Protomyofibroblast Pathway in Early Thermal Burn Healing

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**Key Words**
Burn injury \cdot Myofibroblast \cdot Wound healing \cdot Protomyofibroblast \cdot Skin

**Abstract**
Wound healing following partial thickness thermal burns is commonly hampered by the risk of hypertrophic scarring. Skin myofibroblast (MF) density is commonly increased in postburn healing. The transition between fibroblast-like cells and α-smooth muscle actin (SMA)+ MF possibly begins with CD14+ monocytes, evolving to CD14+ CD34+ fibrocytes, followed by β-SMA+ protomyofibroblast (PMF) maturation. Skin biopsies from 25 burn patients were collected about 1 and 4 weeks after injury. Immunohistochemistry was performed using monoclonal antibodies to α-SMA, β-SMA, factor XIIIa, lysozyme, Mac 387, CD14, CD117 and Ulex europaeus agglutinin-1 (UEA-1). The set of Mac 387+ and CD14+ monocytes was accompanied by both CD34+ fibrocytes and factor XIIIa+ dendrocytes. By contrast, β-SMA+ PMF were rare. Of note, α-SMA+ MF were more abundant at week 4 than at week 1 (p < 0.01). The UEA-1+ endothelial cells showed marked variations in their dermal distribution, irrespective of the densities in the other scrutinized cells. In conclusion, healing of partial thickness thermal burns involves a diversity of cell types including PMF. In the present samples, the PMF density remained low.

**Introduction**

The regular dermal healing of wounds following partial thickness thermal burns is a complex process, particularly involving angiogenesis, extracellular matrix (ECM) deposition, connective tissue cell accumulations and inflammatory cell infiltration. One typical cell maturation process taking place in wound healing resides in the differentiation of peculiar circulating monocytes into ECM-producing fibrocytes and possibly protomyofibroblasts (PMF). The latter cells represent precursors of myofibroblasts (MF). In these instances, hypertrophic and retractile scars are at risk of development [1].

MF is a peculiar connective tissue cell present in abundance in wound healing and many other fibrotic conditions [1–4]. They contain contractile proteins corresponding to α-smooth muscle actin (α-SMA). In some experimental and clinical settings, MF cells possibly express...
such a complex ing burn healing wounds where fibroblast migration
place during skin healing and in some fibrotic lesions as
cursor of the monocyte lineage
mature mesenchymal cells for a bone marrow-derived pre-
mandatory intermediate stage of differentiation into ma-
local recruitment of fibroblast-looking cells from the
proximal dermis and hypodermis, or from the circulating
monocyte-macrophage lineage [5, 6]. Such a complex
condition is supported by the presence of many fibroblasts
engaged in the cell cycle of proliferation at wound edges.
Pericytes and perivascular smooth muscle cells potentially
represent other MF sources. In addition, it was suggest-
ed that fibroblasts arise in large numbers by local epithelial
to mesenchymal transdifferentiation [7, 8]. Such a pro-
cess is characterized by loss of epithelial adhesion and gain
of mesenchymal characteristics. Furthermore, circulating
precursor CD14+ monocytes and CD34+ dendrocytes/
dendrocyte/fibrocyte possibly migrate towards wounds, and they
contribute to the genesis of the MF population in granu-
lation tissue [9–12]. Collagen-producing CD14+ CD34+
dendrocytes/fibrocytes possibly differentiate into contractile MF
and a series of ECM molecular components
possibly by fibroblast-like cells [17–19]. Thus, MF differ-
entiation is regulated by the combination of specific cells
and a series of ECM molecular components [20–22].
Cell-cell contacts [23] and mechanical force transduc-
tions [24–27] participate in the process.
The aim of the study was to scrutinize using immuno-
histopathology the early step of wound healing following partial
thickness thermal burns. In particular, we looked at identifying monocytes, PMF, MF and dermal dendrocytes.

**Patients and Methods**

The study was approved by the Ethics Committees of Liège
University, Belgium, and of the Percy Military Hospital in Clamart,
France. It represented in part an extension to two previous inves-
tigations about thermal burn healing [28, 29]. A total of 25 burn
patients (21 men and 4 women) were enrolled over 9 years. Their
median age was 43 years (range 21–58). The median burn surface
area extended over 34% (range 7–40) of the total body surface. The
most severely affected patients benefited from regular care proce-
dures, including fluid replacement, sedation, analgesia, early nu-
tritional supply, thromboprophylaxis, wound care including anti-
septics (chlorhexidine or povidone-iodine), and early excision of
necrotic tissues followed by grafting. None of the patients de-
veloped a skin infection and they were not under sustained compres-
sive garments.

In each patient, a 4-mm punch biopsy was performed at the end
of week 1 and week 4 after injury in unhealed partial thickness
burn areas that had not been covered by skin grafting. Histopatho-
logical 6-μm-thick sections were cut from the formalin-fixed par-
affin-embedded biopsies. A panel of 9 antibodies (table 1) was used
in a Ventana® procedure. After a 1-hour incubation time with any
of the primary antibodies, the slides were washed in a buffer solu-
tion. Slides were rinsed and covered by the Ventana polymer-
based revelation system. After washing, fast red was used as chro-
rogen substrate. The last steps consisted of counterstaining with
Mayer’s haemalum before mounting. Negative immunohistoches-
tical controls omitted or substituted the antibodies in the labora-
tory procedure.

Blind histopathological examinations were performed. In each
section, the total number of immunoreactive cells was counted per
millimetre length and over 0.2 mm dermal thickness from three
contiguous sectors. These trebly counts were averaged as means
per patient and per square millimetre of vertical sections at both
evaluation times. Global data were presented as medians and rang-
es. Comparisons between the two samplings in time were assessed
using the Wilcoxon test for paired data. A p value < 0.05 was con-
sidered statistically significant.

**Results**

Data are presented in table 2. In the superficial dermis,
blood microvasculature highlighted by Ulex europaeus
agglutinin-1 (UEA-1) exhibited a trend in increase over

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**Table 1. Panel of antibodies**

<table>
<thead>
<tr>
<th>Biomolecule/antibody</th>
<th>Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD14</td>
<td>monocyte</td>
</tr>
<tr>
<td>CD34</td>
<td>dendrocyte/fibrocyte</td>
</tr>
<tr>
<td>CD117</td>
<td>mast cell</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>macrophage</td>
</tr>
<tr>
<td>Mac 387</td>
<td>monocyte/macrophage</td>
</tr>
<tr>
<td>UEA-1</td>
<td>endothelial cell</td>
</tr>
<tr>
<td>α-SMA</td>
<td>MF</td>
</tr>
<tr>
<td>β-SMA</td>
<td>PMF</td>
</tr>
<tr>
<td>Factor XIIIa</td>
<td>dermoepidermal cell</td>
</tr>
</tbody>
</table>

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Table 2. Cell populations in the superficial dermis of partial thickness thermal burns

<table>
<thead>
<tr>
<th>Biomolecule/antibody</th>
<th>D5</th>
<th>p value</th>
<th>D26</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD14</td>
<td>8.8 (2.5–9.7)</td>
<td>0.4103</td>
<td>7.1 (1.5–8.3)</td>
</tr>
<tr>
<td>CD34</td>
<td>17.2 (6.7–27.4)</td>
<td>0.2318</td>
<td>22.4 (13.7–29.7)</td>
</tr>
<tr>
<td>CD117</td>
<td>15.3 (5.6–22)</td>
<td>0.8540</td>
<td>17.2 (10.1–22.2)</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>13.9 (7.7–23.6)</td>
<td>0.9113</td>
<td>13.3 (8.7–19.2)</td>
</tr>
<tr>
<td>Mac 387</td>
<td>16.6 (12.5–36.1)</td>
<td>0.8862</td>
<td>15.9 (7.5–26.9)</td>
</tr>
<tr>
<td>UEA-1</td>
<td>38.7 (13.8–42.9)</td>
<td>0.0878</td>
<td>46.9 (28.5–69.7)</td>
</tr>
<tr>
<td>α-SMA</td>
<td>3.8 (1–11.2)</td>
<td>0.0419</td>
<td>6.2 (1.8–9.3)</td>
</tr>
<tr>
<td>β-SMA</td>
<td>1.4 (0.8–3.1)</td>
<td>0.0314</td>
<td>4.8 (1.1–6.8)</td>
</tr>
<tr>
<td>Factor XIIIa</td>
<td>31.8 (22.0–59.4)</td>
<td>0.0324</td>
<td>47.4 (36.3–58.5)</td>
</tr>
</tbody>
</table>

Median numbers and ranges per square millimetre of section at day 5 (D5) and day 26 (D26) after injury.

time with large variations in the patterns of extension. They were cuffed by dispersed α-SMA+ MF. In addition, at week 1, rare scattered α-SMA+ MF and β-SMA+ PMF were present at a distance from the microvasculature. Both of these cell types were more abundant (p < 0.05) at week 4. Of note, the CD14+ and Mac 387+ precursor monocytes were not significantly influenced (p > 0.05) by the duration of the healing phase. Factor XIIIa+ dermal dendrocytes and lysozyme+ macrophages significantly outnumbered both the PMF and MF at both sampling times. The density in CD117+ mast cells was highly variable among patients both at week 1 and week 4.

Discussion

Partial thickness thermal burn injury is responsible for a peculiar wound process possibly showing unique clinical features leading to a long-term trend in developing abnormal hypertrophic scarring and contractures. The undesirable presentations of such an evolution are attributed to the presence of an excessive amount in ECM produced by a dense population of connective tissue cells. These cells are likely boosted in a hyperactivity state following diverse cytokine stimulations including IL-1β overexpression at the post-transcriptional level. The combined overexpression of IL-1β and tumour necrosis factor (TNF) type I receptor possibly maintain the fibro-genetic potential of hypertrophic scars [30].

Hypertrophic scars contain an increased density in MF. These cells promote collagen network contraction and synthesize increased amounts in both ECM biomolecules and matrix metalloproteinases. The MF lineage represents a heterogeneous population of α-SMA+ profibrogenic, pro-inflammatory, pro-angiogenic and contractile cells [3]. They originate from a process of activation and transdifferentiation mainly involving the monocyte lineage, mesenchymal stem cells and circulating fibrocytes. The switch from PMF to MF is possibly related to TGF-β1 produced by a large set of cell types involved in wound healing [17–19]. Natural resolution of abnormal scarring possibly follows the complete epithelialization. The apoptosis induction mechanisms in scars are possibly related to a reduced production in local growth factors and to a change in MF adhesion to the ECM fibres. Different responses to apoptotic inducers were shown in MF of regular and hypertrophic scars. Such findings support the concept of altered growth and apoptosis during abnormal scarring impeding MF withdrawal at completion of the healing process [31]. In particular, TGF-β1 possibly delays the onset of MF apoptosis.

Scar growth control is of importance in clinical practice. In order to abate any excessive healing process, inflammation must be down-regulated. Early excision of necrotic tissue and wound coverage represent the fundamental strategy. Growing knowledge on healing physiopathology has provided the rationale for some innovative therapies. Despite such advances, only few clinical modalities are currently used for mitigating scar growth.

Human dermis contains dendritic cells exhibiting various distinct phenotypes. Immunohistochemistry using specific monoclonal antibodies directed to Mac 387 and lysozyme is routinely used for assessing the macrophage lineage. The monoclonal Mac 387 antibody recognizes the cytosolic L1 protein complex (calprotectin). The L1 antigen is a calcium-binding protein associated with the cytoskeleton of neutrophils, eosinophils, monocytes [32] and in a subset of reactive macrophages during the early stage of monocyte/macrophage differentiation [33].

Intracytoplasmic factor XIIIa transglutaminase is expressed in specific type 1 dermal dendrocytes derived from the monocyte-macrophage lineage [34, 35]. They are increased in number or size in scars and some other fibrotic processes [36], particularly when TNF is overexpressed. By contrast, their density is reduced in Ehlers-Danlos syndrome [37], where the intrinsic tension forces in the skin are lowered. Their function appears mainly related to phagocytosis and to ECM homoeostasis [38]. Factor XIIIa+ dermal dendrocytes appear to be related functionally to CD117+ mast cells. They increase in num-
er after mast cell degranulation, linked to the release of TNF. These particular phenotypic and functional characteristics are relevant to cellular interactions involved in cutaneous healing [39, 40].

Monocyte stimulation by the granulocyte-macrophage colony-stimulating factor and IL-4 induces factor XIIIa+ type 1 dermal dendrocyte differentiation [34] as well as in the transformation of monocytes into collagen-producing fibrocytes and a-SMA+ MF within a period of 14 days [12]. In the present study, the monocyte-derived MF possibly corresponds to CD14+ CD34+ fibrocytes. As the CD16 expression was not tested in the present study, the extent by which CD16+ monocytes and CD14+ CD16++ monocytes are involved is not clarified in the MF hyperplasia developed in burn healing. Similarly, the epithelial-to-mesenchymal transition [41] has not be presently scrutinized and remains unsettled in human wound healing. The phenotypic alterations or plasticity of epithelial lineages resulting in a gain of mesenchymal characteristics have been reported in wound healing following the induction by TNF through bone morphogenetic protein-2 [41].

The design of the study extending over several years allowed some variations in the nature of the antiseptics. Chlorhexidine and povidone-iodine were used. They possibly altered the density in dermal cells [42]. In particular, their potential effect on PMF is unknown. Future investigations should probably be focused on the effects of distinct antiseptics. The influence of the extent in burn surface area and the duration of the healing phase should also focus the attention of investigators on adequate distinctions between the various cell types in the dermis [43]. In any instance, ethical considerations remain mandatory, and the risk of hypertrophic scarring [44] and contractions should always be minimized.

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Disclosure Statement

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