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Cutaneous wound healing

The primary function of the skin is to serve as a protective barrier against the environment. Loss of the integrity of large portions of the skin as a result of injury or illness may lead to major disability or even death. Wound healing is the restoration of integrity to an injured tissue. It is a dynamic, interactive process involving soluble mediators, blood cells, extracellular matrix and parenchymal cells. Wound healing is a complex biologic event involving three phases: inflammation, tissue formation (proliferation) and tissue remodelling (maturation) which overlap in time.

INFLAMMATORY PHASE (DAY 0–5)

Healing is initiated at the moment of injury. The earliest event in this process is the inflammatory phase which comprises vascular and cellular response. Inflammation is a reaction of the microcirculation characterized by movement of fluid and leukocytes from the bloodstream into extravascular tissues. The clinical signs of this process are caused by changes in blood vessels with dilation leading to erythema and endothelial cell separation allowing plasma extravasation that produce localized swelling.

PROLIFERATIVE PHASE (DAY 3–14)

Proliferative phase is characterized by the formation of a new epithelium at the wound surface and formation of granulation tissue deep in the wound. Granulation tissue consists of a combination of cellular elements, including fibroblasts and inflammatory cells, along with new capillaries embedded in a loose extra cellular matrix of collagen, fibronectin and hyaluronic acid. The formation of granulation tissue is a highly regulated process, which involves a number of events, including fibrogenesis and the growth of new capillaries (angiogenesis), and involution during maturation of the scar.

Reepithelization of wounds begins within hours after injury. Epidermal cells from skin appendages such as hair follicles quickly remove clotted blood and damaged stroma from the wound space. At the same time, the cells undergo marked phenotypic alteration including the retraction of intracellular tonofilaments, dissolution of most intercellular desmosomes that provide physical connections between the cells and formation of peripheral cytoplasmic actin filaments, which allow cell movement (8). The dissolution of hemidesmosomal links between the epidermis and the basement membrane allows interaction between cells and extracellular-matrix proteins, e.g. fibronectin and vitronectin. These proteins are interspersed at the margin of the wound with stromal type I collagen and interwoven with the fibrin clot in the wound space (4). The migrating epidermal cells dissect the wound by separation of desiccated eschar from viable tissue. Migration of endothelial cells and development of new capillary vessels depend not only on the cells and cytokines but also on the systems and organization of extracellular matrix components both in granulation tissue and in endothelial basement membrane.

The proliferation and differentiation of epidermis may be regulated by epidermal growth factor (EGF) (10). There are many studies describing the influence of EGF on healing that includes the enhancement of epithelialization (10). There are other data suggesting that a proliferative process such as healing of skin wound could respond to either endogenous or exogenous application of EGF (10). The migration and proliferation of epidermal cells during reepithelialization correlates with the expression of growth factors such as transforming growth factor-α (TGF-α) and keratinocyte growth factor (KGF) (1, 12).

One of the important characteristics of endothelial cells *in vitro* is their ability to respond to inflammatory mediators and vasoactive amines by a major change in morphology. This process is similar to that observed in vivo. On stimulation, skin endothelial cells convert from the typical cuboidal configuration observed in the resting state to a more mesenchymal-like, spindle-shaped cells seen in neovascularization, inflammation and during formation of granulation tissue. This transition is delicately balanced and is closely regulated by the levels of cyclic AMP and calcium present in the cell. Therefore, calcium may play crucial role in skin diseases characterized by excessive fibrosis and spindle cell proliferations. The cultured endothelial cells grow in a characteristic cuboidal configuration and express gap junctions and pinocytotic vesicles similar to endothelial cells *in vivo* (9).

Inflammatory cells such as neutrophils and monocytes are attracted to the site of injury by a variety of chemotactic factors produced and secreted in response to tissue damage. Infiltrating neutrophils cleanse the wounded area from foreign particles and bacteria that are later extruded with the eschar or phagocytosed by macrophages. Adherence to the extracellular matrix and the influence of transforming growth factor-β (TGF-β), monocyte chemoattractant protein-1 enables infiltration of the wound site and the activation of macrophages. After 72 hours the macrophages become a dominant cell type to be found at the wound site. Activated macrophages release growth factors such as platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF). These cytokines initiate the formation of granulation tissue. Thus, macrophages appear to have a pivotal role in the transition between inflammation and repair (8). Macrophages bind to specific proteins of the extracellular matrix by their integrin receptors. This event appears to stimulate phagocytosis of microorganisms and fragments of extracellular matrix by the macrophages (3). The macrophages produce also (fibroblast growth factor –FGF, platelet derived endothelial factor-PGEF), colony-stimulating factor-1, tumor necrosis factor-α (TNF-α), PDGF , TGF-α, TGF-β, interleukin-1 (L-1), insulin-like growth factor I. FGF and PGEF may play important role in modulation of the healing process.

Macrophages, fibroblasts and blood vessels move into the wound space at the same time. The macrophages recruit a new fibroblasts which lay down a network of collagen fibres surrounding the neovasculature of the wound and provide a continuing source of growth factors necessary to stimulate fibroplasia and angiogenesis. Fibroblasts actively secrete components of the extracellular matrix. They are responsible for production, deposition of the majority of structural proteins needed during tissue reconstruction including different types of collagen, a family of triple-chain proteins, which form the main constituent of the extracellular wound matrix and are responsible for imparting tensile strength to the scar. Fibroblasts produce also proteoglycans, that coats the collagen fibers and binds them together, giving them greater flexibility. They also produce fibronectin which holds the collagen and cells together. Fibroplasia begins 3–5 days after injury and may last as long as 14 days. Fibroblasts migrate and proliferate in response to fibronectin, PDGF, basic fibroblast growth factor (basic fibroblast growth factor-β FGF), TGF, and C5a. Fibronectin is a chemotacic agent for endothelial cells, which coalesce and bind fibrin which adds support to the vessel wall. Fibronectin and hyaluronic acid are the first fibroblast glycoproteins to be deposited in the healing wound. Sulfated proteoglycans appears later. The concentrations of proteoglycans and fibronectin in the wound peak 4 to 6 days after injury and then decline to normal levels by day 12 (9). The synthesis of new connective (granulation tissue) is dependent on the formation of the new blood vessels within the wound. This process begins

approximately four days after injury and requires a good supply of oxygen and nutrients. The capillary loops of the new vessels grow into newly formed tissue. Therefore, oxygenated blood reaches the wound bed and the wound becomes less hypoxic and less nutrient deficient.

Increased numbers of mast cells and macrophages play a significant role in angiogenesis. Thus, it is possible that continuous degranulation of mast cells with release of heparin may stimulate vascular proliferation associated with the tumor. Another clue for importance of macrophages in angiogenesis is increased numbers of abnormal mast cells in paravascular space in patient suffering from psoriasis. Macrophages, when activated, are able to secrete several potent cytokines . The formation of new blood vessels correlates with the release of multiple angiogenic growth factors. The growth factors that are required for dermal endothelial cells proliferation differ from those in other tissues and most likely reflect the specific requirements for maintaining skin vessel homeostasis. These differences should be expected because unlike in other organs the microvasculature of the skin needs to react to injury, to control immune response and to play vital role in thermoregulation.

Angiogenesis is defined as a process in which new blood capillaries from surrounding parent vessels grow into a tissue. Newly formed blood vessels participate in provisional granulation tissue formation by providing nutrition and oxygen. Angiogenesis is of crucial interest to the dermatologist, because of its involvement in wound healing and also in the etiopathology of psoriasis, warts and cutaneous malignancy. Other dermatologic conditions wherein angiogenesis is defective or uncontrolled are decubitus ulcers, stasis ulcers, pyogenic granulomas, hemangiomas, Kaposi's sarcomas, and possibly Spitz nevus, hypertrophic scars, and keloids (2). Angiogenesis is strictly regulated by growth factors and inhibitors of angiogenesis derived both from serum and the surrounding extracellular matrix. Among many known growth factors, vascular endothelial growth factor (VEGF) is believed to be most prevalent and potent agent able to stimulate angiogenesis. Work done by several laboratories over the recent years has elucidated the pivotal role of VEGF in the regulation of normal and abnormal angiogenesis (6).

VEGF belongs to the family, which currently consists of six members: VEGF-A (or VEGF), PIGF, VEGF-B, VEGF-C, VEGF-D and orf virus VEGF (VEGF-E) (6). *In vivo*, VEGF has been shown to regulate vascular permeability, which is crucial for the initiation of angiogenesis (6). High VEGF levels were detected during the proliferative phase of wound healing. Transcription of VEGF mRNA is induced by different growth factors and cytokines, including PDGF, endothelial growth factor – EGF, tumor necrosis factor α TNF-α, tumor necrosis factor β(TNF-β) interleukin-1 β (IL- β). Thus, VEGF may function as a mediator for indirect-acting angiogenic factors. VEGF levels are also regulated by tissue oxygen tension. Exposure to hypoxia rapidly and reversibly induces VEGF expression through both increased transcription and stabilization of the mRNA. In contrast, normoxia downregulates VEGF production and even causes regression of some newly formed blood vessels. By opposing processes, the vasculature exactly meets the metabolic demands of the tissue.

Wound angiogenesis is also regulated by several other potent angiogenic cytokines including angiopoietin, fibroblast growth factor (FGF) and transforming growth factor-β (TGF-β). The co--operation between them and extracellular matrix is essential for wound repair. Recent investigations have shown that the wound extracellular matrix can regulate angiogenesis in part by modulating integrin receptor expression (14). In particular, α(v)β3 integrin receptor for fibrin and fibronectin appears to be required for wound angiogenesis.

One of the earliest and most remarkable studies on neovascularization was conducted by Cliff in 1962. This author used transparent chambers inserted into the rabbit ear. That enabled direct visualization of new vessel growth (5). This was the first study to demonstrate clearly that the basement membrane is first perforated by small projections from the basal surface of the endothelial cell while cell junctions at each end of the dividing cell continue to persist.

Based on the observations in both rabbits and humans, angiogenesis can be summarized as a sequential event and divided into several distinct stages: dissolution of the basement membrane by an endothelial cell sprout, migration of the endothelial cell through the basement membrane, realignment of the migrating cells, vacuolization of the endothelial cells to form nascent lumens, formation of basement membranes, regression on return to homeostasis.

MATURATION PHASE (FROM DAY 7 TO 1 YEAR)

The final phase of wound healing is the process of remodeling of the collagen fibers. The transition from granulation tissue to scar is dependent on continued synthesis and degradation of collagen. During this phase collagen type III, a soft gelationous protein laid down in the proliferation phase is replaced with a more highly organized collagen type I. Collagen type III is present mostly in healing skin while in normal skin collagen type I predominates. The degradation of collagen in the wound is controlled by several proteolytic enzymes termed matrix metalloproteinases. These enzymes are secreted by macrophages, epidermal and endothelial cells and fibroblasts. The various phases of wound repair rely on the balance between matrix metalloproteinases and tissue inhibitors of metalloproteinases (11). The differentiation of collagen is a dynamic process and although it takes place predominantly during the maturation phase it may continue for a long period. Matrix remodelling gradually increases the scar tensile strength to 70% to 80% of normal skin (6).

Over the recent years, extensive research has been done to investigate the complexity of wound healing. However, many aspects of this process, especially the roles of numerous growth factors and interplay between them, remain to be elucidated. This is especially important because of possible benefits arising from the use of some properties of growth factors in the clinical medicine.

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SUMMARY

The wound healing is a complex process that can be divided into inflammatory reaction, proliferation and maturation of newly formed tissue. The inflammatory phase involves vascular and cellular events and is best characterized by edema, erythema and marked increase of blood supply. During proliferative phase there is formation of the epithelium with concomitant grow of granulation tissue and new blood vessels (angiogenesis). Angiogenesis seems to be strictly coordinated and regulated by multiple grow factors and cytokines released at the wound site. Once the tissue within the wound is formed the maturation phase begins. The synthesis of collagens and other extracellular matrix components increase tensile strength of the wound. Thus, the final result of the process of healing is the formation of tissue able to replace the normal, uninjured skin.

Proces gojenia ran skórnych

Proces gojenia rany jest złożoną reakcją, którą można podzielić na fazę zapalenia, proliferacji i dojrzewania. Pierwszy etap gojenia rany – faza zapalna – obejmuje zarówno reakcje naczyniowe, jak i komórkowe, czego efektem jest obrzęk, rumień oraz zwiększenie przepływu krwi przez naczynia krwionośne sąsiadujące z raną. W fazie proliferacji dochodzi do powstawania nabłonka, ziarniny oraz naczyń krwionośnych (angiogeneza). Angiogeneza jest ściśle kontrolowana przez obecne w ranie liczne czynniki wzrostu oraz cytokiny. Po zakończeniu fazy proliferacji następuje dojrzewanie rany, charakteryzujące się nasiloną syntezą kolagenu i innych składowych macierzy zewnątrzkomórkowej. Powoduje to zwiększenie wytrzymałości mechanicznej rany i sprawia, że nowa tkanka jest w stanie zastąpić funkcję prawidłowej skóry.