**Tissue Responses:**

1. **Regeneration** = growth of cells and tissues to replace lost structures
   - requires intact connective tissue scaffold (ECM)
2. **Healing** = in response to a wound, inflammation, cell necrosis
   - occurs in organs incapable of regeneration
   - occurs if there is damage to the ECM

Cell populations size is determined by rate of proliferation, Differentiation, and death by apoptosis

- Terminally differentiated cells – not capable of replication
  1. myocytes
  2. neurons

- Quiescent but able to proliferate
  1. liver
  2. kidney

- Proliferative tissues – incapable of replication; have stem cells
  1. bone marrow
  2. epithelium (skin and gut)
**Continuously dividing (labile) tissues:**
- stem cells; unlimited capacity to replicate
  1. Surface epithelial cells (skin, oral cavity, vagina)
  2. Mucosa of excretory glands
  3. GIT epithelium
  4. UB urothelium
  5. Bone marrow

**Quiescent (stable) tissues:**
- in G0 but can be stimulated to enter G1
  - normally have low level of replication
  1. Parenchymal cells (liver, kidney, pancreas)
  2. Mesenchymal cells (fibroblasts, smooth muscle)
  3. Vascular endothelium
  4. Resting lymphocytes and other leukocytes

**Nondividing (permanent) tissues:**
- cells have left the cell cycle and cannot divide
  1. Neurons
  2. Skeletal muscle
  3. Cardiac muscle

---

**Stem cells:**
1. prolonged self-renewal
2. asymmetric replication = some self replicate, others differentiate
3. have niches in various organs
4. broad developmental plasticity; similar to embryonic stem cells

**Embryonic stem cells:**
1. pluripotent ES cells
2. express unique transcription factors (homeobox protein Nanog)
3. utilize Wnt-β-catenin signaling in maintaining pluripotency

**Adult stem cells (tissue stem cells):**
1. more restricted differentiation capacity; lineage specific
2. MAPC (multipotent adult progenitor cell) – can differentiate into mesodermal, endodermal, neuroectodermal cell types; found in the BM, muscle, brain, skin

---

**Figure 3-3**

Continuously dividing labile cells (e.g., epidermis, GI tract epithelium)

Quiescent, stable cells (e.g., hepatocytes)

Permanent cells (e.g., neurons, cardiac myocytes)

Cell cycle

**G1/S = restriction point; point of no return cell committed to mitosis**
Differentiation pathways for pluripotent bone marrow stromal cells. Activation of key regulatory proteins by growth factors, cytokines, or matrix components leads to commitment of stem cells to differentiate into specific cellular lineages.

Myotubes = combined action of several factors (e.g., myoD, myogenin);
Fat cells = PPARy,
Osteogenic lineage = CBFA1 (also known as RUNX2),
Cartilage formation = Sox9
Endothelial cells = VEGF and FGF-2

Adult stem cells reside permanently in most organs:
1. Liver = stem cells in canals of Hering; give rise to the oval cells → hepatocytes, bile epithelium
   - function as a reserve compartment, activated only hepatocyte proliferation is blocked
2. Brain = stem cells in the olfactory bulb and dentate gyrus of the hippocampus
   - neurogenesis does occur in some areas
3. Skeletal and cardiac muscle
   - satellite cells in skeletal muscle proliferate
   - satellite cells → osteogenic or adipogenic
4. Epithelial cells = continuously divide; increase # by
   - increasing # of actively dividing stem cells
   - increasing # of replications
   - decreasing the cell-cycle time
<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Symbol</th>
<th>Source</th>
<th>Functions</th>
</tr>
</thead>
</table>
| Epidermal growth factor                 | EGF    | Platelets, MØ, saliva, urine, milk, plasma  | Mitogenic for keratinocytes and fibroblasts  
Stimulates keratinocyte migration and granulation tissue formation                                                                         |
| Transforming growth factor alpha        | TGF-α  | MØ, T cells, keratinocytes, many tissues   | Similar to EGF; stimulates replication of hepatocytes and certain epithelial cells                                                     |
| Hepatocyte growth factor                | HGF    | Mesenchymal cells                           | Enhances proliferation of epithelial and endothelial cells and hepatocytes  
Increases cell mobility                                                                                                                   |
| Vascular endothelial growth factor     | VEGF   | Mesenchymal cells                           | Increases vascular permeability  
Mitogenic for endothelial cells                                                                                                              |
| Platelet derived growth factor          | PDGF   | Platelets, MØ, endothelial cells, keratinocytes, smooth muscle | Chemotactic for PMNs, MØ, fibroblasts, smooth muscle cells  
Activates PMNs, MØ, fibroblasts  
Mitogenic for fibroblasts, endothelial cells, smooth muscle cells  
Stimulates production of MMPs, fibronectin, HA  
Stimulates angiogenesis, wound contraction and remodeling  
Inhibits platelet aggregation  
Regulates integrin expression                                                                                                                |
| Fibroblast growth factor-1 (acidic)    | FGF-1  | MØ, mast cells, T cells, endothelial cells, keratinocytes, smooth muscle | Chemotactic for fibroblasts  
Mitogenic for fibroblasts and keratinocytes  
Stimulates keratinocyte migration  
**Angiogenesis, wound contraction and matrix deposition**  
**Role in development of skeletal muscle, lung maturation, and hematopoiesis**                                                                 |
| Fibroblast growth factor-2 (basic)     | FGF-2  | MØ, mast cells, T cells, endothelial cells, keratinocytes, smooth muscle | Chemotactic for fibroblasts  
Mitogenic for fibroblasts and keratinocytes  
Stimulates keratinocyte migration  
Angiogenesis, wound contraction and matrix deposition  
Role in development of skeletal muscle, lung maturation, and hematopoiesis  
Stimulates production of MMPs, keratinocyte proliferation, and leukocytes  
Regulates integrin expression  
Induces TGF-β production                                                                                                                   |
| Transforming growth factor beta         | TGF-β  | Platelets, T cells, MØ, endothelial cells, keratinocytes, smooth muscle cells, fibroblasts | Chemotactic for PMNs, MØ, lymphocytes, fibroblasts, and smooth muscle cells  
Stimulates TIMP synthesis, keratinocyte migration, angiogenesis, and fibroplasia  
**Inhibits production of MMPs, keratinocyte proliferation, and leukocytes**  
Regulates integrin expression and other cytokines  
Induces TGF-β production                                                                                                                   |
| Keratinocyte growth factor (FGF-7)      | KGF    | Fibroblasts                                 | Stimulates keratinocyte migration, proliferation, and differentiation                                                                 |
| Insulin-like growth factor-1            | IGF-1  | MØ, fibroblasts, others                    | Stimulates synthesis of PSGAGs, collagen, keratinocyte migration, and fibroblast proliferation  
Endocrine effects similar to growth hormone                                                                                                 |
| Tumor necrosis factor                   | TNF    | MØ, mast cells, T cells                    | Activates MØ  
Regulates other cytokines                                                                                                                |
| Interleukins                            | IL     | MØ, mast cells, keratinocytes, lymphocytes, many tissues | Many functions  
IL-1 = chemotactic for PMNs, stimulation of MMP-1  
IL-4 = chemotactic for fibroblasts  
IL-6 = TIMP synthesis  
IL-8 = angiogenesis                                                                                                                         |
| Interferons                             | IFN    | Lymphocytes, fibroblasts                   | Activates MØ  
Inhibits fibroblast proliferation and synthesis of MMPs  
Regulates cytokines                                                                                                                      |
**Receptors with intrinsic tyrosine kinase activity:**

1. EGF
2. FGF
3. HGF
4. VEGF
5. PDGF
6. TGF-α
7. c-KIT ligand

- ligands have a ligand-binding domain, a transmembrane region, and a cytoplasmic tail that has intrinsic tyrosine kinase activity.
- binding of ligands induces dimerization of the receptor, tyrosine phosphorylation, and activation of the receptor tyrosine kinase.
- the active kinase then P and activates many downstream effector molecules.
  - phospholipase Cγ (PLCγ)
  - PI-3 kinase

**Seven transmembrane G-protein-coupled receptors:**

1. Vasopressin
2. Serotonin
3. Histamine
4. Epinephrine
5. Norepinephrine
6. Calcitonin
7. Glucagon
8. Parathyroid hormone
9. Corticotropin
10. Rhodopsin

- constitute the largest family of plasma membrane receptors.
- transmit signals into the cell through trimeric GTP-binding proteins (G-proteins).
- binding of the ligand induces change in the receptor causing activation → interaction with G-proteins.
- activation of G-proteins occurs by exchange of GDP with GTP.
- cAMP is a second messenger.
- activation of this receptor can produce IP3 which releases Ca++ from the ER.

**Receptors lacking intrinsic tyrosine kinase activity:**

1. IL-2
2. IL-3
3. other IL
4. INF-α, INF-β, INF-γ
5. Erythropoietin
6. G-CSF
7. Growth hormone
8. Prolactin

- these receptors transmit extracellular signals to the nucleus by activating members of JAK (Janus kinase) family of proteins.
- JAKs link the receptors with and activate cytoplasmic transcription factors (STATs – signal transducers and activation of transcription), which directly shuttle into the nucleus and activate gene transcription.
- cytokine receptors can also activate other signaling pathways such as MAP kinase pathways.

**Figure 3-9**

- Figure showing the interaction between receptors and signaling pathways.
- Diagram illustrates the activation of receptors with intrinsic tyrosine kinase activity and those without.
- Key pathways include PLCγ, PI-3 kinase, and JAK/STAT pathways.
- Diagram highlights the activation of transcription factors and various intracellular signaling components.
Figure 3-10

Binding of ligand → dimerization of the receptor → tyrosine P → activation of tyrosine kinase receptor → P of effector molecules

1. PLCγ → breakdown of membrane inositol phospholipids → IP3 → increase Ca++ concentration

2. PLCγ → breakdown of membrane inositol phospholipids → diacylglycerol → activ. of serine-threonine kinase protein C (PKC) → activation of various transcription factors

3. PI-3 kinase → P of a member phospholipid → generates products that activate AKt → cell proliferation, inhibition of apoptosis

4. P residues in the receptor = docking stations for adaptor proteins (GRB-2) → GRB-2 binds SOS (a GTP:GDP exchange factor) → catalyzes the formation of RAS-GTP → triggers MAP kinase cascade → synthesis and P of FOS and JUN (transcription factors) → stimulate production of GFs and proteins → control entry of cell into cell cycle

Receptors w/ intrinsic tyrosine kinase activity:
1. EGF
2. FGF
3. HGF
4. VEGF
5. PDGF
6. TGF-α
7. c-KIT ligand

Effector molecules:
1. PLCγ
2. PI-3 kinase
Transcription factors:
1. c-MYC
2. c-Jun
3. p53

- cellular events requiring rapid responses do not rely on new synthesis of transcription factors but depend on post-transcriptional modifications that cause transcription factor activation and migration into the nucleus
- modifications include
  - heterodimerization (c-FOS, c-JUN)
  - phosphorylation (P) of factors (STATs)
  - release of inhibition to permit nuclear migration (NFκB)

Regulation of the cell cycle:
1. proto-oncogenes are directly involved in regulation
2. stimulated by GFs or signaling by ECM through integrins
3. has multiple control points and redundancies

G0-G1 = first decision step; transcriptional activation
G1-S = restriction point; rate-limiting step; irreversibly committed to DNA replication; regulated by CDKs checks integrity of DNA before replication
S-G2 =
G2-M = checkpoint; checks integrity of DNA after replication monitors whether cell can undergo mitosis
Liver regeneration:

G0-G1 = TNF, IL-6
G1-S = HGF, TGF-α

Hepatocyte in quiescence → TNF, IL-6 → transition from G0 to G1 → activation of c-FOS, c-JUN → dimerization to form AP-1 transcription factor, c-MYC → encodes transcription factors such as NFκB, STAT-3, C/EBP → progression through G1 → expression of anti-apoptotic genes such as Bcl-X → progression from G1 to S → formation of cyclin D-CDK4 complex → P of RB → activation of cyclin E-CDK2 → replication becomes autonomous
**Extracellular Matrix**
- serves many functions
  1. sequesters water and provides turgor
  2. reservoir for GFs
  3. important in cell-to-cell interactions
- groups of macromolecules
  1. Collagen
  2. Elastic fibers = made of central core of elastin, surrounded by fibrillin
  3. Adhesive glycoproteins = CAMs
  4. Proteoglycans and HA

**Interstitial or fibrillar** collagens = I, II, III, V, XI

**type IV collagen** = nonfibrillar, main component of BM

**Elastic fibers** = central core of elastin, surrounded by a peripheral network of microfibrils (fibrillin)
<table>
<thead>
<tr>
<th>Collagen Type</th>
<th>Tissue Distribution</th>
<th>Genetic Disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fibrillar Collagens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Ubiquitous in hard and soft tissues</td>
<td>Osteogenesis Imperfecta Ehlers-Danlos syndrome-arthrochalasias type</td>
</tr>
<tr>
<td>II</td>
<td>Cartilage, intervertebral disk, vitreous</td>
<td>Achondrogenesis type II, spondyloepiphyseal dysplasia syndrome</td>
</tr>
<tr>
<td>III</td>
<td>Hollow organs, soft tissues</td>
<td>Vascular Ehlers-Danlos syndrome</td>
</tr>
<tr>
<td>V</td>
<td>Soft tissues, blood vessels</td>
<td>Classical Ehlers-Danlos syndrome</td>
</tr>
<tr>
<td>XI</td>
<td>Cartilage, vitreous</td>
<td>Stickler syndrome</td>
</tr>
<tr>
<td><strong>Basement Membrane Collagens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Basement membranes</td>
<td>Alport syndrome</td>
</tr>
<tr>
<td><strong>Other Collagens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>Ubiquitous in microfibrils</td>
<td>Bethlem myopathy</td>
</tr>
<tr>
<td>VII</td>
<td>Anchoring fibrils at dermal-epidermal junctions</td>
<td>Dystrophic epidermolysis bullosa</td>
</tr>
<tr>
<td>IX</td>
<td>Cartilage, intervertebral disks</td>
<td>Multiple epiphyseal dysplasias</td>
</tr>
<tr>
<td>XVII</td>
<td>Transmembrane collagen in epidermal cells</td>
<td>Benign atrophic generalized epidermolysis bullosa</td>
</tr>
<tr>
<td>XV and XVIII</td>
<td>Endostatin-forming collagens, endothelial cells</td>
<td>Knobloch syndrome (type XVIII collagen)</td>
</tr>
</tbody>
</table>
Cell adhesion molecules (CAMs) =
located in the cell membrane- function as receptors; or they are stored in the cytoplasm
1. **Immunoglobulin family CAMs** (ICAM, VCAM, PECAM) – homotypic (interaction b/t same cell) and hetertypic
2. **Cadherins** – Ca++ homotypic interactions; Cadherin = Ca++ dependent adherence protein
   - form two types of cells junctions: zonula adherens and desmesomes
   - major role in regulating cell motility, proliferation, differentiation
   - linked to the cytoskeleton via catenins (α-catenin, β-catenin): β-catenin links cadherin to α-catenin, which connects to actin
   - β-catenin can act independently of cadherins; functions as a regulator of nuclear transcription factors in the Wnt signaling pathway
3. **Integrins** – bind to matrix proteins (fibronectin, laminin) to bind cells to ECM; and bind to adhesive proteins in other cells
   - integrins provide mechanical transmission of intracellular signal transduction pathways: ligand binds integrins → formation of focal adhesion complex → trigger signal transduction pathways (**MAP kinase, PKC, PI-3**)
   - *talin, vinculin, paxillin* = cytoskeletal proteins that colocalize with integrins
   - fibronectin = binds to collagen, fibrin, proteoglycans, cell-surface receptors
   - laminin = most abundant glycoprotein in the BM; binds ECM and cell surface receptors
   - adhesive proteins =
4. **Selectins**

**Cadherins and integrins link the cell surface with the cytoskeleton**

---

**Extracellular Matrix**
1. Collagen
2. Elastic fibers (elastin, fibrillin)
3. Cell adhesion proteins = CAMS
4. Proteoglycans and Hyaluronic acid

In addition to the 4 families of CAMs, there are **secreted adhesion molecules** that are often important in disease processes
1. **SPARC (osteonectin)** – tissue remodeling; inhibit angiogenesis
2. **Thrombospondins** – some inhibit angiogenesis
3. **Osteopontin** – regulates calcification; mediates leukocyte migration; ligand for CD44
4. **Tenacin** – involved in morphogenesis and cell adhesion

**Proteoglycans**: regulate ct structure and permeability;
Core protein of 1+ GAGs
- heparan sulfate
- chondroitin sulfate
- dermatan sulfate

**HA**: a polysaccharide of the GAG family; found in ECM
- binds H20
- found in cartilage in joints
- inhibits cell-cell adhesion
- facilitates cell motility
- binds to CD44 on leukocytes
Table 3-3  Vascular Endothelial Growth Factor (VEGF)

| Proteins | Family members: VEGF (VEGF-A), VEGF-B, VEGF-C, VEGF-D  
|          | Dimeric glycoprotein with multiple isoforms  
|          | Targeted mutations in VEGF result in defective vasculogenesis and angiogenesis |
| Production | Expressed at low levels in a variety of adult tissues and at higher levels in a few sites, such as podocytes in the glomerulus and cardiac myocytes |
| Inducing Agents | Hypoxia  
|          | TGF-β  
|          | PDGF  
|          | TGF-α |
| Receptors | VEGFR-1  
|          | VEGFR-2 (restricted to endothelial cells)  
|          | VEGFR-3 (lymphatic endothelial cells)  
|          | Targeted mutations in the receptors result in lack of vasculogenesis |
| Functions | Promotes angiogenesis  
|          | Increases vascular permeability  
|          | Stimulates endothelial cell migration  
|          | Stimulates endothelial cell proliferation  
|          | VEGF-C selectively induces hyperplasia of lymphatic vasculature  
|          | Up-regulates endothelial expression of plasminogen activator, plasminogen activator inhibitor-1, tissue factor, and interstitial collagenase |
**Repair by Healing, Scar, Fibrosis**
1. Induction of inflammation, removal of damaged/dead tissue
2. Proliferation and migration of parenchyma and connective tissue
3. Angiogenesis and granulation tissue
4. Synthesis of ECM proteins; dep collagen
5. Tissue remodeling
6. Wound contraction
7. Acquisition of wound strength

**HALLMARK** of healing = granulation tissue

---

**Angiogenesis**
- angioblasts
- angioblast like cells = EPCs (endothelial precursor cells)
- these express:
  - vascular endothelial-cadherin
  - E-selectin
  - Tie2 receptor
- **VEGFR-2**, a tyrosine kinase receptor is the MOST important in angiogenesis and is largely restricted to the endothelium
- Angiopoietin 1 and 2, PDGF, TGF-β contribute to stabilization

**Migration / proliferation of fibroblasts:**
- TGF-β
- PDGF
- EGF
- FGF
- IL-1
- TNF

**Collagen synthesis:**
- PDGF
- FGF
- TGF- β
- IL-1
- IL-13

**of these TGF-β is the most important:**
1. fibroblast migration and proliferation
2. increased synthesis of collagen and fibronectin
3. decreased degradation of ECM by MMP
4. chemotactic for monocytes
5. stimulates angiogenesis

---

**ECM Proteins as Regulators of Angiogenesis**
1. Integrins (α5β3) = formation and maintenance of new vessels
2. Matricellular proteins = destabilize cell-matrix interactions
   - thrombospondin 1
   - SPARC
   - tenasin C
3. Proteinases
   - plasminogen activators
   - matrix metalloproteinases – important in tissue remodeling
   - endostatin – inhibits endothelial proliferation and angiogenesis
4. VEGF, FGF-2 – stimulate angiogenesis
5. α5β3 integrin – released from endothelial cells in response to hypoxia; interacts wth MMP-2, VEGFR-2, fibronectin, thrombospondin, osteopontin

---

**VEGF =** stimulates proliferation and motility of endothelial cells
**Ang1 =** binds to Tie2 – recruits periendothelial cells
**PDGF =** recruits smooth muscle cells
**TGF-β =** stabilizes new vessels; enhances production of ECM
**Degradation of collagen and other ECM proteins is achieved by MMPs, which are dependent on zinc ions for their activity.**

---

**Table 3-4  GFs and Cytokines Affecting Wound Healing**

<table>
<thead>
<tr>
<th>Response</th>
<th>Growth Factors (GFs) and Cytokines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocyte chemotaxis</td>
<td>PDGF, FGF, TGF-β</td>
</tr>
<tr>
<td>Fibroblast migration</td>
<td>PDGF, EGF, FGF, TGF-β, TNF, IL-1</td>
</tr>
<tr>
<td>Fibroblast proliferation</td>
<td>PDGF, EGF, FGF, TNF</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>VEGF, FGF, Ang</td>
</tr>
<tr>
<td>Collagen synthesis</td>
<td>PDGF, TGF-β</td>
</tr>
<tr>
<td>Collagenase secretion</td>
<td>PDGF, EGF, FGF, TNF, TGF-β inhibits</td>
</tr>
</tbody>
</table>
**HALLMARK** of chronic interstitial lung diseases, known as pneumoconiosis is **FIBROSIS**

<table>
<thead>
<tr>
<th><strong>Local Factors</strong></th>
<th><strong>Systemic Factors</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood supply</td>
<td>Age</td>
</tr>
<tr>
<td>Denervation</td>
<td>Anemia</td>
</tr>
<tr>
<td><strong>Local infection</strong></td>
<td>Drugs (steroids, cytotoxic meds, Abx)</td>
</tr>
<tr>
<td>Foreign body</td>
<td>Genetic disorders (osteogenesis imperfecta, Ehlers-Danlos syndrome, )</td>
</tr>
<tr>
<td>Hematoma</td>
<td>Hormones</td>
</tr>
<tr>
<td>Mechanical stress</td>
<td>Diabetes</td>
</tr>
<tr>
<td>Necrotic tissue</td>
<td>Malignant disease</td>
</tr>
<tr>
<td>Protection (dressings)</td>
<td>Malnutrition</td>
</tr>
<tr>
<td>Surgical techniques</td>
<td>Obesity</td>
</tr>
<tr>
<td>Type of tissue</td>
<td>Systemic infection</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
</tr>
<tr>
<td></td>
<td>Trauma, hypoglycemia, hypoxia</td>
</tr>
<tr>
<td></td>
<td>Uremia</td>
</tr>
<tr>
<td></td>
<td>Vitamin deficiency (Vit C)</td>
</tr>
<tr>
<td></td>
<td>Trace metal deficiency (zinc, copper)</td>
</tr>
</tbody>
</table>

**INFECTION** is the single most important cause of delay in healing – persistent injury and inflammation

**First Intention Healing** = primary union; wounds with opposed edges

**Second Intention Healing** = secondary union; regeneration of parenchymal cells cannot be completely restore the original architecture, hence abundant granulation tissue grows in from the margina

**WOUND CONTRACTION** is the feature that most clearly differentiates primary from secondary healing
Wound strength:
1 week = 10%
3 months = 70-80%

Complications of Cutaneous Wound Healing:
1. deficient scar formation
2. excessive formation of wound repair components
3. formation of contractures