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Dermal fibroblasts—A heterogeneous population with regulatory function in wound healing

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ABSTRACT

Dermal fibroblasts, which make up the major cell type in the dermis, have, historically, been considered to be relatively 'passive' cells which are responsible for the synthesis and remodeling of extracellular matrix proteins. However, the dermal fibroblast population is composed of heterogeneous and distinct cell types, and it has been established that, under the stress conditions of healing wound environments, dermal fibroblasts participate in the regulation of ongoing inflammation and cell proliferation by secreting a variety of signaling molecules that modulate the functions of immune cells, keratinocyte, endothelial cells and mast cells via both direct cell to cell communication and autocrine and paracrine interactions. This review describes the capacity of dermal fibroblasts to sense and respond to signals from the micro-environment and to communicate with surrounding cells during cutaneous wound healing. The review further emphasizes the, to date, poorly understood roles of heterogeneous dermal fibroblast populations in the wound healing process.

1. Acute wounds

The acute wound healing process commences with hemostasis and, during the subsequent hours, days and even weeks, healing proceeds via neutrophil and monocyte invasion to the wound site, followed by the formation of granulation tissue, re-epithelization, scar formation and eventual remodeling. The orchestration of these various events is regulated by means of intercellular communication. Individual cells and groups of cells interact via direct contact or local wound signal release. All the cells present at the injury site produce signaling molecules; thus, immune cells, keratinocytes, endothelial cells and even fibroblasts contribute to the regulation of wound healing. Importantly, the significance of fibroblasts in the healing process should not be underestimated [1,2].

Immediately following trauma, platelets aggregate at the injury site and form a blood clot via the conversion of fibrinogen to fibrin. The blood clot fills the tissue discontinuity and ensures hemostasis; further, it provides a provisional matrix for the recruitment of immune cells and facilitates the migration of other resident cells from local tissue and blood circulation toward the wound. The tissue debris, dead cells and molecules secreted by the platelets activate inflammatory leukocytes which are crucial in terms of wound clearance and healing [1,3–5]. Moreover, the signaling molecules released by the platelets provide a chemotactic gradient which allows for the leukocytes, fibroblasts and endothelial cells to enhance the healing response [6].

Neutrophils are the first immune cells to arrive at the wound site following tissue damage. They either exit passively from damaged capillaries or actively pass through the blood vessels of the wound by means of diapedesis. Further, they clear the wounded area of microbes and tissue debris via phagocytosis, the production of reactive oxygen species (ROS) and the presence of neutrophil extracellular traps, *i.e.* fiber structures that disarm and kill extracellular bacteria [7]. Finally, they secrete signaling molecules that stimulate the functions of nearby cells. Once the cleaning process is complete, neutrophils undergo apoptosis, followed by which the apoptotic bodies are removed by macrophages [8,9].

Natural killer T cells (NK T cells) infiltrate cutaneous wounds at the same time as do neutrophils, *i.e.* during the early inflammatory phase, and regulate the production of chemokines. However, neither neutrophils nor NK T cells are strictly pro-inflammatory. For example, such cells produce cytokines and chemokines that accelerate the fibroproliferative aspects of wound healing [10].

Mast cells follow the inflow of neutrophils and, immediately following injury, they release several types of pro-inflammatory molecules such as histamine and vascular endothelial growth factor (VEGF), and increase the degree of vascular permeability [11]. Histamine creates pores in the blood vessels which facilitate protein and leukocyte extravasation into the wound site [12]. The fight against infection is also assisted by the release of antimicrobial peptides, *e.g.* cathelicidin [13,14]. Moreover, mast cells exert an effect on endothelial cells,

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keratinocytes and fibroblasts during the proliferative phase of the wound healing process, *i.e.* they stimulate the proliferation and influence the production and remodeling of collagen [15–18].

Macrophages enter the wound site approximately 48 h following injury; these cells originate from resident tissues in the vicinity of the wound or are "recruited" from circulating monocytes. Two macrophage differentiation and activation states have been identified [19]; M1 macrophages are pro-inflammatory, whereas M2 macrophages are associated with tissue repair and scarring [20,21]. Invading M1 macrophages continue the cleaning process via the phagocytosis of the microbes, tissue debris and apoptotic neutrophils related to the early phase of inflammation. The numerous growth factors and cytokines produced by the macrophages are important with respect to angiogenesis, fibroblast chemotaxis and proliferation and the subsequent synthesis and degradation of collagen [8,9,22,23]. Thus, macrophages are essential with concern to both the early inflammatory phase and ensuing phases of the repair process.

The subsequent proliferative phase consists principally of the migration of fibroblasts from various sources resulting in the formation of granulation tissue. Fibroblasts re-populate tissue defects in the wound and provide a new extracellular matrix (ECM), which is followed by the closure of the wound. The ECM formed during the proliferative phase of wound healing serves as a scaffold upon which fibroblasts and other cells migrate into the healing wound. The ECM forms temporary granulation tissue together with the endothelial cells and macrophages that supply the wounded tissue with nutrition and oxygen. Once the granulation tissue has been formed, the fibroblasts produce enhanced amounts of collagen which serve to increase the tensile strength of the more permanent ECM of the wound [2,24]. In addition to their role with respect to the structural support of the healing wound, fibroblasts are capable of responding to a range of tissue damage signals including the presence of inflammatory molecules and pathogens and changes in mechanical properties and oxygen levels [23,25,26]; moreover, they produce a number of cytokines, chemokines and growth factors as a response to the changing wound environment [27,28]. As a result, fibroblasts actively participate in new tissue development and the regulation of wound healing.

The epidermis is repaired from the margins of the wound. The restoration of the skin surface area requires both keratinocyte migration and proliferation in order to provide a cover for the developing granulation tissue. This process, known as re-epithelization, commences as early as at the beginning of the healing process [29]. Keratinocytes undergo phenotypic changes following injury and the contact between the epidermis and the basement membrane is lost which, together with the secretion of a number of proteases, allows for the lateral movement of epidermal cells into the wound [29,30]. Keratinocytes revert to their normal phenotype once the basement membrane and the underlying dermis have been restored [1].

Blood supply is re-established by ongoing angiogenesis, at which time angiogenic factors, *e.g.* VEGF and fibroblast growth factor 2 (FGF-2) secreted by platelets, macrophages, epidermal cells and fibroblasts, allow endothelial cells to invade the wound and, subsequently, to form new blood vessels [1,31].

Some of the fibroblasts differentiate into myofibroblasts during the proliferative phase, principally following stimulation exerted by transforming growth factor-beta (TGF-β). The myofibroblasts, which provide for the contraction of the wound and, eventually, undergo apoptosis, are subsequently replaced by a second wave of fibroblasts which initiate the remodeling of the tissue [32].

The remodeling phase is characterized by the active reorganization of the ECM and a reduction in the total number of capillaries [33]. The major part of type III collagen produced by the fibroblasts during the early stages of wound healing is replaced by type I collagen [29], which matures by means of an increase in the number of cross-links between the collagen fibers; remodeling may take years to reach completion. An increase in wound tensile strength occurs during the remodeling process; however, due to changes in the organization of the matrix [34], completely healed wounds attain only approximately 70 % of the normal strength of unwounded skin. The contraction and subsequent aberrant deposition of collagen leads to the formation of a fibrous scar that lacks the majority of resident dermal cells [1,35]. Moreover, the disorganized elastic fiber network characteristic of scars as opposed to the presence solely of elastin in non-wounded tissue, contributes to the diminished physical properties of completely healed wounds [36].

2. Chronic wounds

Chronic wounds are wounds in which the normal sequence of healing is disrupted which leads to the prolongation of the healing process despite the appropriate therapy. The most common chronic wounds include venous ulcers associated with diabetes mellitus, venous and arterial ulcers resulting from blood hypertension and pressure ulcers. The main factors that lead to the development of chronic wounds consist of a self-perpetuating inflammatory stage, cellular senescence, bacterial infection, a reduction in oxygenation and a lack of nutrition [3,23].

2.1. Inflammatory response disorders

Immune cell infiltration into the bed of the wound is essential in terms of clearing bacteria and foreign particles from the wound; that said, prolonged infiltration may lead to tissue damage. Moreover, the phenotype of immune cells in chronic wounds differs from that of immune cells in acute wounds; thus, the function of such cells in chronic wounds is modified [37], which exerts both positive and negative impacts on the wound healing process.

Polymorphonuclear leukocytes may promote wound healing via the up-regulation of anti-apoptotic genes and the intense production of cytokines and chemokines during the initial wound healing phase. The enforcement of their inflammatory response occurs through the up-regulation of interleukin 1 type I receptor (IL-1R1) and transforming growth factor β receptor (TGF-βR) and their ligands IL-1β and TGF-β, which are secreted by activated macrophages, as well as the up-regulation of VEGF which is considered to make up the most important angiogenic factor [37]. A further example of the positive effect of the presence of immune cells consists of the increased number of Langerhans cells in the epidermis of diabetic foot ulcer patients, which enhances the healing process [38].

The negative effects of modified immune cell phenotypes consist of reduced bactericidal and phagocytic activity compared to their performance in acute wounds, which results in a higher number of apoptotic cells in the wound tissue and the accumulation of bacteria [39–41]. Moreover, excessive neutrophil and macrophage activity in chronic wounds leads to the disruption of the healing process involving changes in terms of cell response, the over-production of ROS and the formation of a non-healing micro-environment characterized by abnormal growth factor profiles, proteolytic imbalance and a generally prolonged and heightened inflammatory state [42–44]. Excessive amounts of ROS originate from immune cell respiratory bursts, particularly those caused by increased NADPH-oxidase (NOX) enzyme activity. While ROS are effective with respect to eliminating wound infection during the normal wound healing process, if the inflammatory phase does not resolve itself during the requisite time period, and the concentration of ROS exceeds the protective concentration, the surrounding tissue and cells may be destroyed, thus disrupting the healing process [45].

2.2. Proliferative phase disorders

Some studies addressing chronic wounds mention piled-up hyperproliferative epithelial edges that fail to re-epithelize [46,47]. Keratinocytes along non-healing edges not only display delayed migration