CHAPTER 1

General Introduction
INTRODUCTION TO THE THESIS

The skin is the largest organ of the human body and fulfils a primary role of protection against assault from outside. Nevertheless, injury of the skin is sometimes inevitable and therefore a well defined repair mechanism is essential. Wound healing is a complicated multistep process following tissue injury. The endpoint of the healing process is development of a scar, which can be excessive when the repair process is disturbed. Wound healing can be divided in four phases: 1) haemostasis, 2) inflammation, 3) proliferation, and 4) remodelling. The velocity of each phase depends on the severity of injury and can be influenced by multiple factors such as inflammation, age, or underlying diseases like diabetes.¹

Phases of wound healing

When skin or tissue integrity is disrupted, restoration of haemostasis must be facilitated to prevent blood loss. Platelets that enter the wounded area will be exposed to extracellular matrix (ECM), and adhesion to matrix ligands promotes aggregation. Simultaneously, the coagulation cascade is activated, resulting in cleavage of fibrinogen by thrombin. Fibrin fibers form a network around platelet aggregates in order to strengthen the blood clot (Fig. 1A).²

Upon secretion of cytokines and growth factors (e.g. transforming growth factor-β (TGF), or platelet derived growth factor) by activated platelets and endothelial cells, inflammatory cells are attracted to the wounded area, which marks the start of the inflammatory phase. Neutrophils are the first type of immune cells entering the wound shortly after injury.³ Their main function is to remove foreign particles and decontaminate the wound by destroying invading bacteria (Fig. 1B). Monocytes migrate to the damaged tissue 24 - 48 hours post-injury via signals such as CC-chemokine Ligand (CCL)-2, and differentiate into macrophages.⁴ These cells phagocytose matrix debris and dead neutrophils, and release cytokines and growth factors to attract fibroblasts to the affected area (Fig. 1C).

Next is the proliferation phase, when new capillaries are formed and reepithelialization takes place. Keratinocytes and fibroblasts proliferate and migrate to the wounded area. Furthermore, activated endothelial cells (from pre-existing vessels) produce proteolytic enzymes thereby finding their way through the matrix, proliferate and migrate to the wound. The formation of granulation tissue is one of the key features of the proliferation phase and functions as replacement for the fibrin clot (Fig. 1D). This tissue consists of ECM proteins, macrophages, keratinocytes
Figure 1: Phases of wound healing.
A) During the haemostasis phase, platelets will bind to exposed collagen, thereby getting activated and form aggregates. Concurrently, the clotting cascade is activated. Fibrinogen will be cleaved to fibrin fibers and form a provisional matrix, to make the blood clot stronger. B) Neutrophils enter the wound during the early inflammation phase, attracted by activated platelets, and bacterial products. Their main task is to remove foreign particles and destroy invading bacteria. Furthermore, neutrophils secrete chemoattractants (e.g. CCL2) for monocytes. C) Approximately 48 hours post wounding, monocytes appear and differentiate into macrophages. Macrophages support neutrophils by phagocytosing bacteria and cell debris. Additionally, macrophages secrete factors, inducing fibroblasts to proliferate and produce ECMs. D) In the proliferation phase, the main focus lies on covering the wound, formation of new capillaries, and granulation tissue formation. Granulation tissue consists of macrophages, neutrophils and fibroblasts. Several fibroblasts start to differentiate into myofibroblasts, which produce abundant amounts of ECM and mediate contractility. Among the ECMs, collagen III will be produced. E) The remodelling phase can last for a year after wounding. In this phase, macrophages, neutrophils, fibroblasts and myofibroblasts become apoptotic. Collagen III will be replaced by the stronger collagen type I. P.w.: post wounding.
and fibroblasts. Fibroblasts can differentiate into myofibroblasts which abundantly produce ECM proteins (mainly collagen type I and III), and provide a contractile force in order to facilitate wound closure.\(^5\)

In the final or remodelling phase, most of the myofibroblasts, endothelial cells and macrophages go into apoptosis. Collagen type III will be digested by matrix metalloproteinases (MMPs), which are secreted by fibroblasts, macrophages and endothelial cells, and the ratio of collagen type I to type III normalizes (Fig. 1E).\(^6\) Remodelling of newly formed tissue can last for a year, although the architecture of a scar will never completely return to the original structure. Appendages such as hair follicles and sweat glands will not reappear in the scarred tissue.

**Altered wound healing**
Dysregulation of the healing process can result in detrimental scar formation. Fibrotic or so-called hypertrophic scars appear as red, elevated, contracting, inelastic lumps of tissues that remain within the boundaries of the original injury.\(^7\) On microscopic level these scars are characterised by excessive and abnormal deposition of ECM components, hypervascularity, hypercellularity, and presence of myofibroblasts.\(^8\) Patients suffering of hypertrophic scars experience an impaired quality of life.\(^7\) Besides pain and itch these scars cause functional and cosmetic impairments, which may lead to psychological problems.

**Dermal and oral repair**
Injury to the skin usually heals by formation of a scar that can vary in degree, depending on the severity of the trauma. Interestingly, wound healing of the oral mucosa is clinically distinguished from dermal healing, although both wounds proceed through the same stages of repair. Oral wounds heal in an accelerated fashion, and scar formation is scarce.\(^9\) Unlike skin, signs of fibrosis are not detected in oral repair. Discrepancies however, exist about the zone of the oral cavity that heals without scarring. Some scientists assert the palatum and gingiva to be the scarfree location,\(^10\) while others found the buccal mucosa to heal without scarring.\(^11\)

Comparable to oral mucosa, fetal wounds generated during first and second trimester of gestation also heal scarless.\(^12\) Fetal wound healing has been studied extensively and a few possible factors have been identified. Fetal wounds differ from adult wounds in e.g. inflammatory responses, ECM composition, and expression of growth factors.\(^13\)-\(^15\) With respect to oral versus dermal healing a few differences are known. For instance, saliva is one of the factors present in the oral cavity that has a
beneficial effect on repair (on both oral and dermal wounds).\textsuperscript{16} On fibroblast-level several genes were differently expressed between oral and dermal fibroblasts.\textsuperscript{17} Furthermore, the pro-fibrotic growth factor TGF-β1 was lower expressed in oral compared to dermal wounds.\textsuperscript{18} Despite the fact that several differences between dermal and oral healing have been identified, the exact mechanism for scarless or scar forming healing remains thus far unclear.

**AIMS AND OUTLINE OF THE THESIS**

The studies described in this thesis were performed to obtain more information about the differences between skin and oral mucosal repair. Eventually, when understanding which factors are responsible for scarless oral healing, these factors can be implemented during dermal wound healing and facilitate more perfect repair.

In **Chapter 2**, a literature survey was performed. Based on this study, differences between dermal and oral wounds were found with respect to immune cells, ECM components and fibroblasts. One of the immune cells important for wound healing is the macrophage. In **Chapter 3**, pro- (M1) and anti-inflammatory (M2) macrophages, which were \textit{in vitro} matured and activated by different protocols, were characterized by means of morphology, marker expression and cytokine production. To investigate whether macrophages are the cells mainly responsible for the different modes of healing observed between skin and oral mucosal wounds, M1 and M2 macrophages were exposed to dermal and gingival fibroblasts (Chapter 4). In addition, the presence of these macrophages and other immune cells in cutaneous and oral scars was determined (Chapter 5). Subsequently, models were established in order to investigate human wound healing. In **Chapter 6A** human skin was transplanted on immunocompromized mice, and this model can be used for future wound healing studies. In another model, \textit{ex vivo} human skin biopsies were used. M1 and M2 macrophages were inserted into wounds of these biopsies and their effect on reepithelialization was observed \textit{in vitro} (Chapter 6B). Besides immune cells and fibroblasts, ECM components were determined in skin and oral mucosa. **Chapter 7** shows that several oral ECM components differ from skin and more closely resemble that of fetal skin. Finally, in **Chapter 8** the results obtained in this thesis are summarized and discussed, as well as the implications these findings may have to improve wound healing in therapeutic approaches.
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REFERENCE LIST