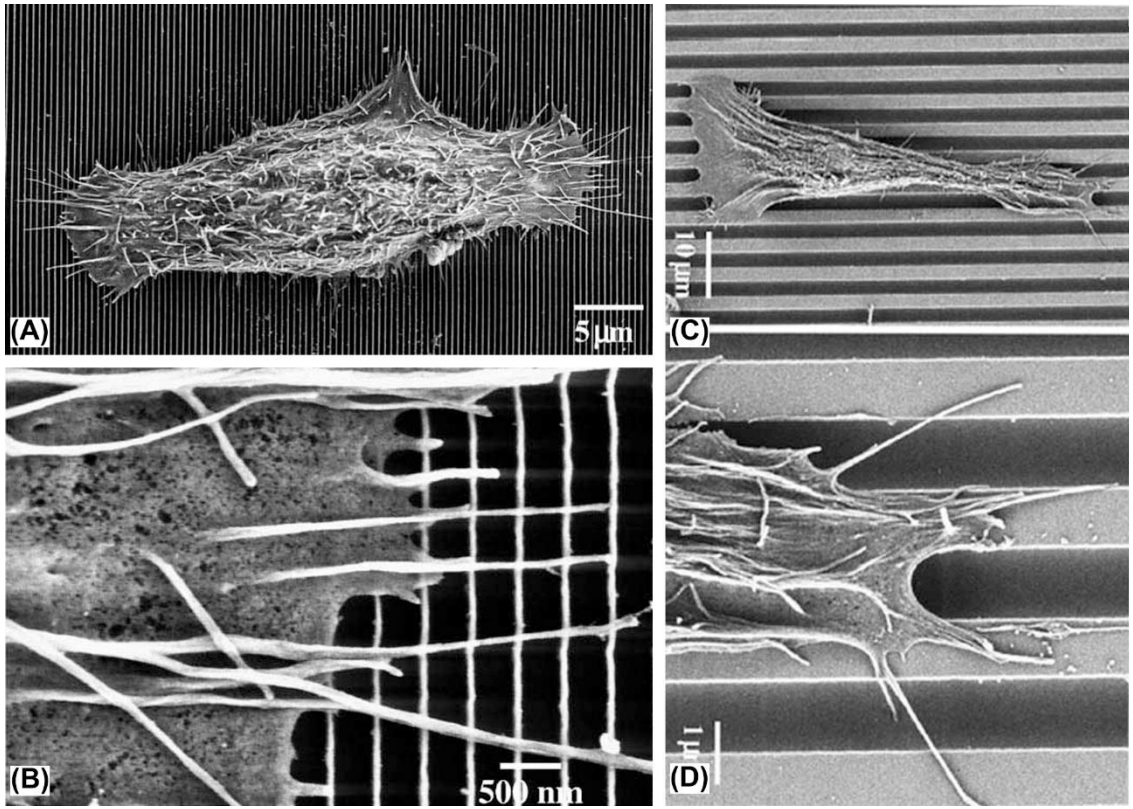
Micrometer scale patterning





- Cell [ ] modulates hMSC differentiation
- Cell binding to the extracellular matrix through specific cell-substratum contacts is critical to cell-growth control through [ ] forces mediated through associated changes in cell shape and cytoskeletal tension

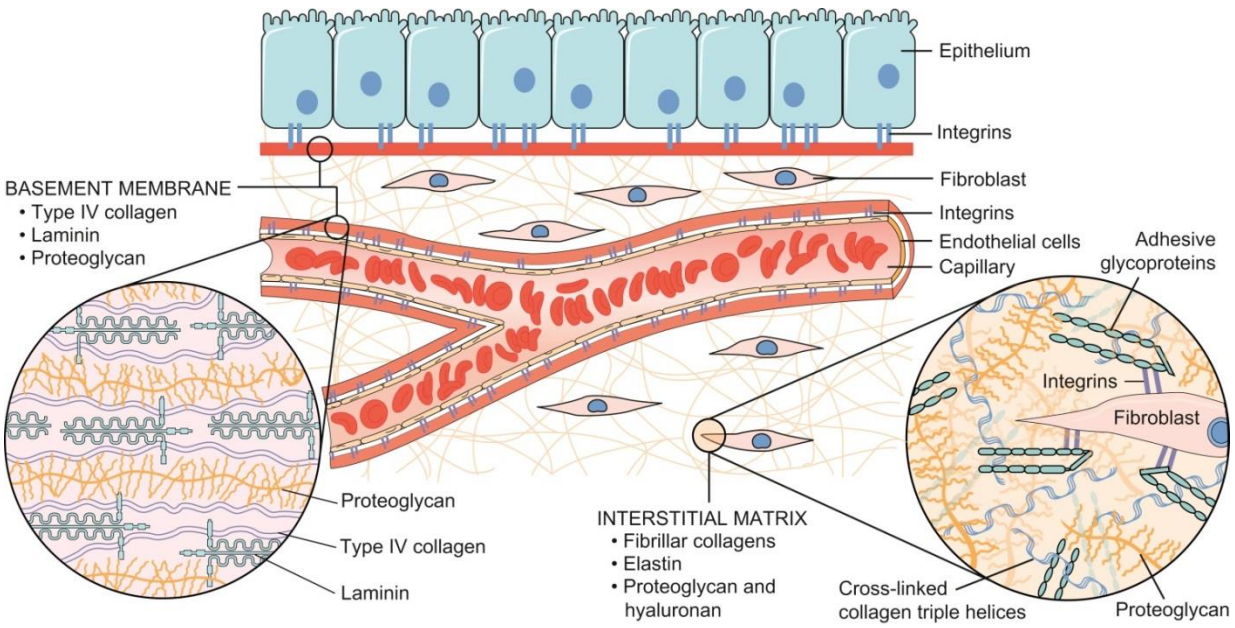
Nanometer scale patterning

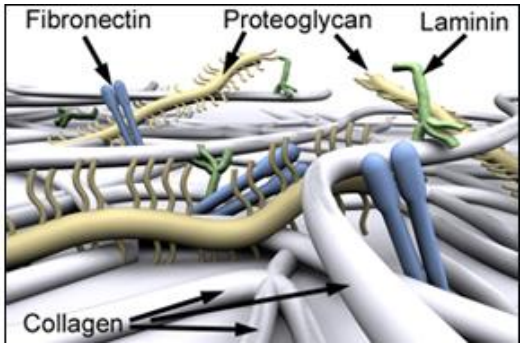


- Cells can [ ] and respond to features as small as 10-30 nm
- At the nanoscale, a finite limit in feature sizes exist with respect to cell alignment to surface topology
- While cells can be profoundly affected by a repetitive nanoscale single feature, their response depends on cell [ ]

Mimicking ECM

Employ biomaterials with surfaces designed to [ ] highly precise reactions with proteins and cells at the molecular level. The binding domains of the extracellular matrix (ECM) environment can be mimicked by a multifunctional cell-[ ] surface created by specific proteins, peptides, and other biomolecules immobilized onto a material.





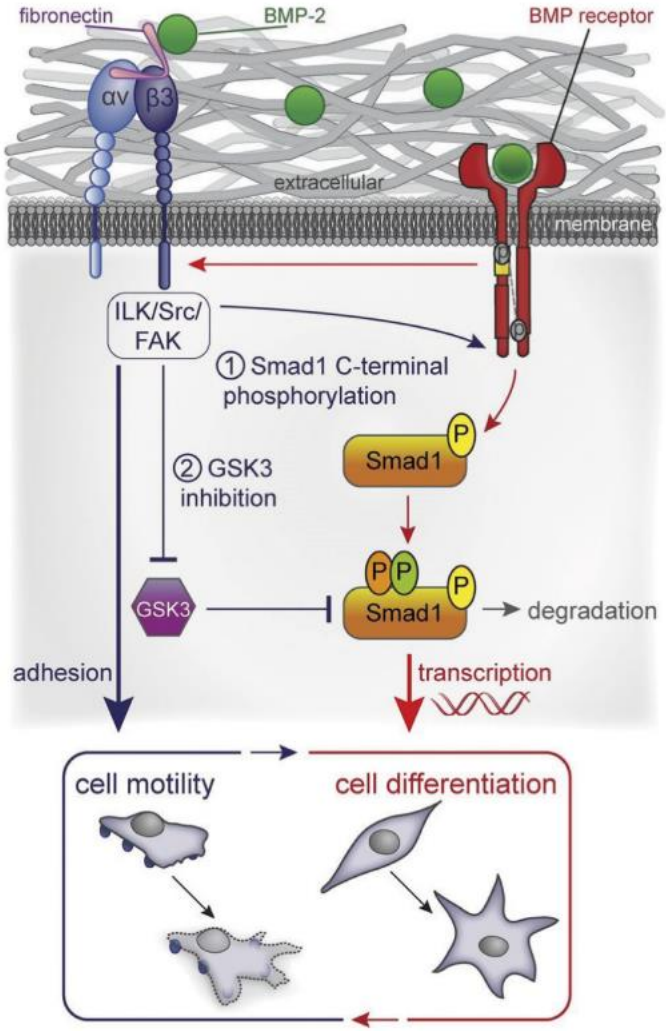
ECM

Characteristics of ECM molecules

Table S1. Diameter and surface charge of primary extracellular matrix components.

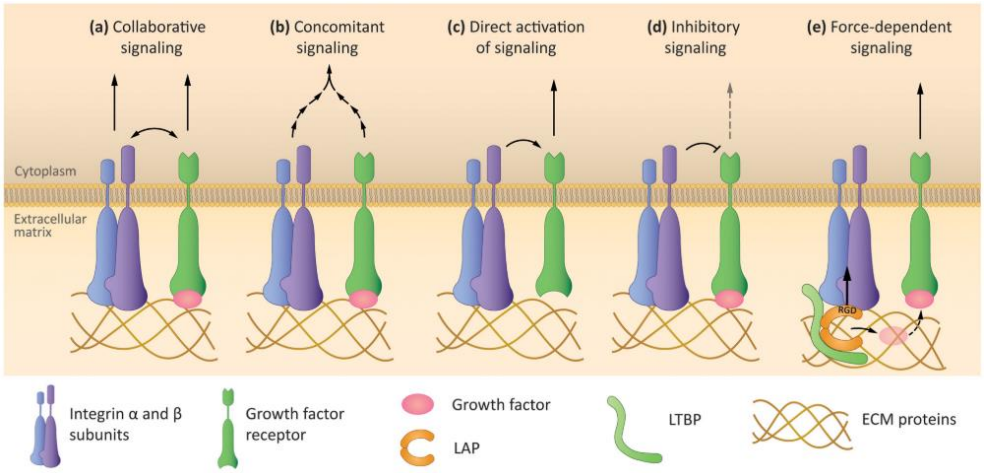
Primary ECM Components	Diameter of components	Surface charge
Collagen molecule (tropocollagen)	1.5 nm [1-6]	−12.2 ± 0.7 mV [7]
Collagen Type I (skin, bone) fibril	67 nm [8-10]	
Type II (cartilage) fibril	80 nm [11, 12]	
Type III (Vessel) fibril	67 nm [13]	
Type IV (Basement membrane) fibril	4 nm [14, 15]	
Laminin	2 nm [16]	−50 mV [17, 18]
Elastin	7 nm [19, 20]	−24 ± 2 mV [21 22]
Vitronectin	6 nm [23]	−20 mV [18]
Glycosaminoglycans (GAGs)	0.7 nm [24]	Negatively charged [25, 26]

Current ECM mimicking materials



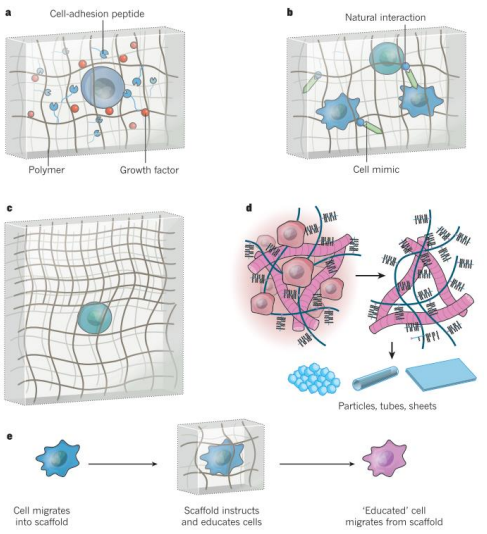
Biointerphases **13**(6) (2018)

Growth Factors (GFs) Modification



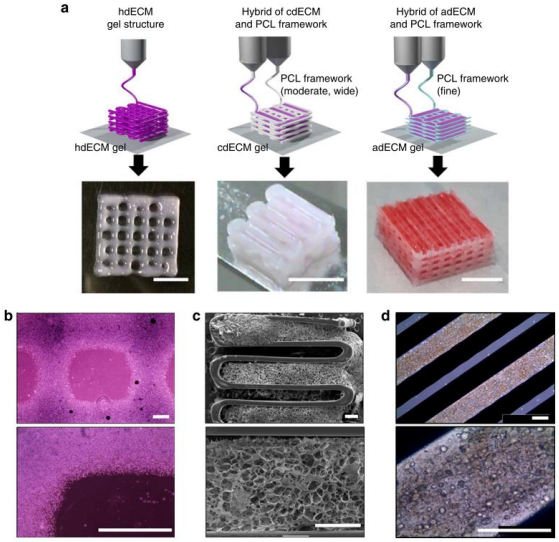
Biointerphases **13**(6) (2018)

Hydrogels



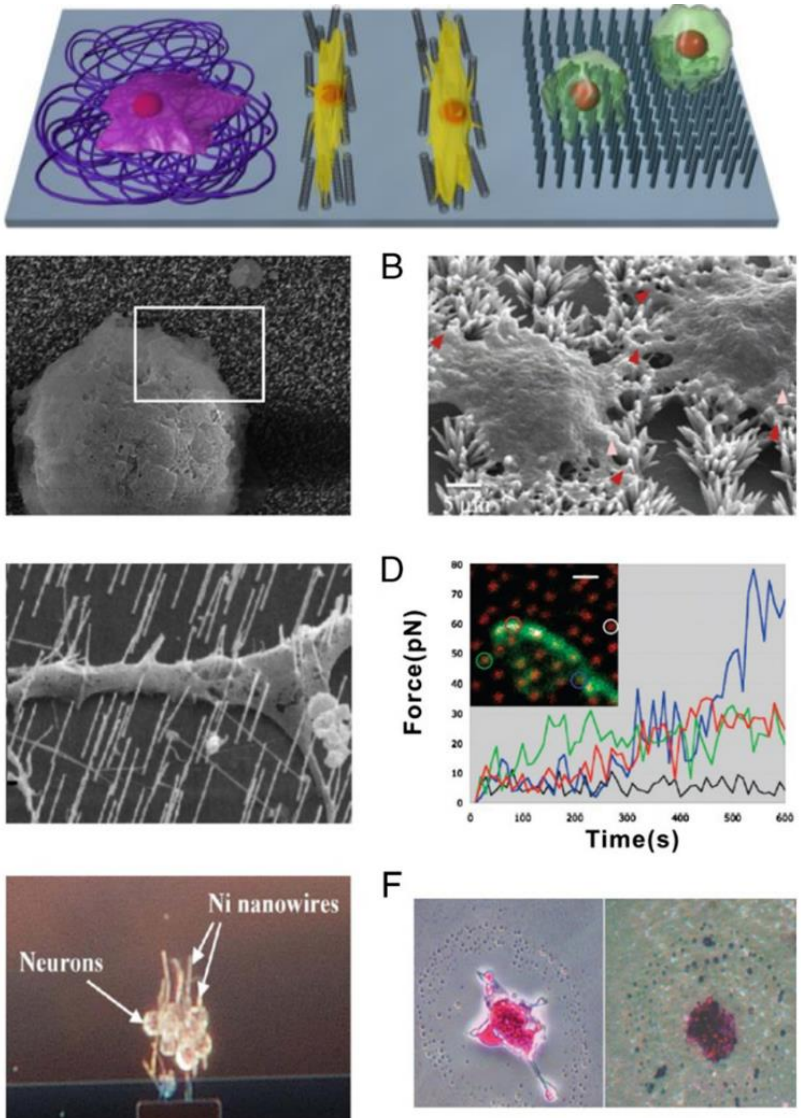
Nature **540**(7633), 386 (2016)

Polymers

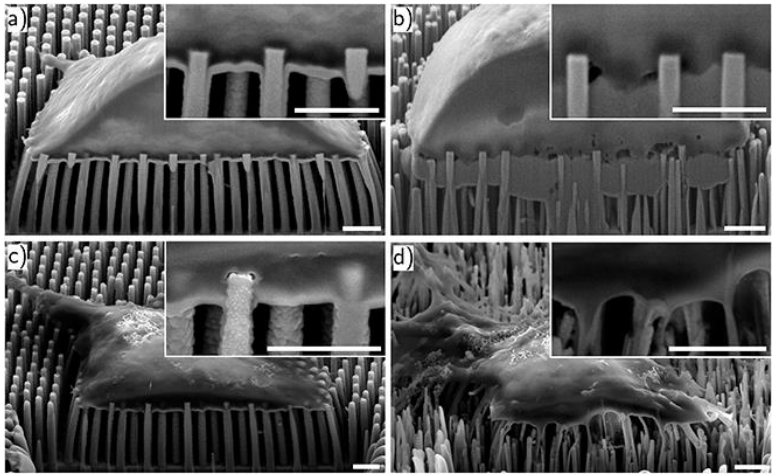


Nature Communications **5**, 3935 (2014)

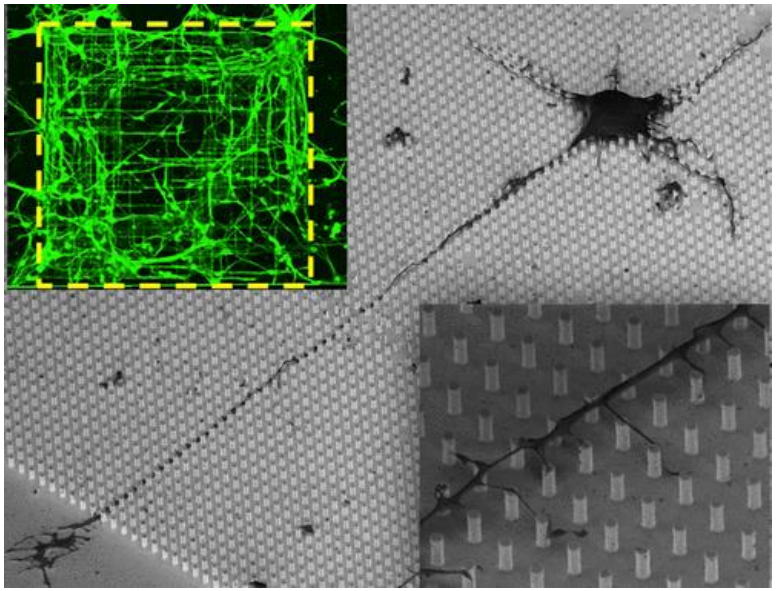
1D nanomaterials for cell growth



Nano Convergence **1**(1) 28 (2014)

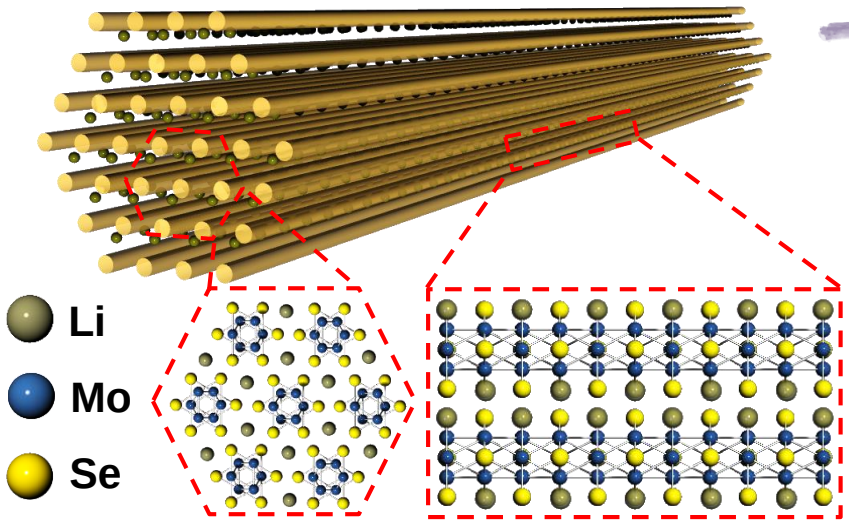


RSC Adv., **9** 11194 (2019)



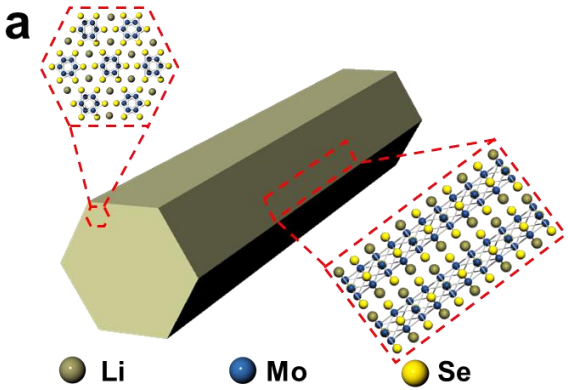
Nano Lett. **17**(6) (2017)

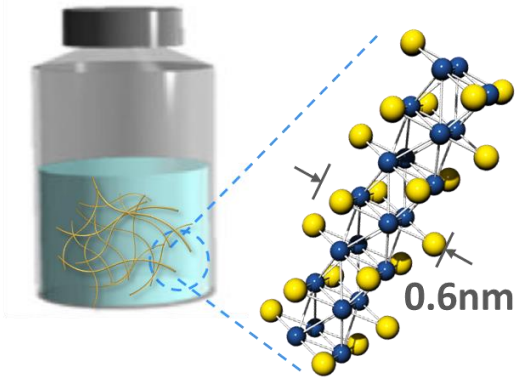
$Mo_3Se_3^-$ SCAC as an ECM mimicking molecule

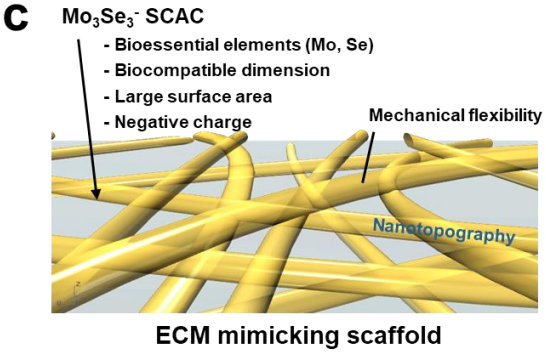


- Negative charge
- Large surface area
- Mechanical flexibility
- Biocompatible/Bioessential elements

→ **ECM mimicking scaffold**

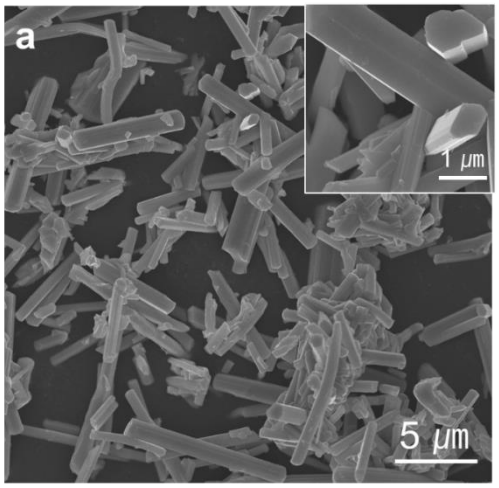


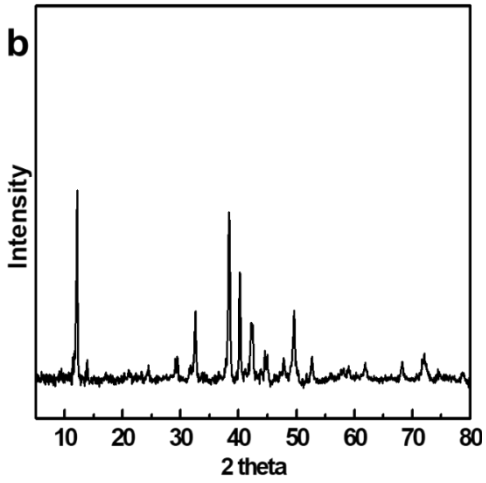


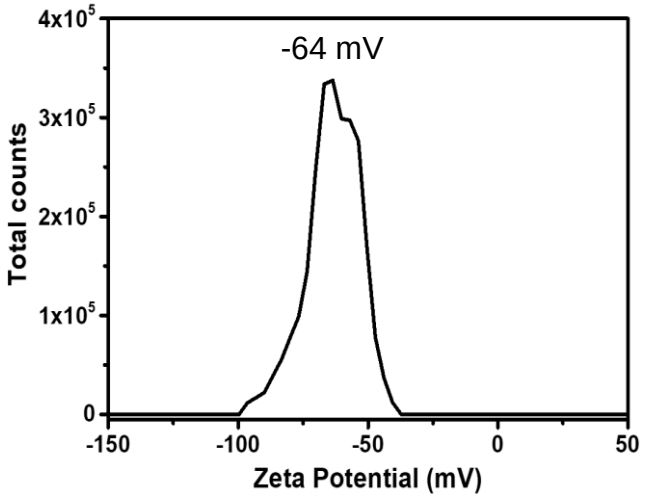


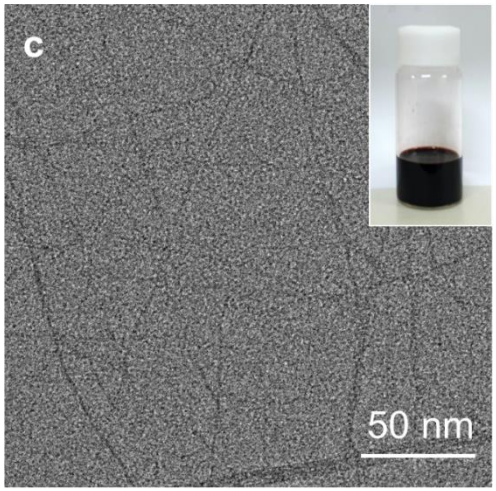
J. W. Lee *et al., Nano Lett.* **18**, 7619−7627 (2018)

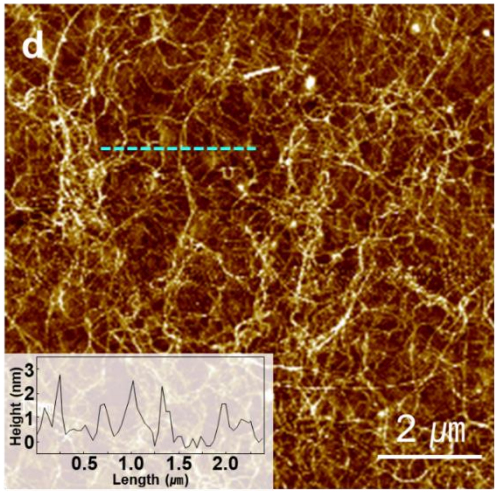
Synthesis of ECM mimicking scaffold film

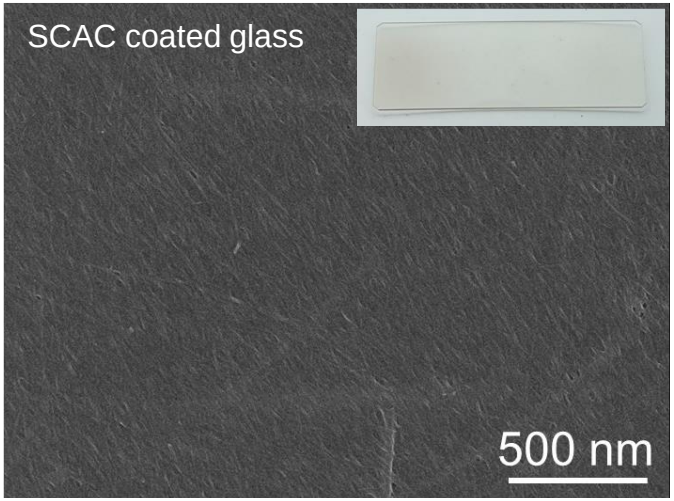




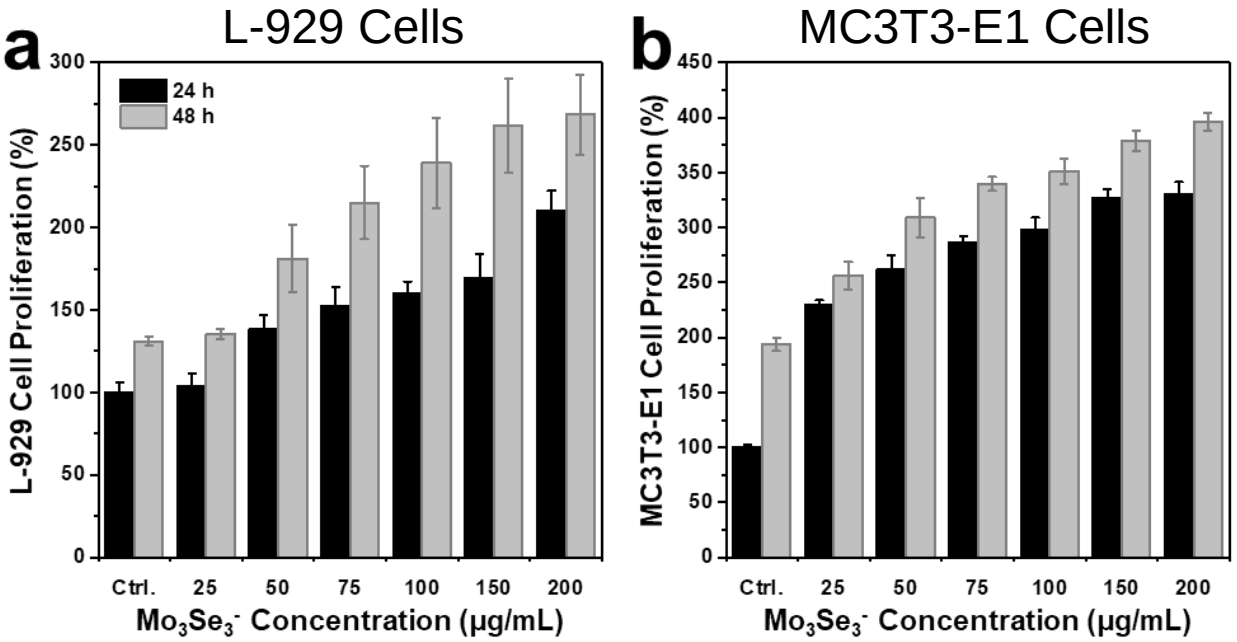


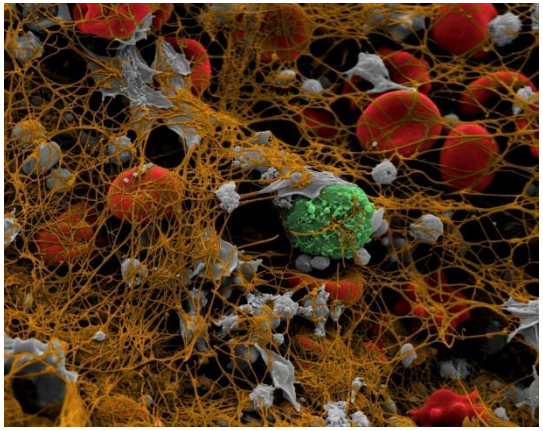






Cell proliferation





- **Mo ions** act as an electron carrier in xanthine oxidase, sulfite oxidase, and aldehyde oxidase, and even prevents dental caries

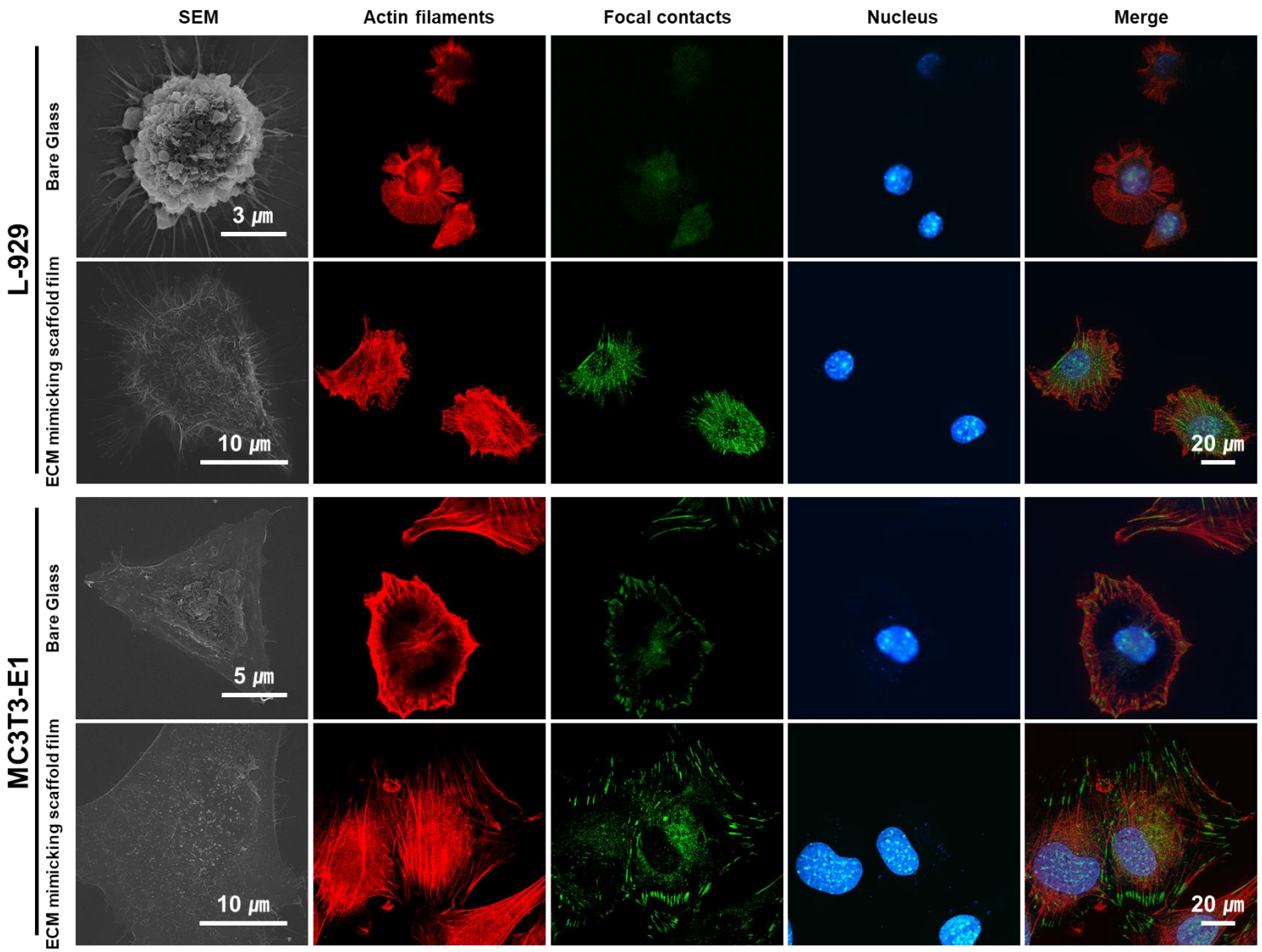
- **Se ions** function as a cofactor for glutathione peroxidases

Sulfite oxidase (mitochondria) enhances activity

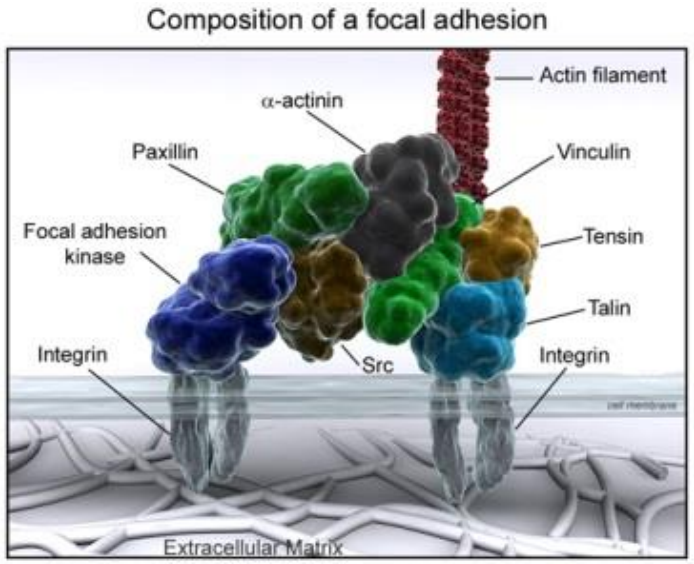
Aldehyde oxidase (cell) removes ROS

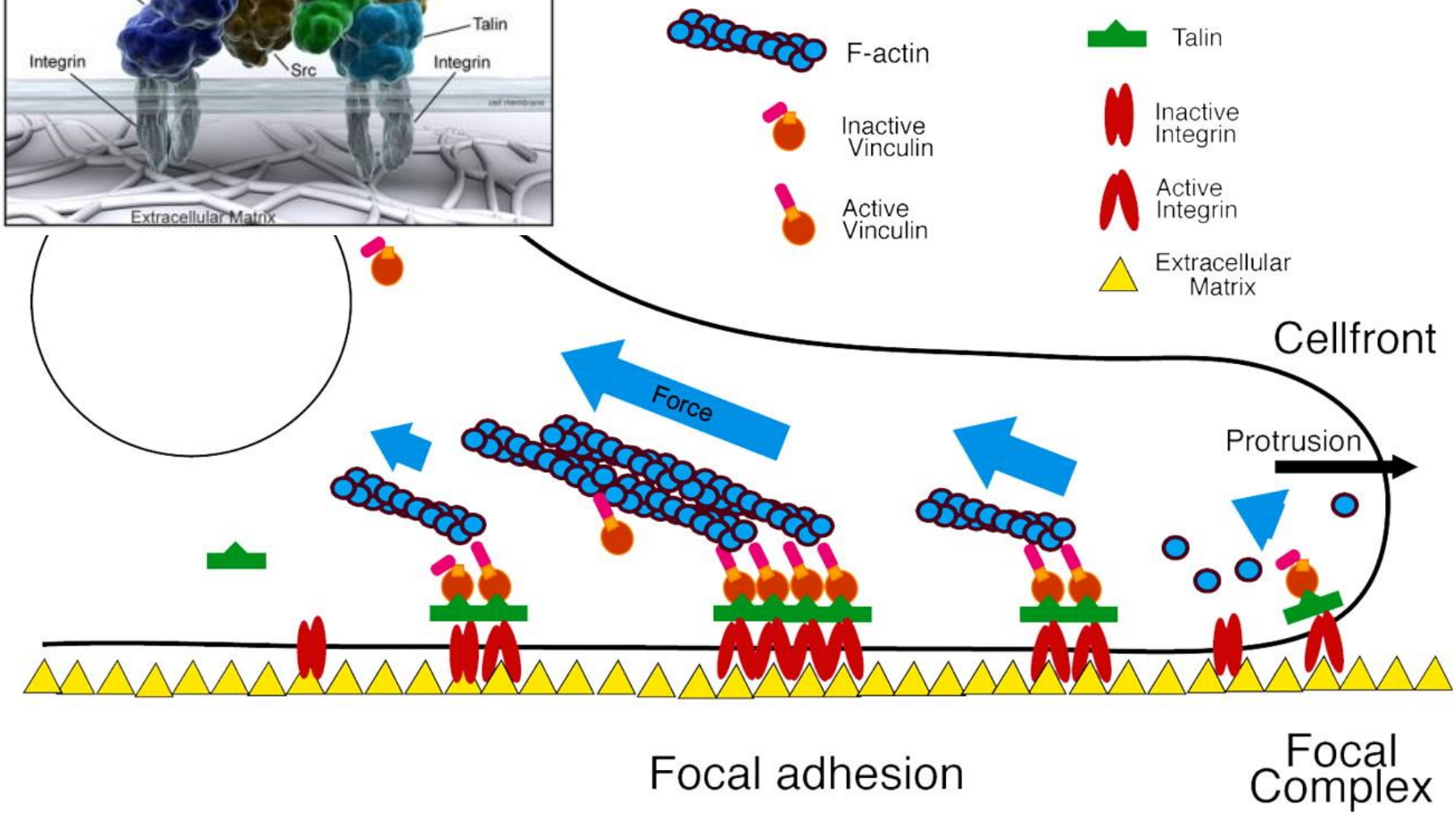
Glutathione peroxidase (cell) removes H_2O_2

Enhanced Cell Adhesion



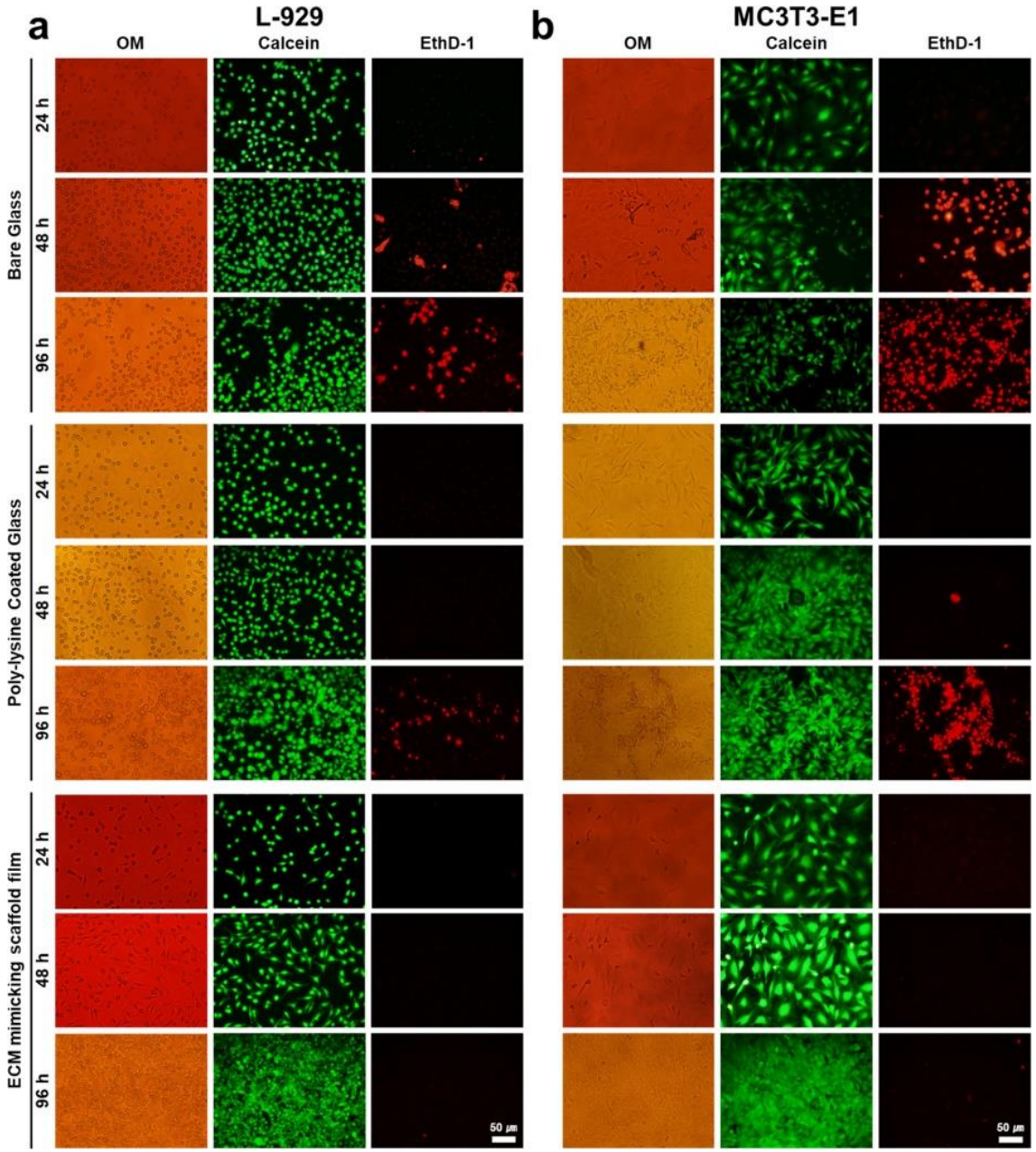
Focal adhesion



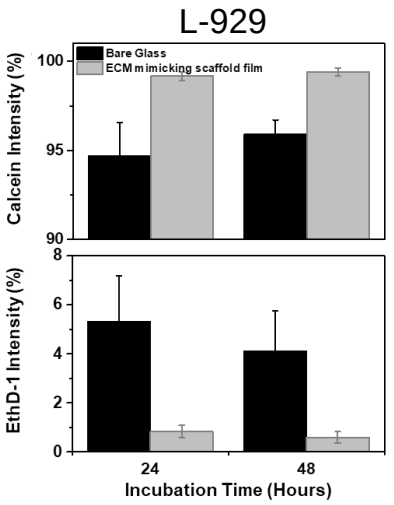


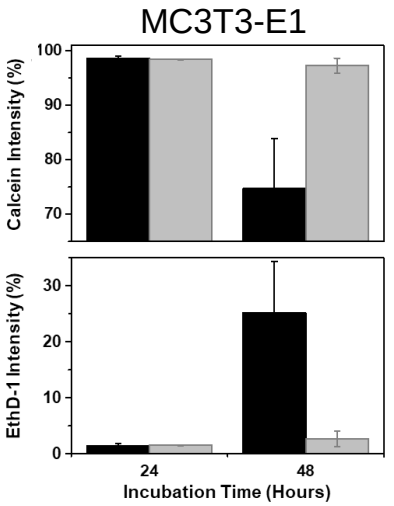
J. of Cell Biol., **179** (5), 1043 (2007)

Live/dead cell assay



Quantification of cell viabilities





Exceptional biocompatibility