Chapter 1

General introduction and outline of the thesis
Skin anatomy

Skin covers the entire body and provides a barrier between the body and the outside world through its mechanical as well as immunological properties. The skin protects the body from dangers such as dehydration, UV radiation and pathogens. The skin consists of two layers: the epidermis and the dermis. The epidermis consists of keratinocytes, but also contains Langerhans cells and melanocytes. The dermis is composed of extracellular matrix (ECM), blood vessels, ECM producing fibroblasts and several types of immune cells. The dermis also contains skin appendices and nerve endings (figure 1). ECM consists of proteoglycans (glycosylated proteins) and several fibrous proteins including collagen and elastin. Collagen, elastin and most of the other proteins are produced by fibroblasts. After assembly, collagen undergoes some changes in order to mature and stabilize. The triple helix is first stabilized by means of hydrogen bonds. Subsequently, several types of cross-links are formed within the triple helices and between triple helices. Cross-links are also formed in elastin fibres. Collagen and elastin fibre content and composition (including cross-linking) and several other proteins including proteoglycans determine the mechanical properties of skin.

Figure 1: anatomy of the skin  Adapted from Nestle OF, Di Meglio P, Qin JZ, Nickoloff BJ (2009).
Skin resident immune cells include dermal T cells and macrophages (Mφ) and epidermal Langerhans cells. These cells are important for immune surveillance, but also prevent the immune system from reacting to harmless agents.

**Cutaneous wound healing**

When the skin is damaged, its barrier function is compromised. Therefore, swift sealing and successive repair of the defect are mandatory. This is achieved through wound healing, where the defect is not resolved by regeneration, but rather by deposition of fibrotic tissue, resulting in scar formation. Fibrosis occurs in many other tissues of the human body besides skin, such as lung and liver. The complex process of wound healing can be subdivided in roughly four successive, but partially overlapping phases: the hemostasis and inflammation phase, the proliferation phase and the remodeling phase. In order to minimize blood loss, hemostasis is achieved directly after wounding through the formation of a fibrin clot by activated platelets and the coagulation cascade. Both activated endothelium and activated platelets kick start inflammation by secreting a variety of inflammatory and chemotactic cytokines. Neutrophils are the first inflammatory cells to arrive in the wound in response to the chemotactic stimuli. These cells scavenge the wound for pathogens and produce cytokines that attract Mφ. Then, the neutrophils undergo apoptosis as Mφ start to enter the wound area after approximately two days. Mφ dispose the wound of dead cells, including neutrophils, resolve the inflammatory phase and direct wound healing towards the granulation phase. After three days, re-epithelialization and neovascularization begin to occur and fibroblasts start to deposit immature (type III) collagen to fill the defect. Subsequently, the newly formed ECM is remodeled to become stronger and more organized. The remodeling phase lasts until approximately one year after trauma (figure 2).

**Excessive cutaneous wound healing**

Derailment of the wound healing cascade results in deposition of excess scar tissue. Two forms of excessive skin scarring exist: keloid- and hypertrophic scars (HTS). This thesis focuses mainly on HTS formation. These types of scars frequently develop as a result of burn wounds, but also after surgical trauma with incidences varying from 40 to 70% after surgical trauma and 91% after burn trauma. HTS is defined as a scar which is raised above skin level, but remains within the boundaries of the original wound, while keloids exceed the wound margins. Other clinical characteristics of HTS include esthetically undesirable features such as erythema and rigidness and co-morbidity such as pain, itch and diminished range of motion when positioned over a joint. Histological properties of HTS...
include increased amounts of collagen compared to normal scars and myofibroblasts which persist in mature HTS as opposed to normal mature scars\textsuperscript{10}.

Figure 2: normal wound healing  
First, hemostasis occurs and immediately starts inflammation, followed by proliferation after approximately 3 days and remodeling which lasts for months.

Regarding possible mechanisms involved in excessive scarring, several risk factors for HTS formation have been suggested, comprising both systemic and local factors. Systemic factors include ethnic background and possibly genetic predisposition; an example of local factors is mechanical force in the form of stretch\textsuperscript{12-15}.

With respect to the wound healing cascade itself, the current opinion states that HTS results from excessive and/or prolonged inflammation, overabundant proliferation and abnormal remodeling\textsuperscript{10}. Alterations of the inflammatory response are thought to play a major role in this derailment of wound healing\textsuperscript{11}. Indeed, altered immune cell populations have been observed in HTS before. For example, HTS contain more Langerhans cells, which is thought to contribute to excessive inflammation in HTS formation\textsuperscript{11}. Mahdavian Delavary and colleagues proposed that a prolonged inflammatory phase in hypertrophic wound healing causes M\textsubscript{φ} to accumulate and induce overabundant ECM production\textsuperscript{8}. Although Niessen and colleagues did not observe differences in M\textsubscript{φ} numbers between HTS and normal human scars of three and 12 months old, this observation does not exclude increased M\textsubscript{φ} numbers during earlier stages of hypertrophic wound healing\textsuperscript{11}.

Despite extensive research on the subject, the exact cause of HTS formation remains unknown and to date no optimal treatment is available. Even though literature suggests that the immune system plays an important role, it is unknown whether systemic or local
immunological predisposing factors (or both) induce or contribute to HTS formation. Next to that, just a few researchers have studied human hypertrophic wound healing as early as two weeks after injury, but most studies focus on later time points in the process of hypertrophic scar formation. Thus, the exact time of onset of the derailment of wound healing leading to HTS formation has not been established yet.

**Outline of the thesis**

Several risk factors for HTS formation have been described of which ethnic background is probably the best known risk factor. Chapter 2 discusses currently known risk factors for HTS formation, their levels of evidence and their influence on the individual phases of the wound healing process. In addition, risk factors for HTS are studied in chapter 3 in patients who received presternal incisions for the purpose of open heart surgery. Most open heart surgeries are performed using extra corporal circulation (ECC; heart lung machine). Surgery as well as ECC induces a systemic inflammatory response. This systemic inflammatory response may affect wound outcome, where a stronger response would result in HTS more frequently. The effect of different types of ECC on scar outcome is examined in chapter 4.

Besides in skin, fibrosis occurs in many other tissues, for example in coronary arteries. HTS and coronary sclerosis are both forms of excessive fibrosis and have several features in common. The possible relation of coronary sclerosis with HTS formation is examined in chapter 5.

Next to several risk factors, the immune response is thought to play an important role in HTS formation, but the precise mechanisms and moment of derailment of the wound healing process are unknown. In order to elucidate whether early inflammation could play a role, the immune response of early hypertrophic wound healing versus normal wound healing on genetic-, protein- and cellular level is examined in chapter 6. No research has been performed previously on alterations of the very early processes of wound healing in association with HTS formation.

With reference to local factors, skin of different anatomic sites is subjected to different influences, for example UV radiation, pathogens, but also mechanical forces such as stretch. Since excessive scarring occurs more frequently in certain anatomic locations, histological and molecular properties of skin of different anatomic sites and predilection sites for excessive scarring are studied in chapter 7.

A general discussion of the results is provided in chapter 8.


Oude Rijn, Leiden; adventurous girl on pedestrian bridge