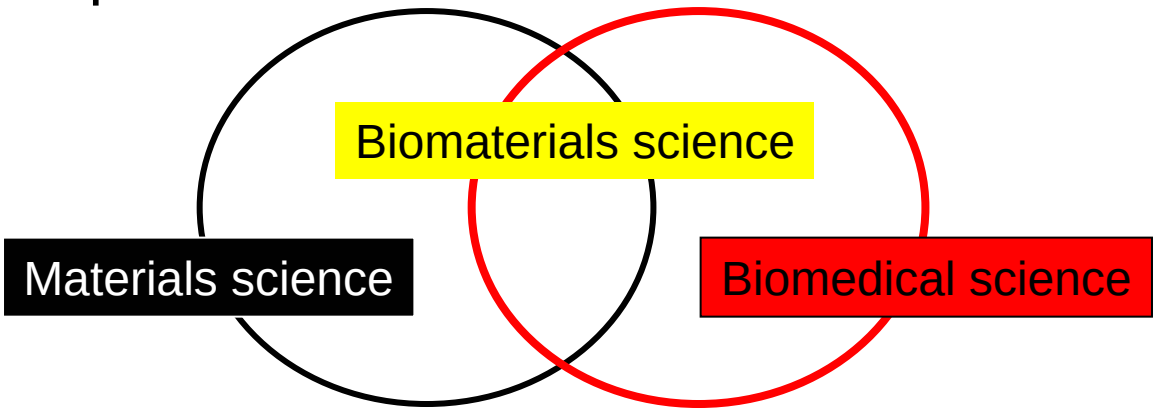
Biological background

- To reasonably follow the arguments on:
  - Biological interaction
  - Biocompatibility
  - Material performance and
  - Biological/clinical performance

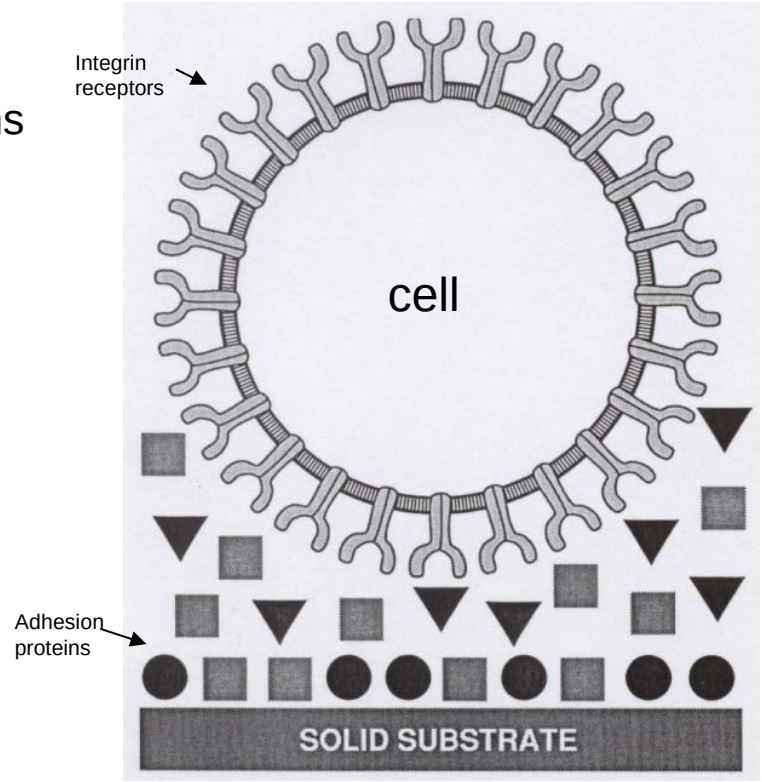


Biological background: Proteins

To avoid immunogenic effects, most biomaterials currently in use are non-biological of origin. Due to proteins attaching to materials surfaces these are then often recognized by the organism as biological factors

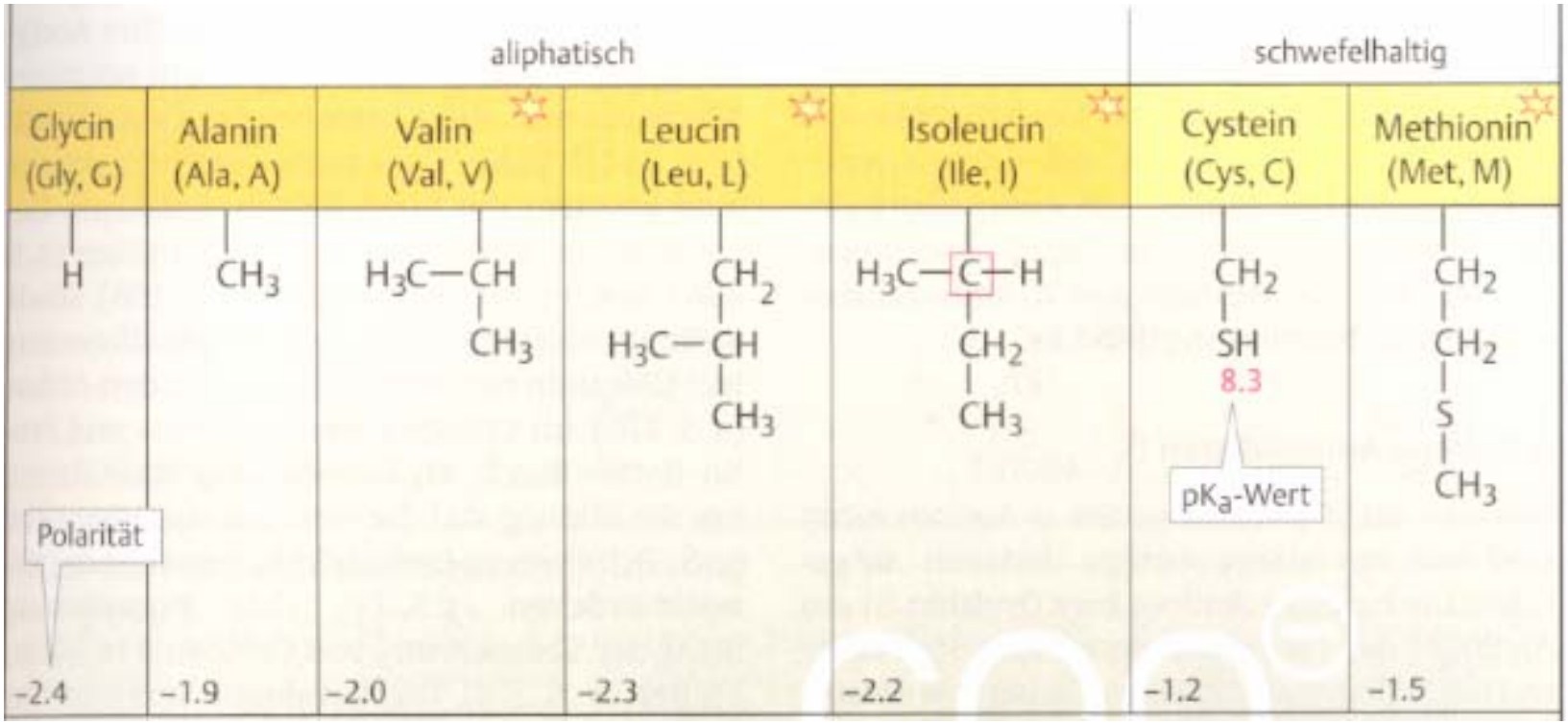
- In seconds after implantation of a biomaterial a monolayer of proteins adsorb to most surfaces.
- Much later (several minutes) cells then recognize the implant as a biological structure, due to the protein coat

  - Through integrin receptors situated at cell surfaces the cells bind the coating proteins
  - Cells respond specifically to proteins; the interfacial protein film may be controlling subsequent bioreactions to implants.
  - Cells can adhere, release active compounds, recruit other cells, commit suicide, or grow in response to the proteins on surfaces.
  - Protein adsorption is also a concern for biosensors, immunoassays and anything else coming into contact with body fluids.

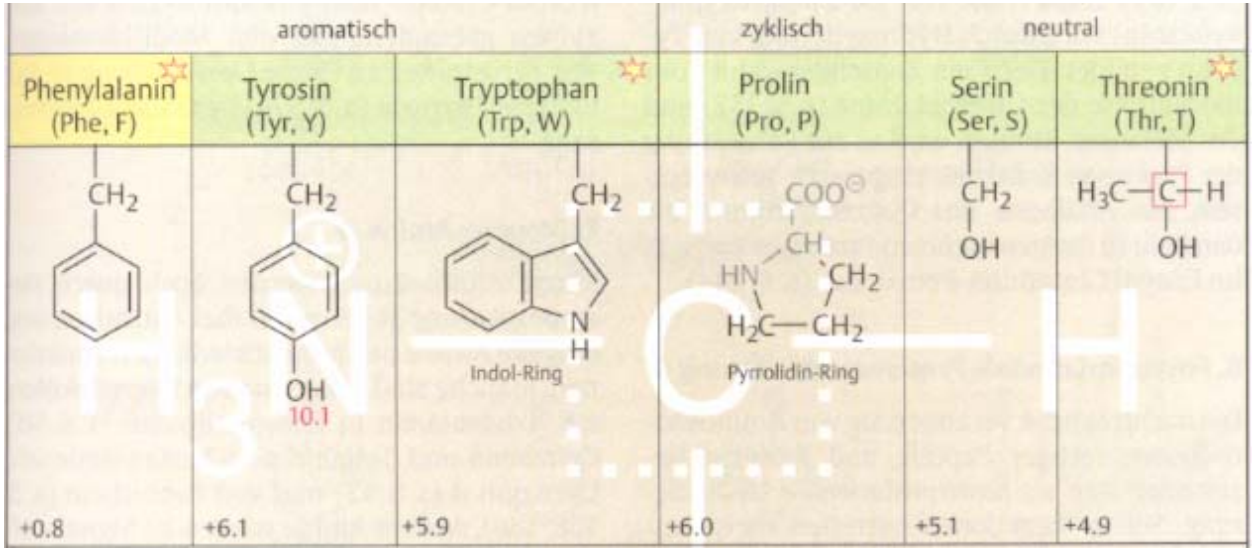


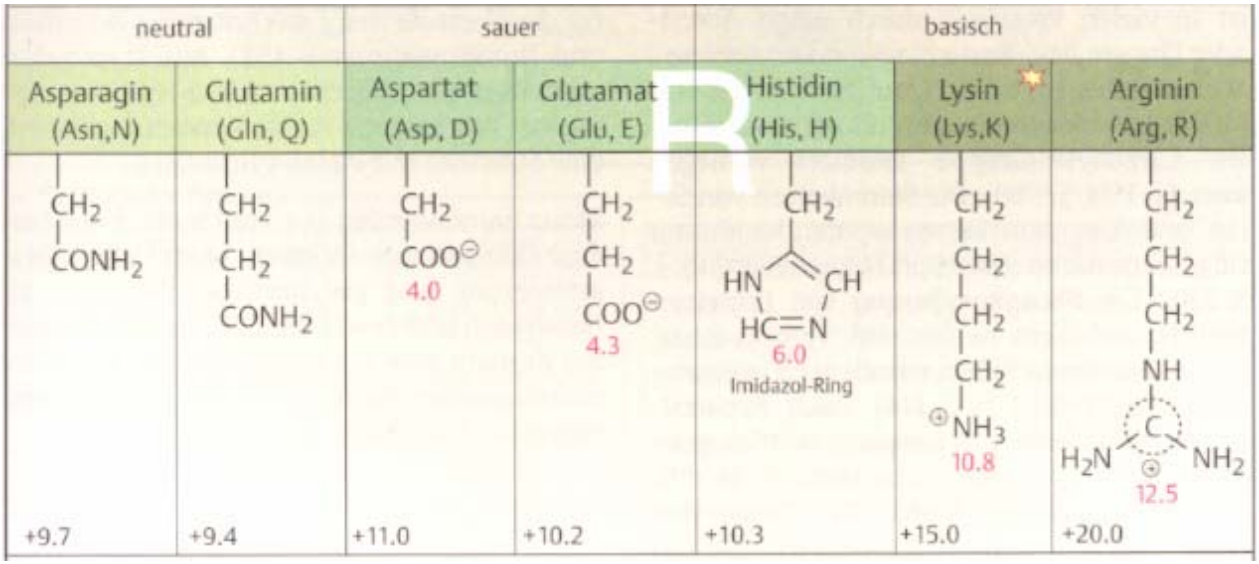
Biological background: Proteins

- Proteins are build from (20) amino acids.
- The side chains of amino acids determine the properties of the proteins:
  - Solubility
  - Interaction with surfaces



Biological background: Proteins





Biological background: Proteins

- Depending on the pH and ionic strength of a media, a large range of charge interactions can be expected between the protein and a surface:
  - Negatively charged proteins adsorb preferentially to positively charged surfaces,
  - and positively charged proteins to negatively charged surfaces.

Protein	Function	Location	Surface activity
Albumin	Carrier	Blood	Low on PE
Fibrinogen	Clotting	Blood	High on PE
IgG	Antibody	Blood	Low on PE
Lysozyme	Bacterial lysis	Tear; hen egg	High on negatively charged surfaces
Hemoglobin	Oxygen carrier	Red cells	Very high on PE
Hemoglobin S	Oxygen carrier	Sickle red cells	Oxy form of HbS has much higher air–water activity than normal Hb
Myoglobin	Oxygen carrier	Muscle	Unknown
Collagen	Matrix factor	Tissue	

Biological background: Proteins

TABLE 3 Enrichment of Proteins Adsorbed on Polyethylene Exposed to Blood Plasma

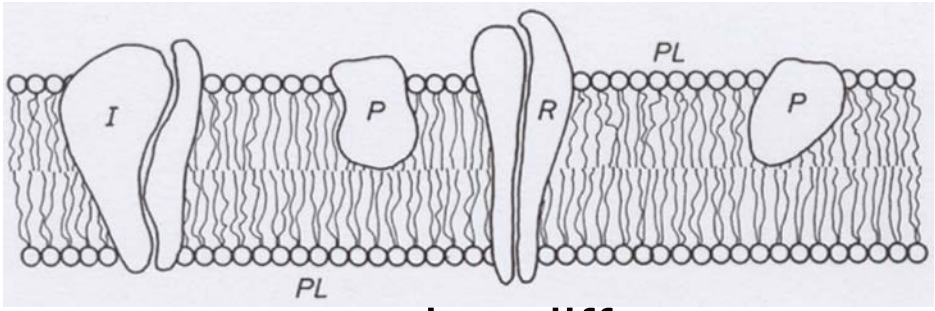
Protein	Enrichment[a]
Fibrinogen	1.3
γ-globulin	0.53
Albumin	0.88
Hemoglobin[b]	79

- Depending on their properties different proteins have different adsorption rates to surfaces.
- The adsorption of proteins to solid surfaces is largely irreversible and leads to the immobilization of the protein in the surface phase.
  - Platelets and cells adhere to adsorbed fibrinogen but do not bind to dissolved fibrinogen.
  - Other adhesion proteins in plasma for cells and platelets:
    - Fibrinogen, fibronectin, vitronectin, von Willebrand factor

    Adhesion can be reduced by precoating materials with proteins that do not interact with cell-integrins, eg. Albumin or IgG
  - Biological activity of the adsorbed protein varies on different surfaces

Biological background: Cells

- The cell membrane surrounds organells and cytoplasm.
- The membrane is a dynamic structure of a double layer of phospholipids in which
  - Proteins
  - Glycoproteins
  - Lipoproteins
  - and carbohydrates float



- Different membrane regions correspond to different functions:
  - Absorption
  - Secretion
  - Fluid transport
  - Mechanical attachment
  - Communication with other cells and ECM components

Biological background: Cells

- Mikrofilaments in the cyto-plasma (made of actin, myosin, actinin, and tropo-myosin) can be connected with the cell membrane via integrin structures.

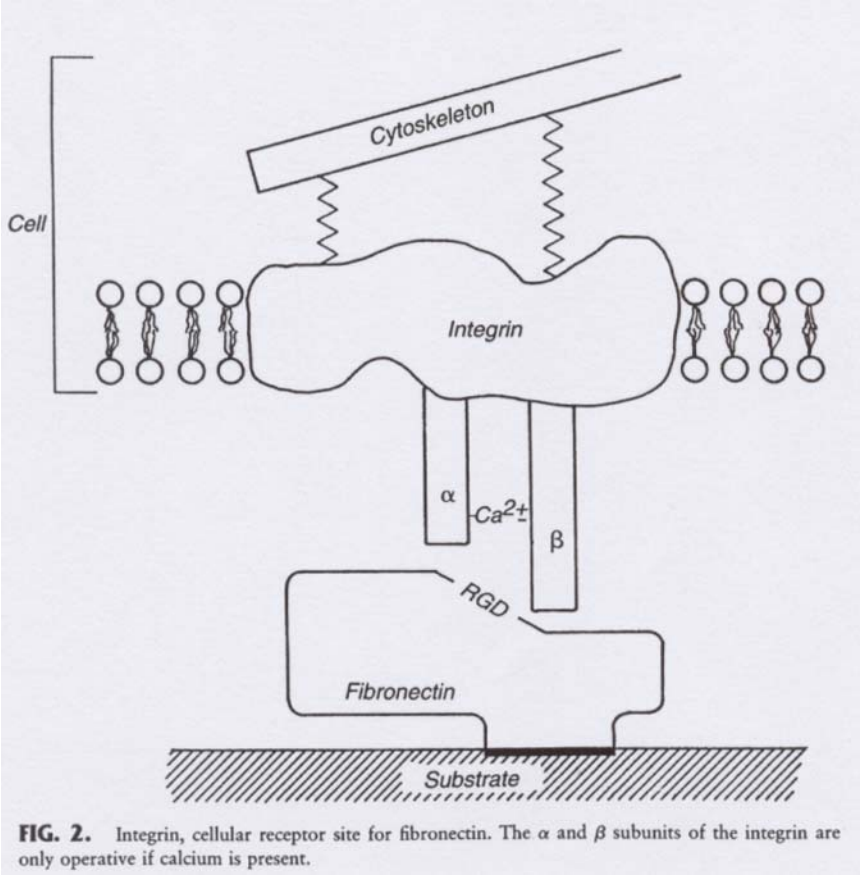


FIG. 2. Integrin, cellular receptor site for fibronectin. The α and β subunits of the integrin are only operative if calcium is present.

Integrins are receptors binding specific sequences in specific proteins:

e.g. RGD (Arg-Gly-Asp) in fibronectin or other adhesive proteins

Biological background: Cells

- The adhesive proteins can bind to solid substrate, ECM-components, and other cells.
- The ECM (extracellular matrix) has collagen, glyco-proteins, elastin, proteoglycans as major constituents.
- ECM-functions:
  - Mechanical support for cellular anchorage
  - Determination of cell orientation
  - Control of cell growth
  - Maintenance of cell differentiation
  - Scaffolding for tissue renewal
  - Establishment of tissue environment
  - Specialized functions:
    - Strength (tendon)
    - Filtration (kidney glomerulus)

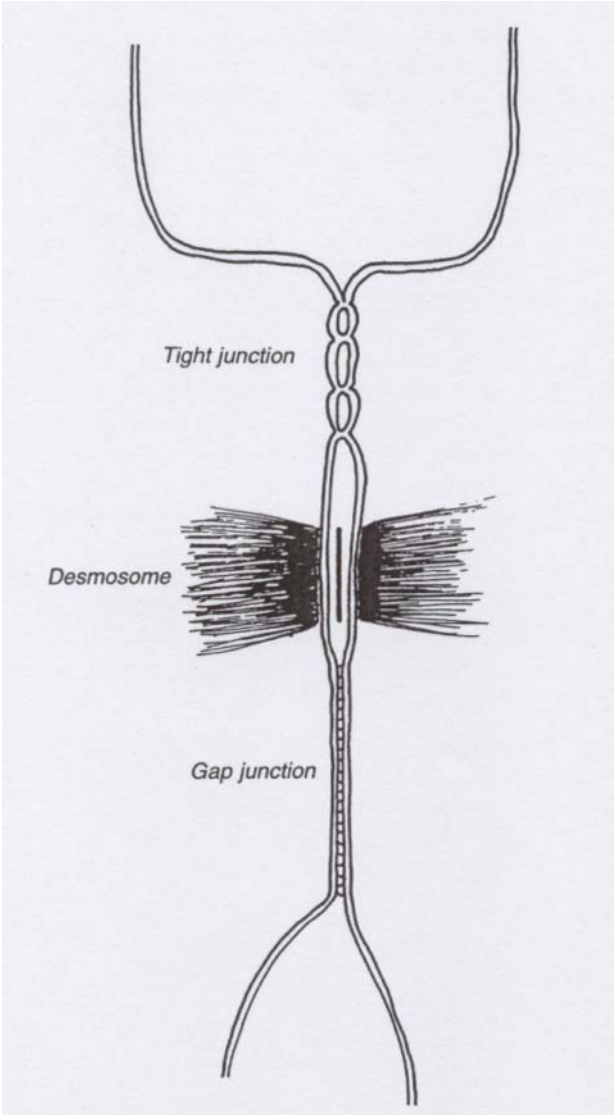
Biological background: Cells

- There are two types of ECM:
  - The interstitial matrix:
    - Is produced by mesenchymal cells and contains
    - Fibrillar collagen, fibronectin, hyaluronic acid and fibril-associated proteoglycans
  - The basal lamina:
    - Is produced by overlying parenchymal cells
    - Contains a meshlike collagen framework, laminin, and heparan sulfate proteoglycan

    In bone and teeth the ECM becomes calcified to produce additional mechanical strength.

Biological background: Cells

- There are four regular adhesive sites between cells and between cells and ECM:
  - **Gap junctions**: connections between the membranes of adjacent cells (pores).
  - **Desmosomes**: mechanical attachment formed by the thickened membrane of two adjacent cells.
  - **Hemidesmosomes**: structure similar to desmosomes between cells and ECM.
  - **Tight junctions**: adhesion sites between adjacent cells; create a barrier to diffusion.



Biological background: Cells

- Adhesive sites between cells and solid substrate:
  - **Focal adhesions**: very strong, often at cell boundaries, involves fibronectin
  - **Close contact**: often surrounding focal adhesions
  - **ECM-contact**: strands and cables of ECM material connect the ventral cell wall with the underlying substratum.

Preabsorbed proteins and cell proteins determine the strength and type of adhesion.

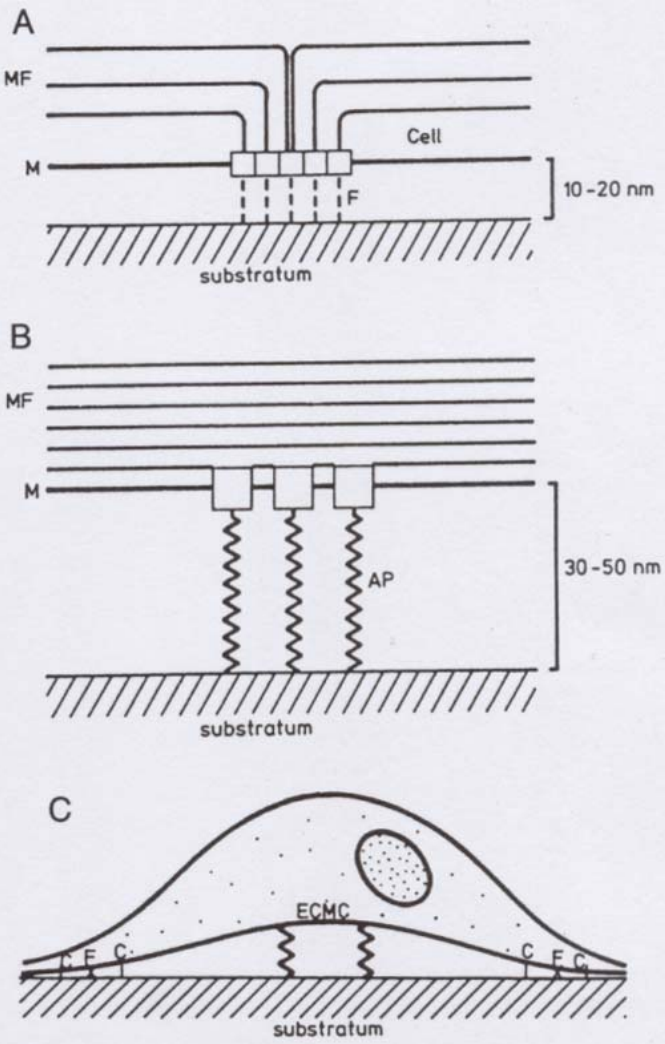


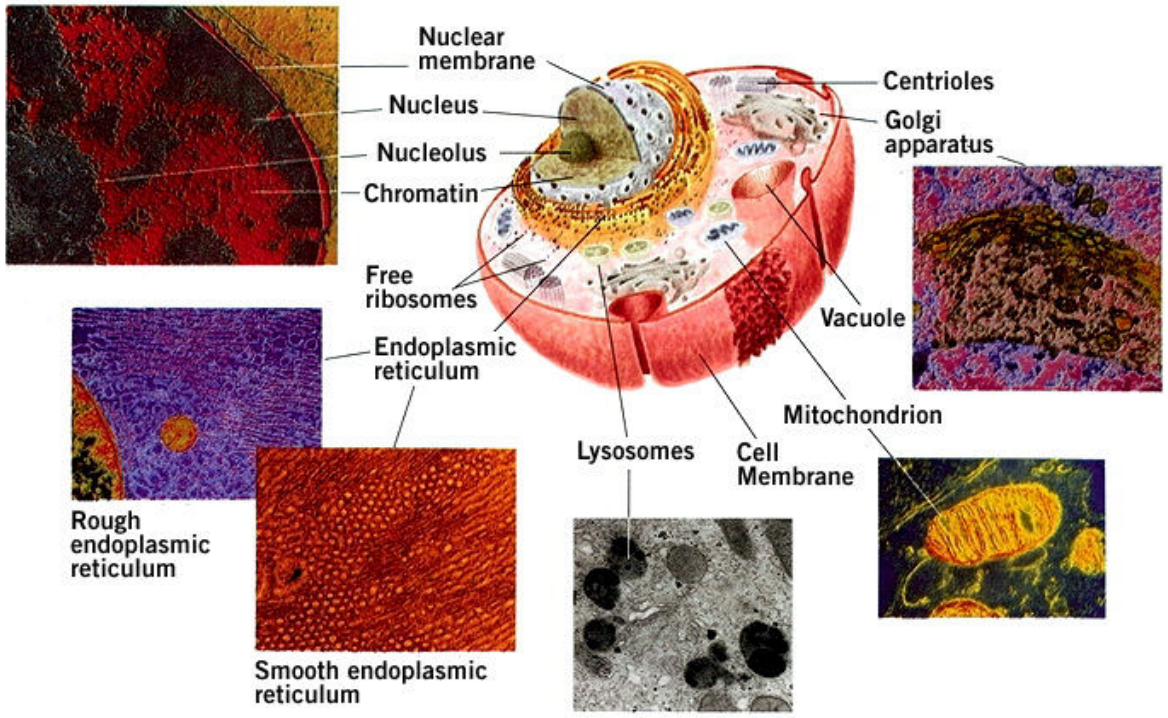
FIG. 4. Cell–substratum contact sites. (A) Focal adhesion sites are predominantly found at the boundaries of cellular extentions. The integrin connects the cytoskeleton with the substratum via fibronectin. (B) Close contacts represent less strong adhesive sites. (C) Localization of the different adhesive sites. MF, microfilaments; M, cell membrane; AP, adhesive protein; F, fibronectin; ECMC, extracellular matrix contact; C, close contact; F, focal adhesion.

Protein and Cell absorption

- The surface topology of a biomaterial can be classified according to roughness and porosity:
  - Roughness at a level of cell adhesion (1µm) is different from roughness at the level of protein adsorption (50nm).
  - Reported that smooth surfaces are more blood compatible than rough surfaces; rough surfaces promote adhesion.
  - For optimal biocompatibility: pore size 1-2µm.
    - Smaller pore sizes cause poor adhesion and increased inflammatory response with little collagen formation.
    - Larger pore size allow ingrowth and anchorage, but can cause a more severe foreign body reaction.
    - Pores in ridge-form can cause muscle and nerve precursor-cells to align and form “tissues” in then direction of the ridges.
    - By topographing material surfaces particular cell-adhesions, proliferations, “tissue”-formations can be engineered.

Organization of cells and tissues

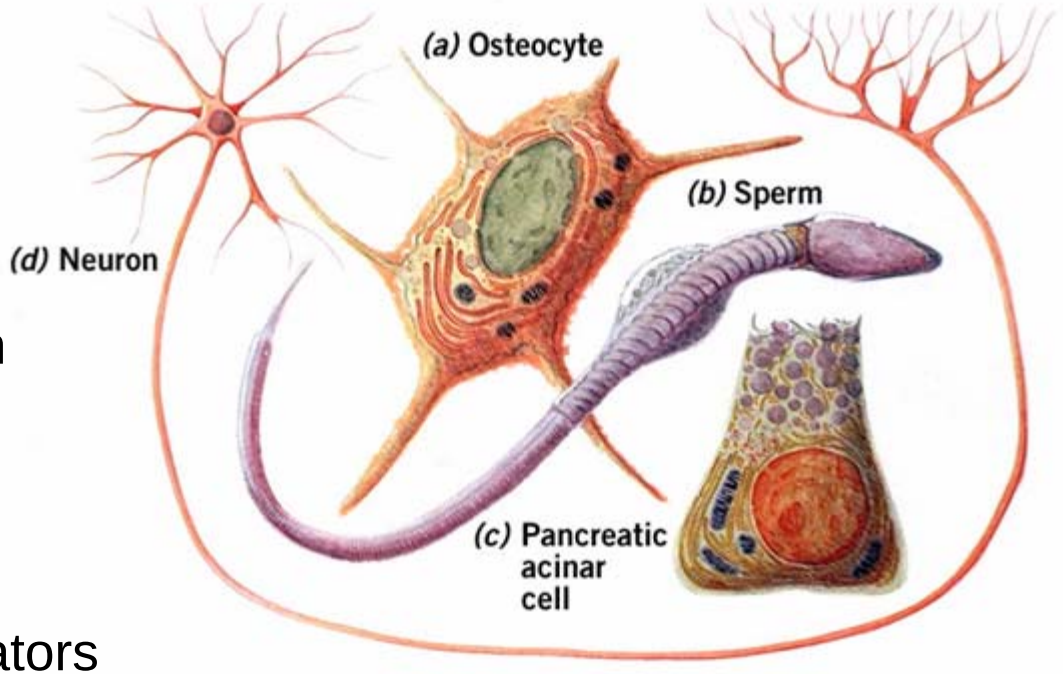
- The relative amount and type of organelles reflect and support the specific functions of the cells:
  - Nucleus
  - Mitochondria
  - Endoplasmatic reticulum
  - Golgi complex
  - Lysosomes
  - Cytoskeleton



Cells functions

- Basic functions (nearly all cells):
  - Nutrient adsorption and assimilation
  - Respiration
  - Synthesis of macromolecules
  - Growth and reproduction

- Specialized functions:
  - Irritability
  - Conductivity
  - Absorption or secretion of specific molecules:
    - Hormones
    - Structural proteins
    - Inflammatory mediators



Cells functions

- Cells either proliferate or differentiate (specialize)
- Humans are composed of differentiated cells (>200).
- Differentiation goes along with loss in capacity for specialization in other ways.
- Cellular differentiation in-volves an alteration in gene expression.
- Differentiation is induced or inhibited by extracellular stimuli (including substances released from implants)

TABLE 1 The Basic Tissues: Classification and Examples

Basic Tissues	Examples
Epithelial tissue	
Surface	Skin epidermis, gut mucosa
Glandular	Thyroid follicles, pancreatic acini
Special	Retinal or olfactory epithelium
Connective tissue	
Connective tissue proper	
Loose	Skin dermis
Dense (regular, irregular)	Pericardium, tendon
Special	Adipose tissue
Hemopoietic tissue, blood and lymph	Bone marrow, blood cells
Supportive tissue	Cartilage, bone
Muscle tissue	
Smooth	Arterial or gut smooth muscle
Skeletal	Limb musculature, diaphragm
Cardiac muscle	Heart
Nerve tissue	Brain cells, peripheral nerve