GENERAL INTRODUCTION
HYPERTROPHIC SCAR FORMATION

The skin is our largest organ and serves as a protective barrier against infection and excessive water loss, and helps our bodies to maintain the right temperature. The skin can be divided into an epidermal and a dermal layer. The epidermis mainly contains keratinocytes, whereas the dermal part consists of two compartments. The first is the cellular compartment, which is typically composed of fibroblasts. The second is the acellular compartment, which mainly contains the extracellular matrix (ECM). All these compartments and different components have their own specific function and work closely together.

The skin is also one of the most easily injured organs. When cutaneous integrity is violated wound healing is crucial to restore this barrier. The healing process of the injured site, which normally results in the formation of a scar, is an extremely complex process involving numerous cell types, cytokines and ECM components. Excessive blood loss from injured blood vessels is prevented by the formation of a blood clot, which further acts as a provisional wound matrix that attracts and guides inflammatory cells, endothelial cells, fibroblasts, and keratinocytes. Together they form new blood vessels, produce ECM and create a new layer that covers the surface of the wound. Many fibroblasts transform into myofibroblasts, which initiate collagen deposition and wound contraction.

Except for superficial (burn) wounds that heal within a few days, most wounds will become a visible scar. Moreover, in some individuals, and particularly in burn victims, the wound healing processes may lead to excessive production of ECM, resulting in a raised hypertrophic scar. They are easily identified by color mismatch, stiffness and rough texture. Patients frequently complain about itching and pain, and experience serious functional and cosmetic problems, which are caused by a myriad of complications, including compression, stiffness sensation, loss of joint mobility and anatomic deformities. These complications may require several surgical corrections, unfortunately not always with satisfying results.

Hypertrophic scars are different from keloids, which also raise above skin level, but proliferate or originate beyond the confines of the original lesion. Most of the available literature on hypertrophic scars and keloids still does not precisely differentiate between both scar types, although several pathological and biochemical differences between hypertrophic scars and keloids suggest that different mechanisms are responsible for their development. Therefore, it is important to clearly differentiate between these two types of scars when trying to unravel the pathogenesis of either of these. In this thesis, we focused specifically on the identification of factors, both at a clinical and molecular level, that are involved in human hypertrophic scar formation.
AIMS AND OUTLINE OF THE THESIS

Research on the molecular and cellular mechanisms of wound healing and scar formation has led to a better understanding of mechanisms that are involved in hypertrophic scar formation. Presently, most research on wound healing is performed using animal models and in vitro cell systems. Animal models include the mouse, guinea pig, rabbit and pig. Experiments performed in vitro are performed in a controlled environment like a test tube or tissue culture flask. It is well established that in wound healing, all cells involved in the process strongly respond to local changes and quickly change their environmentally imprinted behavior. Intricate interactions in scar tissue, both via cell-cell contact and secreted products, are spatiotemporally controlled and difficult to mimic in in vitro cell systems, thereby largely hampering extrapolation of in vitro observations to the wound in a patient. Furthermore, wound healing in other species presents significant differences when compared with wound healing in humans. Moreover, hypertrophic scar formation is a condition that naturally only occurs in humans. Therefore, studying cell (dys)function during hypertrophic scar formation in the complex microenvironment of the human wound remains a prerequisite. This thesis focuses on unraveling both clinical and molecular differences between normotrophic and hypertrophic scar formation in humans specifically.

The current knowledge concerning molecular and cellular causes of hypertrophic scar formation is reviewed in chapter 2. Despite this knowledge, hypertrophic scars remain difficult to treat and an actual preventive treatment is still lacking. The currently used modalities for both preventive and curative management of hypertrophic scar formation are reviewed in chapter 3.

Over the past decades, a considerable amount of research has been performed concerning the predisposition and risk factors for keloid formation. Little is known about risk factors specific for hypertrophic scar formation, as hypertrophic scars and keloids are yet not always well differentiated. In chapter 4 we describe the incidence of hypertrophic scar formation in standardized human wound healing models and investigated its association with several patient characteristics.

When looking at hypertrophic scars at a cellular level, these scars display epidermal abnormalities that strongly resemble the epidermal alterations observed in the skin disorder psoriasis. Psoriasis can be effectively treated with topical application of calcipotriol, a synthetic derivative of vitamin D. We thus hypothesized that topical application of calcipotriol could prevent hypertrophic scar formation by altering the biochemical properties of the epidermis. Therefore, a randomized, double-blind, placebo-controlled trial was performed to investigate the preventive effect of topical calcipotriol on hypertrophic scar formation, and to further analyze the biochemical properties of the epidermis associated with hypertrophic scar formation (chapter 5).

To study the influence of the early inflammatory response on hypertrophic scar formation we conducted a prospective cohort study using a standardized model of presternal scars caused by cardiothoracic surgery through a median sternotomy incision (chapter 6). Presternal scars have a high incidence of hypertrophic scar formation. It is custom to administer dexamethasone systemically at high dose before and after
cardiac surgery that requires the use of cardiopulmonary bypass. Hypertrophic scar formation in the study group was assessed and measured, and differences between patients who did and who did not receive dexamethasone perioperatively were analyzed.

In chapter 7, first steps are made towards identifying the macrophage phenotype during the development of normotrophic versus hypertrophic scars. Macrophages are considered key players in wound healing, as they serve as a source for various cytokines and chemokines essential for orchestrating the wound healing process and ECM production. Since the role of macrophage phenotype has been highlighted in several studies, while other studies underline the importance of macrophages in wound healing, the interest in macrophage function in wound repair is rising. The aim of this study was to determine the macrophage phenotype associated with normotrophic and hypertrophic scar formation in time.

Besides differences in the phenotype of inflammation, it has been previously reported that, compared with normal skin, hypertrophic scars have an increased number of blood vessels and a higher blood flow. This implies the presence of an increased vascular network in hypertrophic scars compared with normotrophic scars. We thus hypothesized that new blood vessel formation is increased during hypertrophic scar formation. In chapter 8 we provide insight in, and identify new molecular details about the angiogenic profile during normotrophic and hypertrophic scar formation. Our study is the first to detail on the time course of the angiogenic response during hypertrophic scar formation in humans in comparison with normotrophic scar formation.

Finally, in chapter 9 the findings described in this thesis are summarized and discussed in the context of the current developments in wound healing research. Conclusions are presented and future directions for research on wound healing and hypertrophic scar formation are proposed.

REFERENCES

9. Niessen, F.B., Schalkwijk, J., Vos, H. & Timens, W. Hypertrophic scar formation is associated with an increased number of


