- **ECM proteins** participate in the process of vessel sprouting in angiogenesis, largely through interactions with integrin receptors in endothelial cells and by providing the scaffold for vessel growth.
- **Enzymes** in the ECM, notably the matrix metalloproteinases (MMPs), degrade the ECM to permit remodeling and extension of the vascular tube.

Deposition of Connective Tissue

The laying down of connective tissue occurs in two steps: (1) migration and proliferation of fibroblasts into the site of injury and (2) deposition of ECM proteins produced by these cells. These processes are orchestrated by locally produced cytokines and growth factors, including PDGF, FGF-2, and TGF-β. The major sources of these factors are inflammatory cells, particularly alternatively activated (M2) macrophages, which are present at sites of injury and in granulation tissue. Sites of inflammation are also rich in mast cells, and in the appropriate chemotactic milieu lymphocytes may also be present. Each of these can secrete cytokines and growth factors that contribute to fibroblast proliferation and activation.

***Transforming growth factor-β (TGF-β)* is the most important cytokine for the synthesis and deposition of connective tissue proteins.** It is produced by most of the cells in granulation tissue, including alternatively activated macrophages. The levels of TGF-β in tissues are primarily regulated not by the transcription of the gene but by the posttranscriptional activation of latent TGF-β, the rate of secretion of the active molecule, and factors in the ECM, notably integrins, that enhance or diminish TGF-β activity. TGF-β stimulates fibroblast migration and proliferation, increased synthesis of collagen and fibronectin, and decreased degradation of ECM due to inhibition of metalloproteinases. TGF-β is involved not only in scar formation after injury but also in the development of fibrosis in lung, liver, and kidneys that follows chronic inflammation. TGF-β is also an antiinflammatory cytokine that serves to limit and terminate inflammatory responses. It does this by inhibiting lymphocyte proliferation and the activity of other leukocytes.

As healing progresses, the number of proliferating fibroblasts and new vessels decreases; however, the fibroblasts progressively assume a more synthetic phenotype, and hence there is increased deposition of ECM. Collagen synthesis, in particular, is critical to the development of strength in a healing wound site. As described later, collagen synthesis by fibroblasts begins early in wound healing (days 3 to 5) and continues for several weeks, depending on the size of the wound. Net collagen accumulation, however, depends not only on increased synthesis but also on diminished collagen degradation (discussed later). Ultimately, the granulation tissue evolves into a scar composed of largely inactive, spindle-shaped fibroblasts, dense collagen, fragments of elastic tissue, and other ECM components (Fig. 3-27*B*). As the scar matures, there is progressive vascular regression, which eventually transforms the highly vascularized granulation tissue into a pale, largely avascular scar. Some of the fibroblasts also acquire features of smooth muscle cells, including the presence of actin filaments, and are called *myofibroblasts*. These cells contribute to the contraction of the scar over time.

Remodeling of Connective Tissue

The outcome of the repair process is influenced by a balance between synthesis and degradation of ECM proteins. After its deposition, the connective tissue in the scar continues to be modified and remodeled. The degradation of collagens and other ECM components is accomplished by a family of *matrix metalloproteinases (MMPs),* so called because they are dependent on metal ions (e.g., zinc) for their activity. MMPs should be distinguished from neutrophil elastase, cathepsin G, plasmin, and other serine proteinases that can also degrade ECM but are not metalloenzymes. MMPs include interstitial collagenases, which cleave fibrillar collagen (MMP-1, -2 and -3); gelatinases (MMP-2 and 9), which degrade amorphous collagen and fibronectin; and stromelysins (MMP-3, -10, and -11), which degrade a variety of ECM constituents, including proteoglycans, laminin, fibronectin, and amorphous collagen.

MMPs are produced by a variety of cell types (fibroblasts, macrophages, neutrophils, synovial cells, and some epithelial cells), and their synthesis and secretion are regulated by growth factors, cytokines, and other agents. The activity of the MMPs is tightly controlled. They are produced as inactive precursors (zymogens) that must be first activated; this is accomplished by proteases (e.g., plasmin) likely to be present only at sites of injury. In addition, activated collagenases can be rapidly inhibited by specific tissue inhibitors of metalloproteinases (TIMPs), produced by most mesenchymal cells. Thus, during scar formation, MMPs are activated to remodel the deposited ECM and then their activity is shut down by the TIMPs.

A family of enzymes related to MMPs is called ADAM (a disintegrin and metalloproteinase). ADAMs are anchored to the plasma membrane and cleave and release extracellular domains of cell-associated cytokines and growth factors, such as TNF, TGF-β, and members of the EGF family.

KEY CONCEPTS

Repair by Scar Formation

- Tissues are repaired by replacement with connective tissue and scar formation if the injured tissue is not capable of proliferation or if the structural framework is damaged and cannot support regeneration.
- The main components of connective tissue repair are angiogenesis, migration and proliferation of fibroblasts, collagen synthesis, and connective tissue remodeling.
- Repair by connective tissue starts with the formation of granulation tissue and culminates in the laying down of fibrous tissue.
- Multiple growth factors stimulate the proliferation of the cell types involved in repair.
- TGF-β is a potent fibrogenic agent; ECM deposition depends on the balance between fibrogenic agents, metalloproteinases (MMPs) that digest ECM, and the tissue inhibitors of MMPs (TIMPs).

Factors That Influence Tissue Repair

Tissue repair may be altered by a variety of influences, frequently reducing the quality or adequacy of the reparative process. Variables that modify healing may be extrin-