

STEM CELLS AND TISSUES ENGINEERING

(TEUFL, STROBL)

HAFNER: Part I

STRUCTURE, ORGANIZATION AND CONTROL OF CELLS AND TISSUE

Overview of Cellular Physiology

- The cell is the fundamental **working unit** of all organisms.
- The specialization of the cells in the various organs is considerable, and no cell can be called "typical" of all cells in the body.
- However, a number of structures (**organelles**) are common to most cells.

Organelles

- Cells contain a variety of organelles that perform **specialized cell functions**.
- The **nucleus** is an organelle that contains the cellular DNA and is the site of transcription.
- The **endoplasmic reticulum** and the **Golgi apparatus** are important in protein processing and the targeting of proteins to correct compartments within the cell.
- **Lysosomes** and **peroxisomes** are membrane-bound organelles that contribute to protein and lipid processing.
- **Mitochondria** are organelles that allow for oxidative phosphorylation in eukaryotic cells and also are important in specialized cellular signalling.



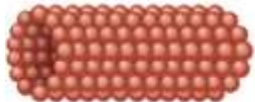
Cell Membranes

- The cell and the intracellular organelles are surrounded by semipermeable membranes.
- Biological membranes have a lipid bilayer core that is populated by structural and functional proteins. These proteins contribute greatly to the semipermeable properties of a biological membrane.

Cytoskeleton

The cytoskeleton is a network of **three types of filaments** that provide structural integrity to the cell as well as a means for trafficking of organelles and other structures around the cell.

1. **Actin filaments** are important in cellular contraction, migration, and signalling. Actin filaments also provide the backbone for muscle contraction.
2. **Intermediate filaments** are primarily structural.
3. **Microtubules** provide a dynamic structure in cells that allows for the movement of cellular components around the cell.

Cytoskeletal filaments	Diameter (nm)	Protein subunit
 Microfilament	7	Actin
 Intermediate filament	10	Several proteins
 Microtubule	25	Tubulin

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Molecular Motor Proteins

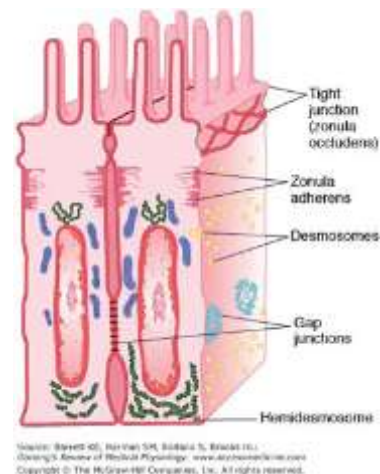
There are **three superfamilies** of molecular motor proteins in the cell that use the energy of ATP to generate force, movement, or both: **Myosins, kinesins, and cellular dyneins.**

Cell Adhesion Molecules

- Cellular adhesion molecules aid in tethering cells to each other or to the extracellular matrix as well as providing for initiation of cellular signalling.
- There are **four main families** of these proteins:
 - **Integrins**
 - **Immunoglobulins**
 - **Cadherins**
 - **Selectins**

Intercellular Connections

- Cells contain distinct protein complexes that serve as cellular connections to other cells or the extracellular matrix.
- **Tight junctions** provide intercellular connections that link cells into a regulated tissue barrier and also provide a barrier to movement of proteins in the cell membrane.
- **Gap junctions** provide contacts between cells that allow for direct passage of small molecules between two cells.
- **Desmosomes** and **adherens junctions** are specialized structures that hold cells together.
- **Hemidesmosomes** and **focal adhesions** attach cells to their basal lamina.



Transport Across Cell Membranes

- **Exocytosis** and **endocytosis** are vesicular fusion events that allow for movement of proteins and lipids between the cell interior, the plasma membrane, and the cell exterior.
- **Exocytosis** can be constitutive or non-constitutive; both are regulated processes that require specialized proteins for vesicular fusion.
- **Endocytosis** is the formation of vesicles at the plasma membrane to take material from the extracellular space into the cell interior.

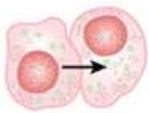

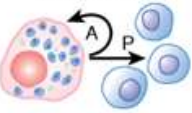
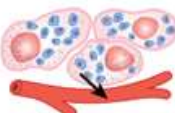
There are various types of **endocytosis** named for the size of particles being ingested as well as the regulatory requirements for the particular process.

These include **phagocytosis, pinocytosis, clathrin-mediated endocytosis, caveolae-dependent uptake, and non-clathrin/non-caveolae endocytosis.**

- **Phagocytosis** ("cell eating") is the process by which bacteria, dead tissue, or other bits of microscopic material are engulfed by cells such as the polymorphonuclear leukocytes of the blood.

Intercellular Communication

- Cells can communicate with one another via chemical messengers.
- Individual messengers (or ligands) typically bind to a plasma membrane receptor to initiate intracellular changes that lead to physiologic changes.
- **Plasma membrane receptor families** include:
 - Ion channels
 - G protein-coupled receptors
 - A variety of enzyme-linked receptors (eg. tyrosine kinase receptors).
- There are additional **cytosolic receptors** (e.g. steroid receptors) that can bind membrane-permanent compounds.
- Activation of receptors leads to cellular changes that include changes in membrane potential, activation of heterotrimeric G proteins, increase in second messenger molecules, or initiation of transcription.

	GAP JUNCTIONS	SYNAPTIC	PARACRINE AND AUTOCRINE	ENDOCRINE
				
Message transmission	Directly from cell to cell	Across synaptic cleft	By diffusion in interstitial fluid	By circulating body fluids
Local or general	Local	Local	Locally diffuse	General
Specificity depends on	Anatomic location	Anatomic location and receptors	Receptors	Receptors

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Organization of Vertebrate Body

There are **four levels of organization**:

- Cells
- Tissues
- Organs
- Organ systems

Bodies of vertebrates are composed of **different cell types**. Humans have **210**.

Tissues are groups of cells that are similar in structure and function. In adult vertebrates, there are **four primary tissues**:

- Muscle
- Nerve
- Epithelial
- Connective

A Muscle Tissue

Muscle is generally divided into **three types**: skeletal, cardiac, and smooth

1 Skeletal Muscle

- Skeletal muscle is a true **syncytium** (a multinucleate cell which results from multiple cell fusions of uninuclear cells) under voluntary control.
- Skeletal muscles receive electrical stimuli from neurons to elicit contraction: "excitation–contraction coupling."
- **Action potentials** in muscle cells are developed largely through coordination of Na^+ , K^+ , and Ca^{2+} channels.
- **Contraction** in skeletal muscle cells is coordinated **through Ca^{2+} regulation** of the actomyosin system that gives the muscle its classic striated pattern under the microscope.

2 Cardiac Muscle

- Cardiac muscle is a collection of individual cells (**cardiomyocytes**) that are linked as a syncytium by gap junctional communication.
- Cardiac muscle cells also undergo excitation-contraction coupling.
- **Pacemaker cells** in the heart can initiate propagated action potentials.
- Cardiac muscle cells also have a striated, actomyosin system that underlies contraction.

3 Smooth Muscle

- Smooth muscle exists as **individual cells** and are frequently under control of the **autonomic nervous system**.
- There are two broad categories of smooth muscle cells: **unitary and multiunit**.

Unitary smooth muscle contraction is synchronized by gap junctional communication to coordinate contraction among many cells. Unitary smooth muscle is found primarily in the walls of hollow viscera (musculature of the intestine, the uterus, and the ureters).

Multiunit smooth muscle contraction is coordinated by motor units, functionally similar to skeletal muscle. Multiunit smooth muscle is made up of individual units with few (or no) gap junctional bridges. It is found in the iris of the eye.

- Blood vessels have both unitary and multiunit smooth muscle in their walls.

Smooth muscle cells contract through an **actomyosin system**, but do not have well organized striations. Unlike skeletal and cardiac muscle, Ca^{2+} regulation of contraction is primarily through phosphorylation-dephosphorylation reactions.

B Nerve Tissue

The human central nervous system (CNS) contains about 10¹¹ (100 billion) **neurons**. It also contains 10-50 times this number of **glial cells**.

Cellular Elements in the CNS

- There are **two main types of glia**: Microglia and macroglia.
- **Microglia are scavenger cells** (cells that engulf and digests debris and invading microorganisms).
- **Macroglia** include oligodendrocytes, Schwann cells, and astrocytes. The first two are involved in myelin formation; astrocytes produce substances that are tropic to neurons, and they help maintain the appropriate concentration of ions and neurotransmitters.

Neurons are composed of a cell body (soma) that is the metabolic center of the neuron, dendrites that extend outward from the cell body and arborize extensively, and a long fibrous axon that originates from a somewhat thickened area of the cell body, the axon hillock.

Nervous system is divided into:

- **Central nervous system (CNS)**
 - Brain and spinal cord
 - Integration and interpretation of input
- **Peripheral nervous system (PNS)**
 - Nerves and ganglia (collections of cell bodies)
 - Communication of signal to the body

C Connective Tissue

Adipose cells (fat cells) also occur in loose connective tissue.

Develop in large groups in certain areas, forming adipose tissue.

Dense connective tissue

Loose connective tissue

Bone

- Osteocytes (bone cells) remain alive in a matrix hardened with calcium phosphate.
- Communicate through canaliculi.

Blood

- Extracellular material is the fluid plasma
- Erythrocytes = red blood cells
- Leukocytes = white blood cells
- Thrombocytes = platelets

D Epithelial Tissue

- An epithelial membrane, or epithelium, covers every surface of the vertebrate body
 - Can come from any of the 3 germ layers.
 - Some epithelia change into glands.
- Provides a protective barrier.
- Epithelia possess remarkable regenerative powers replacing cells throughout life.
- Two general classes:
 - **Simple** = One layer thick
 - **Stratified** = Several layers thick
- Subdivided into:
 - **Squamous cells** = Flat, lines lungs and blood capillaries
 - **Cuboidal cells** = Cube-shaped, lines kidney tubules and several glands
 - **Columnar cells** = Cylinder-shaped, lines airways of respiratory tract and most of the gastrointestinal tract, contains **goblet cells**: secrete mucus

Organs

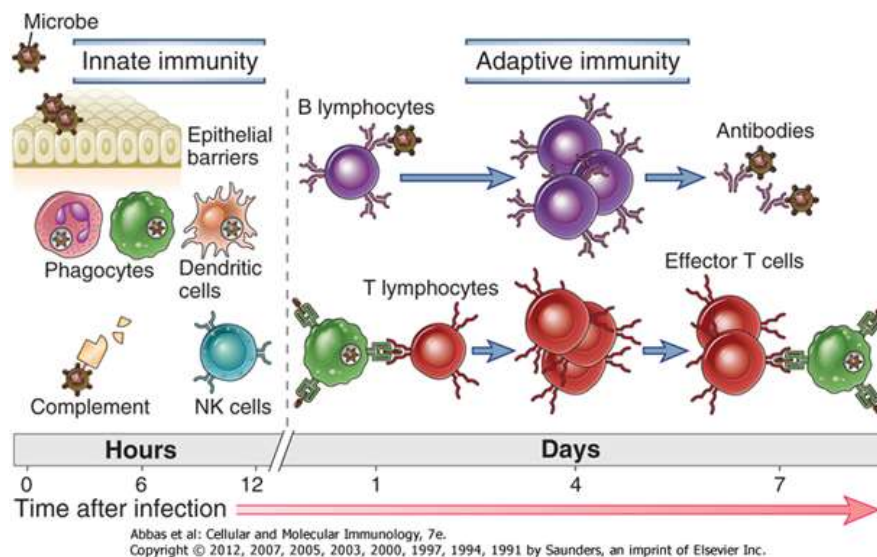
- Organs are **combinations of different tissues** that form a **structural and functional unit**.
- **Organ systems** are groups of organs that cooperate to perform the major activities of the body.
- The vertebrate body contains 11 principal organ systems.

- **Communication and integration**
 - **Three organ systems** detect external stimuli and coordinate the body's responses.
 - **Nervous, sensory** and **endocrine** systems
- **Support and movement**
 - The **musculoskeletal system** consists of two interrelated organ systems.
- **Regulation and maintenance**
 - Four organ systems regulate and maintain the body's chemistry
 - **Digestive, circulatory, respiratory** and **urinary systems**
- **Defense**
 - The body defends itself with two organ systems: **integumentary** and **immune**

IMMUNOLOGY, TOLERANCE, TRANSPLANT IMMUNOLOGY

Properties and Overview of Immune Responses

The physiological function of the immune system is defense against infectious microbes. However, even non-infectious foreign substances can elicit immune responses.



Innate Immunity: An Evolutionary View

All multicellular organisms have defense mechanisms against microbial and viral infections. For **vertebrates**, immune defense can be divided into **innate immunity** and **adaptive immunity**. Vertebrate innate immune elements are closely related to components of immunity in invertebrates.

Innate immunity retains importance as ...

- A **first line of defense**, slowing growth of infectious agents until adaptive immunity kicks in.
- A means of **directing adaptive immunity** (induction of inflammation, activation of dendritic cells, and production of cytokines that specialize immune responses).

Distinctive Features of Innate versus Adaptive Immunity

Innate Immunity	Adaptive Immunity
Germline-encoded, non-clonal receptors	Site-specific somatic recombination, clonally distributed Ag receptors
Limited diversity	Immense diversity and specificity
Responses in minutes and hours	Responses in days and weeks
No memory	Memory

Distinctive Features of Innate versus Adaptive Immunity

Innate Immunity	Adaptive Immunity
Physical barrier: skin, gut, mucus membranes, lung cilia	Physical barrier: none
Soluble factors: AMP, cytokines, complement	Soluble factors: Immunoglobulins
Cells: Macrophages, dendritic cells, NK cells, mast cell, neutrophils	Cells: B and T lymphocytes

Recognition Mechanisms of Innate Immunity: "Sensor Systems"

Microbes evolve rapidly, so innate immunity **must focus on broadly expressed molecules** characteristic of broad groups of microbes.

Pattern recognition receptors (PRR):

- Toll-like receptors (TLRs)
- C-type lectins
- CD14
- IL-1 family
- Nucleotide-binding oligomerization domain

Recognition Mechanisms of Innate Immunity: PAMPs

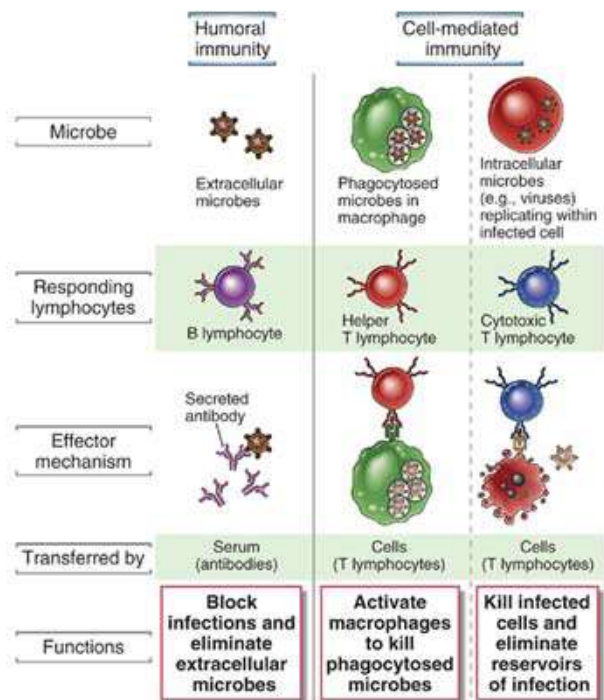
Pathogen-associated molecular patterns

Requirements for the recognition of targets by the innate immune system:

- Molecular structures recognized must be shared by large groups of pathogens.
- PAMPs must be conserved products of microbial metabolism not subject to antigenic variability.
- The recognized structure must be distinct from self antigens: discrimination of self vs. nonself.

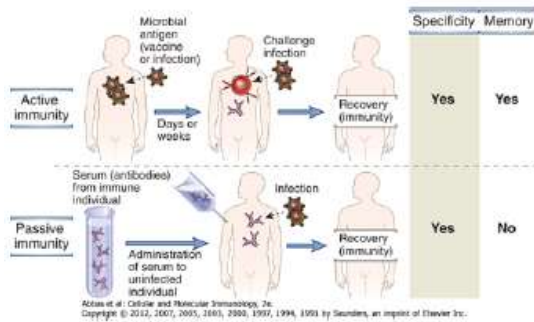
Types of Adaptive Immune Responses

There are **two types** of adaptive immune responses, called **humoral immunity** and **cell-mediated immunity**. They are mediated by different components of the immune system.

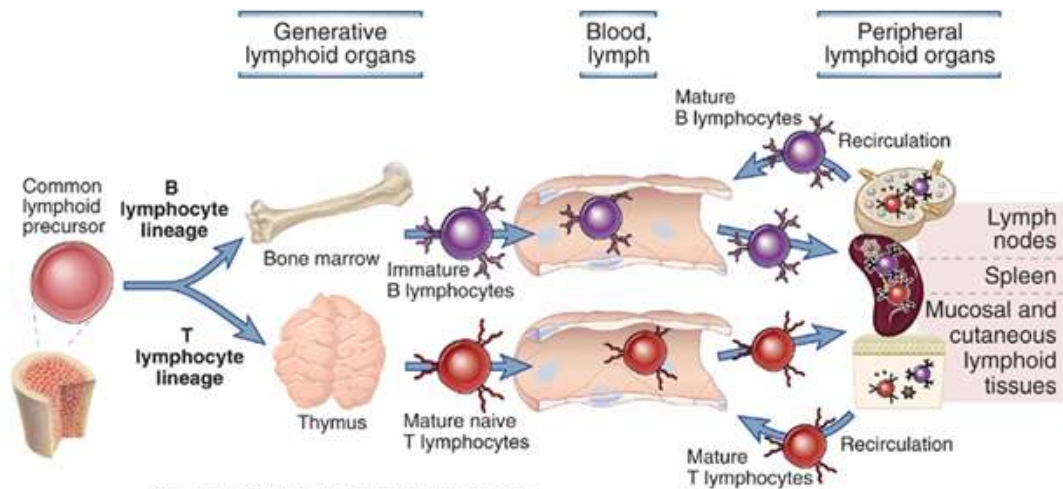
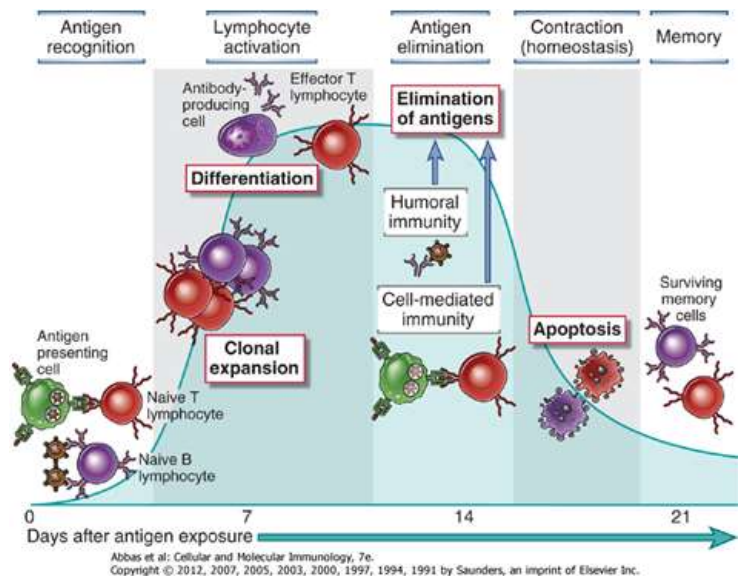
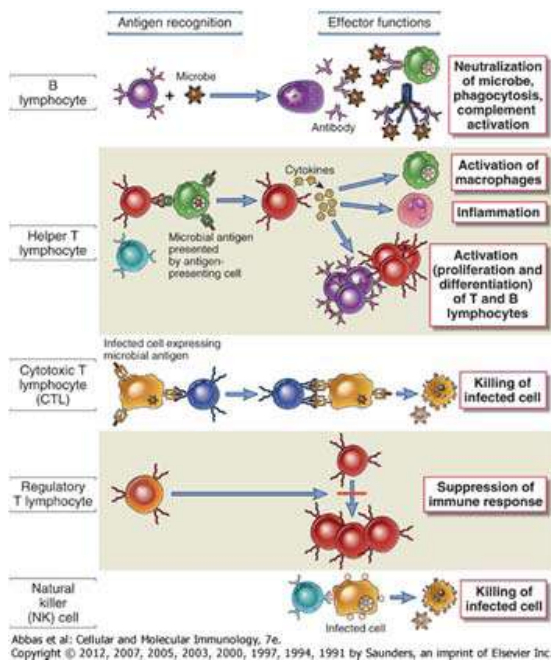
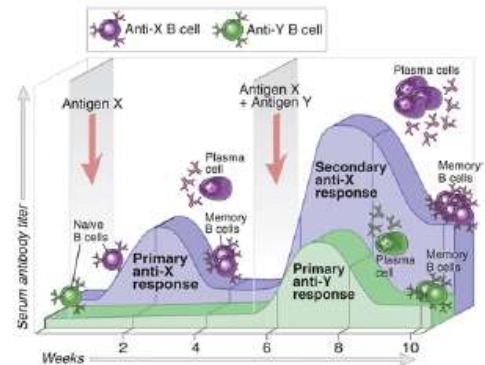


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Active and Passive Immunity



Specificity and Memory of Adaptive Immune Responses



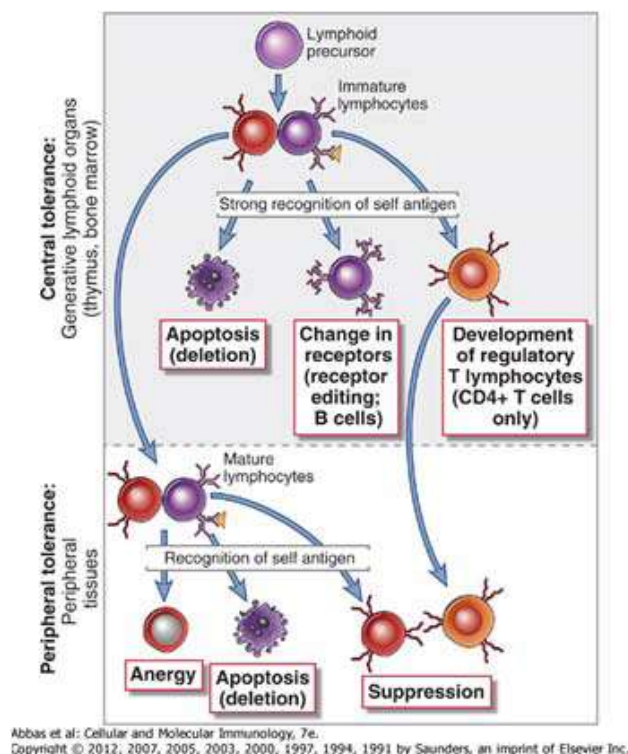
Immunological Tolerance

- Immunological tolerance is defined as **unresponsiveness to an antigen** that is induced by previous exposure to that antigen.
- Antigens that induce tolerance are called **tolerogens**, or tolerogenic antigens to distinguish them from **immunogens**, which generate immunity.

Tolerance to self-antigens is a fundamental property of the normal tissue and failure of self-tolerance results in immune reactions against self (autologous) antigens. Such reactions are called **autoimmunity** and the diseases are called **autoimmune diseases**.

General Features of Immunological Tolerance

- Normal individuals are tolerant to their own (self) antigens because the lymphocytes that recognize self-antigens are killed or inactivated or the specificity of these lymphocytes is changed.
- **Tolerance results from the recognition of antigens by specific lymphocytes.**
- Self-tolerance may be induced in immature self-reactive lymphocytes in the generative lymphoid organs (**central tolerance**) or in mature lymphocytes in peripheral sites (**peripheral tolerance**).



Central tolerance occurs during the maturation of lymphocytes in the central (generative) lymphoid organs, where all developing lymphocytes pass through a stage at which encounter with antigen may lead to cell death or replacement of a self-reactive antigen receptor with a new one.

Peripheral tolerance occurs when, as a consequence of recognizing self-antigens, mature lymphocytes become incapable of responding to that antigen, or are induced to die by apoptosis, or mature T cells are actively suppressed by regulatory T cells.

Whether lymphocytes that recognize antigens become activated or tolerant is determined by the **properties of the antigens**, the state of **maturation of the antigen-specific lymphocytes**, and the **stimuli** received when these lymphocytes encounter self-antigens.

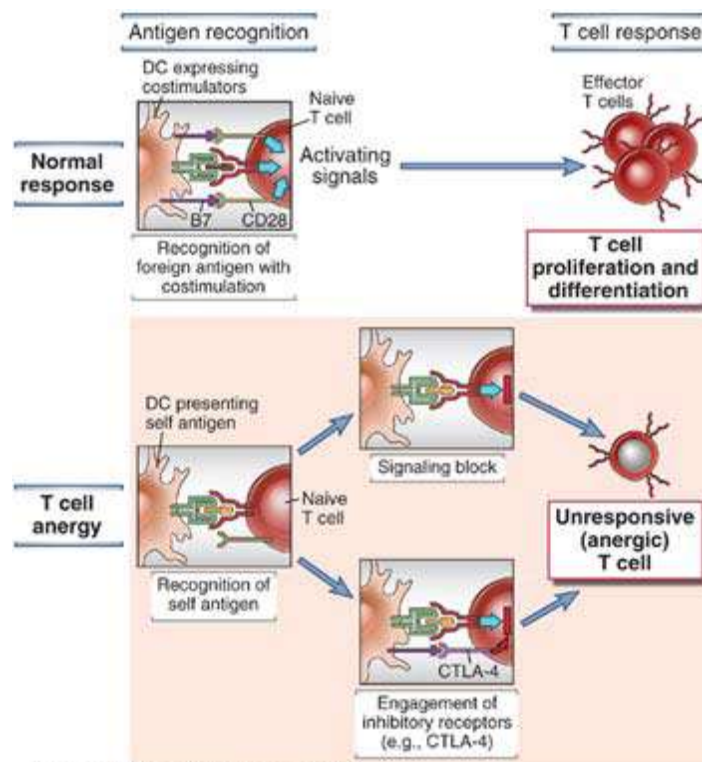
- Some self antigens may be ignored by the immune system.
- Foreign antigens in the absence of costimulatory signals may inhibit immune responses by inducing tolerance in specific lymphocytes.
- The induction of immunologic tolerance may be exploited as a therapeutic approach for preventing harmful immune responses.

Central Tolerance in T cells

Recognition of self-antigens by immature T cells in the thymus may lead to death of the cells (negative selection or deletion) or the development of regulatory T cells that enter peripheral tissues.

Anergy

- Exposure of mature CD4+ T cells to an antigen in the absence of costimulation or innate immunity may make the cells incapable of responding to that antigen.
- Anergic cells show a block in TCR-induced signal transduction.
- When T cells recognize self-antigens, they may engage inhibitory receptors of the CD28 family, whose function is to terminate T cell responses.



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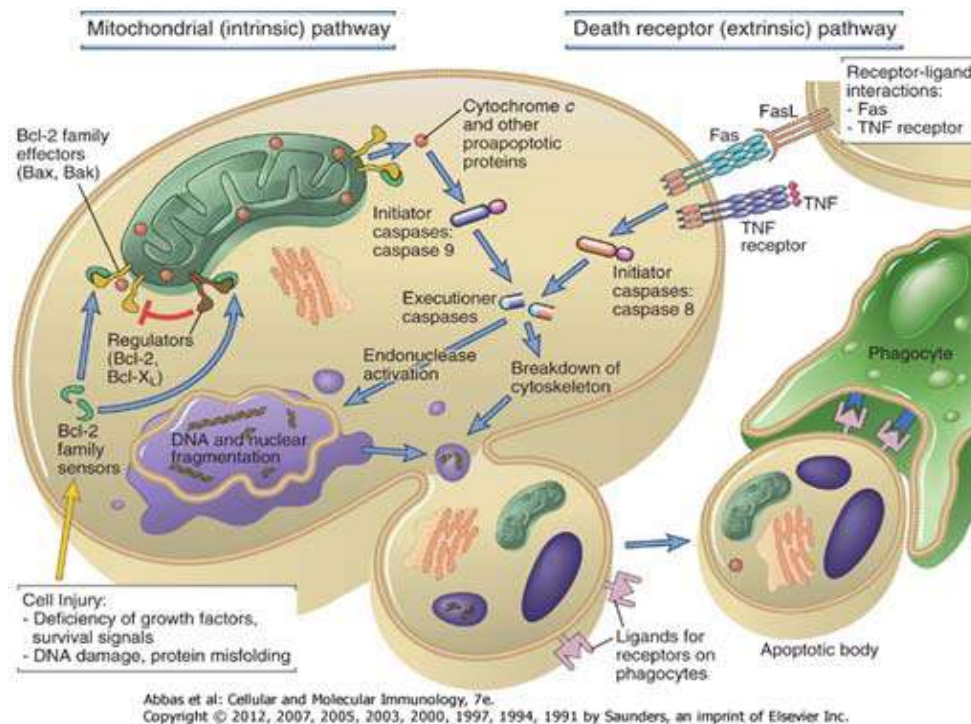
Suppression

Regulatory T lymphocytes are a subset of CD4⁺ T cells whose function is to suppress immune response and maintain self-tolerance.

- Regulatory T cells are generated mainly by self-antigen recognition in the thymus and by recognition of self and foreign antigens in peripheral lymphoid organs.
- The generation and survival of regulatory T cells are dependent on the cytokines TGF- β and IL-2:
- Regulatory T cells produce IL-10 and TGF- β both of which inhibit immune responses.
- Regulatory T cells inhibit the ability of APCs (antigen presenting cell) to stimulate T cells.

Deletion of T Cells by Apoptotic Cell Death

- T lymphocytes that recognize self-antigens without inflammation or that are repeatedly stimulated by antigens die by apoptosis.
- The mitochondrial (or intrinsic) pathway is regulated by the Bcl-2 family of proteins.
- In the death receptor (or extrinsic) pathway, cell surface receptor homologous to tumor necrosis factor (TNF) receptors are engaged by their ligands, which are homologous to the cytokine TNF.



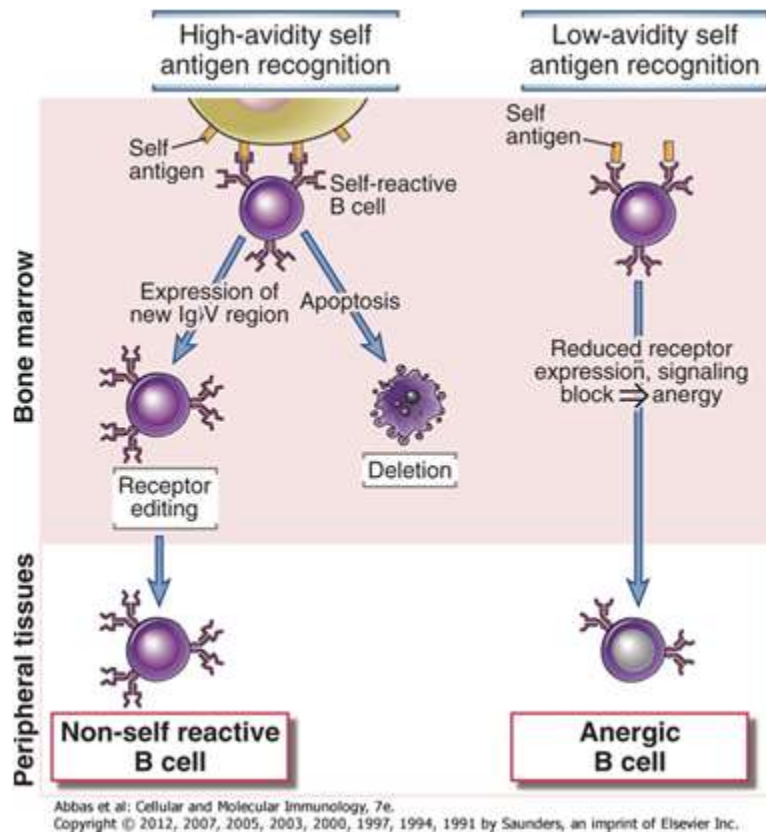
B Lymphocyte Tolerance

Tolerance in B lymphocytes is necessary for maintaining unresponsiveness to thymus independent self-antigens, such as polysaccharides and lipids. B cell tolerance also plays a role in preventing antibody responses to protein antigens.

Central Tolerance in B Cells

Immature B lymphocytes that recognize self-antigens in the bone marrow with high affinity either change their specificity or are deleted:

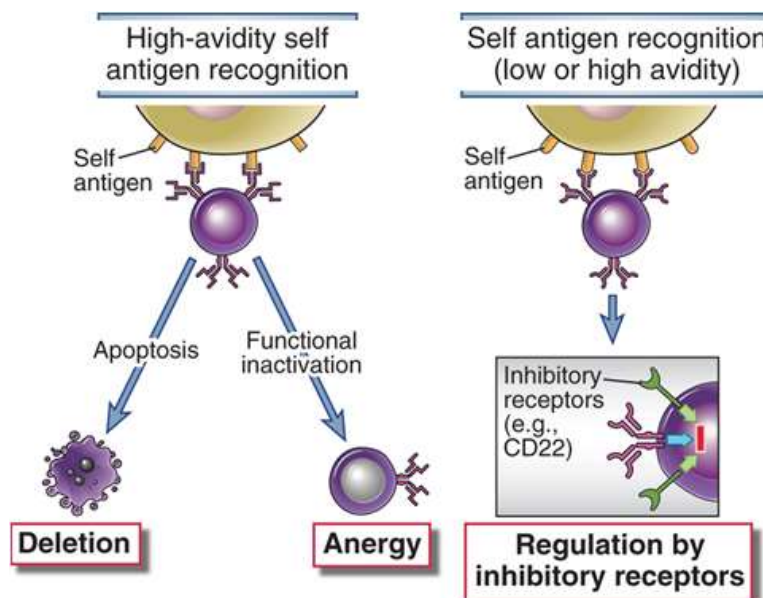
- Receptor editing
- Deletion
- Anergy



Peripheral B Cell Tolerance

Mature B lymphocytes that recognize self-antigens in peripheral tissues in the absence of specific helper T cells may be rendered functionally unresponsiveness or die by apoptosis.

- Anergy and deletion
- Signaling by inhibitory receptors



Pathogenesis of Autoimmunity

Autoimmunity results from a **failure of the mechanism of self-tolerance** in T or B cells, which may lead to an imbalance between lymphocyte activation and control mechanisms.

General mechanisms

- Defects in deletion (negative selection) of T or B cells or receptor editing in B cells during the maturation.
- Defective numbers and functions of regulatory T lymphocytes.
- Defective apoptosis of mature self-reactive lymphocytes.
- Inadequate function of inhibitory receptors.
- Activation of APCs, which overcomes regulatory mechanisms and result in excessive T cell activation.

The major factors that contribute to the development of autoimmunity are **genetic susceptibility** and **environmental triggers** such as infections and local tissue injury. Autoimmune disease may be either systemic or organ specific, depending on the distribution of autoantigens that are recognized.

Autoimmune diseases tend to be chronic, progressive, and self-perpetuating.

Transplantation Immunology

Transplantation is a widely used treatment for replacement of nonfunctioning organs and tissues with healthy organs and tissue. Technically, transplantation is the process of taking cells, tissues, or organs called **graft**, from one individual and placing them into a different individual. The individual who provides the graft is called the **donor**, and the individual who receives the graft is called the **recipient** or **host**.

Transfusion refers to the transfer of circulating blood cells or plasma from one individual to another.

Transplantation of cells or tissues from an individual to a genetically nonidentical individual invariably leads to **rejection** of the transplant due to an adaptive immune response.

A graft transplanted from one individual to the same individual is called an **autologous graft**. A graft transplanted between two genetically identical or syngeneic individuals of the same species is called a **syngeneic graft**.

A graft transplanted between two genetically different individuals of the same species is called an **allogenic graft** (or **allograft**). A graft transplanted between individuals of different species is called **xenogeneic graft** (or **xenograft**).

Immune Responses to Allografts

- Recognition of transplanted cells as self or foreign is determined by polymorphic genes, called **histocompatibility genes**, which differ among different members of a species.

Basic rules

- Cells/organs transplanted between genetically identical individuals are never rejected.
- Cells/organs transplanted between genetically non-identical people or members of two different inbred strains of a species are almost always rejected.
- The offspring of a mating between two different inbred strains of animal will typically not reject grafts from either parent.
- A graft derived from the offspring of a mating between two different inbred strains will almost always be rejected by either parent.
- The molecules responsible for almost all strong (rapid) rejection reactions are called **major histocompatibility complex** (MHC) molecules.
- Allogenic MHC molecules of a graft may be presented for recognition by the T cells of the recipient in two fundamentally different ways.

Direct Alloantigen Recognition

In **direct presentation**, an intact MHC molecule is displayed by donor APCs in the graft and recognized by recipient T cells without a need for host APCs.

Indirect Alloantigen Recognition

In the **indirect pathway**, donor (allogeneic) MHC molecules are captured and processed by recipient APCs that enter grafts, and peptides derived from the allogenic MHC molecules are presented in association with self MHC molecules.

T Cell Recognition of Alloantigens

The T cell response to an organ graft may be initiated in the lymph nodes that drain the graft. As many as 1% to 2% of an individual's T cells are capable of recognizing and responding to a single foreign MHC molecule, and this high frequency of T cells reactive with allogenic MHC molecules is one reason that allografts elicit strong immune responses. Many of the T cells that respond to an allogenic MHC molecule, even on first exposure, are memory T cells.

Effector Functions of Alloreactive T Cells

- **Alloreactive CD4+ and CD8+ T cells** that are activated by graft alloantigens cause rejection by distinct mechanisms.
- Only CTLs that are generated by direct allogenic MHC recognition can kill graft cells, whereas CTLs or helper T cells generated by either direct or indirect alloantigen recognition can cause cytokine mediated damage to grafts.

Alloreactive B Cells

- Antibodies are produced by helper T cell dependent activation of alloreactive B cells.
- Antigens most frequently recognized by alloantibodies in graft rejection are donor HLA molecules (human leukocyte antigen).

Pattern and Mechanisms of Allograft Rejection

Hyperacute rejection is characterized by thrombotic occlusion of the graft vasculature that begins within minutes and hours after host blood vessels are anastomosed to graft vessels and is mediated by preexisting antibodies in the host circulation that bind to donor endothelial antigens.

Acute rejection is a process of injury to the graft parenchyma and blood vessels **mediated by alloreactive T cells** and **antibodies**. The principal mechanism of acute cellular rejection is **CTL-mediated killing** of cells in the graft.

Alloantibodies cause acute rejection by binding to alloantigens (HLA molecules), on vascular endothelial cells, causing **endothelial injury** and **intravascular thrombosis** that results in graft destruction.

As therapy for acute rejection has improved, the major cause of the failure of vascularized organ allografts has become **chronic rejection**. A dominant lesion of chronic rejection is **arterial occlusion** as a resulting in ischemic damage. The pathogenesis remains **poorly understood**, but involves a combination of immunological and nonimmunological processes.

Xenogeneic Transplantation

A major immunologic barrier to xenogeneic transplantation is the presence of natural antibodies that cause hyperacute rejection. The majority of human anti-pig natural antibodies are directed against a pig α -galactosidase. Allogenic hematopoietic stem cells are rejected by even a minimally immunocomponent host, and therefore the donor and recipient must be carefully matched at all MHC loci.

Graft-Versus Host Disease

Graft-versus host disease (GVHD) is caused by the reaction of grafted mature T cells in the marrow inoculum with alloantigens of the host.

Immune Privilege of the Mammalian Fetus

The mammalian fetus expresses paternally inherited genes that are allogenic to the mother, but fetuses are not normally rejected by the mother. Experimental observation indicate that the anatomic location of the fetus is a critical factor in the absence of rejection. The uterine decidua may be a site where immune responses are functionally inhibited.

HAFNER: Part II

INTRODUCTION TO REGENERATION, WOUND HEALING, STRATEGIES FOR THE RECONSTRUCTION OF SPECIFIC TISSUES

An Introduction to Regeneration

If there were no regeneration, there could be no life. If everything regenerated, there would be no death. – Richard J. Goss (1969)

The word **regeneration** means different things to different people. Stedman's Medical Dictionary: Regeneration is the "reproduction or reconstitution of a lost or injured part" or "a form of asexual reproduction."

In recent years, there has been considerable interest in building missing or damaged tissues and organs in humans through the application of bioengineering principles or through the use of stem cell technology. This field is now being called **regenerative medicine**.

Physiological Regeneration

Physiological regeneration, the natural replacement of extruded or worn-out body parts, is a process that occurs in many of our body systems and is often studied in the form of cellular turnover.

- Shedding cycles of the epidermis or the epithelial cells lining the gut
- The renewal of the endometrium after a menstrual period
- The replacement of blood cells

It is important to understand that the term **physiological regeneration does not imply a specific type of mechanism**. There is a tremendous difference between the relatively simple replacement cycle of an epidermal cell and that of a deer antler, which involves much of the complexity of the regeneration of an entire limb.

Physiological regeneration should, therefore, be looked at as a term of convenience that embodies a variety of processes designed to maintain the normal equilibrium of the body tissues.

Reparative Regeneration

- Reparative regeneration is the term that has been applied to most varieties of posttraumatic regeneration.
 - Regeneration of the amputated limb or tail of a salamander or newt
 - Reconstitution of the entire body of a planarian from a fragment less than 1/200 of the original mass.
- The common element in reparative regeneration is the **replacement of a lost or damaged part of the body**.

Epimorphic Regeneration

Phenomena that are characterized by the formation of a regeneration blastema that arises through epithelial mesenchymal interactions and that contains and expresses intrinsic morphogenetic information. The classic example of epimorphosis is the regenerating amphibian limb.

Tissue Regeneration

The term tissue regeneration is applied to the repair of damaged tissues within the body, with the greatest emphasis on mammalian tissues because of the medical implications. Tissue regeneration can be initiated by a wide variety of trauma. Regardless of the nature of the initial traumatic stimulus, the reactions of the traumatized tissues follow a similar course.

1. Trauma (e.g., mechanical, chemical, thermal)
2. Localized posttraumatic ischemia and edema
3. Local inflammation and the removal of damaged tissues by phagocytosis
4. Activation of the cellular precursors of regeneration
5. Revascularization of the traumatized region
6. The extracellular matrix as a substrate for regeneration
7. Increase in the number of regenerating cells by proliferation
8. Differentiation of the regenerating tissue
9. Morphogenesis of the regenerating tissue
10. Functional restoration

Cellular Regeneration

Cellular regeneration refers to the reconstruction of a single cell that has been traumatized. Classic examples are the reconstitution of protozoa after resection or natural fission (a form of asexual reproduction) and the regeneration of transected or otherwise damaged axons of peripheral nerves.

Regeneration by Induction

The essence of this largely experimental approach is that tissue-specific regeneration can be stimulated by the application of tissues or materials with specific inductive properties.

- Simple removal of bone results in the formation of a connective tissue scar.
- If the defect is filled in with a mass of finely ground bone, new bone regenerates within a few weeks.
- If the dura mater is removed, regeneration fails to occur despite the addition of ground bone into the defect.
- Poleshaev's experiments

Hypertrophy

Many internal organs have the capacity to increase their mass after damage or partial removal, or if one member of a pair (e.g. kidneys) is removed. A key element to the hypertrophy of most internal organs is that an increase in functional mass, rather than a restoration of external form.

Basic Biology of Wound Repair

Wound repair is not a simple linear process in which growth factors released activate parenchymal cell proliferation and migration. It is an integration of dynamic interactive processes involving soluble mediators, formed blood elements, extracellular matrix, and parenchymal cells.

These wound repair processes follow a specific time sequence and can be temporally categorized into three major groups:

- **Inflammation**
- **Tissue formation**
- **Tissue remodeling**

The three phases of wound repair are not mutually exclusive but overlap in time.

Inflammation

The primary cell types involved in the process of inflammation are platelets, neutrophils, and monocytes. Upon injury, successful reestablishment of homeostasis depends on platelet adhesion to interstitial connective tissue, which leads to their aggregation, coagulation, and activation.

Activated platelets release several adhesive proteins to facilitate their aggregation, chemotactic factors for blood leukocytes, and multiple growth factors to promote new-tissue formation.

Re-epithelialization

Re-epithelialization of a wound begins within hours after injury by the movement of epithelial cells from the surrounding epidermis over the denuded surface. If a wide expanse of the epidermis is lost, epidermal cells regenerate from stem cells in pilosebaceous follicles.

Migrating epithelial cells markedly alter their phenotype by retracting their intracellular filaments, dissolving most of their desmosomes, and forming peripheral actin filaments. These migrating cells also undergo dissolution of their hemidesmosomal links between the epidermis and the dermis.

Migrating epidermal cells possess a unique phenotype that is distinct from both the terminally differentiated keratinocytes of normal epidermis and the basal cells of stratified epidermis. It is now appreciated that the signals that control wound healing in the adult animal are similar to those that control epithelial fusion during embryogenesis.

Stages in the Epithelialization of a Simple Skin Wound

1. Retraction of the edges of the wound.
2. Rapid covering of the wound by an exudate or a blood clot.
3. Detachment of marginal epidermal cells from their underlying basal lamina.
4. Mobilization of epithelial cells closest to the wound margins.
5. Migration of epidermal cells over a simple exudate or under a blood clot.
6. Cessation of migration when migrating epithelial cells from opposite sides of the wound meet.
7. Initiation of mitotic activity in epidermal cells along the edge of the wound.
8. Thickening of wound epithelium by further migration or mitotic activity of the wound epithelium itself.
9. Re-formation of basal lamina.
10. Final structural and functional remodeling.

Granulation Tissue

New stroma, often called granulation tissue, begins to form approximately four days after injury. Macrophages, fibroblasts, and blood vessels move into the wound space as a unit, which correlates well with the proposed biologic interdependence of these cells during tissue repair.

Fibroplasia

Components of granulation tissue derived from fibroblasts, including the cells themselves and the extracellular matrix, are collectively known as fibroplasia. Growth factors, especially PDGF and TGF- β , stimulate fibroblasts of the periwound tissue to proliferate, express appropriate integrin receptors, and migrate into the wound space.

Neovascularization

Fibroplasia would halt if neovascularization failed to accompany the newly forming complex of fibroblasts and extracellular matrix. The process of new blood vessel formation is called angiogenesis.

Wound Contraction and Extracellular Matrix Organization

During the second and third weeks of healing, fibroblasts begin to assume a myofibroblast phenotype characterized by large bundles of actin-containing microfilaments disposed along the cytoplasmic face of the plasma membrane and the establishment of cell–cell and cell–matrix linkages.

Collagen remodeling during the transition from granulation tissue to scar is dependent on continued collagen synthesis and collagen catabolism. The degradation of wound collagen is controlled by a variety of collagenase enzymes from macrophages, epidermal cells, and fibroblasts.

Wound Repair

Wounds gain only about 20% of their final strength by the third week, during which time fibrillar collagen has accumulated relatively rapidly and has been remodeled by myofibroblast contraction of the wound. Thereafter the rate at which wounds gain tensile strength is slow, reflecting a much slower rate of collagen accumulation.

Definition: Chronic Wound

Wounds showing no response to specific therapy within 6 weeks are classified as chronic wounds.

→ Ulcers (Geschwür)

Most frequent causes (Germany)

- Venous leg ulcers (ca. 1 Mio. per year)
- Diabetic foot ulcer (ca. 900.000 per year)
- Ischemic ulcers (ca. 250.000 per year)
- Pressure ulcer (ca. 1 Mio. per year)

Engineering Skin Tissue

An engineered skin graft should incorporate as many of the following factors as possible:

- The extracellular matrix
- Dermal fibroblasts
- The epidermis
- A naturally occurring semipermeable membrane, the stratum corneum.

These components may act alone, but, more importantly, they act synergistically as part of a fully integrated tissue to protect the underlying tissues of a wound bed and to direct healing of the wound.

Bioengineered Skin Constructs

Two general categories:

1. Cellular based products: Actively stimulate wound healing

- Autologous epidermal cell sheets (Epicel®)
- Allogenic dermal substrates (Dermagraft®)
- Human skin equivalent (HSE) (Apligraf®)

2. Acellular products: Provide a substrate or covering to facilitate wound healing

- Integra®, Alloderm®, ...

Skin Structure and Function

Epidermis:

- Is composed primarily of keratinocytes, which form a stratified squamous epithelium.
- The most superficial keratinocytes in the epidermis form the stratum corneum, the outmost structure that provides the physical barrier of the skin.
- Other cell types: Langerhans cells, melanocytes.

Dermis:

- Underlies the epidermis.
- Provides physical strength and flexibility to the skin as well as the connective tissue scaffolding that supports the extensive vasculature, lymphatic system and nerve bundles.
- Fibroblasts, the major cell type of the dermis, produce and maintain most of the extracellular matrix.
- Other cell types: Endothelial cells (blood vessels), macrophages, lymphocytes, nerve fibres.

Engineering Skin Tissue (2nd)

An engineered skin graft should incorporate as many of the following factors as possible:

- The extracellular matrix
- Dermal fibroblasts
- The epidermis
- A naturally occurring semipermeable membrane, the stratum corneum.

These components may act alone, but, more importantly, they act synergistically as part of a fully integrated tissue to protect the underlying tissues of a wound bed and to direct healing of the wound.

The Key Features to be Replicated in an Engineered Skin Construct are...

1. A dermal or mesenchymal element capable of aiding appropriate dermal repair and epidermal support
2. An epidermis capable of easily achieving biologic wound closure
3. An epidermis capable of rapid re-establishment of barrier properties
4. A permissive milieu for the components of the immune system, nervous system, and vasculature
5. A tissue capable of achieving normalization of structure and additional function, such as reduction of long-term scarring and reestablishment of pigmentation
6. Active cellular component(s) capable of responding to different wound types and conditions
7. Sufficient mechanical strength to allow for clinical manipulation
8. Persistence of cells in the wound for multiple weeks to stimulate the healing process through delivery of cytokines and matrix proteins

Epidermal Regeneration

- Re-epithelialization of the wound is a paramount concern
- Approaches to re-establishing epidermis are numerous:
 - Use of cell suspensions to full-thickness skin equivalents possessing a differentiated epidermis.
 - Silicone membranes have been used as temporary coverings in conjunction with dermal templates
- Regardless of approach, living epidermal keratinocytes are necessary to achieve permanent, biologic wound closure.

The mouse 3T3 fibroblast feeder cell system allows substantial expansion of epidermal keratinocytes and can be used to generate enough thin, multilayered epidermal sheets to resurface the body of a severely burned patient. Once transplanted, the epidermal sheets quickly form epidermis and re-establish epidermal coverage.

With time, the **cultured epithelial autograft (CEA)** stimulates formation of new connective tissue (neodermis) immediately beneath the epidermis, but scarring and wound contraction remain significant problems. Cultured epithelial autografts (Epicel[®]) have been available since the late 1980s.

Dermal Replacement

Human cadaver allograft skin has been used when autologous skin grafts are not possible. Problems associated with human cadaver allografts include the possibility of an immune rejection reaction, potential for infection, and problems of supply and variability in the quality of the material.

Cadaver allograft dermis can be processed to make an immunologically inert, acellular dermal matrix with an intact basement membrane to aid the take and healing of ultrathin autografts (AlloDerm[®]).

- Currently, only the upper papillary layer of dermis is used clinically (AlloDerm[®]).
- One limitation to this approach is that deep dermis and the more superficial papillary layer differ in architecture.
- The deep reticular dermis is needed to prevent wound contraction.
- Providing an appropriate scaffold for deep dermal repair remains a challenge.

There have been advances in the design of artificially grown dermal tissues using human neonatal fibroblasts grown on rectangular sheets of biodegradable mesh (Dermagraft[®]). The fibroblasts propagate among the degrading fibers, producing extracellular matrix in the interstices of the mesh.

Composite Skin Grafts

- Human skin autograft has been the gold standard for resurfacing the body and closing wounds that are difficult to heal.
- Cultured epidermal grafts are more likely to take when the dermal bed is relatively intact, probably because dermal factors influence epithelial migration, differentiation, attachment, and growth.
- The epidermis and dermis act synergistically to maintain homeostasis.

Human Skin Equivalent

One of the first attempts to replicate a full thickness skin graft was by Bell et al. (1981), who described a bilayered skin equivalent. The dermal component consisted of a lattice of type I collagen contracted by tractional forces of rat dermal fibroblasts trapped within the gelled collagen.

This contracted lattice was then used alone or as a substrate for rat epidermal keratinocytes. This technology has now advanced to enable the production of large amounts of Apligraf[®] from a single donor.

Immunological Aspects

The immunology of allogeneic tissue engineered skin grafts is poorly understood. The complexity of biological and immunologic factors, which determine the ability of a graft to take and to persist over time is poorly understood. Properties that determine the immunogenicity of an engineered allogeneic graft are: The purity of cell populations, the antigen-presenting capabilities of graft cells, and the vascularity of the graft.

The antigen-presenting capabilities of keratinocytes and fibroblasts are critical to determining the immunogenicity of the HSE grafts. Fibroblasts and keratinocytes are not professional antigen-presenting cells and fail to stimulate the proliferation of allogeneic T-cells.

These cells do not express HLA class II molecules or co-stimulatory molecules such as B7-1. The inability of keratinocytes and fibroblasts to induce proliferation of allogeneic T-cells is due primarily to the lack of expression of costimulatory molecules, even though aberrant antigen processing and invariant chain expression may also contribute.

The ability to utilize allogeneic cells rather than autologous cells as in CEA therapy enables the reproducible manufacture of consistent Apligraf[®] HSE. The inability of epidermal keratinocytes and dermal fibroblasts to stimulate a T-cell response permits their use in allogeneic applications.