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The number of immune cells is lower in healthy oral mucosa compared to skin and does not increase after scarring

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ABSTRACT

Objective: Depending on the location of injury, wounds can heal with different outcomes. In addition foetal wounds heal fast without scar formation, while scars are a common feature of regular skin repair. Since inflammation is very limited in these wounds reduced numbers or even absence of immune cells might be responsible for scarless foetal wound healing. It is thought that various immune cells, such as macrophages, neutrophils and T-cells, play a role in aberrant wound healing and the fibrotic process seen in scar formation in the adult skin. Similar to the foetus, oral wounds show comparable healing properties by means of accelerated reepithelialization and negligible scar formation. It is possible that reduced inflammatory reaction as a result of lower numbers of immune cells are present in oral wounds compared to skin wounds.

Design: Here we investigated the presence of various immune cells in human skin and oral mucosa, with or without scars. The presence or absence of these cells may play a role in the different modes of healing observed between the two types of tissue. Mast cells, neutrophils, M1/M2 macrophages, T-cells and blood vessels were localized in healthy and scarred skin and oral mucosa (scars >1 year old).

Results: Oral mucosa had significantly fewer neutrophils, macrophages, mannose receptor-positive M2 macrophages, but more blood vessels. Scars contained similar numbers of immune cells compared to healthy tissues.

Conclusions: Less immune cells in the healthy oral mucosa may induce a diminished immune reaction when wounding occurs, and could explain the better healing capacity of the oral mucosa.

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Abbreviations: α-SMA, alpha smooth muscle actin; MR, mannose receptor; OM, oral mucosa; Stab-1, stabilin-1; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor; PDGF, platelet derived growth factor; IL, interleukin; TGF, transforming growth factor.

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1. Introduction

A plethora of cells and molecules participate in the process of wound healing. The sequence of events during wound healing passes different phases (inflammation, proliferation, and remodelling), and immune cells fulfil a notable task during each phase. Both mast cells and neutrophils enter the wound quickly and accumulate at the site of injury.[1] Upon activation, mast cells secrete mediators that increase vascular permeability, *e.g.* interleukin (IL)-6, IL-8, vascular endothelial growth factor (VEGF) and recruit leukocytes, such as neutrophils, attracted by chymase.[2] Furthermore, mast cells contributed to angiogenesis during the proliferation phase, by secretion of VEGF and fibroblast growth factor-2.[3]

Neutrophils form a first line of defence against invading pathogens and release antimicrobial peptides, phagocytose pathogens, and produce reactive oxygen species. Additionally, neutrophils secrete cytokines and chemokines to recruit additional immune cells. Neutrophils also contribute to angiogenesis by secretion of angiogenic factors such as VEGF and IL-8.[4] In addition, neutrophils mediated proliferation of fibroblasts and keratinocytes.[4] Neutrophil depletion had no effect on collagen deposition and wound strength, but led to accelerated reepithelialization.[5] However, in burn wounds, wounds that generally heal with extensive scarring, it was shown that neutrophils remain present in large numbers for at least 6 weeks, whereas in normal wound healing these cells disappear after a few days.[6]

Monocyte-derived macrophages enter the wound approximately 48 h post-injury. During the inflammatory phase, macrophages accomplish their main function: phagocytosis of damaged tissue components and apoptotic neutrophils. Early macrophages (classically activate d or M1 macrophages) secrete pro-inflammatory cytokines such as IL-1, tumour necrosis factor (TNF)-α, and IL-6. Impaired reepithelialization and minimally vascularised granulation tissue were consequences of macrophage depletion during the inflammatory phase, although scar tissue was reduced.[7] As the proliferation phase progresses macrophages switch to an alternative M2 phenotype. The cells start to produce anti-inflammatory cytokines and growth factors like VEGF, IL-10, and transforming growth factor (TGF)-β. Phenotype switching may be induced by phagocytosis of damaged tissue and dead cells.[8] In chronic wounds, persistence of M1 macrophages together with an incomplete switch to M2 macrophages led to tissue breakdown and impaired healing.[9]

The role of T-cells during wound healing is not well defined yet, although a few effects of these cells during repair have been described. T-cell suppression and depletion resulted in impaired wound healing, while enhancement of T-cell function increased wound strength and collagen deposition.[10–12]

Depending on the location, wounds can heal with different outcomes. Oral wounds similar to foetal wounds heal fast without scar formation, while the adult cutaneous healing process progresses slower and may cause scars.[13] The absence of immune cells is one of the factors hypothesized to be responsible for scarless foetal wound healing since foetal wounds had very limited, if any, inflammation.[14] Foetal mice deficient for IL-10 generated scars after wounding, whereas addition of IL-10 mediated tissue regeneration in adult mice.[15,16] On the contrary, immune cells were associated with fibrotic scarring. Increased numbers of mast cells, macrophages, and $CD4^+$ T-cells were found in hypertrophic scars as compared to normotrophic scars.[17–19] Furthermore, mast cells contributed to scar formation in a foetal wound healing model.[20]

Because of the similarities between foetal and oral repair, it is conceivable that oral wounds contain reduced numbers of immune cells compared to skin wounds. Therefore, presence and amount of various immune cells in human tissue (healthy and scarred skin and oral mucosa) was investigated. Presence or absence of these cells possibly plays a role in the different modes of healing observed between the two types of tissue.

2. Materials and methods

2.1. Tissue samples

Human skin was obtained from five healthy individuals undergoing abdominal dermolipectomy (mean age: 48 (range 41–58 years)), and scars (>1 year old; mean age: 37 (range 11–61 years)) were derived after scar revision. Oral mucosa was obtained from five patients (mean age: 10 (range 4–27 years)) with a history of open cleft palate undergoing pharyngoplasty. These oral tissues contain both healthy structures as well as scarred (>1 year old) regions. Tissues were embedded in Tissue Tek® OCT™ Compound (Sakura Finetek, Alphen aan den Rijn, The Netherlands) and stored at −80 °C until sectioning. All donors provided informed consent according to institutional and national guidelines.

2.2. Immunofluorescence

Of all tissues cryosections (5 μm) were made and mounted on collagen coated glass slides. Sections were fixed in acetone for 10 min and blocked with normal goat serum. Next, sections were incubated with primary antibodies (Table 1) or non-relevant isotype control antibodies (negative controls) for 1 h at room temperature. Mast cells were identified by tryptase, neutrophils by CD66b, and T-cells by CD3. CD68 was used as pan-macrophage marker, CD40 and CD64 for M1 macrophages, and mannose receptor (MR or CD206), CD163, stabilin-1, and platelet derived growth factor (PDGF)-CC for M2 macrophages. Blood vessels were stained for CD31 and α-smooth muscle actin (α-SMA). After washing with PBS-tween, corresponding Alexa Fluor® labelled secondary antibodies (Molecular Probes®, Life Technologies, Bleiswijk, The Netherlands) were applied to the tissue sections. Hoechst (Invitrogen, Breda, The Netherlands) was used for nuclei staining and sections were embedded in glycergel (Dako, Glostrup, Denmark).

2.3. Analysis

Five randomly taken pictures were made of every staining, by use of a Leica CTR6000 microscope (Leica-microsystems, Wetzlar, Germany). The number of positive cells was counted