

## **TISSUE REMODELING DURING HEALING, ROLE OF APOPTOSIS**

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Normal wound healing includes a number of overlapping phases. After injury, there is an early inflammatory step characterized by haemorrhage and clotting. During this phase, platelet degranulation occurs, releasing a cocktail of growth factors into the local environment that helps to attract inflammatory cells to the site of damage. These cells in turn release numerous factors which stimulate the repair process. At this time, the wound has a provisional extracellular matrix consisting largely of fibrin. This provisional matrix serves to seal the wound temporarily and allows the invasion of cells that carry out the repair process by fibrogenesis and angiogenesis. It also provides a substrate on which epithelial cells migrate to re-epithelialize the wound. In the next phase, consisting of development of granulation tissue, fibroblasts invade the wound and commence replacing the provisional matrix with a more mature wound matrix containing collagen, fibronectin and other matrix molecules. In this phase, angiogenesis is also occurring, predominantly from the damaged pre-existing vessels. The fibroblasts present during the early granulation tissue phase resemble immature fibroblasts with a highly synthetic appearance, containing abundant cisternae of rough endoplasmic reticulum. However, as the granulation tissue phase proceeds, fibroblasts start showing a new phenotype with prominent microfilament bundles and dense bodies visible by electron microscopy (Gabbiani et al, 1971). The work of many laboratories has contributed to define this cell morphologically, by showing that its contractile structures are represented by microfilament bundles or stress fibers, and biochemically, by showing that stress fibers express contractile proteins typical of smooth muscle cells, particularly of vascular smooth muscle cells, such as  $\alpha$ -smooth muscle actin (Darby et al, 1990). Presently it is accepted that the myofibroblastic modulation of fibroblastic cells begins with the appearance of the protomyofibroblast, whose stress fibers contain only  $\beta$ - and  $\gamma$ -cytoplasmic actins. This first transition is not yet well explored, but it probably depends on the development of mechanical tension. Subsequently, myofibroblastic differentiation evolves, but not necessarily always, into the appearance of the differentiated myofibroblast, the most common variant of this cell, with stress fibers containing  $\alpha$ -smooth muscle actin (Tomasek et al, 2002).

Recently, it has been shown that  $\alpha$ -smooth muscle actin is largely responsible for force production by the myofibroblast both in vitro, using models involving fibroblasts cultured on flexible substrates or within floating and attached collagen gels (Hinz et al, 2001a), and in vivo, using experimental wound healing in the rat (Hinz et al, 2001b).

The appearance of the differentiated myofibroblast is influenced by mechanical tension as well as by chemical mediators, such as transforming growth factor- $\beta$  (TGF- $\beta$ , Desmoulière et al, 1993). It should be noted that the action of TGF- $\beta$  in stimulating both collagen type I and  $\alpha$ -smooth muscle actin synthesis strictly depends on the presence of cellular fibronectin and in particular of the ED-A splice variant of this glycoprotein (Serini et al, 1998). Thus myofibroblast differentiation is a complex process, regulated by at least a cytokine, an extracellular matrix component as well as the presence of mechanical tension. Lastly, in the resolution phase of healing, there is considerable loss of various cell types including myofibroblasts, by apoptosis (Desmoulière et al, 1995). The fibroblasts that remain in granulation tissue after the epithelial defect is closed have reverted to a more quiescent, non-contractile phenotype lacking the microfilament bundles which were present during the contractile phase of healing. It is also conceivable that the residual fibroblasts represent a population of cells which failed to acquire a myofibroblast phenotype during healing and thus survive, while the myofibroblastic cells which appeared during healing represent terminally differentiated cells which undergo apoptosis during the resolution phase. The signal for this cell death is unknown but may be related to reductions in the concentrations of local trophic factors as re-epithelialization and depletion of inflammatory cells occur. The remodeling of the extracellular matrix by metalloproteinases may also play a role by interfering with myofibroblast adhesion to the extracellular matrix as has been suggested by studies on regression of granulation tissue under a vascularized skin flap (Darby et al, 2002) and by studies on resolution of liver fibrosis (Iredale et al, 1998). Early in wound repair, the balance between matrix metalloproteinases such as collagenases and gelatinases and their endogenous inhibitors, tissue inhibitor of metalloproteinases (TIMPs), favours extracellular matrix production. Later in wound healing as remodeling occurs, it is possible that this balance changes and favours matrix degradation and remodeling. As mentioned above, this could potentially result in increased apoptosis. Changes in the physical stress caused by stretch of the granulation tissue may also contribute to the loss of cells via an apoptotic mechanism as has been suggested by in vitro studies of fibroblasts in collagen lattices (Grinnell et al, 1999). Conversely, inappropriate delay of apoptosis, and thus increased survival of myofibroblasts

activated during the healing process, may be a factor which leads to excessive scarring such as that seen in hypertrophic scars or keloids. This latter proposition however, lacks conclusive evidence to date. In hypertrophic scars,  $\alpha$ -smooth muscle actin-positive myofibroblasts are commonly present in nodules of cells, while fibroblasts in keloids are generally  $\alpha$ -smooth muscle actin-negative. Interestingly, when the fibroblasts from these types of scar are cultured, they give rise to similar numbers of myofibroblasts, suggesting that the microenvironment present within the scar is important for regulating the phenotype (Ehrlich et al, 1994). Furthermore, cross talk between the epidermis and dermis exists through the basement membrane. A persistence of activated keratinocytes has been observed in hypertrophic scar epidermis implicating abnormal epidermal-mesenchymal interactions, and suggesting that cellular mechanisms in the pathogenesis of hypertrophic scarring are more complex than isolated dermal phenomena (Machesney et al, 1998). Recently, however, differential responses to apoptotic inducers were observed between normal skin wound and hypertrophic scar myofibroblasts, confirming the hypothesis of defects in apoptosis and growth during pathological scar formation impeding myofibroblast disappearance at the end of healing (Moulin et al, 2004).

In fetal wounds, healing can occur without scarring or contracture. This ability is lost in late gestation. Fetal skin fibroblasts are able to contract collagen lattices but show reduced staining for  $\alpha$ -smooth muscle actin. In vivo, early fetal wounds also show markedly fewer  $\alpha$ -smooth muscle actin staining fibroblasts than in late fetal wounds or in adult wounds where  $\alpha$ -smooth muscle actin-positive myofibroblasts are abundant (Estes et al, 1994).

In conclusion, during the last forty years the concept of granulation tissue contraction and of fibrocontractive pathological situations has been clarified in many aspects (Desmoulière et al, 2003). Further work on the biology of the myofibroblast will definitively contribute to the understanding and the control of normal and pathological connective tissue remodeling.

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