Observations regarding the repair of wounds (i.e., wound healing) date to physicians in ancient Egypt and battle surgeons in classic Greece. The liver’s ability to regenerate forms the basis of the Greek myth involving Prometheus. The clotting of blood to prevent exsanguination was recognized as the first necessary event in wound healing. At the time of the American Civil War, the development of “ludable pus” in wounds was thought to be necessary, and its emergence was not appreciated as a symptom of infection but considered a positive sign in the healing process. Later studies of wound infection led to the discovery that inflammatory cells are primary actors in the repair process. Although scurvy (see Chapter 8) was described in the 16th century by the British navy, it was not until the 20th century that vitamin C (ascorbic acid) was found to be necessary for the function of prolyl hydroxylase, an enzyme required for proper folding and stabilization of collagen into a triple helix. The study of wound healing now encompasses a complex environment containing many cell types, matrix proteins, growth factors, and cytokines, which regulate and modulate the repair process. Nearly every stage in the repair process is redundantly controlled, and there is no single rate-limiting step, with the possible exception of the ingress of inflammatory cells. Successful healing maintains tissue function and repairs tissue barriers, preventing blood loss and infection, but is usually accomplished through collagen deposition or scarring (fibrosis). Advances in our understanding of growth factors, extracellular matrix and stem cell biology are improving healing, and offer the possibility of regenerating tissues to their normal architecture and of engineering replacement tissues.

Successful repair relies upon a crucial balance between the yin of matrix deposition and the yang of matrix degradation. Regenerative processes are favored when the matrix composition and architecture are unaltered. Thus, wounds that do not heal may reflect excess proteinase activity, decreased matrix accumulation, or altered matrix assembly. Conversely, fibrosis and scarring may result from reduced proteinase activity or increased matrix accumulation. Whereas the formation of new collagen during repair is required for increased strength of the healing site, chronic fibrosis is a major component of diseases that involve chronic injury.

The Basic Processes of Healing

Many of the basic cellular and molecular mechanisms necessary for wound healing are found in other processes involving dynamic tissue changes, such as development and tumor growth. Three key cellular mechanisms are necessary for wound healing:
- Cellular migration
- Extracellular matrix organization and remodeling
- Cell proliferation

Migration of Cells Initiates Repair

Cells That Migrate to the Wound

Ingress of cells into a wound and activation of local cells are initiated by mediators that are either released de novo by resident cells or from reserves stored in the granules of platelets and basophils. The contents of these granules include cytokines, chemoattractants, proteases, and mediators of inflammation, which together (1) control vascular delivery, (2) degrade damaged tissue, and (3) initiate the repair cascade. Platelets are activated when bound to collagen exposed at sites of endothelial damage, and their ensuing aggregation, in combination with
fibrin cross-linking, limits blood loss. Activated platelets release platelet-derived growth factor (PDGF) and other molecules that facilitate adhesion, coagulation, vasoconstriction, repair, and clot resorption. Mast cells are bone marrow-derived cells whose granules contain high concentrations of heparin. They reside in connective tissue near small blood vessels and respond to foreign antigens by releasing the contents of their granules, many of which are angiogenic. Resident macrophages, tissue-fixed mesenchymal cells, and epithelial cells release mediators that not only contribute to the early response, but also perpetuate it. Their numbers are increased through proliferation and recruitment to the site of injury (Fig. 3-1). The following are characteristic of skin wounds:

- **Leukocytes** arrive at the wound site early and migrate rapidly by forming small focal adhesions (focal contacts). A family of small peptide chemoattractants (chemokines), are capable of restricted or broad recruitment of particular leukocytes (see Chapter 2).

- **Polymorphonuclear leukocytes** are rapidly recruited from the bone marrow and invade the wound site within the first day. They degrade and destroy nonviable tissue by releasing their granular contents.

- **Macrophages** arrive shortly after neutrophils but persist for days or longer. They phagocytose debris and orchestrate the developing granulation tissue by the release of cytokines and chemoattractants.

- **Fibroblasts, myofibroblasts, pericytes, and smooth muscle cells** are recruited by growth factors and matrix degradation products, arriving in a skin wound by day 3 or 4. These cells are responsible for fibroplasia, synthesis of connective tissue matrix, tissue remodeling, wound contraction, and wound strength.

- **Endothelial cells** form nascent capillaries by responding to growth factors and are visible in a skin wound beyond day 3. The development of capillaries is necessary for the exchange of gases, the delivery of nutrients, and the influx of inflammatory cells.

- **Epithelial cells** in the epidermis move across the surface of a skin wound, penetrate the provisional matrix (see below), and migrate upon stromal collagen, which is coated with plasma glycoproteins, fibrinogen, and fibronectin. The process of reepithelialization is delayed if the migrating epithelial cells must reconstitute a damaged basement membrane. In addition, the phenotype of the epithelial layer is altered in the absence of basement membrane.

- **Stem cells** from bone marrow, the bulb of the hair follicle, and the basal epidermal layer provide a renewable source of epidermal and dermal cells capable of differentiation, proliferation, and migration. Under appropriate conditions, these cells form new blood vessels and new epithelium and regenerate skin structures, such as hair follicles and sebaceous glands.

### Mechanisms of Cell Migration

Cell migration uses the most important mechanism of wound healing, namely the response of cells to chemical signals (cytokines) and insoluble substrates of the extracellular matrix. Locomotion of the rapidly migrating leukocytes is powered by broad, wavelike, membrane extensions called lamellipodia. Slower moving cells, such as fibroblasts, extend narrower, fingerlike membrane protrusions labeled filopodia. Cell polarization and membrane extensions are initiated by growth factors or chemokines, which trigger a response by binding to their specific receptors on the cell surface. Actin filaments polymerize and form a network at the membrane’s leading edge, thereby propelling lamellipodia and filopodia forward, with traction provided via attachments to the extracellular matrix substrate. Actin-related proteins stimulate actin assembly, and numerous actin-binding proteins act like molecular tinker toys, rapidly constructing, stabilizing and destabilizing actin networks.

The leading edge of the cell membrane impinges upon the extracellular matrix and adheres to it through transmembrane adhesion receptors termed integrins (see Chapter 2). These molecules are highly redundant, and many different integrin heterodimer combinations recognize the same matrix components (e.g., collagen, laminin, fibronectin). Focal contacts develop through the adherence of the integrin extracellular domain to the connective tissue matrix. Focal adhesions form under the cell body, whereas smaller focal contacts form at the leading edge of migrating cells. The focal contact anchors the actin stress fibers, against which myosins pull to extend or contract the cell body. As the cell moves forward, older adhesions at the rear are weakened or destabilized, allowing the trailing edge to retract.

More than 50 proteins have been associated with the formation of adhesion plaques. The cytoplasmic domain of integrins is the foundation of a protein cascade that acts to anchor actin stress fibers. The Rho-family of GTPases (Rho, Rac, and Cdc42) act as molecular switches that interact with surface receptors to regulate matrix assembly, generate focal adhesions, and organize the actin cytoskeleton.

Importantly, the integrins transmit intracellular signals to cells that also regulate cellular survival, proliferation, and differentiation. These functional properties are also affected by other cell activators (e.g., cytokines) by signaling through cytoplasmic tails of integrins from inside the cell to the outside matrix. In this manner, cytokines can also influence the organization and tension in matrix and tissue. Thus, growth factors and integrins share several common signaling pathways, but integrins are unique in their ability to organize and anchor the cytoskeleton. Cytoskeletal connections are involved in cell-cell and cell-matrix connections and determine the shape and differentiation of epithelial, endothelial, and other cells.

### Extracellular Matrix Sustains the Repair Process

Three types of extracellular matrix contribute to the organization, physical properties, and function of tissue:

- **Basement membrane**
- **Provisional matrix**
- **Connective tissue (interstitial matrix or stroma)**

### Basement Membranes

Basement membranes, also called basal lamina, are thin, well-defined layers of specialized extracellular matrix that separate the cells that synthesize it from connective tissue (Fig. 3-2). By light microscopy, a basement membrane appears as a thin lamina that is stained by the periodic acid-Schiff stain (PAS). Epithelium, adipocytes, muscle cells, Schwann cells, and capillary endothelium produce basement membranes.

- Basement membranes are constructed from extracellular matrix molecules, including collagen IV, laminin, entactin/
FIGURE 3.1. Cell migrations during repair. (1) Leukocytes attach to and migrate between capillary endothelial cells, penetrate the basement membrane, and enter the matrix. (2) Capillary endothelial cells, released from the basement membrane, migrate through the matrix to form new capillaries. (3) Pericytes detach from endothelial cells and their basement membranes to migrate into the matrix. (4) Fibroblasts become bipolar and migrate through the matrix to the site of injury. (5) Epithelial keratinocytes detach from neighboring cells and basement membranes and migrate between the scab and the wound along the provisional matrix of the dermis. FGF = fibroblast growth factor; VEGF = vascular endothelial growth factor.
nidogen, and perlecan, a heparan sulfate proteoglycan (Table 3-1). They self-assemble into a sandwich-like structure composed of two interacting networks.

- Within different tissues and during development, the expression of unique members of the collagen IV and laminin families imparts diversity to the basement membrane and the many structures and functions it supports.
- Basement membranes act as filters, cellular anchors, and a surface for migrating epidermal cells after injury. They also serve to reestablish the neuromuscular junction after nerve damage. Basement membranes also determine cell shape, contribute to developmental morphogenesis, and, notably, provide a repository for growth factors and chemotactic peptides.

Provisional Matrix

Provisional matrix is a term that describes the temporary extracellular organizations of plasma-derived matrix proteins and tissue-derived components that accumulate at sites of injury (e.g. hyaluronan, tenascin, and fibronectin). These molecules associate with the preexisting stromal matrix and serve to stop blood or fluid loss. They also support the migration of monocytes, endothelial cells, epidermal cells, and fibroblasts to the wound site. Plasma-derived provisional matrix proteins include fibrinogen, fibronectin, and vitronectin. These proteins become insoluble by binding to the stromal matrix and by forming cross-links.

### TABLE 3–1

<table>
<thead>
<tr>
<th>Basement Membrane Constituents and Organization</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basement Membrane Components</strong></td>
</tr>
<tr>
<td>Perlecan (heparan sulfate proteoglycan)</td>
</tr>
<tr>
<td>Laminin</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Nidogen/Entactin</td>
</tr>
<tr>
<td>Collagen IV</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Minor Collagens VIII, XV, XVIII</td>
</tr>
</tbody>
</table>

FGF = fibroblast growth factor, SPARC = secreted protein acidic and rich in cysteine; VEGF = vascular endothelial growth factor.
**Stromal (Connective Tissue) Matrix**

Connective tissue forms a continuum between tissue elements such as epithelia, nerves, and blood vessels and provides physical protection by conferring resistance to compression or stretching. The connective tissue stroma is also an important medium for the storage and exchange of bioactive proteins.

Connective tissue contains both extracellular matrix elements and individual cells that synthesize the matrix. The cells are primarily of mesenchymal origin and include fibroblasts, myofibroblasts, adipocytes, chondrocytes, osteocytes, and endothelial cells. Bone marrow-derived cells (e.g., mast cells, macrophages, and transient leukocytes) also populate connective tissue.

The extracellular matrix of connective tissue, commonly referred to as stroma or interstitium, is defined by fibers formed from a large family of collagen molecules (Table 3-2). Of the fibrillar collagens, type I collagen is the major constituent of bone. Type I and type III collagens are prominent in skin; type II collagen is the predominant form in cartilage. Elastin fibers, which impart elasticity to skin, large blood vessels, and lungs, are decorated by microfibrillar proteins such as fibrillin. The so-called ground substance represents a number of molecules, including glycosaminoglycans (GAGs), proteoglycans, and fibronectin, which provide for many important biological functions of connective tissue in addition to the support and modulation of cell attachment.

**Collagens**

Collagen is the most abundant protein in the animal kingdom; it is essential for the structural integrity of tissues and organs. When collagen synthesis is reduced, delayed, or abnormal, the result is failed wound healing, as seen in scurvy. Excess collagen deposition leads to fibrosis. Fibrosis is the basis of connective tissue diseases such as scleroderma and keloids, and also accounts for the compromised tissue function that accompanies chronic damage to many organs, including kidney, lung, and liver.

The collagen superfamily of insoluble extracellular proteins comprises the constituents of connective tissue in all organs, most notably cornea, arteries, dermis, cartilage, tendons, ligaments, and bone. There are more than 20 collagen proteins, assembled from at least 38 different polypeptide chains. Common to all collagen chains are helical segments, largely composed of glycine, proline, hydroxyproline, and hydroxylysine, in which every third amino acid is glycine (Gly-X-Y). The collagen domain that codes for the glycine repeat is important for the formation of the triple helical structure.

Collagen synthesis is complex and is often used as an example of the complexity of post-translational protein modification. Each molecule is made by self-association of three α chains that wind around each other to form a triple helix. The triple helix includes members from an α chain family that is unique for each collagen type. Collagen chains lose stability when errors occur that change the Gly-X-Y sequence, in which case the molecule is more vulnerable to proteinase activity. In general, successful collagen synthesis results from a series of post-translational modifications: (1) alignment of the three chains; (2) formation of the triple helix; (3) cleavage of noncollagenous terminal peptides; (4) molecular alignment and association; and (5) covalent cross-linking, which is mediated by the copper-dependent enzyme lysyl oxidase. Mutational alterations of fibrillar collagen structure are responsible for diseases of bone (osteogenesis imperfecta), cartilage (chondroplasias), skin, joints, and blood vessels (Ehlers-Danlos syndrome) (see Chapters 6 and 26).

**Fibrillar collagens** include types I, II, and III, V, and XI. Types I, II, and III are the most abundant collagens and appear as long fibrils that are formed from a staggered packing of long, cross-linked collagen molecules, whose triple helix is uninterrupted (see Table 3-2). These fibrillar collagens turn over slowly and are generally resistant to proteinase digestion, except by specific matrix metalloproteinases (MMPs). Type I fibril size and structure can be modified via incorporation of type V molecules or association with type III molecules, while type XI collagen modifies type II fibril size. Mutations in fibrillar collagens, which do not contain nonhelical interruptions, range from lethal to minor and involve skin, blood vessels, bone, or cartilage. Type I collagen is the most abundant collagen, and mutations in the gene that encodes this molecule, as seen in osteogenesis imperfecta, result in assembly defects in the triple helix, leading to increased bone fractures, thin dermis, and easy bruising (see Chapter 6).

**Nonfibrillar** collagens (see Table 3-2) contain globular domains that prevent fibril formation. These collagens contain varied numbers of nonhelical domains that interrupt the triple helical segments and confer structural variability and flexibility not possessed by the fibrillar collagens. Nonhelical domains enable small collagens (IX, XII) to associate with fibrillar collagens, modulating fiber packing or a linear collagen. Collagen VI forms beaded filament structures (VI) that encircles fibrillar collagens I and II, is found close to cells, associates with elastin in elastic fibers, and mutations are associated with certain myopathies, as it helps bind muscle cells to basement membrane. Other nonfibrillar collagens act as transmembrane proteins (XVII) in the hemidesmosome that attaches epidermal cells to basement membrane and as a fibrillar anchors (VII) connecting the hemidesmosome and basement membrane to the underlying stroma. **Network-forming collagens** facilitate the formation of flexible chicken-wire networks of basement membrane collagen (IV) or more ordered hexagonal networks (VIII, X) in other tissues. Mutations in collagen IV cause the abnormal glomerular basement membrane formation seen in Alport’s syndrome. Proteolytic fragments of collagen exhibit a different set of biological properties that are also important in tissue remodeling. For example, fragments of basement membrane collagens IV, XV, and XVIII inhibit angiogenesis and tumor growth.

The collagens are called scleroproteins, meaning both white and hard; yet in one circumstance, layers of collagen can be translucent, as exemplified by the transparent cornea. The cornea consists of 10 to 20 orthogonally-stacked layers of heterodimERIC complexes of Type I and Type V collagens (Fig. 3-3), the fibrils being uniform and smaller sized than Type I collagen fibers in skin. Each layer contains parallel, uniform-sized collagen fibers that are oriented at right angles to the underlying one. In healing, the injured cornea forms disorganized white collagenous scars, which are opaque and interfere with vision. The structure of the cornea suggests those who have seen only dermal collagen, in a loose, random, basket weave-like network. Yet structured orientation of collagen in human skin has long been known. Plastic surgeons use wrinkle lines to promote inconspicuous healing, the wrinkles indicating the primary direction of the underlying dermal collagen. The tensile strength of skin that is broken parallel to creases and wrinkle lines exceeds that which is broken perpendicular to these lines, further suggesting a structured orientation of dermal collagen.
<table>
<thead>
<tr>
<th>Type</th>
<th>Macromolecular Association</th>
<th>Aggregate Form</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Fibril-forming</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Self-association in staggered array</td>
<td>I &amp; II Fibrils</td>
</tr>
<tr>
<td>II (cartilage)</td>
<td></td>
<td>III Fibrils</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V, XI</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B. Nonfibril-forming</strong> (Interrupting noncollagen domains)</td>
<td></td>
<td>Beaded filament</td>
</tr>
<tr>
<td>VI</td>
<td></td>
<td>Type II fibril</td>
</tr>
<tr>
<td>IX (cartilage, also a proteoglycan)</td>
<td></td>
<td>Type I fibril</td>
</tr>
<tr>
<td>XII</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XV &amp; XVIII (also proteoglycans)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Network-forming</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV (basal lamina)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIII</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X (hypertrophic cartilage)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Anchoring (epithelium)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td></td>
<td>Hemidesmosome and basement membrane</td>
</tr>
<tr>
<td>Transmembrane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XVII (BP180, BPAG2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Elastin and Elastic Fibers

Elastin is a secreted matrix protein that, unlike other stromal proteins, is not glycosylated (Table 3-3). Elastin allows deformable tissues such as skin, uterus, ligament, lung, elastic cartilage, and aorta to stretch and bend, and yet recoil. Its lack of carbohydrate and its hydrophobic amino acid sequence make it the most insoluble of all vertebrate proteins. It may seem surprising, therefore, that elastin fibers are damaged by aging and sun exposure, conditions that lead to age-related loss of dermal suppleness. The elastic fiber is crucial for the function of several vital tissues, yet it is not efficiently replaced during repair of skin and lung. The slow accumulation of functional elastin following damage to skin or lung is offset by the fact that it is degraded with difficulty and turns over slowly.

Elastin stability results from its (1) hydrophobicity, (2) extensive covalent cross-linking (mediated by lysyl oxidase, the same enzyme that cross-links collagen), and (3) resistance to most proteolytic enzymes. Arterial wall injury, unlike skin and lung damage, leads to rapid re-formation of the concentric rings of elastic lamellae. This observation illustrates the difference in the elastin synthetic capabilities of the vascular smooth muscle cell and those of dermal or lung fibroblasts.

Elastin is deposited as fibrils, which are complexed with several glycoproteins (microfibrils) that decorate the perimeter of the elastic fiber. The best-characterized microfibrillar protein is fibrillin (see Table 3-3). When mutated, abnormal fibrillin causes Marfan syndrome, whose pleomorphic manifestations include dissecting aortic aneurysm (see Chapter 6).

Matrix Glycoproteins

The matrix glycoproteins contribute essential biological functions to basement membrane and stromal connective tissue. In general, these molecules are large (150,000 to 1,000,000 kd) multimeric and multidomain proteins, with long arms that bind other matrix molecules and support or modulate cell attachment. Matrix glycoproteins help to (1) organize tissue topography, (2) support cell migration, (3) orient cells, and (4) induce cell behavior. The principal matrix glycoprotein of basement membrane is laminin, and that of stromal connective tissue is fibronectin.

LAMININS: The laminins are a biologically versatile family of basement membrane glycoproteins whose cross-like structure is formed by products of three related gene subfamilies to form α, β, and γ heterotrimers (see Table 3-1). There are 15 known laminin isotypes, which are formed from varying combinations of the 5α, 3β, and 3γ chains. The expression of laminin isotypes in specific tissues contributes to the heterogeneity of tissue morphology and function, in part, by supporting cell attachment. Laminin molecules self-assemble into two-dimensional sheets that associate with type IV collagen sheets and other basement membrane proteins.

The appropriate expression of epidermal laminin is key for both normal epidermal function and reepithelialization of wounds. Epidermal strength is imparted by hemidesmosomes, which develop from the binding of basement membrane laminin to epithelial integrin and collagen VII. The latter is the anchoring fibril that connects the epidermal cell and basement membrane to the dermal connective tissue. Mutations in epidermal laminin, integrin, or collagen VII produce a potentially fatal skin blistering disease, termed epidermolysis bullosa.

FIBRONECTINS: Fibronectins are versatile, adhesive glycoproteins widely distributed in stromal connective tissue and deposited in wound provisional matrix (see Table 3-3). Fibronectin chains form a V-shaped homo- or heterodimer that is connected at the C terminus by two disulfide bonds. Specific domains within fibronectin bind bacteria, collagen, heparin, fibrin, fibrinogen, and the cell matrix receptor, integrin. Indeed, the integrin receptor family has been partly defined by studies demonstrating its specific binding to fibronectin. The multifunctional dimer is designed to link matrix molecules to one another or to cells. Thrombi support cell migration on the fibronectin that links fibrin strands and are stabilized by cross-linking of factor XIII (transglutaminase) to other provisional and dermal matrix components.

Two classes of fibronectin are formed by a single gene but from different sources: (1) the less soluble cellular form and (2) a hepatocyte-derived, soluble form in plasma. Though they are coded by one gene, as many as 24 variants may be formed by alternative splicing. The plasma-derived thrombus, contains high concentrations of fibronectin, which is cross-linked to fibrin by activated coagulation factor XIII (transglutaminase). Clot-bound fibronectin supports platelet adhesion and promotes reepithelialization of corneal and cutaneous wounds by promoting keratinocyte attachment and migration. Fibronectin synthesized by mesenchymal cells, such as fibroblasts, is polymerized into insoluble fibrils, which are found in granulation tissue and loose connective tissue. Excisional wound clotting and reepithelialization are unaffected by experimentally knocking out plasma fibronectin, suggesting that cellular fibronectin can compensate for its absence.

Glycosaminoglycans (GAGs)

GAGs are long, linear polymers of specific repeating disaccharides arranged in sequence that are also known as mucopolysaccharides. The name of the GAG chain is determined by the disaccharide subunits in the polymer. GAG chains are negatively charged, owing to the presence of carboxylate groups and, with the exception of hyaluronan, the attachment of N- or O-linked sulfate groups to the disaccharide. GAG
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of several lysosomal hydrolases that function to degrade GAGs. The twelve known mucopolysaccharidoses are slowly evolving disorders of connective tissue that significantly decrease life expectancy and affect ossification of cartilage, skeletal structure, and stature facies and may cause psychomotor problems or even mild retardation.

sequences have the potential for exceptional diversity owing to epimerization and variability in modifications such as acetylation and sulfation. When the sulfated GAG chains are O-linked to serine residues of protein cores they are called proteoglycans (see below). Glycosaminoglycan storage disorders result from an autosomal recessive (or in one case X-linked) deficiency of one of several lysosomal hydrolases that function to degrade GAGs. The twelve known mucopolysaccharidoses are slowly evolving disorders of connective tissue that significantly decrease life expectancy and affect ossification of cartilage, skeletal structure, and stature facies and may cause psychomotor problems or even mild retardation.

<p>| Table 3-3: Noncollagenous Matrix Constituents of Stroma |
|---------------------------------|----------------|---------------------------------|----------------|</p>
<table>
<thead>
<tr>
<th>Stromal Connective Tissue Components</th>
<th>Molecular Structure</th>
<th>Molecular Associations</th>
<th>Tissue Structures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fibronectin</strong></td>
<td>Dimeric protein Chains chosen from ~20 splice variants of one gene</td>
<td>Integrin receptors of many cells (RGD-binding site)</td>
<td>Plasma fibronectin is soluble Cellular fibronectin can self-associate into fibrils at cell surface</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Collagen, heparin, decorin, fibrin, certain bacteria (opsonin), LTBP (latent transforming growth factor-β binding proteins)</td>
</tr>
<tr>
<td><strong>Elastin</strong></td>
<td>Elastin cross-links to form fiber Monomer with several splice variants, one gene</td>
<td>Self-association to form cross-linked fibers</td>
<td>Elastin fiber decorated with microfibrils</td>
</tr>
<tr>
<td><strong>Fibrillin</strong></td>
<td>2 members, 2 genes</td>
<td>Other components of microfibrils (LTBP), laminin, versican,</td>
<td></td>
</tr>
<tr>
<td><strong>Versican</strong> (hyaluronan-binding proteoglycans)</td>
<td>Family of 4 related genes 10–30 chondroitin sulfate and dermatan sulfate GAG chains</td>
<td>Linked to hyaluronan via CD-44 (link protein)</td>
<td></td>
</tr>
<tr>
<td><strong>Decorin</strong> Small leucine-rich proteoglycans</td>
<td>1 protein core, 1 gene</td>
<td>Collagen I and II, fibronectin, TGF-β, thrombospondin</td>
<td></td>
</tr>
</tbody>
</table>

GAG = glycosaminoglycan, TGF = transforming growth factor.
**TABLE 3-4**

<table>
<thead>
<tr>
<th>Tissue or Body Fluid</th>
<th>Primary Mesodermal Cell</th>
<th>Prominent Collagen Types</th>
<th>Noncollagenous Matrix Proteins</th>
<th>Glycosaminoglycans Proteoglycans (PGs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td></td>
<td>I, III, V, VI, XII</td>
<td>Fibronectin, elastin, fibrillin</td>
<td>Hyaluronan, decorin, biglycan, versican</td>
</tr>
<tr>
<td><strong>Dermis</strong></td>
<td></td>
<td>I, III, V, VI, XII</td>
<td>Fibronectin, elastin, fibrillin</td>
<td>Hyaluronan, decorin, biglycan, versican</td>
</tr>
<tr>
<td>Reticular/papillary</td>
<td>Fibroblast</td>
<td>I, III, V, VI, XII</td>
<td>Fibronectin, elastin, fibrillin</td>
<td>Hyaluronan, decorin, biglycan, versican</td>
</tr>
<tr>
<td>Epidermal junction</td>
<td></td>
<td>I, III, V, VI, XII</td>
<td>Fibronectin, elastin, fibrillin</td>
<td>Hyaluronan, decorin, biglycan, versican</td>
</tr>
<tr>
<td><strong>Muscle</strong></td>
<td>Muscle cell</td>
<td>I, III, V, VI, XII</td>
<td>Fibronectin, elastin, fibrillin</td>
<td>Aggrecan, biglycan, decorin, fibromodulin</td>
</tr>
<tr>
<td><strong>Peri, epicardium</strong></td>
<td>Fibroblast</td>
<td>I, III, V, VI, XII</td>
<td>Fibronectin, elastin, fibrillin</td>
<td>Aggrecan, biglycan, decorin, fibromodulin</td>
</tr>
<tr>
<td>Aortic media/adventitia</td>
<td></td>
<td>I, III, V, VI, XII</td>
<td>Fibronectin, elastin, fibrillin</td>
<td>Aggrecan, biglycan, decorin, fibromodulin</td>
</tr>
<tr>
<td><strong>Tendon</strong></td>
<td>Fibroblast</td>
<td>I, III, V, VI, XII</td>
<td>Fibronectin, tenascin (myotendon junction), elastin, fibrillin</td>
<td>Decorin, biglycan, fibromodulin, lumican, versican</td>
</tr>
<tr>
<td><strong>Ligament</strong></td>
<td>Fibroblast</td>
<td>I, III, V, VI, XII</td>
<td>Fibronectin, elastin, fibrillin</td>
<td>Decorin, biglycan, fibromodulin, lumican, versican</td>
</tr>
<tr>
<td><strong>Cornea</strong></td>
<td>Fibroblast</td>
<td>I, III, V, VI, XII</td>
<td>Fibronectin, elastin, fibrillin</td>
<td>Decorin, biglycan, fibromodulin, lumican, versican</td>
</tr>
<tr>
<td><strong>Cartilage</strong></td>
<td>Chondrocyte</td>
<td>II, IX, VI, VIII, XI X hypertrophic cartilage</td>
<td>Anchorin CII, fibronectin, tenascin</td>
<td>Hyaluronan, aggrecan, biglycan, decorin, fibromodulin, lumican, perlecan (minor)</td>
</tr>
<tr>
<td><strong>Bone</strong></td>
<td>Osteocyte</td>
<td>I, V</td>
<td>Osteocalcin, osteopontin, bone sialoprotein, SPARC (osteonecrist)</td>
<td>Decorin, fibromodulin, biglycan</td>
</tr>
<tr>
<td><strong>Basement membrane zones</strong></td>
<td>Epithelial, endothelial adipocytes, Schwann cell, muscle cells (endomysium), pericytes</td>
<td>IV, XV, XVIII</td>
<td>Laminin, Nidogen/entactin</td>
<td>Collagen XVIII (vascular), agrin (neuromuscular junctions)</td>
</tr>
</tbody>
</table>

**Table 3-4: Tissue Expression of Extracellular Matrix Molecules**

**Hyaluronan**

Hyaluronan, the only GAG that is not covalently linked to a protein, exists as a random coil of 2,000 to 25,000 disaccharides. Hyaluronan can associate with proteoglycans (defined below) that contain hyaluronan-binding regions. Certain proteoglycans bind ionically via a linking protein along the hyaluronan backbone to form large, hyaluronan/proteoglycan composites, such as aggrecan and versican (see Table 3-3), molecules that are found in cartilage and stromal tissues. The negatively charged carboxylate backbone of hyaluronan binds large amounts of water, creating a viscous gel that produces turgor in the matrix. The large size and hydrated viscosity of hyaluronan impart resilience and lubrication to joints and connective tissue, and pericellular accumulation of these molecules enables cell migration through the extracellular matrix.

**Proteoglycans**

Proteoglycans consist of varying numbers of GAGs, heparan, chondroitin sulfate, and keratan sulfate, linked by O-glycosidic bonds to serines or threonines on specific core proteins. They have a higher carbohydrate content than matrix glycoproteins, and though not branched, demonstrate varied modifications such as sulfation, unique linkages, and varying sequences. Individual proteoglycans differ in size, core proteins, choice of GAG chains, and tissue distribution.

Like the matrix glycoproteins, proteoglycans participate in matrix organization, structural integrity, and cell attachment. Though the protein core of proteoglycans often contains biological activity, the properties of several proteoglycans are largely mediated by the GAG chains themselves. Heparan sulfate GAG chains of basement membrane (perlecan and collagen XVIII) and cell-associated proteoglycans modulate the availability and actions of heparin-binding growth factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and heparin-binding epidermal growth factor (EGF). A group of small proteoglycans, which share a core protein domain of leucine-rich repeats, regulates transforming growth factor-β (TGF-β) activity and fibril formation in collagens I and II (see Table 3-3).
The tissue expressions of extracellular matrix proteins and proteoglycans are summarized in Table 3-4.

Remodeling Is the Long-lasting Phase of Repair

In the later stages of the repair process, inflammatory cells diminish in number, and capillary formation is completed. Remodeling indicates that the equilibrium between collagen deposition and degradation has been restored. The MMPs are the main digestive enzymes in remodeling, but neutrophil protease and serine proteases are also present.

A large family of 25 proteinases, the matrix metalloproteinases (MMPs), are crucial components in wound healing, because they enable cells to migrate through the stroma by degrading matrix proteins (see Table 2-3). They are involved in cell–cell communication, the activation or inactivation of bioactive molecules (e.g., matrix fragments and growth factors), and influence cell growth and apoptosis. The MMPs are synthesized as inactive zymogens and require extracellular activation by already activated MMPs or by serine proteinases. They are classified by the MMP acronym followed by a numerical suffix (e.g., MMP-1, MMP-2) or are called by common names such as collagenase, stromelysin, and gelatinase. MMPs cleave numerous extracellular substrates, many of which are degraded by more than one MMP. As with integrins, such redundancy emphasizes the importance of these molecules in regulatory control. The list of molecules needed for wound healing is indistinguishable from the list of MMP substrates. These include:

- Clotting factors
- Extracellular matrix proteins
- Latent growth factors and growth factor-binding proteins
- Receptors for matrix molecules and cell–cell adhesion molecules
- Other MMPs, other proteinases, and protease inhibitors
- Chemotactic molecules

Most MMPs are closely regulated at the transcriptional level, the exception being MMP-2 (gelatinase A), which is often constitutively expressed. Transcription is regulated by (1) integrin signaling, (2) growth factor signaling, (3) binding to certain matrix proteins, or (4) tensional force on a cell. As would be predicted by the location of their substrates, MMPs are secreted into the extracellular matrix or are membrane bound. Membrane-bound MMPs are either transmembrane molecules or are linked to glycosylphosphatidylinositol (GPI). MMP-1 and -2 associate with integrins, thereby facilitating cell migration.

In addition to enhancing migration and matrix remodeling, MMPs can disrupt cell–cell adhesions and release, activate, or inactivate bioactive molecules stored in the matrix. These include growth factors, chemokines, growth factor-binding proteins, angiogenic/antiangiogenic factors, and bioactive cryptic fragments released with degradation of matrix molecules.

Once secreted, MMP activity can be minimized by binding to specific proteinase inhibitors. In addition to the important plasma-derived proteinase inhibitor, α2-macroglobulin, there is a family of endogenous tissue inhibitors of metalloproteinases (TIMPs). Another group of proteolytic enzymes with a metalloproteinase activity that process membrane bound receptors, growth factors, and cytokines are the ADAM (a disintegrin and metalloproteinase) family.

Cell Proliferation Is Evoked by Cytokines and Matrix

A prominent early feature in injured tissue is a transient increase in cellularity, which serves to replace damaged cells. Cell proliferation also serves to initiate and perpetuate granulation tissue, which is specialized vascular tissue that is formed transiently during repair (discussed below). Cells of granulation tissue accumulate from labile cell populations, including circulating leukocytes and basal epithelial cells, and from stable cells, such as capillary endothelia and resident mesenchymal cells (fibroblasts, myofibroblasts, pericytes, and smooth muscle cells). Local and marrow-derived stem or progenitor cells may also populate wounds, differentiating into endothelial and fibroblast populations. Cells that are terminally differentiated (e.g., cardiac myocytes, neurons) do not contribute to repair or regeneration (discussed below).

Growth factors and small chemotactic peptides (chemokines) provide soluble autocrine and paracrine signals for cell proliferation, differentiation, and migration. Signals from soluble factors and extracellular matrix also work collectively to influence cell behavior.

The behaviors of cells in healing wounds—proliferation, migration, and altered gene expression—are largely initiated by three receptor systems that share integrated signaling pathways:

- Protein tyrosine kinase receptors for peptide growth factors
- G protein-coupled receptors for chemokines and other factors
- Integrin receptors for extracellular matrix

Tyrosine kinase receptors, growth factors matrix integrin receptors, and G protein-coupled receptors act in concert to direct cell behavior. These distinct receptor families bind unrelated ligands yet transmit signals within a network of cascading and intersecting intracellular signaling pathways that amplify the messages, often activating similar processes. Even different processes, such as proliferation, differentiation, and migration may share signals, such as those that initiate cytoskeletal changes. A discussion of the myriad intracellular signaling mechanisms that regulate cell growth, survival and proliferation is beyond the scope of the current discussion.

Repair

Outcomes of Injury Include Repair and Regeneration

Repair and regeneration develop following inflammatory responses, inflammation itself being the primary response to tissue injury (see Chapter 2). To understand how inflammation influences repair, it is useful to review the various possible outcomes of acute inflammation. Transient acute inflammation may resolve completely, with locally injured parenchymal elements being regenerated without significant scarring. For example, in recovery from a moderate sunburn, small numbers of acute inflammatory cells temporarily accompany transient vasodilation beneath the solar-injured epidermis. By contrast, progressive acute inflammation, with emergence of macrophage-predominant inflammation, is intrinsic to the sequence of collagen elaboration and repair. Complete regeneration may be a feature of hepatic injury. For example, normal liver histology is the expected outcome following self-limited toxic drug injury.

Organization represents a pathological outcome of an inflammatory response. It occurs in serous cavities such as the
pericardium and peritoneal cavities. In pericarditis, fibroblasts secrete and organize collagen within fibrin strands, thereby binding the visceral and parietal pericardium together (Fig. 3-4). This results in constricted ventricular filling of the heart and may require surgical intervention. Fibrin strands sometimes become organized within the peritoneal cavity following intraabdominal surgery. Such adhesions (threads of collagen) can trap loops of bowel and cause intestinal obstruction.

**Wound Healing Exhibits a Defined Sequence**

Wound healing resulting in scar formation remains the predominant mode of repair. Given that wounds in the skin and the extremities are easily accessible, they have been extensively used as models. Though more difficult to study, healing within hollow viscera and body cavities generally parallels the repair sequence in skin (Table 3-5 and Fig. 3-5).

**Thrombosis**

A thrombus (clot)—referred to as a **scab** or **eschar** after drying out—forms a barrier on the wounded skin to invading surface microorganisms. This barrier also prevents the loss of plasma and tissue fluid. Formed primarily from plasma fibrin, the thrombus is rich in fibronectin. At the site of injury, fibronectin is soon cross-linked by transglutaminase to provide local tensile strength and maintain closure. The thrombus also contains contracting platelets, an initial source of growth factors. Transglutaminase cross-links collagens and fibronectin, which aids in storage of latent growth factors and affects MMP degradation of matrix. Overabundance of transglutaminase may lead to excessive scarring. Much later, the thrombus undergoes proteolysis, after which it is penetrated by regenerating epithelium. The scab then detaches.

**Inflammation**

Repair sites vary in the amount of local tissue destruction. For example, the surgical excision of a skin lesion leaves little or no devitalized tissue. Demarcated, localized necrosis accompanies medium-sized myocardial infarcts. By contrast, widespread, irregularly defined necrosis is a feature of a large third-degree burn. Initially, an acute, neutrophil-dominated, inflammatory response liquefies the necrotic tissue. Acute inflammation persists as long as necessary, since repair cannot progress until necrotic structures are liquefied and removed. Subsequently, plasma-derived fibronectin binds to collagen and cell membranes to facilitate phagocytosis. Fibronectin and cellular debris are chemotactic for macrophages and fibroblasts (see Fig. 3-5). The appearance of macrophages as the predominant cell at the site of injury signals the onset of the repair process. Macrophages ingest proteolytic products of neutrophils and secrete collage-

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**TABLE 3-5**

**Repair in Skin**

| EARLY | 1. Thrombosis: Formation of a growth factor-rich barrier having significant tensile strength  
|       | 2. Inflammation: Necrotic debris and microorganisms must be removed by neutrophils; the appearance of macrophages signals and initiates repair  
|       | 3. Reepithelialization: Newly formed epithelium establishes a permanent barrier to microorganisms and fluid  |
| MID | 4. Granulation tissue formation and function: This specialized organ of repair is the site of extracellular matrix and collagen secretion; it is vascular, edematous, insensitive, and resistant to infection  
|       | 5. Contraction: Fibroblasts and possibly other cells also transform to actin-containing myofibroblasts, link to each other and collagen, and contract, stimulated by TGF-β1 or β2  |
| LATE | 6. Accretion of final tensile strength results primarily from the cross-linking of collagen  
|       | 7. Remodeling: The wound site devascularizes and conforms to stress lines in the skin  |

TGF = transforming growth factor
FIGURE 3-5. Summary of the healing process. The initial phase of the repair reaction, which typically begins with hemorrhage into the tissues. (1) A fibrin clot forms and fills the gap created by the wound. Fibronectin in the extravasated plasma is cross-linked to fibrin, collagen, and other extracellular matrix components by the action of transglutaminases. This cross-linking provides a provisional mechanical stabilization of the wound (0–4 hours). (2) Macrophages recruited to the wound area process cell remnants and damaged extracellular matrix. The binding of fibronectin to cell membranes, collagen, proteoglycans, DNA, and bacteria (opsonization) facilitates phagocytosis by these macrophages and contributes to the removal of debris (1–3 days). (3) Fibronectin, cell debris, and bacterial products are chemoattractants for a variety of cells that are recruited to the wound site (2–4 days). The intermediate phase of the repair reaction. (4) As a new extracellular matrix is deposited at the wound site, the initial fibrin clot is lysed by a combination of extracellular proteolytic enzymes and phagocytosis (2–4 days). (5) Concurrent with fibrin removal, there is deposition of a temporary matrix formed by proteoglycans, glycoproteins, and type III collagen (2–5 days). (6) Final phase of the repair reaction. Eventually the temporary matrix is removed by a combination of extracellular and intracellular digestion, and the definitive matrix, rich in type I collagen, is deposited (5 days–weeks).
nase, thereby promoting further liquefaction. They also provide growth factors that stimulate fibroblast proliferation, collagen secretion, and neovascularization.

Fibroblasts are also early responders to injury. These collagen-secreting cells are involved in inflammatory, proliferative, and remodeling phases of wound repair. Fibroblasts are capable of further differentiation to contractile myofibroblasts.

Granulation Tissue
Granulation tissue is the transient, specialized organ of repair, which replaces the provisional matrix. Like the placenta, granulation tissue is only present where and when needed. It is deceptively simple, with a glistening and pebbled appearance (Fig. 3-6). Microscopically, a mixture of fibroblasts and red blood cells first appears, followed by the development of provisional matrix and patent single cell-lined capillaries, which are surrounded by fibroblasts and inflammatory cells.

A key step in the development of granulation tissue is the recruitment of monocytes to the site of injury by chemokines and fragments of damaged matrix. Later, plasma cells are conspicuous, even predominating. Activated macrophages coordinate the development of granulation tissue through the release of growth factors and cytokines (Table 3-6, and see below). These molecules direct angiogenesis, activate fibroblasts to form new stroma, and continue the degradation and removal of the provisional matrix. However, recent studies challenge established concepts regarding the central role of the macrophage in wound repair. Neonatal mice lacking a transcription factor (PU.1) necessary for hematopoietic maturation of macrophages and functioning neutrophils are able to repair skin wounds normally and scar-free.

Granulation tissue is fluid-laden, and its cellular constituents supply antibacterial antibodies and growth factors. It is highly resistant to bacterial infection, allowing the surgeon to create anastomoses at such nonsterile sites as the colon, in which fully one third of the fecal contents consist of bacteria.
Redundancy and interaction among growth factors, other cytokines, and MMPs are illustrated in Figures 3-8 and 3-9. The actions of growth factors are not entirely redundant, since each has a predominant function in repair. Specificity derives from (1) selective expression from members of large families, such as FGF and TGF-β; (2) temporal expression of different tyrosine kinase receptors and isoforms in unrelated cell populations; (3) variation in the response pathways or intensity by distinct receptors; and (4) latency or activation of growth factors (Table 3-6). Tables 3-7 and 3-8 exhibit how growth factors control the specific events in repair.

Several growth factor ligands are presented to their receptors bound to extracellular matrix components such as heparan sulfate proteoglycans. Interestingly, certain matrix molecules within the laminin, collagen, tenascin and decorin families contain domains with binding affinity to growth factor receptors. Signals presented in this way are spatially restricted, more persistent, and present lower affinity but concentrated signals that may influence proliferation or migration differently than soluble ligands.

Growth factors expressed as an early wound response (VEGF, FGF, PDGF, EGF, and keratinocyte growth factor [KGF]) support migration, recruitment, and proliferation of cells involved in fibroplasia, re-epithelialization and angiogenesis. Growth factors that peak later (TGF-β, and insulin-like growth factor-1 [IGF-I]) sustain the maturation phase and

### TABLE 3-6

**Extracellular Signals in Wound Repair**

<table>
<thead>
<tr>
<th>Phase</th>
<th>Factor(s)</th>
<th>Source</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulation</td>
<td>Xilla, TGF-α, TGF-β, PDGF, EGF, FGF</td>
<td>Platelets</td>
<td>Thrombosis; Chemotraction of subsequently involved cells</td>
</tr>
<tr>
<td>Inflammation</td>
<td>TGF-β, Chemokines, TNF, IL-1</td>
<td>Neutrophils, macrophages, keratinocytes,</td>
<td>Attract monocytes and fibroblasts; differentiates fibroblasts and stem cells</td>
</tr>
<tr>
<td>Granulation tissue formation</td>
<td>Basic FGF, TGF-β</td>
<td>Monocytes then fibroblasts</td>
<td>Various factors are bound to proteoglycan matrix</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>VEGFs, FGF</td>
<td>Monocytes, Macrophages, keratinocytes, fibroblasts</td>
<td>Development of blood vessels</td>
</tr>
<tr>
<td>Contraction</td>
<td>TGF-β1, β2</td>
<td>Various</td>
<td>Myofibroblasts appear, bind to each other and collagen, and contract</td>
</tr>
<tr>
<td>Reepithelialization</td>
<td>EGF, TGF-α</td>
<td>Macrophages, keratinocytes</td>
<td>Epithelial proliferation and migration</td>
</tr>
<tr>
<td>Maturation arrest of proliferation</td>
<td>TGF-β1</td>
<td>Platelets, monocytes, fibroblasts</td>
<td>Accumulation of extracellular matrix, fibrosis, tensile strength</td>
</tr>
<tr>
<td></td>
<td>Heparin sulfate proteoglycan (HSPG) Decorin proteoglycan</td>
<td>Endothelium, Secretory fibroblasts</td>
<td>HSPG: Capture of TGF-β and of VEGF and basic FGF in basement membrane</td>
</tr>
<tr>
<td></td>
<td>Interferon</td>
<td>Plasma monocytes</td>
<td>Decorin: Capture TGF-β, stabilize collagen structure, downregulate migration, proliferation</td>
</tr>
<tr>
<td></td>
<td>Increased local oxygen</td>
<td>Repair process</td>
<td>Supresses proliferation of fibroblasts and accumulation of collagen</td>
</tr>
<tr>
<td>Remodeling</td>
<td>PDGF-FGF</td>
<td>Platelets, fibroblasts</td>
<td>Induction of MMPs</td>
</tr>
<tr>
<td></td>
<td>MMPs, t-PAs, u-PAs</td>
<td>Sprouted capillaries, epithelial cells</td>
<td>Remodeling by permitting in growth of vessels and restructuring of ECM</td>
</tr>
<tr>
<td></td>
<td>Tissue inhibitors of MMPs</td>
<td>Local, not further defined</td>
<td>Balance the effects of MMPs in the evolving repair site</td>
</tr>
</tbody>
</table>

ECGF = endothelial cell growth factor; ECM = extracellular matrix; EGF = epithelial growth factor; FGF = fibroblast growth factor; IL = interleukin; MMPs = matrix metalloproteinases; PDGF = platelet-derived growth factor; TGF = transforming growth factor; TNF = tumor necrosis factor; t-PA = tissue plasminogen activator; u-PA = urokinase-type plasminogen activator; VEGF = vascular endothelial growth factor.

**Fibroblast Proliferation and Matrix Accumulation**

The temporary early matrix of granulation tissue contains proteoglycans, glycoproteins, and type III collagen (see Fig. 3-5). The release of cytokines from fixed cells in the damaged tissue causes hemorrhage and attracts inflammatory cells to the site. About 2 to 3 days after injury, activated fibroblasts and capillary sprouts are detected. The shape of fibroblasts in the wound changes from oval to bipolar, as they begin to form collagen (Fig. 3-7) and synthesize other matrix proteins, such as fibronectin. The secretion of type III collagen, initially 20% of the total collagen, is transient and a forerunner to the formation of type I collagen, which imparts greater tensile strength. Approaching the peak of matrix accumulation in 5 to 7 days, the release of TGF-β increases the synthesis of collagen and fibronectin and decreases metalloproteinase transcription and matrix degradation. Extracellular cross-linking of newly synthesized collagen progressively increases wound strength. In the PU.1 null mice, which lack macrophages, phagocytic fibroblasts substitute for macrophages engulfing dying cells of skin wounds.

**Growth Factors and Fibroplasia**

The initial discovery of EGF and the subsequent identification of at least 20 other growth factors have provided explanations for many of the rapidly changing events in repair and regeneration. Redundancy and interaction among growth factors, other
remodeling of granulation tissue. Tissue regeneration is also driven by signaling networks, which in cooperation with matrix, support self-renewal, maintenance, and differentiation of stem cells.

Wound outcomes vary after exogenous growth factors are added to wounds, depending on the experimental protocol and wound type. PDGF is approved and used to accelerate healing in neuropathic diabetic foot ulcers. In general, however, topical growth factor application does not prevent scars and has not yet been consistently demonstrated to speed or improve healing compared to accepted methods of chronic wound management. Progress in cell culture, matrix, and growth factor biology have advanced in vitro engineering of skin substitutes and improved clinical results for chronic wounds.

Although the roles of growth factors in the initiation and progression of repair are reasonably well understood, the limiting and terminating events are not well defined. Diminishing anoxia as repair progresses may be key to the arrest of the repair process. Repair may also cease because of reduced turnover of extracellular matrix. Finally, increased storage and decreased availability of growth factors may stabilize the matrix, which may then transmit signals that reduce the effects of growth factors. Granulation tissue eventually transitions to scar tissue, as the homeostasis between collagen synthesis and collagen breakdown begins to balance within weeks of injury. Fibroblasts remain active at the wound site, continuing to alter scar appearance over several years.

| TABLE 3–7 |
|-----------------|-----------------|
| **Growth Factors Control Specific Stages in Repair** | |
| Attraction of monocytes/macrophages | PDGFs, FGFs, TGF-β |
| Attraction of fibroblasts | PDGFs, FGFs, TGF-β, CTGF, EGFs |
| Proliferation of fibroblasts | PDGFs, FGFs, EGFs, IGF, CTGF, TNFs |
| Angiogenesis | VEGFs, FGFs |
| Collagen synthesis | TGF-β, PDGFs, IGF, CTGF, TNFs |
| Collagen secretion | PDGFs, FGFs, CTGF, TNFs |
| Migration and proliferation of epithelium-epidermis | KGF, TGF-α, IGF |

CTGF = connective tissue growth factor; EGF = epidermal growth factor; FGF = fibroblast growth factor; IGF = insulin-like growth factor; KGF = keratinocyte growth factor; PDGF = platelet-derived growth factor; TGF = transforming growth factor; TNF = tumor necrosis factor.
FIGURE 3-8. Cutaneous wound. A) 2–4 days. Growth factors controlling migration of cells are illustrated. Extensive redundancy is present, and no growth factor is rate limiting. Most factors have multiple effects, as listed in Table 3-7. Blood vessels are proliferating, and the epidermis is penetrating the thrombus, but not at its surface. The upper portion will become an eschar or scab. FGF = fibroblast growth factor; IGF = insulin-like growth factor; TGF = transforming growth factor; PDGF = platelet-derived growth factor; VEGF = vascular endothelial growth factor; MMPs = matrix metalloproteinases; t-PA = tissue plasminogen activator; u-PA = urokinase-type plasminogen activator.
Angiogenesis

The Growth of Capillaries

At its peak, granulation tissue has more capillaries per unit volume than any other tissue. New capillary growth is essential for the delivery of oxygen and nutrients to the cells. New capillaries form by angiogenesis (i.e., sprouting of endothelial cells from preexisting capillary venules) and create the granular appearance for which granulation tissue is named. Less often, new blood vessels form de novo from angioblasts. The latter process is known as vasculogenesis and is primarily associated with developmental processes.

Angiogenesis in wound repair is tightly regulated. Quiescent capillary endothelial cells are activated by the local release of cytokines and growth factors. The endothelial cells and pericytes are bordered by basement membranes, which must be locally degraded before endothelial cells and pericytes migrate into the provisional matrix. Endothelial passage through the matrix requires the cooperation of plasminogen activators, matrix MMPs, and integrin receptors. The growth of new capillaries is supported by the proliferation and fusion of endothelial cells and recent studies suggest that bone marrow-derived endothelial progenitor cells may also be recruited to support the growing vessel. Migration of cells into the wound site is directed by soluble ligands (by chemotaxis) and proceeds along adhesive matrix substrates (by haptotaxis). Once capillary endothelial cells are immobilized, cell–cell contacts form, and an organized basement membrane develops on the exterior of the nascent capillary. Association with pericytes and signals from angiopoietin, TGF-β, and PDGF establish a mature vessel phenotype and help form nonleaky capillaries. New capillaries that have not matured may undergo endothelial apoptosis.

Experimentally, stimulation of angiogenesis in cell culture requires extracellular matrix and growth factors. VEGF is the key driver of angiogenesis; the loss of even one allele results in lethal defects in the embryonic vasculature. In vivo angiogenesis is initiated by hypoxia and a redundancy of cytokines, growth factors, and various lipids, which stimulate or regulate VEGF. Activated granulation tissue macrophages and endothelial cells produce beta-FGF and VEGF, and wound epidermal cells release VEGF in response to keratinocyte growth factor (KGF or FGF-7). Because the chief target of VEGF is the endothelial cell, this molecule is a critical regulator of embryonic vascular development and angiogenesis, regulating endothelial survival, differentiation, and migration. Splicing variants of VEGF concentrate along both soluble and matrix bound gradients of attraction and help ensure appropriate vessel branching.

The binding of angiogenic growth factors to heparan sulfate-containing GAG chains is a crucial feature of angiogenesis. Association with heparan sulfate chains affects the availability and action of growth factors and vessel pattern formation by (1) creating a storage reservoir of VEGF and beta-FGF in capillary basement membranes and (2) using cell surface proteoglycan receptors to regulate VEGF and beta-FGF receptor conformation, as well as signal delivery and intensity.

Angiogenesis and Receptor Cross-Talk

Surface integrin receptors sense changes in the extracellular matrix and can react by modulating the cellular response to growth factors. This cross-talk is possible because integrin and growth factor signals converge to trigger many of the same signaling cascades that support survival, cell proliferation, differentiation, and migration. Unlike growth factors, integrin receptors drive cell locomotion by organizing cytoskeletal changes at the membrane. When exposed to growth factors or the loss of an organized basement membrane, quiescent endothelial cells express new integrins that modulate endothelial migration on provisional matrix proteins. Capillary sprouting relies principally on β1-type integrins, although the survival and spatial organization of the capillary network is regulated by different integrins responding to the composition and structure of their extracellular matrix ligands. Without the appropriate matrix or sufficient growth factor signaling, endothelial cells are vulnerable to apoptotic cues.

Reepithelialization

Epidermis constantly renews itself by keratinocyte mitosis at the basal layer. The squamous cells then cornify or keratinize as they mature, move toward the surface, and are shed a few days later. Maturation requires an intact layer of basal cells that are in direct contact with one another and the basement membrane. If cell–cell contact is disrupted, basal epithelial cells reestablish contact with other basal cells through mitosis. In the skin, the hair follicle is the primary source of the regenerating epithelium. Epithelial regeneration is illustrated in Figure 3-8. Once reestablished, the epithelial barrier demarcates the scar from the newly covered wound. When epithelial continuity is reestablished, the epidermis resumes its normal cycle of maturation and shedding.

Epithelialization provides a protective barrier against infection and fluid loss. In addition to epithelial cells, the epidermis includes important immune cells, such as dendritic cells and Langerhans cells. In general, epithelial cells close wounds either by migrating to cover the damaged surface or, less often, by a cinching process called purse-string closure. Skin provides the best-studied example of epithelial repair. The basal layer of skin epithelial cells, also called epidermal cells or keratinocytes, contributes important cytokines (interleukin [IL]-1, VEGF, TGF-α, PDGF, TGF-β) for the initiation of healing and the immune response. To begin migration, keratinocytes must undergo cellular differentiation before forming a new covering over the wound. Normally, these
cells are attached to laminin in the underlying basement membrane by hemidesmosome protein complexes containing $\alpha_6\beta_4$ integrin. Among the molecules associated with the hemidesmosome complex are several members of the collagen family, namely, type XVII collagen (BP-180) and collagen type VII, also termed anchoring fibril (see Table 3-2). The anchoring fibril connects the hemidesmosome–basement membrane complex to the dermal connective tissue collagen fibers. Mutations in collagen XVII, epidermal laminin, integrin $\alpha_6\beta_4$, or collagen VII produce a potentially fatal skin blistering disease, termed epidermolysis bullosa, while autoantibodies against the transmembrane collagen XVII (BP180, BPAG2) causes acquired blistering disorders like bullous pemphigoid (see Chapter 24).

Epithelial cells are connected at their lateral edges by tight junctions and by adherens junctions composed of cadherin receptors. Cadherins are calcium-dependent, integral membrane proteins that form extracellular cell–cell connections and anchor intracellular cytoskeletal connections. Cadherins in the adherens junctions bind stable actin bundles to a cytoplasmic complex of $\alpha$-, $\beta$-, and $\gamma$-catenins. The layer of actin that encircles the epithelial cytoplasm creates lateral tension and strength and is referred to as the adhesion belt. The shape and the strength of epithelial sheets result from tension created by cytoskeletal connections to basement membrane and cell-to-cell connections.

Cellular migration is the predominant means by which the wound surface is recapitIALIZED. Migrating epidermal cells originate at the margin of the wound and in hair follicles or sweat glands. If the basement membrane is lost, cells come in contact with unfamiliar stromal components, an effect that stimulates cell locomotion and protease expression. Experimentally, cells are seen to migrate along a soluble chemical gradient (chemotaxis), according to matrix concentration or adhesion (haptotaxis), and according to matrix pliability or stiffness (durotaxis).

Activation of epithelial motility is driven by the assembly of actin fibers at focal adhesions organized by integrin receptors. Different integrins bind to components of the wound, stromal, or basement membrane matrices and direct the migrating cells along the margin of viable dermis. Movement through cross-linked fibrin apposed to the dermis also requires the activation of plasmin from plasminogen to degrade fibrin. Wound margin epithelial cells of mice lacking plasminogen fail to move through the fibrin matrix. In addition to degrading fibrinogen and fibrin, plasmin aids in the activation of specific MMPs. Proteolytic cleavage of stromal collagens I and III and laminin at focal adhesion contacts can release adhesion or enable keratinocyte migration. Migrating keratinocytes eventually resume their normal phenotype after re-forming a confluent layer and attaching to their newly formed basement membrane.

### Wound Contraction
As they heal, open wounds contract and deform. The means by which wounds contract was a mystery until the discovery of a specialized cell of granulation tissue, namely, the myofibroblast (Fig. 3-9). This modified fibroblast cannot be distinguished from the collagen-secreting fibroblast by conventional light microscopy. Unlike the fibroblast, the myofibroblast expresses $\alpha$-smooth muscle actin, desmin, and vimentin, and it responds to pharmacological agents that cause smooth muscle to contract or relax. In short, it is a fibroblast that reacts like a smooth muscle cell. The myofibroblast is the cell responsible for wound contraction as well as the deforming pathological process termed wound contracture. The appearance of the myofibroblast, usually about the third day of wound healing, is associated with the sudden appearance of contractile forces, which then gradually diminish over the next several weeks. Myofibroblasts have an increased presence and persistence in fibrosis and in hypertrophic scars, particularly burn scars. Myofibroblasts exert their contractile effects by forming syncitia in which the myofibroblasts are bound together by tight junctions. By contrast, fibroblasts tend to be solitary cells, surrounded by collagen fibers. The myofibroblast may originate as a pericyte, fibroblast, or stem cell.

### Wound Strength
Skin incisions and surgical anastomoses in hollow viscera ultimately develop 75% of the strength of the unwounded site. Despite a rapid increase in tensile strength at 7 to 14 days, by the end of 2 weeks the wound has acquired only about 20% of its ultimate strength. Most of the strength of the healed wound results from intermolecular cross-linking of type 1 collagen. The 2-month-old incision, although healed, is still visibly obvious. The incision line and suture marks are distinct, vascular, and red. By 1 year, the incision is white and avascular but usually still identifiable. As the scar fades further, it is often slowly deformed into an irregular line by stresses in the skin.

### Regeneration
Regeneration is the renewal of a damaged tissue or a lost appendage that is identical to the original one. Regeneration requires a population of stem or precursor cells with the potential to differentiate and replicate. The adult human body is made up of several hundred types of well differentiated cells, yet it maintains the remarkable potential to rebuild itself by replenishing dying cells and to heal itself by recruiting or activating cells that repair or regenerate injured tissue. Some regenerative processes may be thought of as a partial recapitulation of embryonic morphogenesis from pluripotent stem cells. Unlike the newt, humans cannot regenerate limbs, but there are notable examples of regenerative processes. Tissues are adept at healing injury but the regenerative potential is unfortunately restricted to a limited number of adult tissues. Scientists have long recognized that unique cells within bone marrow, epidermis, intestine, and liver maintain sufficient developmental memory to orchestrate tissue specific regeneration. There is a great need to develop similar capabilities in joint cartilage, brain neural tissue, heart myocardium, and pancreatic beta cells. The power to replenish or regenerate tissue is derived from a small number of unspecialized cells, or stem cells, unique in their capacity for self-renewal and for producing clonal progeny that differentiate into more specialized cell types.

### Embryonic and Adult Stem Cells are Key to Regeneration
Embryonic stem (ES) cells, up to the stage of the pre-implantation blastocyst, are able to differentiate into all cells of the adult organism and preserve small populations of more restricted stem cells. Cells able to divide indefinitely, without terminally differentiating, continue to inhabit many adult tissues and have even been identified in tissues not observed to regenerate. These adult stem cells may exist in a specific tissue or be seeded in that tissue from circulating cells of bone marrow origin. Either way, the recently appreciated presence of stem cells within a broader variety of tissues underscores the importance of a permissive and supportive environment for stem cell-driven regeneration (see Table 3-9). Stem cells of adult tissues possess a more restricted
range of cell differentiation than ES cells, yet provide a promising balance between the medical hopes and ethical concerns associated with therapeutic applications using ES stem cells.

Classifying cells by their distinguishing characteristics normally aids our understanding of complex systems. Adult stem cells, however, present challenges in both identification and classification. Their classification typically involves imperfect choices between characteristics such as morphology, developmental tissue of origin, the organ or tissue the cells were isolated from, genetic or immunologic markers, or the capacity to differentiate into multiple or restricted lineages. Exceptions arise for several reasons: (1) any organ or tissue may contain more than one type of stem cell, (2) similar stem cells may be found in different organs, and (3) a stem cell found in tissue may have originated in the bone marrow. Distinguishing between stem cell types using morphology and genetic or phenotypic markers for identification can be difficult and misleading because of the small number of stem cells present in a tissue, shared features between the cells, variance in marker expression through stages of development, and the inherent phenotypic plasticity of stem cells.

Stem cells may be more generally defined by common properties that reflect their exquisite regulation, including:

- The ability to divide without limit and to avoid senescence and maintain genomic integrity
- The ability to intermittently undergo division or to remain quiescent
- The ability to propagate by self-renewal and differentiation
- The absence of lineage markers
- The shared presence of certain growth and transcription markers common to uncommitted cells

Self-Renewal

Self-renewal is the defining in vivo property of adult stem cells and an impermanent property of early ES cells. Relevant stem cell markers based on common traits are experimentally challenging to identify because the populations are small and the cells are difficult to purify. Stem cells may modify their phenotype with changes in cell culture conditions and tissue microenvironments. The presence of stem cells is commonly demonstrated by labeling cellular DNA in the S phase of the cell cycle, while the movement of bone marrow-derived cells to tissue is often studied by observing marker specific transplanted cells in tissues of the irradiated host. Stem cells achieve self-renewal through asymmetric cell division, which produces a new stem cell and a daughter cell able to transiently proliferate and terminally differentiate. In contrast to stem cells, progenitor cells have little or no capability for self-renewal. Immortal cancer cells, like stem cells, are capable of self-renewal, however their renewal is unregulated due to their inability to terminally differentiate or revert to quiescence.

This begs the question: Is there a connection between stem cells and cancer cells? Not all cancer cells are capable of regener-
ataing a tumor, suggesting that within a tumor, some cells are more differentiated and do not exhibit stem-like behavior. The cancer cell’s capacity for self-renewal may be acquired early in tumorigenesis; or tumors may arise from resident stem cells, multipotent hematopoietic stem cells, or even partially committed progenitor cells that reacquire, through transforming events, stem cell characteristics. Tumors have been likened to wounds that do not heal. Perpetuation of inflammatory processes could provide triggers that release stem or progenitor cells from their normal controls by modifying soluble and matrix-based signals of the microenvironment.

**Stem Cell Differentiation Potential**

The potential of ES cells to differentiate into all lineages diminishes with advancing stages of embryo development. Cells established from the first few divisions of the fertilized egg are **totipotent**, that is, they are capable of forming any of approximately 200 different cell types in the adult body and the cells of the placenta. Nuclei of adult somatic cells can be totipotent, as dramatically proven by nuclear transplantation cloning experiments in amphibians and mammals, but this should not be confused with stem cell potency. ES cells from the inner cell mass of the blastocyst are **pluripotent**, meaning they may differentiate into nearly all cell types. Pluripotent stem cells of the postfertilization zygote are **multipotent**, meaning they may differentiate into nearly all cell types, but are not totipotent. Many adult stem cells, which must self-renew through the life of the organism, are **multipotent**, or able to differentiate into several cell types within specialized tissues. Hematopoietic stem cells are an example of lineage-restricted multipotent stem cells, capable of forming all the cells found in blood (see Table 3-9). The terms multipotent and pluripotent are often used reciprocally, especially when referring to mesenchymal stem cells from the bone marrow, which appear capable of differentiating into multiple cells of different tissues.

**Progenitor cells** are **stable cells** distinguished from stem cells by their lack of significant capacity for self-renewal; however, they maintain the potential for differentiation and rapid proliferation. They are sometimes referred to as unipotent stem cells, as exemplified by the basal keratinocyte of skin, but some may be multipotent or oligopotent.

In addition to normal differentiation pathways within a single tissue, stem cells of one tissue may **transdifferentiate** into cells of another tissue. Transdifferentiation, or **plasticity**, may be induced experimentally using specific in vitro culture conditions or by the seeding of transplanted bone marrow stem cells in different tissue microenvironments. Somatic stem or progenitor cells, resident in a several mesenchymal tissues, but of uncertain origin, maintain the capacity to transdifferentiate. Another means of tissue specific differentiation by a circulating cell involves fusion of a circulating stem cell with a resident injured cell, as has been described in animal experiments.

Bone marrow contains hematopoietic, mesenchymal, and endothelial stem cells, providing a multifaceted regenerative capacity. Bone marrow stem cells, which are set aside during embryonic development, replenish the hematopoietic population. Endothelial stem cells from bone marrow have been implicated in tissue angiogenesis and may supplement endothelial hyperplasia during regeneration of blood vessels. Moreover, bone marrow-derived mesenchymal stem cells may populate repairing tissue in other parts of the body (see Table 3-9).

Skin epithelium and hair follicles regenerate from stem cells if the wound does not disrupt the epidermal basement membrane or the hair bulbs. Intestinal epithelium turns over rapidly and is replenished by intestinal stem cells that reside in the crypts of Lieberkühn. Liver regeneration is partly a misnomer, since the regeneration of liver following partial hepatectomy is a hyperplastic response by mature differentiated hepatocytes and, for the most part, does not involve stem cells. However, there is evidence for stem cell-driven liver regeneration when hepatocytes are damaged by viral hepatitis or toxins. This regenerative potential is thought to arise from “oval cells” in the epithelium of small bile ducts. These putative stem cells have characteristics of both hepatocytes (α-fetoprotein and albumin) and bile duct cells (γ-glutamyl transferase and duct cytokeratins) and may reside in the terminal ductal cells in the canal of Hering.

**Influence of Environment on Stem Cells**

Stem cells exist in **microenvironments** or **niches** that provide sustaining signals from matrix and neighboring cells to ensure their perpetuation. The mere presence of adult stem cells or progenitor cells is not solely sufficient for tissue regeneration when tissue is damaged. The method of repair is also influenced by the environment of the injury, that is, the growth factors, cytokines, proteases, and the composition of the extracellular matrix. Whether a wound is repaired by regeneration or scarring and fibrosis is at least partly determined by the concentration, duration, and composition of environmental signals present during inflammation. Epidermal healing during the first or second fetal trimester and maintenance regeneration of adult skin or intestinal epithelium, generally occur without inflammation present and within an innate extracellular matrix. In such instances, normal structures and architecture are regenerated without fibrosis or scarring. Wounds, however, disfigure physical damage, inflammatory growth factors, and matrix changes that influence the response away from regeneration to scarring. Spinal cord injury, as an example, provides a particularly difficult challenge. Injury-induced cellular reactions lead to the glial scar development, blocking axonal regeneration and complicating the possibility of introducing an appropriately differentiated stem cell that could drive regeneration and reestablish normal tissue function.

**Cells Can Be Classified by Their Proliferative Potential**

The cells of the body divide at different rates. Some mature cells do not divide at all, whereas others complete a cycle every 16 to 24 hours.

**LABILE CELLS**: Labile cells are found in tissues that are in a constant state of renewal. Tissues in which more than 1.5% of the cells are in mitosis at any one time are composed of labile cells. However, not all the cells in these tissues are continuously cycling. Stable cells are also constituents of labile tissues that are programmed to divide continuously. Rapidly self-renewing (labile) tissues are typically tissues that form physical barriers between the body and the external environment. These include epithelia of the gut, skin, cornea, respiratory tract, reproductive tract, and urinary tract. Notable in this group are hematopoietic cells of the bone marrow and lymphoid organs involved in immune defense. Polymorphonuclear leukocytes are the best example of a terminally differentiated cell that is rapidly renewed. Under appropriate conditions tissues composed of labile cells regenerate after injury, provided that enough stem cells remain.

**STABLE CELLS**: Stable cells populate tissues that normally are renewed very slowly but are populated with progenitor cells capable of more rapid renewal after tissue loss. The liver and the proximal renal tubules are examples of stable cell populations. Stable cells populate tissues in which fewer than 1.5% of the cells...
are in mitosis. Stable tissues (e.g., endocrine glands, endothelium, and liver) do not have conspicuous stem cells. Rather, their cells require an appropriate stimulus to divide. It is the potential to replicate and not the actual number of steady state mitoses that determines the ability of an organ to regenerate. For example, the liver, a stable tissue with less than one mitosis for every 15,000 cells, regenerates rapidly after a loss of as much as 75% of its mass.

**PERMANENT CELLS:** are terminally differentiated, have lost all capacity for regeneration, and do not enter the cell cycle. Traditionally, neurons, cardiac myocytes, and cells of the lens were considered permanent cells, though recent studies are challenging previous dogma. If lost, permanent cells cannot be replaced. Although permanent cells do not divide, most of them do renew their organelles. The extreme example of permanent cells is the lens of the eye. Every lens cell generated during embryonic development and postnatal life is preserved in the adult without turnover of its constituents.

**Conditions That Modify Repair**

**Local Factors May Influence Healing**

**Location of the Wound**

In addition to the size and shape of the wound, its location also affects healing. Sites in which skin covers bone with little intervening tissue, such as skin over the anterior tibia, are locations where skin cannot contract. Skin lesions in such areas, particularly burns, often require skin grafts because their edges cannot be apposed. Complications or other treatments, such as infection or ionizing radiation, also slow the repair process.

**Blood Supply**

Lower extremity wounds of diabetics often heal poorly or even require amputation when it otherwise would not be necessary. In such cases, advanced atherosclerosis in the legs compromises blood supply and impedes repair. Varicose veins of the legs slow the venous return and can also cause ulceration and nonhealing. Bed sores (decubitus ulcers) result from prolonged, localized, dependent pressure, which diminishes both arterial and venous blood flow. Joint (articular) cartilage is largely avascular and has limited diffusion capacity; often it cannot mount a vigorous inflammatory response. As a result, articular cartilage repairs poorly, a phenotype that usually worsens with age.

**Systemic Factors**

No specific effect of age alone on repair has been found. Although the skin of a 90-year-old person—which exhibits reduced collagen and elastin—may heal slowly, the same person’s cataract extraction or colon resection heals normally because the bowel and the eye are practically unaffected by age.

Coagulation defects, thrombocytopenia, and anemia impede repair. Local thrombosis decreases platelet activation, thereby reducing the supply of growth factors and limiting the healing cascade. The decrease in tissue oxygen that accompanies severe anemia also interferes with repair. Exogenous corticosteroids retard wound repair by inhibiting collagen and protein synthesis and by exerting antiinflammatory effects.

**Fibrosis and Scarring Contrasted**

Successful wound repair that leads to localized scarring is a transient, not chronic, process that leads to rapid resolution of local injury. By contrast, many chronic diseases involve persistent, unresolved inflammation with progression of the repair response culminating in diffuse fibrosis. Inhaled smoke or inhaled silica particles induce persistent inflammation in the lung. Immunologically mediated inflammation of joints initiates rheumatoid arthritis. Inflammatory and noninflammatory factors lead to glomerulosclerosis in the kidney, including infection, hypertension, and diabetes.

Continuing insult or inflammation, mediated through the interplay of monocytes and lymphocytes, results in persistent high levels of cytokines, growth factors, and locally destructive enzymes such as collagenases. Whatever the cause, fibrosis of parenchymal organs such as the lung, kidney, or liver disrupts the normal architecture and reduces or destroys function. The functional unit (alveolus, hepatic lobule, or renal glomerulus or tubule) is replaced by disordered collagen. Fibrosis of parenchymal organs is generally irreversible, calling for measures to prevent exposure to the cause, or therapeutic measures, as in rheumatoid arthritis, to suppress the inflammatory process and so minimize ultimate destruction of joints.

**Fibrosis should be viewed as the pathological end result of persistent injury, causing loss of function.** Often it is the common final result of diverse diseases or injuries the causes of which cannot be ascertained from the end result. As an example, scars of former glomeruli developed following bacterial or immunological injury to the kidney, the specific cause being no longer identifiable. Scarring, however, is often beneficial: the scar resulting from a surgical incision in skin, though cosmetically unattractive, holds the skin together.

Prevention of fibrosis requires either blocking the stimulus of matrix production or increasing the level of matrix degradation. Approaches to controlling fibrotic progression to end-stage kidney disease have therefore targeted profibrotic factors such as TGF-β and plasminogen activator inhibitor (PAI-1). When PAI-1 is inhibited, it fails to activate plasminogen and the resulting plasmin degrades the extracellular matrix either directly or through activating matrix MMPs. Thus matrix deposition in the glomerulus is reduced, protecting the glomerulus from scarring and obliteration. Interestingly, inhibition of PAI could also reduce the incidence of intra-abdominal adhesions, a persistent problem of abdominal surgery and the main cause of intestinal obstruction. The adhesions are initiated by fibrin deposition when mesothelial lining is disrupted. If the fibrin matrix is not dissolved by plasmin within a few days, the fibrinous adhesion is invaded by fibroblasts and eventually transformed into a permanent fibrotic adhesion.

**Specific Sites Exhibit Different Repair Patterns**

**Skin**

Healing in the skin involves both repair, primarily dermal scarring, and regeneration, principally of the epidermis and vasculature. The salient features of primary and secondary healing are provided in Figure 3-10.

**Primary healing** occurs when the surgeon closely approximates the edges of a wound. The actions of myofibroblasts are minimized, and regeneration of the epidermis is optimal, since epidermal cells need migrate only a minimal distance.

**Secondary healing** proceeds when a large area of hemorrhage and necrosis cannot be completely corrected surgically. In this situation, myofibroblasts contract the wound, and subsequent scarring repairs the defect.
HEALING BY PRIMARY INTENTION (WOUNDS WITH APPOSED EDGES)

(A) A wound with closely apposed edges and minimal tissue loss. (B) Such a wound requires only minimal cell proliferation and neovascularization to heal. (C) The result is a small scar.

HEALING BY SECONDARY INTENTION (WOUNDS WITH SEPARATED EDGES)

(A) A gouged wound, in which the edges are far apart and in which there is substantial tissue loss. (B) This wound requires wound contraction, extensive cell proliferation, and neovascularization (granulation tissue) to heal. (C) The wound is reepithelialized from the margins, and collagen fibers are deposited in the granulation tissue. (D) Granulation tissue is eventually resorbed and replaced by a large scar that is functionally and esthetically unsatisfactory.
The success and method of healing following a burn wound depends on the depth of the burn injury. If the burn is superficial or does not extend beyond the upper dermis, stem cells from the sweat glands and hair follicles will regenerate the epidermis. If deep dermis is involved, the regenerative elements are destroyed and surgery and engraftment are necessary to cover or heal the wound site and reduce scarring and severe contractures.

**Cornea**
The cornea differs from skin in its stromal organization, vascularity, and cellularity. Like skin, the stratified squamous epithelial covering is continually renewed by a stem cell population, which is located in the corneal limbus. Chemical injury to the cornea results in scarring, the white corneal scar effectively blinding the eye. Parenthetically, the cornea, because of its relative avascularity, was the first organ or anatomical structure to be successfully transplanted. Trachoma, an infectious human disease caused by an inflammatory response to *Chlamydia trachomatis*, is the world’s most common cause of blindness, resulting from scarring and opacity of the cornea (see Fig 29-1).

**Liver**
Acute chemical injury or fulminant viral hepatitis causes widespread necrosis of hepatocytes. However, if liver failure is not fatal, and if the connective tissue stroma, vasculature, and bile ducts survive, the parenchyma regenerates and normal form and function are restored. Small cells at the canal of Hering, termed oval cells, are thought to be the stem cell responsible for this method of liver regeneration (see Table 3-9). By contrast, chronic injury in viral hepatitis or alcoholism is associated with the development of broad collagenous scars within the hepatic parenchyma, termed **cirrhosis** of the liver (Fig. 3-11). The hepatocytes form regenerative nodules that lack central veins and expand to obstruct blood vessels and bile flow. Portal hypertension and jaundice ensue despite adequate numbers of regenerated but disconnected hepatocytes.

![FIGURE 3-11. Cirrhosis of the liver.](image URL)

In the Greek myth of Prometheus, a vulture tore out his liver every evening, only to have it grow back by morning. It required more than another two millennia for the demonstration that the liver indeed possesses tremendous regenerative capacity, even though the normal hepatic parenchyma is almost devoid of mitoses and virtually all hepatocytes are in cell cycle phase G0. This method of liver regeneration, after resection, is actually compensatory hyperplasia of hepatocytes. The necessary conditions for hepatic regeneration are complex and beyond the scope of this discussion. Suffice it to say that regeneration is arrested when the normal ratio of liver-to-total body weight is reestablished; the molecular switch that regulates this ratio is obscure. In human liver transplantation, a partial donation of the right lobe of the liver from a living donor is followed by complete regeneration of the normal liver in both the recipient and the donor.

**Kidney**
Although the kidney has limited regenerative capacity, the removal of one kidney (nephrectomy) is followed by compensatory hypertrophy of the remaining kidney. In the case of renal injury, if it is not extensive and the extracellular matrix framework is not destroyed, the tubular epithelium regenerates. In most renal diseases, however, there is some destruction of the framework. Regeneration is then incomplete, and scar formation is the usual outcome. The regenerative capacity of renal tissue is maximal in cortical tubules, less in medullary tubules, and nonexistent in glomeruli. Recent data suggest tubule repair occurs not from bone-marrow derived cells but due to proliferation of endogenous renal progenitor cells.

**Cortical Renal Tubules**
Normally, there is some turnover of tubular epithelium, leading to shedding of cells in the urine. No reserve cell has been identified, and simple division accomplishes replacement. The outcome of injury depends on whether the tubular basement membrane is ruptured. If the injury does not produce discontinuities in the basement membrane, the surviving tubular cells in the vicinity of the wound flatten, acquire a squamous appearance, and migrate into the necrotic area along the basement membrane. Mitoses are frequent, and occasional clusters of epithelial cells project into the lumen. Soon, the flattened cells are more cuboidal, and differentiated cytoplasmic elements appear. Tubular morphology and function are normal in 3 to 4 weeks.

**Tubulorrhesis**
*Tubulorrhesis* refers to the rupture of the tubular basement membrane. The sequence of events resembles that of tubular damage, in which the basement membrane is intact, except that interstitial changes are more prominent. Proliferation of fibroblasts, increased deposition of extracellular matrix, and collapse of the tubular lumen are seen. The final result is regeneration of some tubules and fibrosis of others, usually causing focal losses of functional nephrons.

**Medullary Renal Tubules**
Medullary diseases of the kidney are often associated with extensive necrosis, which involves tubules, interstitium, and blood vessels. If the lesion is not fatal, the necrotic tissue sloughs into the urine. Healing by fibrosis produces urinary obstruction within the kidney. Although there is some epithelial proliferation, there is no significant regeneration.
Glomeruli
Unlike tubules, glomeruli do not regenerate. Injuries that produce necrosis of glomerular endothelial or epithelial cells, whether focal, segmental, or diffuse, heal by scarring (Fig. 3-12). Mesangial cells are related to smooth muscle cells and seem to have some capacity for regeneration. Following unilateral nephrectomy, the glomeruli in the remaining kidney undergo hypertrophy and hyperplasia to produce greatly enlarged glomeruli.

Lung
The epithelium lining the respiratory tract has an effective regenerative capacity, provided that the underlying extracellular matrix framework is not destroyed. Superficial injuries to tracheal and bronchial epithelia heal by regeneration from the adjacent epithelium. The outcome of alveolar injury ranges from complete regeneration of structure and function to incapacitating fibrosis. As is the case with the liver, the degree of cell necrosis and the extent of the damage to the extracellular matrix framework determine the outcome (Fig. 3-13).

Alveolar Injury with Intact Basement Membranes
Alveolar injury follows a number of insults, for example, infections, shock, and oxygen toxicity. The injury produces a variable degree of alveolar cell necrosis. The alveoli are flooded with an inflammatory exudate particularly rich in plasma proteins. As long as the alveolar basement membrane remains intact, healing is by regeneration, and neutrophils and macrophages clear the alveolar exudate. If these cells fail to liquefy the alveolar exudate, it is organized by granulation tissue, and intra-alveolar fibrosis results. Alveolar type II pneumocytes (the alveolar reserve cells) migrate to denuded areas and undergo mitosis to form cells with features intermediate between those of type I and type II pneumocytes. As these cells cover the alveolar surface, they establish contact with other epithelial cells. Mitosis then stops and the cells differentiate into type I pneumocytes. Regeneration may occur via bone-marrow derived cells or by putative lung bronchioalveolar progenitor or stem cells that differentiate to bronchiolar Clara cells and alveolar cells (see Table 3-9).

Alveolar Injury with Disrupted Basement Membranes
Extensive damage to the alveolar basement membrane evokes scarring and fibrosis. Mesenchymal cells from the alveolar septa proliferate and differentiate into fibroblasts and myofibroblasts. The role of macrophage products in inducing fibroblast proliferation in the lung is well documented. The myofibroblasts and fibroblasts migrate into the alveolar spaces, where they secrete extracellular matrix components, mainly type I collagen and proteoglycans, to produce pulmonary fibrosis. The most common chronic pulmonary disease is emphysema, which involves airspace enlargement and the destruction of alveolar walls. Ineffective replacement of elastin is associated with irreversible loss of tissue resiliency and function.

Heart
Cardiac myocytes are permanent, nondividing, terminally differentiated cells. Recent studies, however, have provided evidence for minimal regeneration of cardiac myocytes from previously unrecognized stem or reserve cells. The origin of these cells, whether they reside in the myocardium or migrate there following injury from sites unknown, is not resolved. For practical purposes, myocardial necrosis, from whatever cause, heals by the formation of granulation tissue and eventual scarring (Fig. 3-14). Not only does myocardial scarring result in the loss of contractile elements, but the fibrotic tissue also decreases the effectiveness of contraction in the surviving myocardium.

Nervous System
Mature neurons have been described as permanent and postmitotic cells, and recent studies suggesting possible regenerative capacity have not altered well-established observations about injury in the nervous system. Following trauma, only regrowth and reorganization of the surviving neuronal cell processes can reestablish neural connections. Although the peripheral nervous system has the capacity for axonal regeneration, the central nervous system lacks this ability. The olfactory bulb and hippocampal dentate gyrus regions of adult mammalian brain are now known to regenerate via neural precursor or stem cells, although the physiological significance of this finding remains to be determined. Multipotent precursor cells have also been demonstrated in other parts of the brain, raising hope that repair of neural circuitry may eventually be possible (see Table 3-9).

Central Nervous System
Any damage to the brain or spinal cord is followed by the growth of capillaries and gliosis (i.e., the proliferation of astrocytes and
FIGURE 3-13. Overview of repair. This figure provides an overview that interrelates the early dynamic events in repair. The time scale in this figure is not linear; initial tensile strength, the first phase, develops almost immediately. Remodeling is ill defined, extending from its early beginning in repair for weeks or months.
Pathology

microglia). Gliosis in the central nervous system is the equivalent of scar formation elsewhere; once established, it remains permanently. In spinal cord injuries, axonal regeneration can be seen up to 2 weeks after injury. After 2 weeks, gliosis has taken place and attempts at axonal regeneration end. In the central nervous system, axonal regeneration occurs only in the hypothalamohypophysial region, where glial and capillary barriers do not interfere with axonal regeneration. Axonal regeneration seems to require contact with extracellular fluid containing plasma proteins.

Peripheral Nervous System

Neurons in the peripheral nervous system can regenerate their axons, and under ideal circumstances, interruption in the continuity of a peripheral nerve results in complete functional recovery. However, if the cut ends are not in perfect alignment or are prevented from establishing continuity by inflammation or a scar, a traumatic neuroma results (Fig. 3-15). This bulbous lesion consists of disorganized axons and proliferating Schwann cells and fibroblasts. The regenerative capacity of the peripheral nervous system can be ascribed to (1) the fact that the blood–nerve barrier, which insulates peripheral axons from extracellular fluids, is not restored for 2 to 3 months; and (2) the presence of Schwann cells with basement membranes. Laminin, a basement membrane component, and nerve growth factor (NGF) guide and stimulate neurite growth.

Fetal Wound Repair

Progress in surgery now permits corrective surgical operations to be performed in utero. Wounds produced in the first and second trimesters of pregnancy heal without scarring; at birth, healed cutaneous incisions are not visible. Fetal wounds also heal more rapidly than adult wounds. Adult-type healing occurs in late pregnancy. Fetal healing is characterized by the virtual absence of acute inflammation, by the regeneration of skin appendages such as hair follicles (not seen in adult healing) and by the absence of TGF-β in fetal skin. Hyaluronan is preserved at higher levels for a longer time in fetal repair as compared with adult repair, apparently inhibiting scarring. MMPs are also increased in fetal skin, promoting scarless healing. The fibroblasts of fetal wounds do not appear to require activation to secrete collagen, in contrast to adult fibroblasts.

The epidermis of the fetal skin is bilayered, as contrasted with the multiple layers of the stratified adult epidermis. Embryonic epidermal cells around the margin of the wound are pulled over the wound via contraction of a thick cable of actin along the leading edge of the cells. Fetal epidermal cells are drawn toward one another as if gathered by a purse-string, whereas adult wound keratinocytes crawl along the matrix using lamellipodia to attach to the provisional matrix glycoproteins. Fetal wound repair continues to provoke study, because to recapture the environment of fetal repair could be extremely useful in circumstances of unwanted scarring, such as the healing of burns. The appearance of a newborn who has undergone a surgical operation in utero, but who has no visible evidence of this at birth, is arresting.

Effects of Scarring

In the absence of the ability to form scars, mammalian life would hardly be possible. Yet scarring in parenchymal organs modifies their complex structure and never improves their function. For example, in the heart, the scar of a myocardial infarction serves to prevent rupture of the heart but reduces the amount of contractile tissue. If extensive enough, it may cause congestive heart failure or the formation of a ventricular aneurysm. Similarly, the aorta that is weakened and scarred by atherosclerosis is prone to dilate as an aneurysm. Scarring of mitral and aortic valves as a result of local inflammation caused by rheumatic fever are often stenotic, regurgitant, or both, leading to congestive heart failure. Persistent inflammation within the pericardium produces fibrous adhesions, which result in constrictive pericarditis and heart failure.

Alveolar fibrosis in the lung causes respiratory failure. Infection within the peritoneum or even surgical exploration may lead to adhesions and intestinal obstruction. Immunological injury to the renal glomerulus eventuates in its replacement by a collagenous scar and, if this process is extensive, renal failure. Scarring in the skin following burns or surgical excision of lesions produces unsatisfactory cosmetic results. An important goal of therapeutic intervention is to create optimum conditions for “constructive” scarring and prevent pathological “overshoot” of this process.
Wound Repair Is Often Suboptimal
Abnormalities in any of three healing processes—repair, contraction, and regeneration—result in unsuccessful or prolonged wound healing. The skill of the surgeon is often of critical importance.

Deficient Scar Formation
Inadequate formation of granulation tissue or an inability to form a suitable extracellular matrix leads to deficient scar formation and its complications.

Wound Dehiscence and Incisional Hernias
Dehiscence (the wound splitting open) is most frequent after abdominal surgery and can be a life-threatening complication. Increased mechanical stress on the wound from vomiting, coughing, or bowel obstruction sometimes causes dehiscence of the abdominal wound. Systemic factors predisposing to dehiscence include metabolic deficiency, hypoproteinemia, and the general inanition that often accompanies metastatic cancer. An incisional hernia of the abdominal wall refers to a defect caused by prior surgery. Such hernias resulting from weak scars are often the consequence of insufficient deposition of extracellular matrix or inadequate cross-linking in the collagen matrix. Loops of intestine are sometimes trapped within incisional hernias.

Ulceration
Wounds can ulcerate when there is an inadequate intrinsic blood supply or insufficient vascularization during healing. For example, leg wounds in persons with varicose veins or severe atherosclerosis often ulcerate. Nonhealing wounds also develop in areas devoid of sensation because of persistent trauma. Such trophic or neuropathic ulcers are commonly seen in diabetic peripheral neuropathy. Occasionally they occur in patients with spinal involvement from tertiary syphilis and leprosy.

Excessive Scar Formation
Excessive deposition of extracellular matrix, mostly excessive collagen, at the wound site results in a hypertrophic scar. Keloid is an exuberant scar that tends to progress beyond the site of initial injury and recurs after excision (Fig. 3-16). Histologically, both of these types of scars exhibit broad and irregular collagen bundles, with more capillaries and fibroblasts than expected for a scar of the same age. More clearly defined in keloids than in hypertrophic scars, the rate of collagen synthesis, the ratio of type III to type I collagen, and the number of reducible cross-links, remain high. This situation that indicates a “maturation arrest,” or block, in the healing process. Further support for maturation arrest as an explanation for keloid and hypertrophic scars is the overexpression of fibronectin in these lesions. In addition, unlike normal healing tissue, these scar tissues fail to downregulate collagen synthesis when glucocorticoids are administered. Keloids are unsightly, and attempts at surgical repair are always problematic, the outcome likely being a still larger keloid. Dark-skinned persons are more frequently affected by keloids than light-skinned people, and the tendency is sometimes hereditary. By contrast, the occurrence of hypertrophic scars is not associated with skin color or heredity.

Excessive Contraction
A decrease in the size of a wound depends on the presence of myofibroblasts, development of cell–cell contacts, and sustained cell contraction. An exaggeration of these processes is termed contracture and results in severe deformity of the wound and surrounding tissues. Interestingly, the regions that normally show minimal wound contraction (e.g., the palms, the soles, and the anterior aspect of the thorax) are the ones prone to contractures. Contractures are particularly conspicuous in the healing of serious burns and can be severe enough to compromise the movement of joints. In the alimentary tract, a contracture (stricture) can result in obstruction to the passage of food in the esophagus or a block in the flow of intestinal contents.

Several diseases are characterized by contracture and irreversible fibrosis of the superficial fascia, including Dupuytren contracture (palmar contracture), Lederhosen disease (plantar contracture), and Peyronie disease (contracture of the cavernous tissues of the penis). In these diseases, there is no known precipitating injury, even though the basic process is similar to contracture in wound healing.
Excessive Regeneration and Repair

In addition to the many responses to injury described thus far, an additional lesion merits consideration, namely **pyogenic granuloma**. This lesion is a localized, persistent, exuberant overgrowth of granulation tissue, most commonly seen in gum tissue in pregnant women. It also develops in the squamo-columnar junction of the uterine cervix and at other sites. An injury preceding the development of pyogenic granuloma cannot usually be found. Like injury-induced granulation tissue, it lacks nerves and can be surgically trimmed without anesthesia. Conceptually, pyogenic granuloma is a transitional lesion, resembling granulation tissue but behaving almost as an autonomous benign neoplasm.