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Review

Fibrocytes: a unique cell population implicated in wound healing

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Abstract. Following tissue damage, host wound healing ensues. This process requires an elaborate interplay between numerous cell types which orchestrate a series of regulated and overlapping events. These events include the initiation of an antigen-specific host immune response, blood vessel formation, as well as the production of critical extracellular matrix molecules, cytokines and growth factors which mediate tissue repair and wound closure. Connective tissue fibroblasts are considered essential for successful wound healing; however, their origin remains a mystery. A unique cell population, known as fibrocytes, has been identified and characterized. One of the unique features of these blood-borne cells is their ability to home to sites of tissue damage. This article reviews the identification and characterization of fibrocytes, summarizes the potential role of fibrocytes in the numerous steps of the wound-healing process and highlights the potential role of fibrocytes in fibrotic disease pathogenesis.

Key words. Tissue repair; fibrosis; tissue remodeling; TGFβ; angiogenesis; antigen presentation.

What are fibrocytes?
The discovery, isolation and initial characterization of fibrocytes

Wounds or tissue injuries caused by trauma, burns, inflammation, infection, and metabolic deficiencies result in the physical disruption of the normal cellular architecture of the tissue. In response to tissue injury, the host commences a repair process that is regulated by cellular, humoral and connective tissue mediators. The cell types implicated in the repair of tissue injury include platelets, monocytes/macrophages, T lymphocytes, endothelial cells and connective tissue fibroblasts. Connective tissue fibroblasts found at the sites of tissue injury and in areas of tissue remodeling are believed to play an essential role in the healing process. Although it is unclear whether these connective tissue fibroblasts found in wounds and scar tissues originate from the circulation or from the surrounding tissue areas, the concept that fibroblast-like cells found within the wound originate from the peripheral blood dates back almost 100 years (reviewed in [1]). Fibroblasts found at sites of tissue injury may originate from different sources depending on the wound/injury type. For example, in minor wounds fibroblasts may migrate from surrounding undamaged tissue, whereas fibrocytes may be recruited to deep tissue wounds where they differentiate into fibroblasts.

The investigations by Bucala and colleagues in the early 1990s that led to the discovery of 'fibrocytes' were based on the hypothesis that specialized 'fibroblast-like cells' present in experimentally implanted wound chambers originated from the circulation. This discovery led to the identification and initial characterization of a distinct population of blood-borne $CD34^+/Col\ I^+$ fibroblast-like cells that rapidly enter sites of tissue injury [2]. Termed fibrocytes, these cells comprise ~0.1–0.5% of nonery-

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throcytic cells in the peripheral blood (as determined by spot immunofluorescence staining using anti-CD34-rhodamine and anti-col I-fluorescein 5 (6)-isothiocyanate (FITC) of blood cells following erythrocyte lysis) [2]. Fibrocytes can be isolated from buffy coats prepared from blood and cultured ex vivo [2–4]. These crude fibrocyte preparations obtained from human or mouse blood are grown in Dulbecco's modified Eagle medium containing fetal calf serum, without the addition of other growth factors. After 10–14 days of incubation, ex vivo cultured fibrocytes display an adherent, spindle-shaped morphology. Fibrocytes are then purified from this crude preparation (70–80% pure) following negative selection for other immune cell types (B cells, T cells and monocytes) (see fig. 1). The resulting fibrocyte population (>95% pure based on collagen I and CD11b staining, or collagen I and $CD34^+$ staining) has been characterized based on expression of (i) extracellular surface markers [including cluster of differentiation (CD) antigens, major histocompatibility complex (MHC)-like molecules and extracellular matrix protein markers] (table 1) and (ii) cytokine, chemokine and growth factor expression patterns (table 2).

Fibrocytes are a unique $CD45^+$ cell population [2] They are distinct from monocytes, ($CD14^-$, esterase$^-$, $CD54^-$), dendritic cells ($CD10^-$, $CD25^-$, $CD38^-$), Langerhans cells ($CD1a^-$), T lymphocytes ($CD3^-$, TCR^-, $CD4^-$, $CD25^-$), B cells ($CD19^-$), fibroblasts (collagen I^+, $CD34^+$), epithelial cells (cytokeratin$^-$) and endothelial cells (vWF^-, $CD11b^+$). In addition, when examined by electron microscopy, fibrocytes exhibit unique cytoplasmic extensions intermediate in size between microvilli and pseudopodia which further differentiate fibrocytes from blood-borne leukocytes [2].

Fibrocytes expressing CD34, CD11b and collagen I (but not CD14, CD3 or CD10) found in the peripheral blood, wound sites and areas of tissue remodeling should not be confused with fibrocytes or fibroblast cultures which are

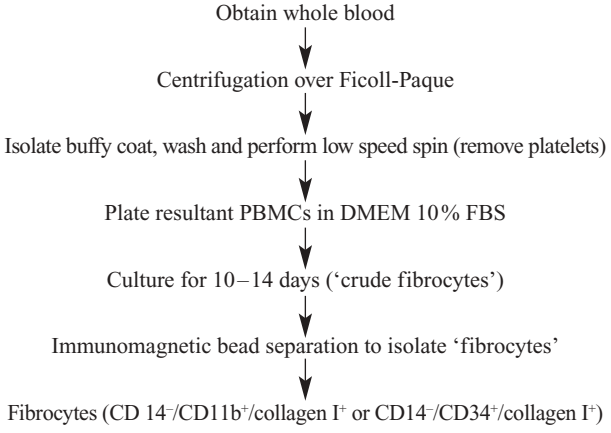

Figure 1. Schematic representation indicating the isolation and growth conditions for peripheral blood fibrocytes. Taken from [4].

Table 1. Fibrocyte-associated surface markers.

Marker type	Reference
ECM markers	
Collagen I	(2–8)]
Collagen III	[2]
Fibronectin	[2]
Vimentin	[2]
CD markers	
CD11a (LFA-1)	[2–3]
CD11b (Mac 1)	[2]
CD13 (pan myeloid antigen)	[2]
CD34 (hemopoetic stem cell antigen)	[2]
CD45 (leukocyte common antigen)	[2]
CD54 (ICAM)	[2]
CD58 (LFA-3)	[2]
CD80 (B7-1)	[3]
CD86 (B7-2)	[2–3]
MHC-related markers	
MHC class II	[2–3]
HLA-DP	[3]
HLA-DQ	[3]
HLA-DR	[3]
Chemokine receptors (ligands)	
CCR3 (MCP-3, MCP-5, RANTES, MIP1α, HCC-1)	[4]
CCR5 (MIP1α, MIP1β, RANTES)	[4]
CCR7 (ELC, SLC)	[4]
CXCR4, fusin (SDF-1)	[4]

Table 2. Fibrocytes secrete chemokines, cytokines and growth factors implicated in wound repair.

Factor	Comments	Ref.
α chemokines		
MIP1α	constitutive; ↑ with TGF-β1 or IL-1β	[8]
MIP1β	constitutive; ↑ with TGF-β1 or IL-1β	[8]
MCP-1	constitutive; ↑ with TGF-β1 or IL-1β	[8]
β chemokines		
IL-8	constitutive; ↑ with TGF-β1 or IL-1β	[8, 31]
GROα	constitutive; ↑ with TGF-β1 or IL-1β	[8]
Cytokines		
TNF-α	with IL-1β stimulation	[8]
IL-6	with IL-1β or TNF stimulation	[8]
IL-10	with IL-1β or TNF stimulation	[8]
Growth Factors		
Angiogenin	constitutive	[31]
CTGF	constitutive	[31]
IGF-1	constitutive	[31]
M-CSF	constitutive	[8, 31]
PDGF	constitutive	[31]
TGFβ	constitutive	[8]
Other		
MMP-9	Constitutive, active and latent	[31]
αSMA	↑ with TGF-β1	[4]

implicated in the amyloid fibrillogenesis described by Harris and colleagues [5]. Nor should they be confused with 'spiral ligament fibrocytes' located within the ear. Spiral ligament fibrocytes are the cells which interconnect with basal cells of the stria vascularis via gap junctions and are postulated to play a critical role in maintaining cochlear homeostasis [6–7]. Both of these cell types appear to be unrelated to the blood-borne fibrocytes implicated in wound healing.

Ex vivo cultured peripheral fibrocytes originate from CD14$^+$ cells and differentiate into a cell population with wound-healing potential

The precise origin of peripheral blood fibrocytes has puzzled investigators since their discovery. Fibrocytes express CD45 (leukocyte common antigen), a marker of bone marrow-derived cells (see table 1). Early studies using sex-mismatched, bone marrow chimeric mice together with DNA amplification of the male-specific SRY gene showed that circulating fibrocytes in vivo arise from radioresistant bone marrow progenitor cells or an unidentified tissue source [2].

Recent studies by Abe and colleagues, examining the origin and differentiation pathway of this cell population, show that peripheral blood fibrocytes isolated from blood differentiate ex vivo from an adherent CD14$^+$ cell population [4]. Although fibrocytes do not require the addition of specific growth factors (other than those present in fetal calf serum), they do proliferate slowly in response to interleukin (IL)-1β and tumor necrosis factor α (TNF-α) [8] and to basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) [C. N. Metz, unpublished observations]. Based on in vitro studies, it is postulated that circulating 'progenitor fibrocytes' undergo phenotypic changes that promote the differentiation of these circulating precursor cells to fibrocytes that are recruited to wound sites where they become mature fibrocytes and play a role in wound contracture and wound healing (fig. 2). Early observations demonstrated that cultured peripheral blood fibrocytes require interaction with activated T cells to permit their early differentiation. The requirement for T cell interaction is similar to that reported for the differentiation of CD1a$^+$ dendritic cells [9]. Following their interaction with T cells, it is proposed that they migrate to the wound site (see fig. 2 and below). Within the wound, these early-differentiated fibrocytes might further interact with recruited T cells and then fully differentiate into mature fibrocytes following exposure to transforming growth factor-β (TGFβ, expressed in early wounds). In response to TGF-β, it is postulated that these mature fibrocytes express increased α smooth muscle actin (αSMA) which provides a contractile force for wound closure and produce collagen and other critical extracellular matrix molecules that promote wound healing (fig. 2).

Where are fibrocytes found?

Although initially identified as a blood-borne cell population (CD34$^+$, CD11b$^+$ and/or collagen1$^+$), fibrocytes have been localized to various tissues under both normal and pathological conditions (see table 3). Fibrocytes initially were localized to scar tissues, as well as in implanted wounded chambers [2]. Since the first report identifying blood-borne fibrocytes, cells with a similar phenotype (CD34$^+$) have been described in cutaneous wounds, keloids, fibrotic tissues, normal tissue, cutaneous tumors and mesenchymal tumors. Because CD34, a marker present on endothelial cells, was the only marker used for these studies, caution must be used in interpreting the results. In our experience, it is relatively easy to differentiate between tissue fibrocytes and endothelial cells because the endothelial cells are CD34Hi and fibrocytes are CD34Lo, and because fibrocytes (elongated) are dispersed within the tissue, whereas endothelial cells form ringlike structures (vessels) and accordingly form a pronounced pattern. Staining serial sections with vWF would further help discriminate between fibrocytes (vWF$^-$) and endothelial cells (vWF$^+$).

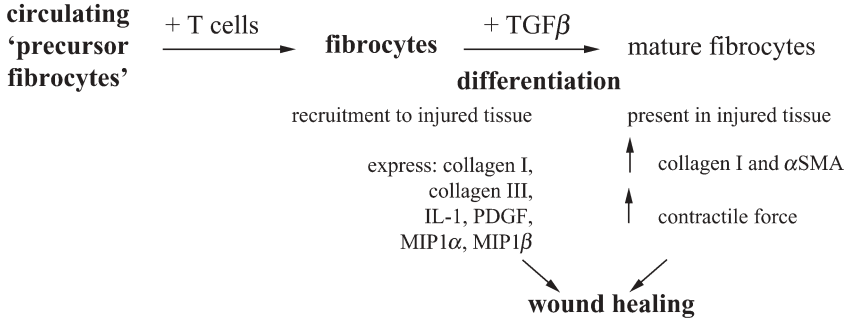

Figure 2. The proposed differentiation pathway of fibrocytes from a circulating precursor population. Modified from [4].

Table 3. In situ localization of fibrocytes.

Tissue type	Reference
Wound tissue	
Subcutaneously implanted wound chambers	[2]
Cutaneous wounds	[4]
Scar tissue	
Cutaneous/subcutaneous	[2]
Red and pink scars	[35]
Keloids/hypertrophic scars	[36]
Normal tissue	
Normal skin	[37]
Normal pancreatic tissue	[18]
Normal breast tissue	[10, 38]
Fibrotic tissue	
Fibrotic granulomatous liver	[8]
Tumors	
Basal cell carcinomas (dermal)	[39]
Mesenchymal tumors	[42–47]

Interestingly, the occurrence of $CD34^+$ fibrocytes in the stroma surrounding skin tumors has been found to be of diagnostic significance. Kirchmann and colleagues report that $CD34^+$ fibroblast like cells are abundant in benign desmoplastic trichoepitheliomas, whereas basal cell carcinomas and adnexal carcinomas lack $CD34^+$ cells [19–20]. Similarly, the presence or absence of $CD34^+$ fibrocytes may be useful in differentiating between invasive breast carcinomas and benign breast lesions [10]. Based on the observations that benign breast lesions (sclerosing adenosis) exhibit stromal $CD34^+$ cells, whereas stroma derived from invasive breast carcinomas is devoid of $CD34^+$ fibrocytes, Barth and colleagues report that the absence of $CD34^+$ fibrocytes favors the diagnosis of human basal cell carcinoma when distinguishing between basal cell carcinomas and benign skin appendage tumors [10]. Together these observations suggest that the presence of $CD34^+$ fibroblast-like cells might prove to be very useful in differentiating between benign (presence of $CD34^+$ fibrocytes) and invasive/malignant lesions (absence of $CD34^+$ fibrocytes). The lack of fibrocytes with antigen-presenting capabilities (see below) in invasive tumor sites suggests that these cells may play a role in local immune surveillance and the loss of these cells may permit an invasive phenotype.

Peripheral blood fibrocytes: recruitment to wound sites

The original report describing fibrocytes details the appearance of blood-borne fibroblast-like cells in subcutaneously implanted wound chambers in mice and in early human cutaneous scar tissues [2]. However, the mechanism(s) by which peripheral blood fibrocytes migrate to specific sites of tissue injury were not understood. Numerous circulating cells, including monocytes, neutrophils and T lymphocytes, migrate to tissue locations as the result of chemokine-chemokine receptor interactions. Based on these observations, the fibrocyte-associated chemokine receptor expression profile was examined [4]. These studies revealed the expression of several chemokine receptors on the surface of fibrocytes, including CCR3, CCR5, CCR7 and CXCR4. Using both in vitro and in vivo fibrocyte chemotaxis techniques, Abe and colleagues revealed that fibrocytes migrate in response to secondary lymphoid chemokine (SLC), the ligand for CCR7. SLC (also known as 6Ckine, Exodus-2 and TCA-4) has been shown to play a role in the organization of lymphoid tissues during development by attracting T lymphocytes and mature dendritic cells [11]. Interestingly, the expression of SLC at sites of inflammation has been reported [12]. Thus, it appears that fibrocytes express several chemokine receptors and can migrate to wound sites in response to specific chemokine gradients.

The role of fibrocytes in wound healing

Fibrocytes have been postulated to play a pivotal role in wound healing and tissue repair processes. Fibrocytes can contribute to wound healing by numerous potential mechanisms: (i) by serving as potent antigen-presenting cells (APCs); (ii) by producing important cytokines, chemokines and growth factors necessary for wound repair (see table 2); (iii) by secreting essential extracellular matrix proteins involved in wound repair (see table 1); (iv) by serving as a contractile force in wound closure via αSMA expression and (v) by promoting angiogenesis, a critical step in the wound repair process.

Fibrocytes are potent APCs able to recruit and activate T cells

The skin is the first immune defense barrier which serves to protect the host against infection [13]. When this critical barrier is damaged, pathogenic bacteria can easily invade. One mechanism by which the body defends itself is the strategic location of APCs at specific sites to initiate antigen-specific host immune responses. Several studies support the critical role of fibrocytes in the initiation of immunity during tissue injury and repair. Isolated human fibrocytes express the cell surface molecules required for antigen presentation, including major histocompatibility complex molecules (HLA-DP, HLA-DQ and HLA-DR), the costimulatory molecules [CD80 (B7-1) and CD86 (B7-2)] as well as adhesion molecules [CD11a (LFA-1), CD54 (ICAM-1) and CD58 (LFA-3)] [3]. The expression level of these specific markers by fi-

brocytes is similar to that expressed by monocytes. Moreover, human fibrocytes localized to cutaneous scar tissues express high levels of HLA-DR in situ [3], suggesting that fibrocytes present in the wounded areas function as APCs.

Because human leukocyte antigen-D-related (HLA-DR) expression is considered a prerequisite for antigen presentation in vivo [14], Chesney and colleagues tested the functional capacity of both human and mouse fibrocytes to present antigen and stimulate antigen-specific T lymphocytes in vitro [3]. Human fibrocytes induce APC-dependent T cell proliferation when cultured with specific antigen, suggesting that fibrocytes play a role in the initiation of antigen-specific immunity. When compared with monocytes and dendritic cells for antigen-presenting capacity in vitro (using the same autologous T cells), fibrocytes were between monocytes (low) and dendritic cells (high) when assessed by peak antigen-dependent T cell proliferation induced by the APCs [3].

Similarly, isolated mouse fibrocytes express major histocompatibility markers, adhesion molecules and costimulatory molecules (I-a, I-E, CD54 and CD86) required for antigen presentation [3]. Mouse fibrocytes cultured ex vivo, pulsed with foreign antigen in vitro, and then injected into mouse skin, migrate into regional lymph nodes where they sensitize naive T cells and/or activate memory T cells in vivo [3]. These observations further support the role of fibrocytes in the initiation of antigen-specific immunity. In addition, human fibrocytes secrete MIP-1α and MIP-1β (see table 2), potent chemoattractant molecules for $CD4^+$ T cells. These $CD4^+$ T cells are considered essential for the generation of an antigen-specific response in vivo [15]. Thus, fibrocytes may contribute to the host defense response during tissue injury/tissue invasion by recruiting and activating T lymphocytes to sites of injury.

More recent studies by Zhu and colleagues [16] characterized fibrocytes isolated from Macaques and found them to be phenotypically similar to human and mouse fibrocytes (i.e. $CD34^+$ and $collagen^+$). The Macaque fibrocytes were transfected with a vector encoding green fluorescent protein or DNA expression vectors encoding the simian immunodeficiency virus (SIVmne) structural and regulatory genes and then tested for their ability to augment antigen presentation for SIV vaccines. These studies suggest that fibrocytes are a readily accessible source of APCs capable of initiating and promoting T cell immunity. Furthermore, they highlight the potential clinical utility of fibrocytes in vaccine development for the treatment of diseases such as human immunodeficiency virus (HIV) or cancer. However, further studies are required to better characterize the functional capacity of fibrocytes as APCs before they can be used for clinical vaccine development.

Fibrocytes secrete extracellular matrix proteins implicated in wound repair/remodeling and disease pathogenesis

Fibrocytes produce extracellular matrix molecules found during tissue repair

Reparative cells contribute to wound healing by secreting extracellular matrix components. The production of extracellular matrix molecules by these cells is regulated by numerous specific growth factors and other mediators. A deficiency in these regulatory factors is postulated to cause delayed healing (insufficient extracellular matrix), whereas an excess of these factors could promote scarring (excessive extracellular matrix). Ex vivo cultured fibrocytes express numerous extracellular matrix molecules, including vimentin, fibronectin, collagen I and collagen III (table 1), and fibrocytes localized to wounds in situ express collagen, suggesting their role in wound repair.

The potential role of fibrocytes in fibrosis and scarring

The regulation of matrix production within the wound is critical to prevent fibrosis during the tissue repair process. Numerous reports identify the presence of fibrocytes in scar tissue (see table 3). In the case of experimental schistosomiasis, a parasitic infection where T-cell-mediated reactions against parasitic eggs sequestered in the lung and liver result in severe fibrosis, $CD34^+$ fibrocytes were found in areas of connective tissue matrix deposition within fibrotic livers [8]. By contrast, no $CD34^+$ cells were present in the normal livers of uninfected mice, suggesting that fibrocytes may contribute to the fibrotic pathology associated with schistosomiasis. Further studies implicate fibrocytes in the pathogenic fibrosis associated with radiation damage [17], Lyme disease [24–26] (see below) as well as pulmonary fibrosis [C. N. Metz, unpublished observations]. Therefore, it is important to understand the regulation of connective tissue molecules by fibrocytes to prevent fibrosis while promoting wound repair.

The presence of fibrocytes in stromal tissue: an indication for differentiating between malignant and noninvasive (benign) tumor lesions

Stromal remodeling is associated with chronic pancreatitis and ductal adenocarcinomas. A very recent investigation reported the distribution of $CD34^+$ fibrocytes in pancreatic diseases (pancreatic adenocarcinoma, endocrine tumors of the pancreas and chronic pancreatitis) [18]. Morphological analysis showed spindle-shaped cells with small centrally located elongated nuclei and long slender dendrite-like projections. In normal pancreatic tissue, there were few $CD34^+$ fibrocytes, whereas there was an increased number of stromal $CD34^+$ fibrocytes in tissue

(predominantly located in areas of diffuse or nodular stromal fibrosis around intralobular ducts and acini) obtained from patients with chronic pancreatitis. By contrast, stroma-associated $CD34^+$ fibrocytes were absent from both pancreatic endocrine tumors and adenocarcinomas. These data suggest an association between $CD34^+$ fibrocytes in pancreatitis and stromal fibrosis, whereas pancreatic endocrine tumors and adenocarcinomas lack $CD34^+$ fibrocytes.

The role of fibrocytes in disease pathogenesis

Lyme disease, transmitted by the spirochete *Borrelia burgdorferi*, is the most common vector-borne illness in the United States (reviewed in [21–22]). It is a multisystem disease that affects the skin, nervous system, heart and joints. Ticks deposit *B. burgdorferi* into the dermis of their host, where eventually they become associated with collagen fibers. Recent studies reveal the interaction between decorin-binding adhesins present on the *B. burgdorferi* and host tissue collagen-associated proteoglycan decorin [23]. Joint diseases associated with untreated *B. burgdorferi* infection include arthritis and fibromyalgia, a chronic pain syndrome with diffuse joint and muscle pain. However, the process by which the bacteria invade the joint tissue is not completely understood. Based on the production of extracellular matrix proteins (collagen I, III, fibronectin and vimentin) by fibrocytes, Grab and colleagues investigated the interaction between isolated fibrocytes and *B. burgdorferi* in vitro [24]. Using electron microscopy, they revealed that *B. burgdorferi* are not phagocytosed by fibrocytes (isolated from humans or rhesus monkeys), but rather sequestered within the cell membrane by tubelike processes extending from the fibrocytes. They postulate that this semiinternalization of the *B. burgdorferi* by fibrocytes serves to enhance the infection by transporting the bacteria from the peripheral circulation to the joint while protecting the bacteria from the host immune system, and thus may play an important role in the pathogenesis of Lyme disease. Based on the observations that fibrocytes localize to areas of matrix deposition in *Schistosoma japonicum*-infected granulomatous livers in mice (implicating persistent fibrocyte-T cell activation responses in the development of fibrotic liver disease [8]), and that the Lyme disease spirochete can invade peripheral blood fibrocytes [25], Grab and colleagues hypothesize that fibrocytes are the active immune cells that contribute to some of the pathologies observed in Lyme neuroborreliosis [26]. Furthermore, the secretion of cytokines/chemokines by fibrocytes (TNF-α, IL-6, MIP1α, MIP1β) which skew the $CD4^+$ Th response could also promote the progression of Lyme disease. Thus, fibrocytes may be an important cell type involved in the pathogenesis of Lyme disease.

Fibrocytes are TGF-β-responsive cells that express αSMA: Their potential role in wound contracture

Based on their presence within wounds and their expression of collagen I and collagen III, fibrocytes have been postulated to mediate wound healing and/or fibrosis. However, their functional role in these activities has not been well characterized. Recently, Abe and colleagues showed that fibrocytes could differentiate into 'myofibroblast-like' cells that express αSMA in response to TGF-β and exhibit a contractile force in vitro [4]. Myofibroblasts are transiently found in early to mid-phase wound tissues and have been proposed to exert a critical contractile force required to close tissue wounds (reviewed in [27–28]). These cells respond to TGF-β with increased αSMA expression, enhanced collagen production and increased contractile activity in vitro. Likewise, treatment of ex vivo cultured fibrocytes isolated from peripheral blood with TGF-β enhanced both collagen production and αSMA expression by these cells. Furthermore, isolated fibrocytes contract collagen gels in vitro, and the treatment of these cells with TGF-β increased their contracture abilities (fig. 3) [4]. Thus, fibrocytes and myofibroblasts appear to share many common features, including their transient presence within the wound, production of numerous extracellular matrix proteins, pro-inflammatory cytokines, chemokines, and enhanced collagen secretion and gel contracture ability following treatment with TGF-β. Of course, the question remains whether fibrocytes and myofibroblasts are two distinct populations. In summary, the addition of TGF-β – a multifunctional growth factor expressed in the early wounds critical for tissue repair – to fibrocytes facilitates

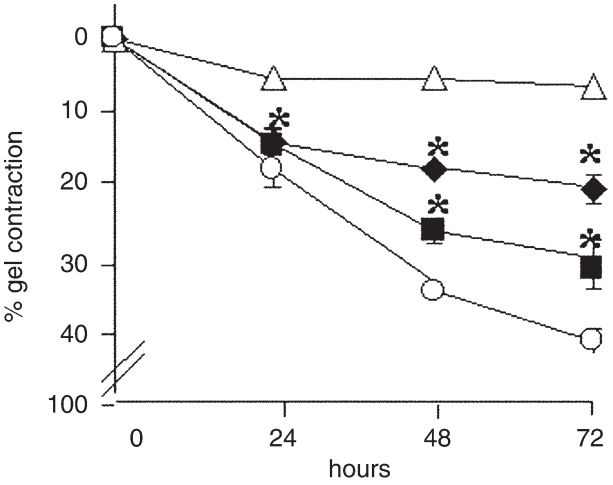

Figure 3. Fibrocytes contract collagen gels in vitro. PBMCs (△), cultured enriched fibrocytes (untreated = ◆; TGFβ-treated (10 ng/ml) = ■), or dermal fibroblasts (○) were resuspended in a collagen I solution at 10^5 cells/ml and subjected to a gel contraction assay (n = 3). The data are shown as percent gel contraction (from the beginning of the experiment) ± SE. * = $P < 0.05$, as determined by the Student's t-test. Taken from [4].

their differentiation toward a wound-healing phenotype similar to that exhibited by myofibroblasts (illustrated in fig. 2).

Fibrocytes promote endothelial cell proliferation, migration and tube formation in vitro and angiogenesis in vivo

Normal wound healing requires angiogenesis that facilitates the removal of debris and prepares the wound bed for development of a critical framework of granulation tissue necessary for wound closure. These newly formed vessels represent over 50% of the granulation tissue mass found in early wounds (reviewed in [29]). Wound-related angiogenesis appears to be regulated by the interaction of endothelial cells with the extracellular matrix within the wound space [30]. Although numerous cellular mediators of wound healing have been identified, few studies have focused on the role of specific cell types that mediate angiogenesis during wound healing.

Based on previous observations that fibrocytes secrete IL-8 and other growth factors, Hartlapp and colleagues characterized the production of numerous pro-and anti-angiogenic factors by ex vivo cultured fibrocytes (table 2). Fibrocytes secrete VEGF, platelet-derived growth factor (PDGF), angiogenin, IL-1β, granulocyte colony-stimulating factor (GCSF) and bFGF. In addition to promoting endothelial cell proliferation in vitro, culture supernatants obtained from fibrocytes promote endothelial cell migration and endothelial cell differentiation (tube formation) in vitro [31]. By contrast, fibrocytes express few anti-angiogenic molecules [31].

A critical event during the invasion stage of angiogenesis is the proteolysis of the basement membrane. Previous studies have shown that matrix metalloproteinases (MMPs) mediate the dissolution of the basement membrane during early tissue repair and initiate angiogenesis. Although MMPs are not present in normal skin, both MMP-2 and MMP-9 are strongly induced within 24 h of wounding [32, 33]. MMP-9 is the main MMP found in wound fluid, with peak activity expressed between 2 and 4 days post-wounding [34]. Consistent with these observations, fibrocytes home to cutaneous wound sites in vivo within 1–4 days [2], and ex vivo cultured fibrocytes constitutively express MMP-9 messenger RNA (mRNA) and secrete high levels of active MMP-9 [31]. Together, these data demonstrate that cultured fibrocytes secrete factors that promote an angiogenic phenotype in endothelial cells in vitro. Clearly, the timing of observation and the local tissue environment will significantly effect the expression of MMPs, growth factors and cytokines by fibrocytes in vivo.

Further studies using the Matrigel implant model of angiogenesis in mice, show that fibrocytes (fig. 4E) (and fibrocyte culture supernatants (fig. 4C) promote blood vessel formation in vivo. Thus, it appears that fibrocytes may play a role in blood vessel formation during the initial stages of wound healing based on their early presence within the wound and their ability to promote endothelial cell proliferation, migration, differentiation, as well as neovascularization in vivo.

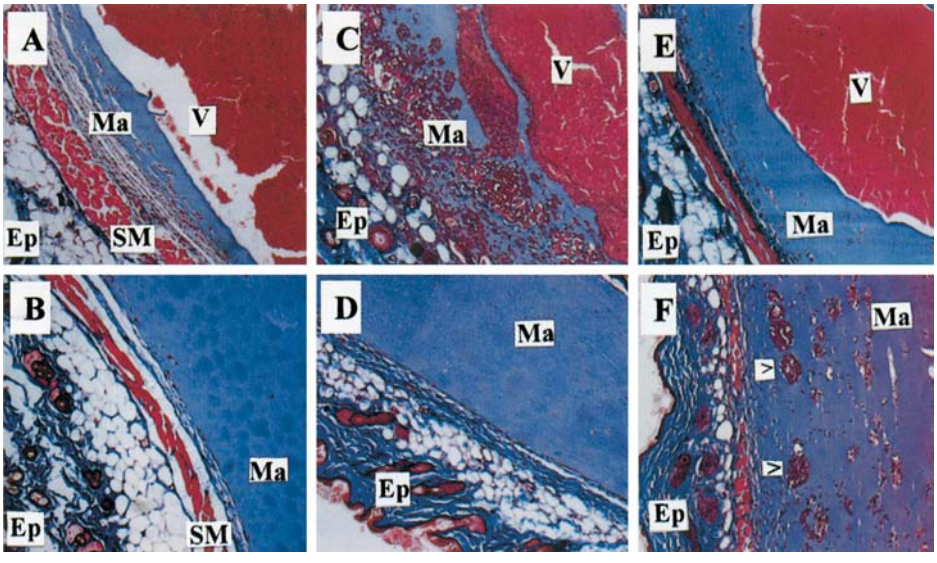

Figure 4. Fibrocytes and fibrocyte culture supernatants induce angiogenesis in vivo. Matrigel was injected into mice (n = 5 per group) containing (A) heparin plus aFGF (positive control); (B) heparin alone (negative control for Matrigel model); (C) heparin plus fibrocyte-conditioned media; (D) heparin plus unconditioned media (negative control for heparin plus fibrocyte-conditioned meda); (E) mouse fibrocytes; or (F) NIH 3T3 cells (negative control for mouse fibrocytes). After 6 days, the Matrigel plugs were removed and examined for blood vessel formation using Masson's Trichrome staining. Stained sections were photographed at 100 ×. Representative Matrigel plugs are shown. Epidermis (Ep); Matrigel (Ma); skeletal muscle (SM); vessels (V); Taken from [31].

Future research directions

Numerous studies demonstrate the potential role of fibrocytes in wound healing. These investigations highlight the localization of fibrocytes at sites of tissue damage and repair where they could (i) initiate antigen-specific immunity as APCs; (ii) secrete extracellular matrix proteins, cytokines and pro-angiogenic molecules, which promote wound repair and (iii) express αSMA, which mediates wound closure. Based on their ability to secrete extracellular matrix molecules and their presence within scar tissue, numerous studies also implicate fibrocytes in fibrosis and scarring, typical in connective tissue disorders such as, schistosomiasis, lung fibrosis, keloids and scleroderma. Also, the localization of APC-associated $CD34^+$ fibrocytes in pancreatic tissue and stroma-associated fibrocytes in pancreatic tissue (during pancreatitis), and the absence of CD34+ fibrocytes in pancreatic endocrine tumors and adenocarcinomas suggest that these cells play a role in local immune surveillance. In other words, the loss of tissue-associated fibrocytes with antigen-presenting abilities may contribute to the invasive nature of the tumor.

In the setting of wound healing, one can predict that too few fibrocytes (or underactive fibrocytes) at the site of tissue damage may result in a weak host immune response, permitting further pathogenic invasion and a delayed wound healing response. By contrast, too many fibrocytes or prolonged, persistent fibrocyte-mediated T cell activation within the damaged tissue region may contribute to fibrosis and scarring. Future studies are required to determine how the delicate balance between the wound-healing properties and pro-fibrotic abilities of fibrocytes is achieved.

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