Cells

1. Brief intro of cell:

(1) Cells are the structural and functional units of all living organisms.

✷ **What do cells need to live?**

1. Nutrients (ie; amino acids)
2. Fuel/energy source (ie; glucose)
3. Oxygen
4. 3D scaffold structure
5. Correct chemical environment (pH, salinity)

(2) Three main regions: nucleus, plasma membrane and cytoplasm

I. Nucleus:

- Contains genetic material (DNA)
- Three regions: Nuclear membrane (double phospholipid membrane), Nucleolus and Chromatin

II. Plasma membrane:

- Double phospholipid membrane
- Composition:

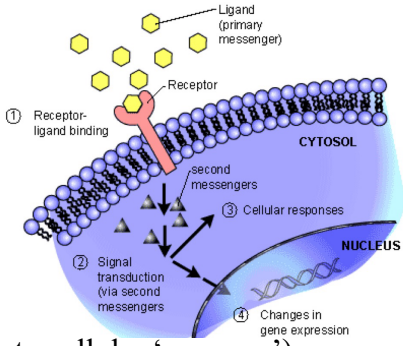
Lipids

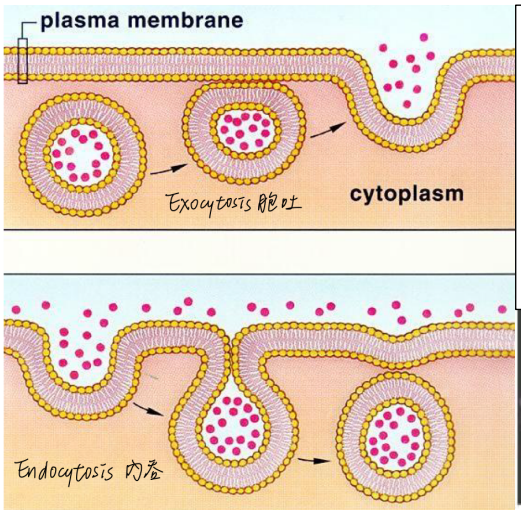
Protein:

transport proteins

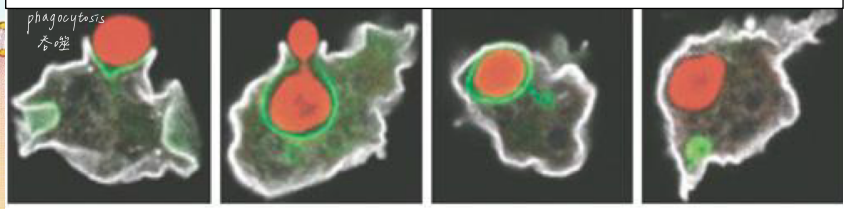
surface membrane proteins (signal receptors)

receptor protein (transduces the signal into an intracellular 'message')





- Endocytosis (內吞) –import materials from the external medium by capturing them in vesicles that pinch off from the plasma membrane.
- Exocytosis(胞吐) – the converse process, by which cells export material synthesized in the intracellular compartments.
- Phagocytosis (吞噬) – a special case of endocytosis, in which the material carried into the cell is a smaller cell or cell fragment



- Phagocytosis

(1) recognition of the target particle,

(2) ingestion of it in a phagosome (phagocytic vacuole),

(3) maturation of this phagosome into a phagolysosome,

(4) final destruction of the ingested particle in the robust antimicrobial environment of the phagolysosome.

III. Cytoplasm (細胞質):

- Mitochondria (粒線體): chemical energy for the cell, cellular respiration (細胞呼吸); double layer membrane

- Endoplasmic reticulum (內質網): protein synthesis

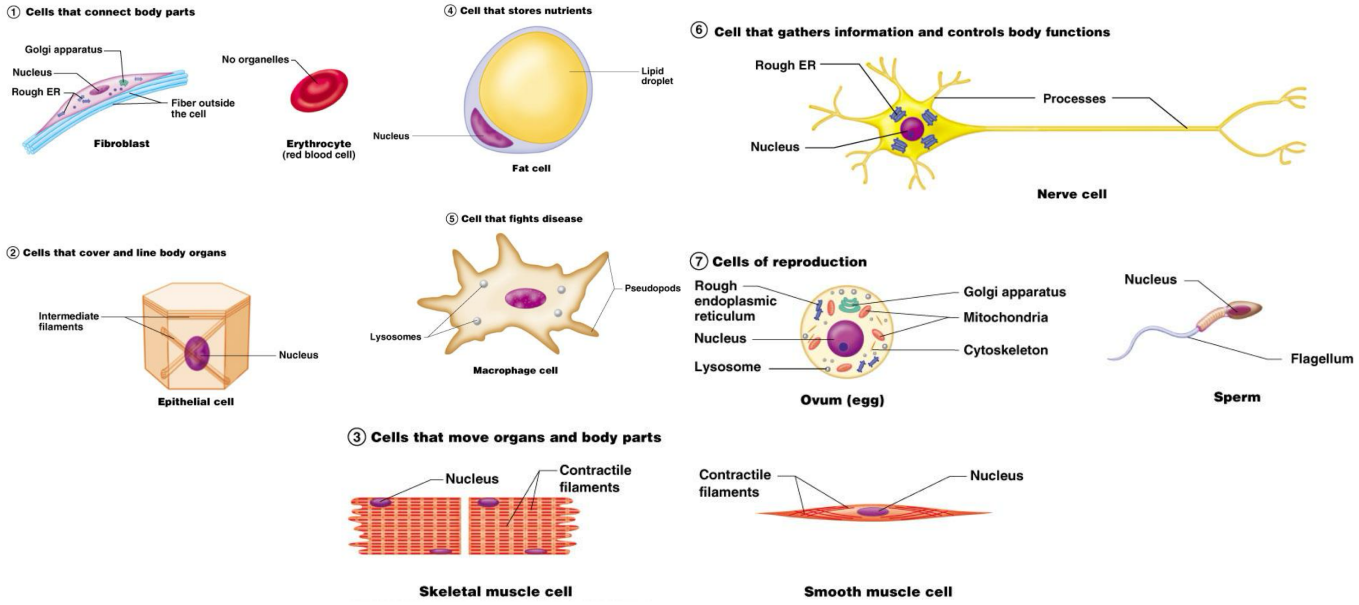
-Ribosomes (核醣體): translation (轉譯)

-Lysosomes (溶小體): Intracellular digestion of food

(3) The essential cell attributes are:

- Protection from the environment
- Acquisition of nutrients (獲取營養)
- Movement
- Communication
- Catabolism (分解代謝)
- Energy generation
- Self-replication（自我複製）

(4) Types of cells:



(5) E.g. Blood cells:

- Erthrocytes (red blood cells): Transport oxygen (using hemoglobin 血紅蛋白) to tissue
- Leukocytes (white blood cells): Defend the body against infection and foreign materials

e.g. Neutrophils (嗜中性球): phagocytosis

Monocytes (單核細胞): Once in tissue, it becomes a macrophage (巨噬細胞)

- Platelets (血小板): initiate blood clotting, repair

(6) Co-culture: Direct cell-to-cell contact→Might have an impact on cell migration, growth, proliferation and differentiation

Tissue

(1) Tissues are groups of cells that are similar in structure and function

✸ **What do tissues need to live?**

Living tissue needs a blood supply – vascularized network of blood vessels

(2) Four primary tissue types:

I. Epithelial: covering

Covering and lining epithelium, Glandular epithelium, Endothelium (the inner lining of blood vessels, the heart, and lymphatic vessels) is a specialized form of epithelium.

II. Connective: Binding and support, Protection, Insulation, Transportation

III. Nervous: control

IV. Muscular: movement

Tissue Type	Description	Subtypes
Epithelial	line inner and outer surfaces	based on shape (squamous, cuboidal, columnar) and number of layers (simple, stratified, pseudostratified)
Connective	provide structure and connection; characterized by noncellular matrix outside the cells	loose, dense, fibrous, cartilage, bone, blood, adipose
Nervous	coordinate receipt of and response to stimuli	neurons, glial cells
Muscle	movement is a consequence of contraction of these cells results in movement	smooth, striated, cardiac

(3) **Extracellular matrix** (細胞外基質, ECM): (下方 scaffold 的地方會在仔細介紹)

ECM is a dynamic structure→need to remodel collagen constantly to maintain structure

- Structural and biochemical support to the surrounding cells
- **Cell Signaling**, cell adhesion (cell-to-cell communication and differentiation) is a common function of ECM.
- Regulation of Cell Behavior (differentiation and function expression)

(4) Cell adhesion (細胞黏附): is the binding of a cell to a surface or substrate (是指細胞之間或細胞與細胞外基質（ECM）之間通過專門的蛋白質相互結合和附著的過程

Common cell adhesion molecules (CAM, 細胞黏附分子): integrins 整合素

(5) Cell signaling:

✸ **How do cells talk to one another?**

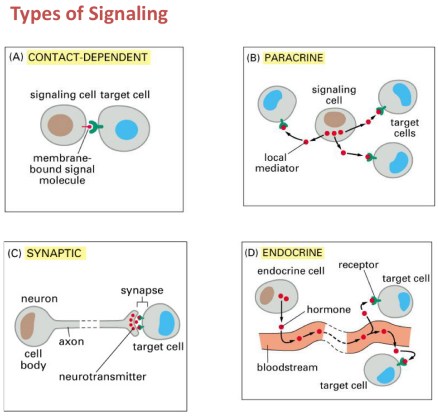
Contact (dependent) - via proteins in the plasma membrane

Autocrine (自分泌) - via growth factors, cell that releases the signal is also the target.

Paracrine (旁分泌) (重要)- via neurotransmitters and cytokines, action on adjacent target cells.

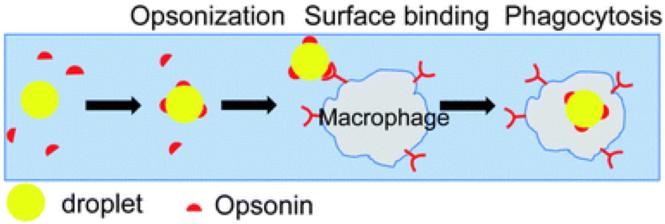
Endocrine (內分泌)- via hormones (for example), action on distant target cells.

Synaptic (突觸) - via neurotransmitters, action on post-synaptic cell in response to electrical stimuli

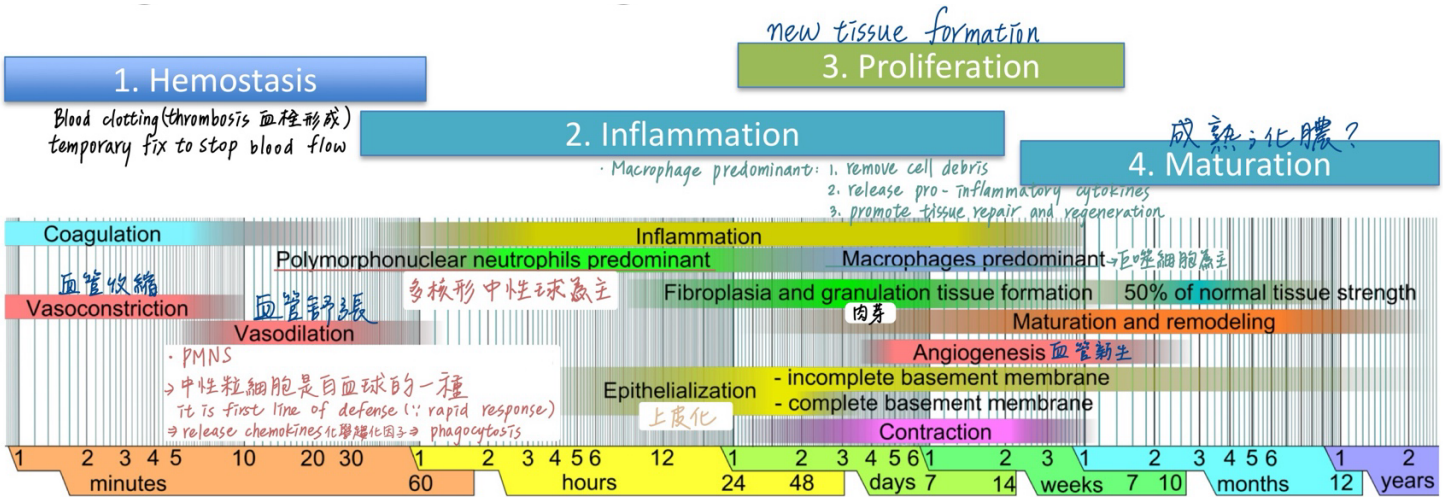


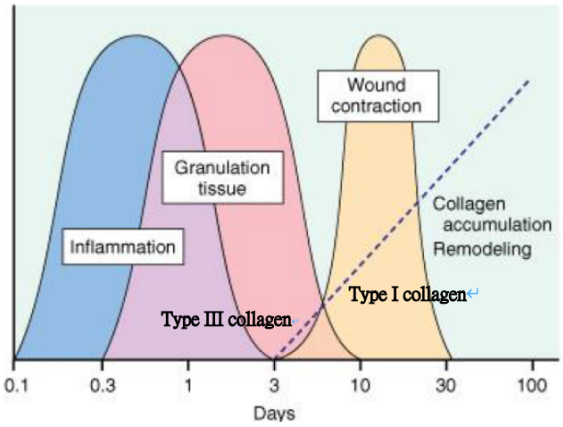
Inflammatory

1. Inflammation 發炎: is the standard, initial response of the body to injury
   (1) Function - control, neutralize or isolate the damaging species.
   (2) Four clinical Sign of inflammation:
      I. Redness: Due to a higher local concentration of red blood cells (erythrocytes), this lead to rapid vasodilation (血管舒張) and an increase in blood flow into the capillary (微血管) beds, in turn cause redness.
      II. Pain: Swelling activates local deep pain receptors.
      III. Swelling: Increased permeability (滲透性)of the capillary endothelium allows fluid (extracellular fluid) to move into the surrounding tissue.
      IV. Heat: May be associated with increased blood flow, increased cellular activity.
2. Inflammatory response: (tissue damage foreign material) –
      I. It is the defensive mechanisms (防禦機制) initiated by the immune system when the body faces infections and injuries.
      II. A localized (局部的) physical condition in which part of the body becomes reddened 紅, swollen 腫, hot, and often painful.
      III. Neutrophils are the first responders, and represent the first line of defense – early/acute inflammation
3. Innate immune response:
      I. Mainly relies on phagocytes (吞噬細胞) to remove pathogens (病原體)
      II. Types of Phagocytes, including several types of white blood cells (leukocytes):
      - Neutrophils (中性粒細胞)(重要): primary role is phagocytosis (swallowing bacteria, foreign material or dead cells) cannot differentiate further or multiply (mitosis)
      - Monocytes (單核細胞)
      - Macrophages (巨噬細胞) (重要): actively phagocytose materials, can continue to differentiate
      - Mast Cells (肥大細胞)
      - Dendritic Cells (樹突細胞)
      III. Identifying foreign materials:
      Opsonins (調理素) are any molecules that enhance phagocytosis
      Mark an antigen (抗原) for an immune response, or identify dead cell/fragments for recycling through enzymatic degradation



4. Wound healing:
   (1) Wound healing is a complicated process where tissue (ie, skin tissue) repairs itself after injury
   (2) The process of wound healing, 4 phases: (重要)

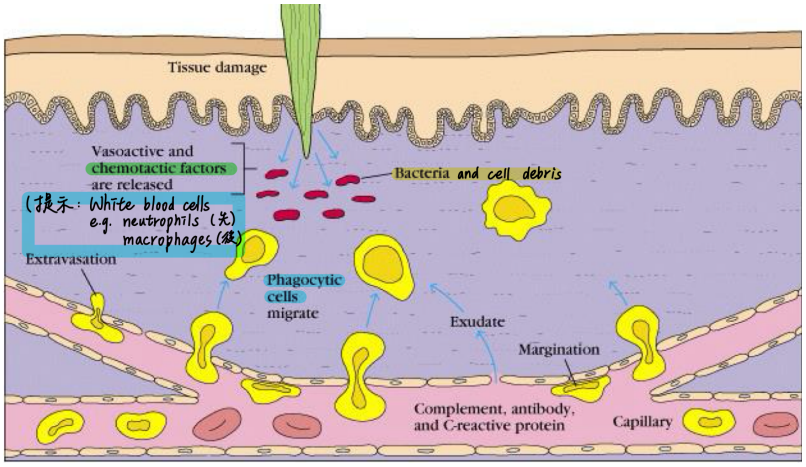


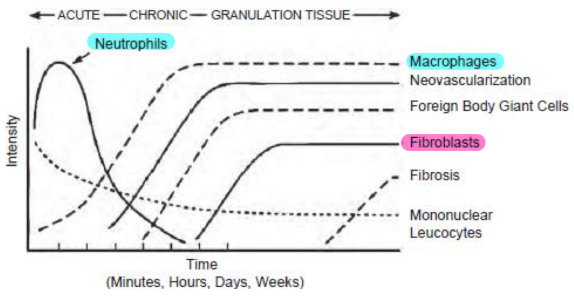


I. Hemostasis (blood clotting)血液凝固: platelets (血小板) adhere to the site of injury, become activated→coagulation (凝固) cascade → forms a clot, stopping active bleeding (hemostasis)→ When clot dries→ Scab (痂) forms reducing the risk of infection

II. Inflammation 發炎:

- Chemical signals (cytokines 細胞因子) are released →attract neutrophils (中性粒細胞) migrate to the wound site
- Removal of toxins and waste: White blood cells remove and phagocytose (吞噬) bacteria and cell debris (碎片) from the wound.
- Stimulate growth of other cells





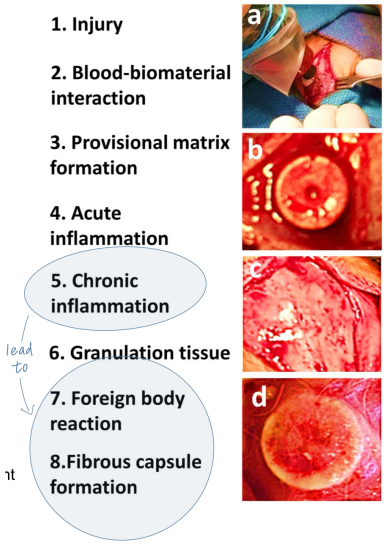
III. Proliferation 增生- fibroplasia and granulation tissue formation

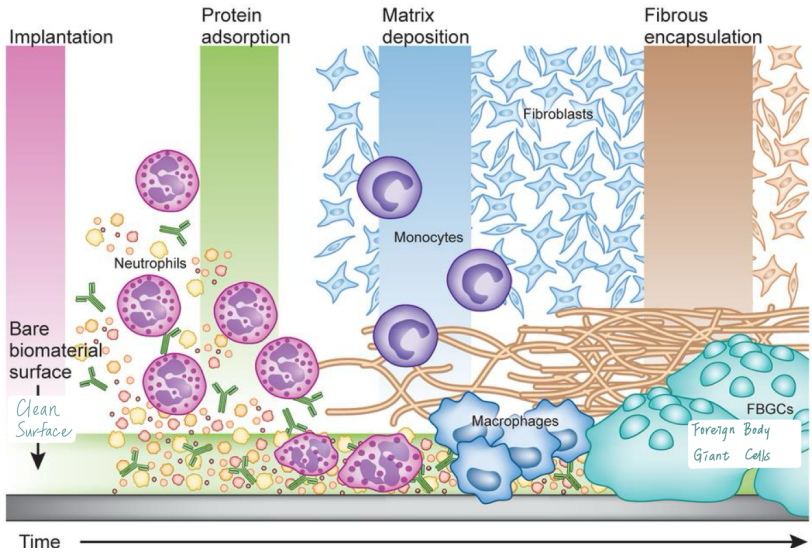
- Growth factors are released by platelets into the wound→ cause the migration and division (分裂) of cells
- Angiogenesis, the growth of new blood vessels, results in vascularization of the new granulation tissue.
- The fibroblasts contract the edges of the wound together by pulling on the collagen network.

IV. Maturation – tissue remodeling

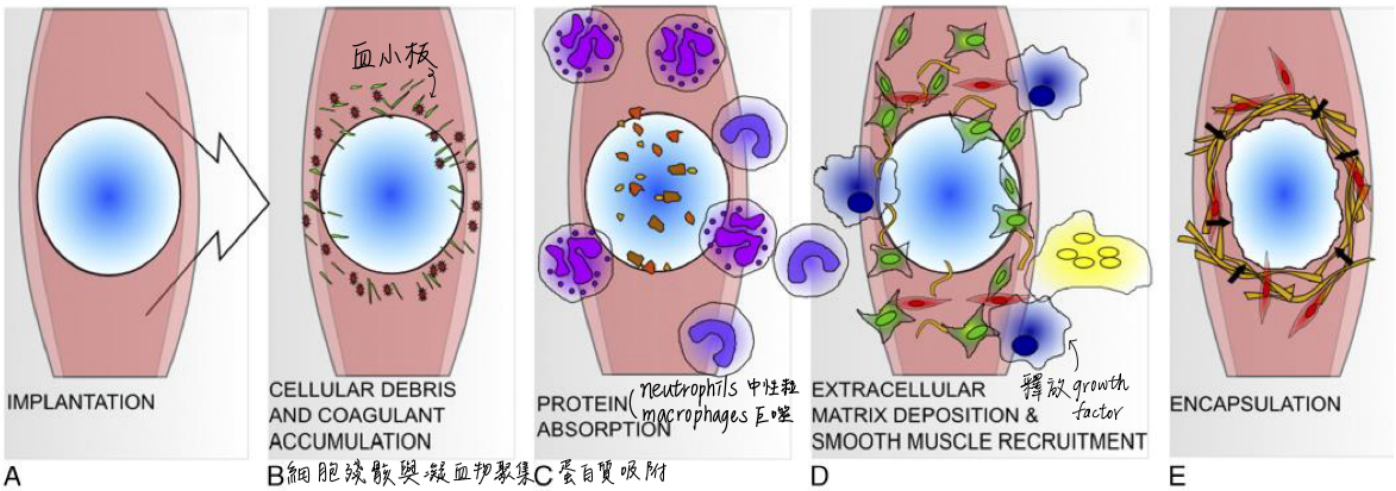
Foreign body response

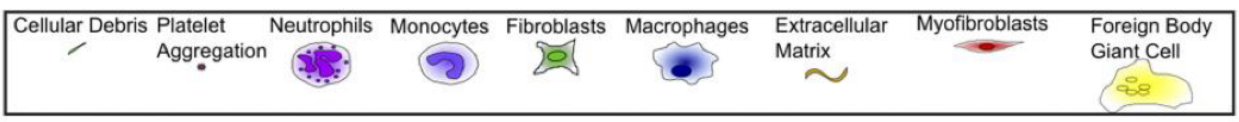
1. Foreign materials implantation:



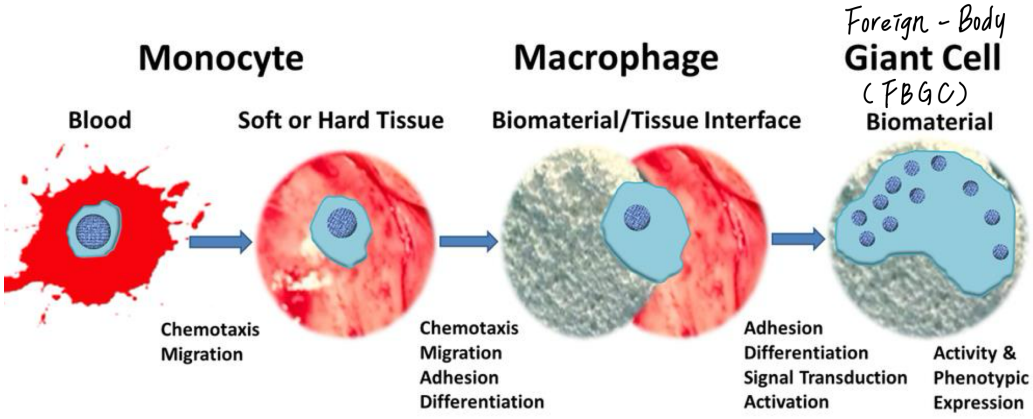


2. Brief intro of foreign body response (reaction):
- Foreign body response is essentially a form of the body's wound response and repair of injury.
- The foreign body reaction is indicated by the presence of foreign body giant cells and the components of granulation tissue (macrophages, fibroblasts, and capillaries in varying amounts)
- A typical outcome of foreign material implantation is the formation of a fibrous encapsulating layer, to form a foreign body granuloma (肉芽腫, an aggregation of macrophages formed due to chronic 慢性 inflammation)





- Foreign-Body Giant Cells (FBGCs): When the particle sizes are beyond the capacity of a single macrophage to internalize, FBGCs are formed to phagocytize.
FBGCs attempt to phagocytize large particles. They remain at the
biomaterial–tissue interface for the lifetime of
the implanted device
• Known as "frustrated Sometimes they fail, which known as "frustrated phagocytize.



- Fibrous capsule (纖維囊) formation: The final stage of the foreign body response and healing process is the development of a fibrous encapsulation of collagen layers, known as fibrosis.

3. Outcome of foreign material in the body:
   I. Fibrous encapsulation (most usual response): fibrous capsule is formed around the implant
   II. Integration: e.g. the implantation of certain metals in bone, there can be a well-integrated growth of normal host tissue to the implant – for bone this is called osseointegration (骨整合)
   III. Resorption: For resorbable implants
   IV. Extrusion (排出、突出): The implant near the skin (epithelial tissue) can be extruded or (externalized) from the body.
4. Tissue response to implants:
   I. Acute inflammation: is short term (minutes to a few days).
      - Once at the injury site, phagocytosis and enzymatic release occurs→the activation of neutrophils and macrophages whose purpose is to digest foreign
      - This involves recognition, attachment, engulfment and degradation.
   II. Chronic inflammation is long term (≥≥ days).
      - Lymphocytes 淋巴球 and plasma cells 漿細胞 are involved in immune reactions- mediate antibody production.
      - Macrophages process and deliver antigen 抗原 to immuno-competent cells 免疫活性細胞

   ✷ **Question: What causes chronic inflammation?**

   Ans:- Non-biocompatible material (chemically unstable, toxic, mechanically weak)
   - Debris due to wear, degradation
   - Infection, due to bacteria (during surgery, or insufficient sterilization)
5. Biocompatibility of implant and other biomaterials, involves:
   I. an acceptable, stable physiological response, in contact with the surrounding tissue
   II. pharmacological acceptability (nontoxic, nonallergenic, nonimmunogenic, noncarcinogenic)
   III. chemically inert and stable (typically no time-dependent degradation, unless by design)
   IV. adequate mechanical properties and fatigue life

V. a minimum susceptibility to infection

- **Question: What determines whether a material is biocompatible or not??**

Ans:

I. Many physical, chemical and structural properties of materials will contribute to the foreign body response.

II. The biomaterial surface will often determine the degree of the foreign body response.

Even for “biocompatible” materials, a high surface to volume ratio of fabrics, porous structures, etc. will result in higher ratios of macrophages and foreign body giant cells than a smooth component made of the identical material.

Protein absorption

- The properties of biomaterials that influence protein absorption:

1. Material composition
2. Environment
3 Chemistry (polar/non-polar)
4. Topography (roughness, porosity)

- Several properties of a protein result in changes to protein adsorption:

1. Size and charge of protein:
   (1) Smaller proteins tend to diffuse faster towards a surface
   (2) Larger proteins have more active sites to bind to a surface
2. Hydrophobicity of protein
   (1) In water, hydrophilic residues 残留物 are more present on the surface of the protein
3. Structural changes in protein – soft vs. hard proteins
4. Chemical composition and surface chemistry

Tissue engineering and regenerative medicine

1. Tissue engineering:
   (1) Application of methods from engineering and life sciences to create artificial constructs to direct tissue regeneration
   (2) Major concepts of tissue engineering: isolate specific cells through a small biopsy from a patient → grow them on a three-dimensional biomimetic scaffold→deliver the construct to the desired site in the patient's body→direct new tissue formation into the scaffold that can be degraded over time
   (3) Three types of tissue engineering:
   - Regeneration - Identify the cues and processes that can allow for whole tissue or organ growth
   - Repair - Stimulate the tissue to repair itself.
   - Replace - A biological substitute is created in the lab that can be implanted to replace the tissue

   Three elements that are essential for regeneration:
   1. biomaterial scaffold with mechanical support
   2. progenitor cells (祖細胞) that can differentiate into specific cells
   3. inductive growth factor

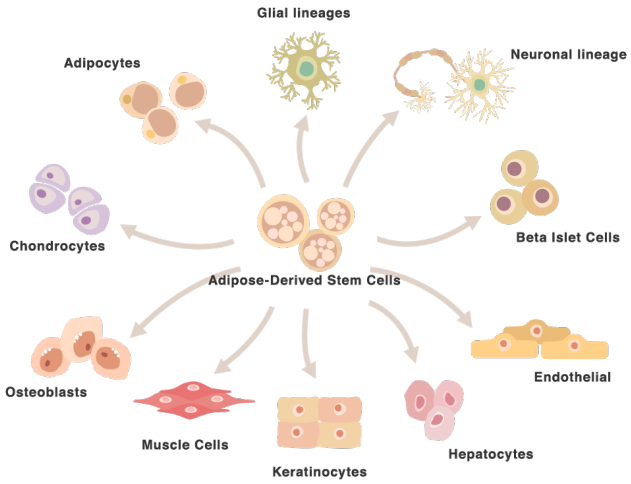
   (4) Types of cells used for tissue engineering:
   - Autologous cells 自體細胞 are obtained from the same individual to which they will be re-implanted.
   - Allogeneic (同種) cells come from another donor
   - Xenogenic (異種) cells are these isolated from another species – e.g. animal cells
2. Scaffolds:
   (1) The feature elements of the scaffolds:
   • Biological components - cells
   • Biochemicals – Signaling cues to direct cell growth and differentiation (ie; growth factors)
   • Non-biological components (Polymer scaffold, Fibers, Plastic, Gels)
   (2) Scaffolds must meet some specific requirements:
   • A high porosity and an adequate pore size → cell seeding and diffusion throughout the whole structure of both cells and nutrients.
   • Vascularization requires relatively large pores
   • Biodegradability→ scaffolds should preferably be absorbed by the surrounding tissues without the necessity of a surgical removal.
   • The rate of degradation should almost be same as the rate of tissue formation
   • Injectability (可注射性) is sometimes useful for certain clinical applications
   (3) Types of scaffold materials:
   • Bioresorbable polymers – synthetic polymers, and different derivatives of the extracellular matrix has been studied to evaluate their ability to support cell growth.
   • Protein type polymers, such as collagen or fibrin
   • Polysaccharide polymers, like chitosan (derived largely from shrimp shells)
   • Polylactic acid (PLA) - a polyester which degrades within the body to form lactic acid, which is easily removed from the tissue

• Polyglycolic acid (PGA) and polycaprolactone (PCL) - have degradation mechanisms like PLA but have a faster and a slower rate of degradation (respectively) compared to PLA.

• Hydrogels have been developed - network of polymer chains that are hydrophilic, to make a water-containing gel

(4) Scaffold stiffness can induce changes in stem cell differentiation:

- Stiffer → osteoblasts
- Softer → neurons



(我找的圖例，不用背)

(5) Cell seeding in engineered scaffolds:

- Advantages:
  - Enhanced tissue regeneration through targeted cell placement
  - Improved cell survival by providing a favorable microenvironment
  - Customizable for various applications
- Limitations and problems:
  - Difficulty in achieving uniform cell distribution (細胞分布不均)
  - Cell viability issues
  - Mechanical properties mismatch between scaffold and native tissue
  - Potential immune response and scaffold rejection

(6) Scaffolds usually serve at least one of the following purposes:

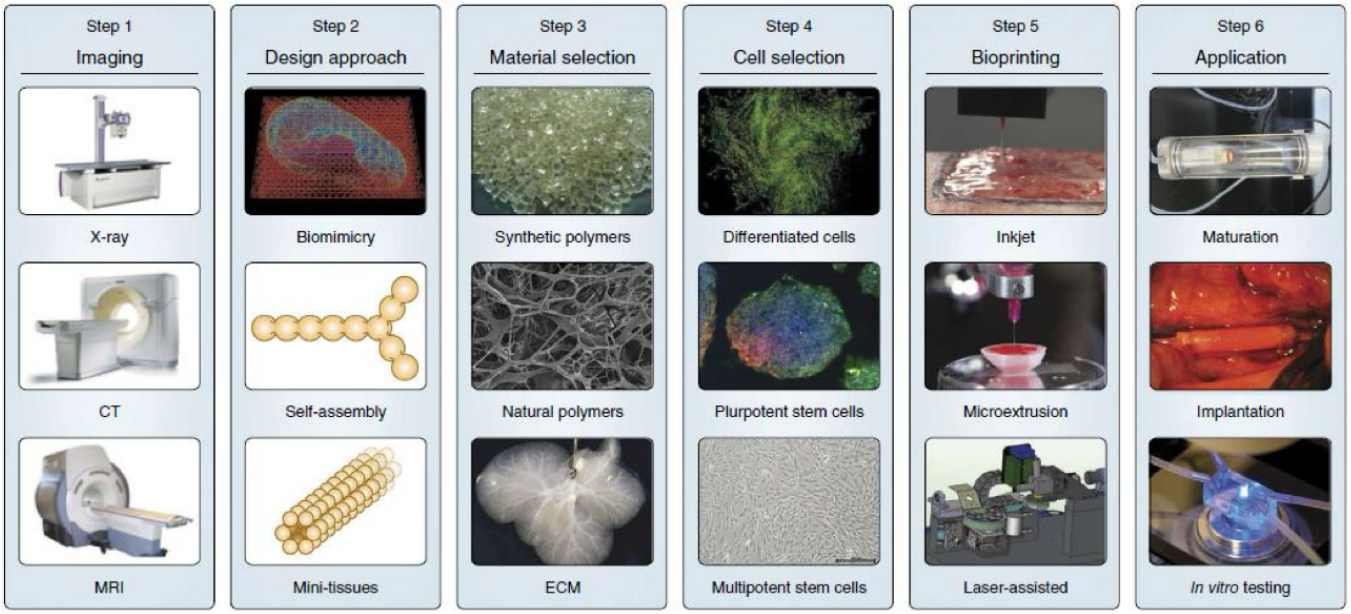
- Allow cell attachment and migration
- Deliver and retain cells and biochemical factors
- Enable diffusion of vital cell nutrients and expressed products
- Exert certain mechanical and biological influences to modify the behaviour of the cell phase

* **Question: PPT 中提到 3D structure can better mimic extracellular matrix (細胞外基質, ECM) to match specific tissue types 我的問題是為什麼要特別提到 mimic ECM environment 呢？**

Ans:

- Cell signaling: ECM with growth factors and cytokines→ induce cell growth and proliferation and tissue regeneration
- Structural and biochemical support to the surrounding cells: 細胞黏附（cell adhesion）的天然環境且提供彈性（elasticity）和強度（strength）
- Regulation of Cell Behavior (differentiation and function expression)

3. Stem cells:
   (1) Stem cells are undifferentiated cells with the ability to divide in culture and give rise to different forms of specialized cells.
   (2) Two different sources of stem cells:
      - Adult stem cells: multipotent 多功能 - potential to differentiate into multiple, but limited cell types
      - Embryonic (胚胎) stem cells: are mostly multipotent – can differentiate into any of the three main types of tissue: endoderm (內胚層), mesoderm (中胚層), or ectoderm (外胚層)
4. 3D bioprinting:
   (1) 3D bioprinting:



   (2) Step 5 bioprinting: