A CLINICAL STUDY OF SCAR FORMATION IN THE HUMAN PALATAL MUCOSA

by

Breanne Elizabeth Joslin

D.M.D., The University of Manitoba, 2005
B.Sc., The University of Manitoba, 2001

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Abstract

Scar formation is a frequent consequence of wound healing and has widespread negative effects on individuals’ quality of life, both physically and psychologically. For most people, scars are unsightly, but in addition to this, they can result in serious morbidities such as pruritus, pain, contracture, and decreased heat tolerance in severe situations. The association between degree of scarring and depth of dermal injury has been recognized by surgeons for many years, however the cellular and molecular basis for these observations remains poorly understood. Interestingly, oral wounds have been shown to heal faster and with less clinical and histological scar formation than similar skin wounds. It was hypothesized that palatal wounds in general show relatively little scarring and also that there is increased scar formation of the palatal mucosa following a connective tissue graft (CTG) harvest (deep wound) than a free gingival graft (FGG) harvest (superficial wound). This was a retrospective clinical study carried out at the University of British Columbia, Faculty of Dentistry. Intraoral photographs were taken of the palate in 37 subjects. Each subject had undergone a CTG and/or FGG harvest by a Graduate Periodontics resident more than six months prior to the study. 23 FGG and 23 CTG scars were assessed. Two independent calibrated blinded examiners assessed the photographs using a modified version of the Manchester Scar Proforma. A value of zero, one, or two was given for each parameter, with no difference from normal tissue scored as zero and gross mismatch scored as two. The values for each parameter were summed to produce a total scar score, zero to six, for each site. The component parameters were also examined individually so that color, contour, and distortion could be evaluated independently. The results of this study demonstrated that scar formation in the palatal mucosa is minimal and in many cases, non-existent. CTG donor sites did not have more severe scar formation than FGG sites.
Preface

This is a clinical study and research approval was granted by the UBC Clinical Research Ethics Board and designated UBC CREB number H12-00315. This work has not been published previously. All parts of the study were completed by Breanne Joslin. Statistical consultation was obtained through Rick White, Department of Statistics, UBC.
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<tr>
<td>CTG</td>
<td>connective tissue graft</td>
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<tr>
<td>DETC</td>
<td>dendritic epidermal T-cells</td>
</tr>
<tr>
<td>ECM</td>
<td>extracellular matrix</td>
</tr>
<tr>
<td>EDA</td>
<td>cellular extradomain A</td>
</tr>
<tr>
<td>EPC</td>
<td>endothelial precursor cells</td>
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<tr>
<td>EGF</td>
<td>epidermal growth factor</td>
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<tr>
<td>EGFR</td>
<td>epidermal growth factor receptor</td>
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<tr>
<td>ERK</td>
<td>extracellular signal regulated kinase</td>
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<td>FGG</td>
<td>free gingival graft</td>
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<td>FGF</td>
<td>fibroblast growth factor</td>
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<td>HGF</td>
<td>hepatocyte growth factor</td>
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<tr>
<td>IFN</td>
<td>interferon</td>
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<tr>
<td>IGF</td>
<td>insulin-like growth factor</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>KGF</td>
<td>keratinocyte growth factor</td>
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<tr>
<td>MMPs</td>
<td>matrix metalloproteinases</td>
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<tr>
<td>MSS</td>
<td>Manchester scar scale</td>
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<tr>
<td>PDGF</td>
<td>platelet derived growth factor</td>
</tr>
<tr>
<td>POSAS</td>
<td>patient and observer scar assessment scale</td>
</tr>
<tr>
<td>SPARC</td>
<td>secreted protein acidic and rich in cysteine</td>
</tr>
<tr>
<td>Stat3</td>
<td>Signal transducer and activator of transcription 3</td>
</tr>
<tr>
<td>TGF</td>
<td>transforming growth factor</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<td>--------------------------------------------------</td>
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<tr>
<td>Th</td>
<td>T-helper</td>
</tr>
<tr>
<td>TIMPs</td>
<td>tissue inhibitors of metalloproteinases</td>
</tr>
<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
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<tr>
<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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<td>VSS</td>
<td>Vancouver scar scale</td>
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Chapter 1: Review of the Literature

1.1 Introduction

Scar formation is a frequent consequence of wound healing and has widespread negative effects on individuals’ quality of life, both physically and psychologically. For most people, scars are unsightly, but in addition to this, they can result in serious morbidities such as pruritus, pain, contracture, and decreased heat tolerance in severe situations (Durani et al., 2008; Gauglitz et al., 2011). The occurrence of each varies amongst individuals and depends on many factors including the size of wound, the individual’s sex, age, and health status (Gauglitz et al., 2011).

The association between degree of scarring, and depth of dermal injury has been recognized by surgeons for many years. While the cellular and molecular basis for these observations remains poorly understood, they are the basis for surgical techniques such as the split-thickness skin graft and also for the treatment of partial thickness burns (Dunkin et al., 2007). The fundamental principle that shallow wounds do not scar serves as the basis for laser treatment of superficial vascular malformations and for facial resurfacing (Ross et al., 2000).

In the skin, penetration of the reticular dermis has been found to be the critical depth of wound that result in scar formation. Those wounds that are confined to the superficial papillary dermis or to the epithelium do not result in scarring (Dunkin et al., 2007). In contrast, wounds that penetrate the lamina propria of palatal mucosa tend to result in minimal scarring (Häkkinen et al., 2013). To date, there are no known clinical studies that compare the type of scar formation in the palatal mucosa to the depth of wounding. A critical depth of wounding is not known.
This study compares the scar formation in healed wounds created from the harvest of free gingival (FGGs) and connective tissue grafts (CTGs). In theory, FGGs involve the removal of much less tissue, and a more shallow depth of penetration than CTG harvests. The FGG harvest involves removal of the epithelium and a small portion of the lamina propria. In contrast, the CTG harvest requires penetration to the deepest layers of the lamina propria, often to the periosteum, in order to obtain adequate graft thickness.

It is hypothesized that there is increased scar formation of the palatal mucosa following a CTG graft harvest than a FGG graft harvest.

1.2 Normal composition of oral mucosa

The human hard palate is covered by oral mucosa. It contains a keratinized stratified squamous epithelium called oral epithelium and an underlying connective tissue layer, called the lamina propria (Gartner, 1994). The lamina propria contains a dense fibro-elastic material with pervasive vascular and neural networks (Stephens and Genever, 2007). Deep to the lamina propria is the submucosa, however this region is poorly defined in the oral mucosa. In many areas of the hard palate, the submucosa is composed of loose, glandular connective tissue, which separates the lamina propria from bone (Bartold et al., 2000). There is typically much less glandular tissue in the posterior area of the hard palate when compared to the anterior.

The most superficial layer of the oral mucosa, oral epithelium, is continuously renewed through mitotic division. The turnover time for cells in the oral epithelium is estimated to be 25 days (Nanci, 2008). There are four cell layers that make up the oral epithelium namely the basal
Epithelium is separated from the connective tissue by the basement membrane that is composed of two distinct layers, namely lamina lucida and lamina densa. The lamina lucida contains laminin-332 anchoring fibrils that link hemidesmosomes to the connective tissue. The lamina densa is closest to the connective tissue and contains a mesh of type IV collagen fibrils as well as multiple laminins, entactin, perlecan, etc. Anchoring fibers (type VII collagen) below the lamina densa connect to the anchoring fibrils to further stabilize the connective tissue epithelium border (Ghohestani et al., 2001).

Basal cells of the epithelium lay in contact with the basement membrane and are connected to the basal lamina via $\alpha_6\beta_4$ integrin. The cells are cuboidal or cylindrical in shape and are capable of mitotic division. Basal cells have a high nuclear to cytoplasmic ratio and contain a large number of organelles including mitochondria and keratin filaments (Menon, 2002). As new cells are produced, older daughter cells move through the epithelium as keratinocytes. The movement from basement membrane to the outer surface of the epithelium takes approximately 1 month and this is typically in harmony with the rate of cell division in the basal layer (Lindhe et al., 2009).

The prickle layer (stratum spinosum) contains cytoplasmic processes, which resemble spines and give the cell layer a prickly type appearance. The cells are relatively large, and polyhedral in shape. Adjacent cells in the stratum spinosum are held together by tight junctions which create an important barrier function (Menon, 2002).
Keratinocytes undergo a number of changes as they move from the basal layer to the epithelial surface. Examples of such changes include an increase in both the number of tonofilaments in the cytoplasm and the number of desmosomes (Menon, 2002). In comparison, the number of organelles such as mitochondria, lamellae of rough endoplasmic reticulum, and golgi complexes decreases as the keratinocytes move toward the cell surface (Garant, 2003; Lindhe et al., 2009).

As cells move through the spinosum towards the granulosum, electron dense keratohyaline bodies are found. Glycogen containing granules are also found in the stratum granulosum and are thought to be involved in keratin synthesis (Garant, 2003). The transition of cells from the granulosum to the stratum corneum is marked by the sudden keratinization of the cytoplasm of the keratinocyte and the associated absence of organelles such as endoplasmic reticulum, mitochondria, nucleus, and Golgi complex. The cells now lack the ability to carry out protein synthesis and energy production and are dependent on other cells in order to differentiate and undergo keratinization (Schroeder and Amstad-Jossi, 1984). While keratin producing cells make up approximately 90% of the total cell population in oral epithelium there are other cells types present including melanocytes, Langerhans cells, Merkel’s cells, and inflammatory cells such as polymorphonuclear leukocytes (Lindhe et al., 2009).

Deep to the epithelial layer of the oral mucosa lies the connective tissue layer, known as the lamina propria. It is composed of collagen fibers (approximately 60% of connective tissue volume), fibroblasts (around 5%), vessels and nerves (around 35%) (Lindhe et al., 2009). The major cell types found in the connective tissue are fibroblasts and they play a vital role in
development, maintenance, and repair of gingival connective tissue (Bartold et al., 2000). In addition to fibroblasts, gingival connective tissue also contains mast cells, macrophages, and inflammatory cells. They exist within an amorphous ground substance containing proteoglycans and glycoproteins (Bartold et al., 2000).

The boundary between the oral epithelium and connective tissue is wavy in nature. There are connective tissue papillae that project into the epithelium forming complementary invaginations called rete pegs within the epithelium. Rete pegs are recognized as a characteristic morphologic feature of epithelium (Lindhe et al., 2009).

1.3 Anatomical differences between skin and gingiva
Most studies of scar formation in the literature examine skin. Skin and palatal mucosa share many similarities, including a keratinized epithelium with an underlying layer of connective tissue. The connective tissue layer is referred to as the dermis in skin and as the lamina propria in mucosa (Stephens and Genever, 2007). The two structures, skin and palatal mucosa, share similar functions acting as a physical barrier to noxious agents, initiating an immune response when needed, providing thermoregulation, osmoregulation, and electrolyte balance (Stephens and Genever, 2007). While skin and mucosa share many common features and functions, there are differences between the two. Mucosa does not contain hair follicles or sweat glands. This difference may affect wound healing as hair follicles are known to aid in the process of re-epithelialization (Ito and Cotsarelis, 2008). In comparison to skin, there is greater variation in the differentiation of oral mucosa at different sites (Schroeder and Amstad-Jossi, 1984). The hard palate and gingiva, for instance, are fully keratinized, while the buccal mucosa is not. An
additional difference between the two structures is the rate of cell turnover; approximately four
to five times faster in the gingiva than the epidermis (Garant, 2003).

1.4 Anatomical composition of scars

On a molecular level, scars are due to the aberrant formation and modulation of the extracellular
matrix of skin. They may form in mucosa as well, however wound healing in the human oral
mucosa results in significantly less scarring than that of skin (Wong et al., 2009). Scars are often
classified as hypertrophic or keloid, but may also be present in more subtle forms such as fine
lines of fibrotic tissue (Slemp and Kirschner, 2006). There are clinical, histological, and
epidemiological differences between the more severe hypertrophic and keloid scar types.
Hypertrophic scars do not extend beyond the original wounding site and consist of mainly wavy,
well-organized type I collagen bundles oriented parallel to the tissue surface (Gauglitz et al.,
2011). They do not exhibit the typical basket-weave arrangement of collagen observed in normal
tissue. This may account for why scar tissue has only approximately 70% of its original tensile
strength even years following the scar formation (Clark et al., 1996). Hypertrophic scar
formation tends to occur four to eight weeks post wounding and may show rapid proliferation for
up to six months (Gauglitz et al., 2011). Keloids, on the other hand, project beyond the original
wound margin and consist of large thick type I and III collagen bundles. They represent an
imbalance between the extracellular matrix (ECM) deposition and degradation, which results in
progressive ECM accumulation. In contrast to hypertrophic scars, keloids may continue to
proliferate indefinitely and may even arise spontaneously without an inciting injury. A common
histological finding between the two fibrotic scar types is the overproduction of multiple
fibroblast proteins, including fibronectin (Gauglitz et al., 2011). Hypertrophic scar incidence
varies from 40-70% following surgery and as high as 91% following burn injury (Gauglitz et al., 2011). Keloid formation is less common, however is found to be more common in dark-skinned individuals (as high as 16% in African populations) and seems to show some genetic predilection (Marneros and Krieg, 2004).

1.5 Overview of wound healing

Wound healing represents a complex balance amongst key cells including neutrophils, macrophages, lymphocytes, endothelial cells, keratinocytes, and fibroblasts. These cells function in overlapping sequence during the wound healing process. Any shift in the balance amongst them can result in the wound healing process being compromised. In general, there are four stages to wound healing including hemostasis and inflammation, re-epithelialization, granulation tissue formation and angiogenesis, and tissue remodeling.

1.5.1 Hemostasis and inflammation

Hemostasis is key in the early stages of wound healing and functions to seal the wound. Primary hemostasis occurs immediately after injury to blood vessels. Spasm within the smooth muscle of vessel walls results in the retraction of severed arteries and vasoconstriction of arteries and veins (Ruggeri, 2009).

Coagulation is initiated by activation of factor XII and by exposure of tissue factor III via wounding. In a series of zymogen activations known as the Waterfall Cascade Model of clotting, activated factor X results in the formation of thrombin. Thrombin affects wound healing through its chemotactic and mitogenic activities upon macrophages, fibroblasts, and endothelial cells.
Thrombin is essential to wound healing and mice which are deficient in factors IX or VIII exhibit delayed wound healing (Monroe et al., 2010).

The production of thrombin catalyzes the formation of fibrin from fibrinogen and results in the formation of a fibrin rich blood clot. Fibrin cross-linked with plasma fibronectin creates a fibrin plug for hemostasis. Fibrin itself serves as adhesion for leukocytes, fibroblasts, and endothelial cells as well as a reservoir for growth factors such as FGF-2 and VEGF (Laurens et al., 2006). The formation of fibrin is closely associated with a simultaneous fibrinolytic mechanism. This prevents untoward clotting at sites distant to the injury and also protects against the development of atherosclerotic vascular disease.

Platelets are derived from megakaryocytes and serve primarily as mediators of hemostasis. Under normal healthy conditions; they are confined to the intravascular spaces. Wounding results in the exposure of subendothelial tissue and types IV and V collagen and promotes the binding and aggregation and platelets (Bilodeau and Hamm, 2007). The initial binding and aggregation of platelets also causes recruitment of additional platelets and different types of leukocytes to the site of injury. This serves to amplify the coagulation response. Platelets also secrete adhesive proteins such as fibronectin, fibrinogen, thrombospondin, and vonWillebrand factor VIII which aid in the clotting process (Dale, 2005).

Platelets release soluble mediators like serotonin, PDGF, transforming growth factor-α, adenosine phosphate, platelet activating factor, factor V, 1, 2-hydroxy eicosatetraenoic acid, and thromboxane A2. They are capable of differential responses to stimuli which may actually reflect differences in the Ca\(^{2+}\) homeostasis or age of the platelets (Alberio et al., 2002; Dale, 2005). α-
granules released by platelets create different microenvironments at the wound site. Through the release of distinct populations of α-granules, both inhibitors and promoters of angiogenesis are released by platelets (Italiano et al., 2008).

O’Brien suggests that the platelet may play an inhibitory role in inflammation in some settings while becoming pro-inflammatory in other settings where inflammatory mediators are present (O'Brien, 2012). An example of their pro-inflammatory role is the release of interleukin-1, which can exacerbate the immune response (Ware et al., 2013).

A robust inflammatory response is regarded as the typical response following wounding. Inflammation has been, and continues to be, an area of significant interest in science with respect to wound healing. Following injury, the innate immune response is the first to be activated (Shaw and Martin, 2009) and includes keratinocytes, mast cells, dendritic cells, macrophages, and in some cases T-cells. The innate immune response is a fast process and in some cases, such as with mast cell degranulation, can occur almost immediately. Innate immune cells initiate the inflammatory process and cause the release and/or production of pro-inflammatory mediators such as cytokines, histamine, leukotriene and prostanoids. Pro-inflammatory mediators drive neutrophil migration to the site of injury, but also causes vasodilation, increased vascular permeability, and activation of endothelial cells (Turabelidze and DiPietro, 2011).

In the early phase of wound healing, leukocytes, the most prominent being neutrophils, are recruited to the wound site in large numbers via chemotaxis. Leukocytes produce cytokines and chemoattractants and so their accumulation drives a positive feedback loop resulting in more and
more leukocyte recruitment. This, in turn, causes amplification of the inflammatory response (Robson et al., 2001). With reference to neutrophils specifically, their function is understood as mainly to prevent bacterial infection. Neutrophils are very effective in their role as a “cleanup cell” and they accomplish this mainly through phagocytosis (Broughton, Janis and Attinger, 2006b). They are capable of producing nets of histones and DNA which trap and kill microbes, however this requires neutrophils to move through intact capillary walls into surrounding tissue (Brinkmann et al., 2004). This serves a useful purpose of decontaminating the wound, however it also results in damage to intact tissue and vasculature. To assist in their microbicidal function, neutrophils produce substances such as reactive oxygen species and proteases which kill not only pathogens, but also healthy host cells (Broughton, Janis and Attinger, 2006a). Thus, there is a cost to the host organism for mounting an inflammatory response. In fact, studies have shown faster wound closure in mice with induced neutropenia than in immunocompetent controls (Dovi et al., 2003).

As mentioned earlier, mast cells also comprise part of the innate immune response. Those present at the site of injury degranulate following wounding, releasing stored inflammatory mediators. Mast cells are also recruited to wounds, but take longer to arrive than the recruited neutrophils. The fate of mast cells following their action at the site of injury is less well understood than that of neutrophils, however a fluid relationship seems to exist between the two cell types. Studies have shown that a decrease in the number of mast cells at a wound site also results in fewer neutrophils (Szpaderska et al., 2003; Weller et al., 2006; Wilgus, 2008).
The reduction of neutrophils following their initial recruitment to the wound site is an important aspect in limiting the inflammatory response. Macrophages play a role in this process and are capable of causing both neutrophil apoptosis and phagocytosis (Meszaros et al., 1999). For this reason, and also because of their ability to produce growth factors necessary for angiogenesis and fibrogenesis, macrophages are regarded as an indispensable cell in the wound healing process (Danon et al., 1989).

While neutrophils and macrophages dominate the early stages of wound healing, T-lymphocytes are present in the late inflammatory phase, reaching their peak eight days post-wounding (Martin and Muir, 1990). T-lymphocytes are thought to be attracted to the wound site by the interleukin-1 (IL-1), complement components, and immunoglobulin G (IgG) breakdown products. Their exact role in wound healing is poorly understood, however the balance of particular types of T-lymphocytes, CD4+ cells and CD8+ cells, affects wound healing differently. CD4+ cells have been shown to improve wound healing while CD8+ cells impair healing (Park and Barbul, 2004). Epithelial tissues contain a unique type of resident T-cell called dendritic epidermal T-cells (DETC). Their increased number, as a result of injured keratinocytes, is thought to improve wound healing (Jameson and Havran, 2007).

As discussed the innate immune response results in the production of vast numbers of neutrophils and macrophages, which are the main producers of pro-inflammatory cytokines. Cytokines are multifunctional and critical to both the inflammatory and remodeling stages of wound healing. Some of these cytokines, including IL-1, IL-6, and TNF-α, are more prevalent during the inflammatory phase of wound healing (Singer and Clark, 1999). IL-1 is produced by a
number of cells including keratinocytes, neutrophils, monocytes, and macrophages. It encourages proliferation of keratinocytes and endothelial cells. In addition, IL-1β helps to activate many of the innate immune cells that are present at the wound site (Barrientos et al., 2008). IL-6 is another pro-inflammatory cytokine that has increased expression after wounding. Similarly to IL-1, IL-6 is produced by neutrophils, monocytes, and macrophages. However, it is also produced by fibroblasts (Gallucci et al., 2004). IL-6 is thought to be of significant importance during the early stages of wound healing and mice that are deficient in IL-6 have a healing time of more than three times that of wild-type controls (Gallucci et al., 2004). A delay in re-epithelialization as well as impaired formation of granulation tissue was found to contribute to the slower healing within the test group (Gallucci et al., 2000). In addition to IL-1β and IL-6, TNF-α shows upregulation during the early stages of wound healing. The nature of its function is dependent on its concentration. At low concentrations it promotes wound healing through indirect stimulation of inflammation and increasing macrophage produced growth factors. However, TNF-α is a hindrance to wound healing when present in high concentrations as it suppresses the production of ECM proteins and TIMPS, and increases productions of MMPs (Barrientos et al., 2008).

1.5.2 Re-epithelialization

Re-epithelialization is a critical process in re-establishing the barrier function of mucosa following wounding. Epithelial keratinocytes have been observed to begin migration from the wound edge to the provisional matrix within 24 hours of wounding. Their migration is dependent on a morphological change, from polarized basal cells to flattened and elongated cells. This change is initiated through their exposure to (1) pro-migratory matrix molecules in the wound
site (2) growth factors and cytokines that are released by inflammatory and other wound cells from the blood clot and surrounding extracellular matrix, and (3) wound generated electric fields (Schultz and Wysocki, 2009).

The basal epithelial cells of unwounded tissue are in close proximity to the extracellular matrix components of the basal membrane including laminin-111, laminin-332, and type IV collagen. A full thickness epithelial wound brings keratinocytes into contact with the extracellular environment which contains type I collagen and fibronectin-rich connective tissue and plasma derived proteins (Davis et al., 2000). Interestingly, mouse models with a knockout of laminin-332 produce a lethal phenotype and people with laminin-332 mutations have severely compromised wound healing ability (Schneider et al., 2007).

Integrins are a family of cell surface glycoproteins that function in a variety of ways. Much of their function is derived from variations in their two subunits α and β, which are covalently associated with one another. Integrins mediate the attachment of a cell to the surrounding matrix. They also serve to convey two-way signaling across the plasma membrane, and influence cell growth, differentiation, and migration (Häkkinen et al., 2000; Jacobsen et al., 2010; Larjava et al., 2011). Animal studies have shown the importance of cellular signaling pathways mediated by integrins and growth factors. Re-epithelialization has been shown to be impaired by deficiencies in any of the following including extracellular signal regulated kinase (ERK2), signal transducer and activation of transcription 3 (Stat 3), and Rac1 (DiPersio, 2007; Tscharntke et al., 2007; Sano et al., 2008; Satoh et al., 2009; Castilho et al., 2010).
Integrin α6β4, which is a receptor for laminin-332 and a part of the hemidesmosome is known to play a substantial role in re-epithelialization. The expression of this integrin seems necessary for the EGF-induced keratinocyte migration. Mouse models lacking the expression of integrin α6β4 show markedly delayed re-epithelialization and reduced keratinocyte migration in skin (Russell et al., 2003; Nikolopoulos et al., 2005).

Existing keratinocyte integrins are affected by the presence of wound matrix proteins and combined with the influence of growth factors and cytokines, they can produce matrix receptors that are specific to wounds. This is most apparent with the three fibronectin receptor integrins α5β1, αvβ1 and αvβ6 (Larjava et al., 1993; Larjava et al., 1996). Each of the above affects the process of re-epithelialization in different ways. The first, α5β1 promotes keratinocyte adhesion and motility and may also add to the increased proliferation of non-migratory keratinocytes behind the leading edge during wound healing through its interaction with EDA fibronectin (Zhang et al., 1995; Bata-Csorgo et al., 1998). αvβ1 is thought to support keratinocyte migration over the underlying fibronectin EDA via a low affinity fibronectin binding which enables cell attachment without decreasing the migration speed (Vogel et al., 1990; Zhang et al., 1993; Koivisto et al., 1999). The third-mentioned fibronectin receptor integrin, αvβ6, is expressed in the late stages of oral mucosal and skin wounds. Along with its substrates fibronectin EDA and tenascin-C, αvβ6 integrin is positioned alongside the fibrin-fibronectin clot and as far as the tip of the migrating cell sheet (Haapasalmi et al., 1996; Häkkinen et al., 2000). Its peak expression takes place at the time of fusion between epithelial sheets (Larjava et al., 2011). It has been speculated that αvβ6 integrin activates matrix-bound TGF-β1 and presents it to adjacent epithelial cells and/or fibroblasts (Larjava et al., 2011). This effectively results in the regulation
of keratinocyte proliferation, inflammation, and deposition of extracellular matrix. Integrin α9β1 seems essential to the keratinocyte proliferation at wound edges as shown by Singh et al. in knockout mice (Singh et al., 2009).

Integrins are expressed in cells other than keratinocytes and changes in this expression can both accelerate and delay wound re-epithelialization. For instance, the absence of integrin Mac-1 which signals neutrophils and monocytes delays skin wound re-epithelialization in knockout mice. Conversely, the elimination of integrin αvβ3 which is expressed in wounds by platelets, endothelial cells, macrophages, and dermal fibroblasts, causes accelerated re-epithelialization (Reynolds et al., 2005; Sisco et al., 2007).

Integrins act as the main mediators of cell-extracellular matrix interaction, however there are a number of other keratinocyte cell surface adhesion receptors that can function alongside integrins and contribute to re-epithelialization. For instance, glycosaminoglycans, specifically epidermal hyaluronan, are expressed at the wound margins (Oksala et al., 1995) and cause keratinocyte proliferation and migration (Kaya et al., 1997). Transmembrane heparan sulfate proteoglycans syndecan-1 and -4 are expressed by keratinocytes in human oral mucosal wounds and are capable of facilitating keratinocyte attachment to unprocessed laminin-332 (Oksala et al., 1995; Gallo et al., 1996; Okamoto et al., 2003). Knockout mice in either syndecan-1 or -4 have not shown decreased keratinocyte migration during epithelialization, however those deficient in syndecan-1 have reduced keratinocyte proliferation (Echtermeyer et al., 2001; Stepp et al., 2002).
In addition to the pro-migratory matrix molecules that are stimulated through the action of integrins, cytokines and growth factors released by wound platelets in the clot are also important for re-epithelialization. The main growth factors that regulate re-epithelialization are from the EGF-family, TGF-β1 and KGF’s (Davis et al., 2000).

Growth factor receptors such as EGF receptor (EGFR) can interact with extracellular matrix molecules via binding to the molecules’ matrikine domains (Swindle et al., 2001; Tran et al., 2004). This facilitates keratinocyte migration. The EGF-like repeats of tenascin-C and laminin-332 bind to EGFR and favor migratory over proliferation pathways (Swindle et al., 2001; Haugh, 2002). EGFR is strongly upregulated in keratinocytes at the wound edges following tissue injury. This continues within the hypertrophic, proliferating zone of epithelium until the proliferative phase is completed (Stoscheck et al., 1992; Wenczak et al., 1992; Schelfhout et al., 2002).

The second-mentioned family of growth factors seen in wound healing is the TGF-β group. TGF-β1 stimulates keratinocyte motility by changing the cells from a differentiating to a regenerative phenotype (Hashimoto, 2000). TGF-β1 function is complex and shows mixed results in animal models. Knockout mice have shown delayed wound repair as a result of increased inflammation (Brown et al., 1995) while the overexpression of TGF-β1 has been associated with severe inflammation in wounds (Wang et al., 2006).

Growth factors that mediate keratinocyte migration specifically include keratinocyte growth factor (KGF), epidermal growth factor (EGF) and insulin growth factor (IGF). They cause
changes in the organization of the cytoskeleton and cell shape (Putnins et al., 1999; Haase et al., 2003).

In order for re-epithelialization to occur, fibrinolysis of the clot ahead of the migrating keratinocytes must take place. Fibrinolysis depends on the activation of plasmin, which occurs via activators that bind to integrins on the keratinocyte cell surface (Vaalamo et al., 1996). Wound re-epithelialization has been observed to be nearly completely absent in mice that are deficient in both plasminogen and uPA/tPA (Romer et al., 1996; Carmeliet, 2003).

Keratinocytes express a number of matrix metalloproteinases (MMPs). MMPs are a family of about 25 zinc and calcium dependent, structurally related proteinases which are capable of breaking down components of the extracellular matrix as well as other molecules in wounds (Steffensen et al., 2001; Illman et al., 2008). While there are a large number of MMPs expressed in early wound healing, MMP-9 seems critical to re-epithelialization. Studies have shown delayed keratinocyte migration and dissolution of the fibrin clot in skin wounds of MMP-9 deficient mice (Kyriakides et al., 2009). Tissue inhibitors of metalloproteinases (TIMPs) are important regulators of MMP activity and work in concert with MMPs. Disruptions in the balance between MMPs and TIMPS are known to result in poor wound healing (Vaalamo et al., 1996; Salonurmi et al., 2004).

Additional modulators of wound re-epithelialization are divalent cations in the wound fluid. Keratinocytes are known to lose to their migratory phenotype when exposed to dialyzed wound fluid. Early wounds show elevated levels of Mg$^{2+}$ and decreased levels of Ca$^{2+}$ and this is known
to correlate with the period of epithelial migration. Increased Mg$^{2+}$ is thought to enhance integrin function, whereas the decreased Ca$^{2+}$ is thought to favor re-epithelialization via keratinocyte mitogenesis (Grzesiak and Pierschbacher, 1995).

Wound re-epithelialization is influenced by wound-induced electric fields. Mammalian cells have established transepithelial voltage differences, which are created by actively pumping Na$^+$ and K$^+$ inwards, and Cl$^-$ outwards across the epithelium. Wounding causes a breakdown of this potential at the wound edges and results in a wound induced laterally oriented electrical field. The wound induced electrical field is known to direct epithelial cell migration and also cell division (Song et al., 2002; Song et al., 2004; Zhao, 2009).

### 1.5.3 Granulation tissue formation

The formation of new connective tissue begins 2-4 days after wounding and is driven mainly by fibroblasts, the principal cellular constituents of connective tissue. Fibroblasts exist in a plethora of phenotypes and can be defined as adherent cells that have the capacity to synthesize and remodel the collagen rich extracellular matrix (Darby and Hewitson, 2007; Sorrell and Caplan, 2009). In addition to fibroblasts, new connective tissue contains the existing blood clot components, new connective tissue ECM, and new and forming blood vessels and inflammatory cells (Clark et al., 1996; Gurtner et al., 2008; Larson et al., 2010).

Wounding results in signals that cause fibroblast proliferation, modulation of fibroblast adhesions to the extracellular matrix, and give directional cues that facilitate cell migration to the provisional wound matrix (Clark et al., 1996). While neutrophils constitute the majority of
leukocytes in the initial inflammatory stage of wound healing, there is a shift in predominant cell type that occurs around day 3 following wounding where macrophages, lymphocyte subsets, and mast cells begin to accumulate in greater numbers (Eming et al., 2007; Shiota et al., 2010). Wound keratinocytes secrete inflammatory mediators including chemokines, such as monocyte chemotactic protein-1 (MCP-1) and cytokines, such as IL-1β and TNF-α. The inflammatory cells that are recruited to the wound site release soluble growth factors, cytokines, and chemokines. There is also release of prostaglandins from damaged cells and these cause the activation and migration of connective tissue cells, into the wound (Werner and Grose, 2003; F Li et al., 2010).

Inflammatory cells also give rise to reactive oxygen species (ROS). In low concentrations, ROS are beneficial to the wound healing process by acting as signaling molecules and which regulate cell survival and gene expression (Novo and Parola, 2008). In addition to ROS, acute short-term hypoxia is known to have a number of beneficial effects on wound healing. It promotes angiogenesis, activates connective tissue cells, induces connective tissue cell and ECM proliferation, and stimulates growth factor release (Boraldi et al., 2007; Grayson et al., 2007; Lee et al., 2009). Other cells present in the tissue that are known to activate fibroblasts in wound healing include sensory and peripheral nerves. They influence the proliferation of fibroblasts via the release of neuropeptides (Roosterman et al., 2006; da Silva et al., 2010).

Hyaluronan production is increased within hours of wounding and it accumulates in the ECM. It functions to regulate the inflammatory response (Mack et al., 2012). In addition, hyaluronan regulates TGF-β mediated fibroblast proliferation in skin (Meran et al., 2011).
Another factor that may add to fibroblast activation and proliferation during the wound healing process is the destruction of gap junction communication between cells (Su et al., 1997). Connexin mediated gap junctions facilitate communication between fibroblasts and it is thought that this communication maintains cells in a quiescent state via cell-to-cell transfer of microRNAs in normal non-wounded tissue. Injury to the connective tissue results in a break in the communication that is mediated by gap junctions (Lim et al., 2011).

Connective tissue cells begin to migrate into the provisional matrix about 3 days after wounding. Compared to inflammatory cell migration, fibroblast migration is slow and moves about 0.5-1.0µm/minute (Schneider and Haugh, 2006). Fibroblasts use integrins to facilitate their migration to the provisional matrix. In order to invade the wound site, cells require modification of their integrin expression. Cytokines, namely TGF-β and PDGF affect the expression of these important integrins (Lin et al., 2005; van Beurden et al., 2005).

Fibroblast migration is also modulated by the expression of MMPs. Cell-surface associated, membrane type MMPs such as MMP-14 are expressed and affect the focalized ECM remodeling during cell migration. Other MMPs such as MT2-MMP and MT3-MMP are expressed when cells arrive at the wound site (Greiling and Clark, 1997; Hotary et al., 2002). In addition to MMPs, soluble mediators such as PDGF are active in granulation tissue formation. PDGF released by platelets and macrophages at the wound site causes PDGF-induced chemotaxis, which aids in the migration of fibroblasts. To assist further in fibroblast migration, proteases such as MMP-2, MMP-9, and plasmin cause release of latent growth factors such as TGF-β which facilitate fibroblast migration (Toriseva and Kähäri, 2009).
Cells are also capable of secreting new ECM proteins, such as a particular type of fibronectin, cellular extra domain A (EDA) – fibronectin. Fibronectin is required for cell migration and EDA-fibronectin supports integrin mediated cell adhesion and migration more effectively than plasma fibronectin (Singh et al., 2004; Singh et al., 2010).

Once in the provisional matrix, cells begin to proliferate again and start producing new granulation tissue ECM. This newly formed granulation tissue acts as a scaffold for subsequent connective tissue healing. The initially formed granulation tissue is highly cellular, unorganized, and structurally weak. The collagen deposition is very fast in the first 21 days after injury and then proceeds at a slower rate for a minimum of 90 days (Hering et al., 1983).

In addition to its role in fibroblast migration, EDA-fibronectin controls the deposition and organization of the extracellular matrix. It is capable of regulating fibroblast gene expression and participates in their differentiation into myofibroblasts (Serini et al., 1998; Leiss et al., 2008; Singh et al., 2010).

There are a large number of matricellular proteins present in the ECM during the later stages of granulation tissue formation. This includes SPARC (osteonectin), hevin, thrombospondin-1 and -2, tenascin-C and –X, periostin, and the Cyr-CTGF-Nov-family (CCN-family) proteins (Chiodoni et al., 2010). These proteins act by modulating the cell-ECM interactions, binding and regulating the activity of cytokines and growth factors, and also possibly interacting with specific growth factor receptors made available by wound cells (Hamilton, 2008; Chiodoni et al., 2010).
Wound contraction is a process that approximates wound edges more closely to one another. It begins around 7 days after wounding and can account for up to 50% of the wound closure in humans. Wound contraction is facilitated mainly by myofibroblasts (Clark et al., 1996; Gurtner et al., 2008; Schultz et al., 2011) which behave like smooth muscle cells, expressing α-smooth muscle actin (Hinz and Gabbiani, 2010). The gradual differentiation of fibroblasts into myofibroblasts is controlled by cell-ECM interactions, growth factors, and mechanosensory signals (Clark et al., 1996).

Remodeling is the transition of poorly organized granulation tissue ECM into mature connective tissue. Type I collagen replaces type III collagen. The collagen fibrils form into thicker bundles, are arranged perpendicularly to the wound edges, and start to form their typical cross linked arrangement. This is a slow process and animal studies have shown that the breaking strength of a wound at 21 days healing may only be 20% of the final strength, which can take months or years to be reached (Clark et al., 1996; Gurtner et al., 2008). There are a number of mechanisms that regulate ECM abundance and organization during remodeling.

One factor that aids in the regulation of ECM abundance and composition is the decreased number of inflammatory cells during the remodeling stage of wound healing. This results in a generalized reduced tissue cellularity. There are fewer pro-inflammatory signals in the wound and also increased expression of mediators that suppress inflammation such as lipoxins, resolvins, maresins, and IL-10 (Sato et al., 1999; Peranteau et al., 2008; Serhan, 2011). The ECM composition changes significantly during remodeling and an example of this is the accumulation of leucine-rich proteoglycans. These act to regulate collagen formation and also to down-regulate
pro-mitogenic TGF-β signaling which results in suppressed cell proliferation (Häkkinen et al., 2000; Iozzo and Schaefer, 2010). Mechanosensory signals also play a role in the regulation of cell proliferation. As the newly forming ECM reaches a similar stress-balance to its neighboring tissues, the mechanosensory input decreases in order to reduce cell proliferation and ECM deposition (Carlson and Thompson, 2004; Grinnell and Petroll, 2010; Provenzano and Keely, 2011; Schultz et al., 2011). Apoptosis of cells such as myofibroblasts and cells forming blood vessels adds to the reduced cellularity of the newly remodeling tissue (Desmoulière et al., 1997).

ECM degradation is affected by proteolytic degradation, a process driven by MMPs. This process results in a reduction of ECM abundance and also increases its subsequent re-organization (Gill and Parks, 2008; Toriseva and Kähäri, 2009). The expression of MMP’s in fibroblasts is regulated by secreted cytokines and growth factors including IL-1β, PDGF, EGF, and TNF-α. MMPs are believed to be more critical during the inflammation stage of wound healing rather than the remodeling stage and in mouse models with multiple MMP knockout genes, there were no major changes observed in the later stages of wound healing. There was also no scar formation (Bullard et al., 1999; Hattori et al., 2009; Kyriakides et al., 2009; Mirastschijski et al., 2010). Numerous MMPs mediate the breakdown of type I and III collagen and therefore a change in the expression of these particular enzymes can impact wound healing. Hypertrophic scars and keloids have been found to have high levels of MMP-2 and low levels of MMP-9 (Neely et al., 1999). Similarly, a shift in the balance between MMP and TIMP expression may determine whether there is abnormal accumulation of ECM during wound healing (Gill and Parks, 2008; Toriseva and Kähäri, 2009).
In addition to proteolytic degradation of ECM during the remodeling stage of wound healing, many wound cells such as fibroblasts and macrophages contribute to the process through phagocytosis. Reduced numbers of fibroblasts and macrophages results in a reduction in their produced growth factors, pro-fibrotic TGF-β1 and TGF-β2. As mentioned previously, the remodeling stage of wound healing is accompanied by decreased cytokine production, and this causes even further reduction in the expression of TGF-β (Levine et al., 1993; Gold et al., 1997; Yang et al., 1999; Beanes et al., 2003; Eslami et al., 2009).

1.5.4 Angiogenesis

Angiogenesis is a process driven by endothelial cells. New vascular formations are known to develop in 3 ways including sprouting from pre-existing vessels, new de novo formation of vessels through endothelial precursor cells (EPCs), and by the incorporation of circulating monocytes, which then differentiate into endothelial cells (Carmeliet, 2000; Carmeliet, 2003; Phelps and Garcia, 2009). Sprouting angiogenesis is controlled by growth factors, chemokines, and proteolytic enzymes. These factors modulate the sprouting process and also help to stabilize the newly formed microvasculature (Bicknell and Harris, 2004; Adams and Alitalo, 2007; Eming et al., 2007). At the wound site, monocytes differentiate into macrophages, important mediators in the process of angiogenesis.

Macrophages facilitate sprouting angiogenesis, introduce EPCs, and produce vascular endothelial growth factor (VEGF) (Pollard, 2009). Studies in mice have shown an improved wound healing response in diabetic mice when VEGF was applied topically to wounds and this is thought to be due to recruitment of EPCs to the wound site (Galiano et al., 2004). In addition
to the above-mentioned actions, macrophages also produce metalloproteinases, which allow modification of the ECM, when needed, for the developing blood vessels. Examples of this are chymase and tryptase (both serine proteases), which are stored in mast cells and are capable of activating a succession of proteolytic enzymes that create space for new vessels to grow into (Lingen, 2001; Ribatti et al., 2009).

A number of other inflammatory cells also promote angiogenesis. Lymphocytes are present in chronic wounds and produce VEGF, bFGF and HGF; mediators which encourage angiogenesis. As well, mast cells contain a number of mediators for angiogenesis including VEGF, FGF-2, TNF-α, and TGF-β. VEGF is the first known endothelial mediator of angiogenesis and much research has focused around it (Otrock et al., 2007; Nieves et al., 2009). There are six isoforms of VEGF and this is suggestive that VEGF has not only a role in the initiation of angiogenesis but also in maintaining the long-term stability and homeostasis of the vasculature (Elçin et al., 2001; Lee et al., 2007; Nieves et al., 2009). Studies have shown that VEGF production is strongly stimulated by hypoxia, a physiological occurrence in wound healing tissues with high metabolic demand (Li et al., 2009).

Neutrophils, key cells in inflammation, also have an important role in angiogenesis. They release proteolytic enzymes, which as mentioned above allow the formation of inroads in the ECM by new vessels. Pro-inflammatory mediators at the site of wounding, such as TNF-α, cause neutrophil breakdown. This releases stored VEGF as well as inflammatory chemokines such as CXCR1 and 8. The chemokines are then thought to stimulate endothelial cell proliferation, as this has been shown in vitro (Murdoch et al., 2008; Tazzyman et al., 2009).
New vessel formation involves interaction with the ECM and requires a number of ECM components including fibronectin, collagen, vitronectin, tenascin, and laminin (Feng et al., 1999; Li et al., 2003). Integrins, specifically α1β1 and αυβ3, are critical in wound angiogenesis. Studies have shown that α1β1 is critical to VEGF mediated angiogenic signaling (Senger et al., 1997). Similarly, blocking the αυβ3 receptor blocks angiogenesis (Christofidou-Solomidou et al., 1997). The inhibition of angiogenesis is an important factor in the wound healing process as the two processes; pro-angiogenic and inhibition of angiogenesis seem closely related. Endogenous inhibitors of angiogenesis are comprised mainly of proteins, or fragments of proteins, that are stored in the ECM and are released following activation of MMPs, cathepsins, or elastases (van Hinsbergh et al., 2006; Ribatti, 2009).

1.6 Scar formation

Scars develop as a result of aberrations in physiologic wound healing. In general, they signify an imbalance between anabolic and catabolic phases of wound healing but more specifically, they represent a problem with one of the 4 key biological processes to wound healing; inflammatory response, TGF-β activity, cell-ECM interaction, and fibroblast phenotype.

1.6.1 Inflammatory response

A prolonged inflammatory stage of wound healing is thought to be a significant contributor to scar formation. Immune cell infiltrate is present in scar tissue of keloids which may result in increased fibroblast activity with more and sustained ECM deposition (Brown and Bayat, 2009). Increased expression of IL-6 has been associated with increased scar formation, specifically in keloid formation (Xue et al., 2000).
In conjunction with the severity of inflammation, the type of immune response may also have a significant impact on the development of scars. CD4$^+$ T cells are thought to be a major immunoregulator in wound healing and it may lead to either a TH1 or TH2 response depending on the cytokine expression profile. A predominantly TH2 response is associated with fibrogenesis while a predominantly TH1 CD4$^+$ expression has been shown to completely stop tissue fibrosis. This occurs as a result of IFN-γ and IL-12 production (Wynn et al., 1995; Wynn et al., 1996).

1.6.2 TGF-β activity

TGF-β plays a vital role in normal wound healing and is first released in large quantities from degranulating platelets at the wound site. TGF-β consists of three isoforms and variations in the quantity and duration of their individual expression have been linked to hypertrophic scar and keloid formation. TGF-β1 and –β2 are potent stimulators of collagen and proteoglycan synthesis. They cause not only apposition of ECM but also prevent its breakdown (Szulgit et al., 2002; Köse and Waseem, 2008). Increased expression of TGF-β1 has been strongly associated with prolonged myofibroblast differentiation, increased matrix accumulation, and subsequent scar formation. Studies of dermal wounds in rats have shown that injection of a neutralizing antibody to TGF-β1 and –β2 into the wound margins results in healing without scar formation (Shah et al., 1992; Shah et al., 1995).

TGF-β3, which reduces connective tissue deposition during the later stages wound healing, seems to prevent myofibroblast differentiation and scarring (Ferguson and O'Kane, 2004; Waddington et al., 2010). There are, however conflicting results in the literature as some studies
have shown significantly reduced TGF-β3 mRNA expression in keloid fibroblasts when compared to fibroblasts obtained from hypertrophic scars or skin (Bock et al., 2005). The variability in these results may serve as further evidence that beyond week 1, it is not merely the presence of TGF-β, its receptors and modulators, but more specifically the differential expression of each that affects hypertrophic scar and keloid formation (Gauglitz et al., 2011).

TGF-β activity is mediated by a specific group of intracellular signaling proteins, which are known together as the SMAD signal transduction pathway. The proteins are further classified as receptor-regulated SMADs (R-SMADs), common-mediator SMADs, and inhibitory SMADs. These proteins, when phosphorylated, are capable of regulating the transcription of specific genes. Two SMADs within the R-SMADs group, R-SMADs 3 and 4, have been shown to be the predominant mediators of autocrine stimulation by TGF-β in hypertrophic scar-derived fibroblasts (Kopp et al., 2005). Down-regulation of SMAD3 expression results in significantly less procollagen gene expression and reduced ECM production by keloid fibroblasts (Wang et al., 2007). SMAD7 applies negative feedback control over TGF-β1 (Okado et al., 2002). SMAD7 overexpression has been shown to have therapeutic benefits in the treatment of scar forming conditions such as post-obstructive renal fibrosis (Terada et al., 2002; Chen and Davidson, 2005).

1.6.3 Cell to ECM interaction

The ECM and its associated proteins play an essential role in wound healing and scar formation. One such example is the small proteoglycan decorin. Decorin is a leucine-rich molecule that binds collagen (Ameye and Young, 2002) and may suppress collagen phagocytosis by fibroblasts
during the remodeling stage of wound healing (Bhide et al., 2005). It is also capable of binding TGF-β1, which minimizes the stimulatory effects of TGF-β1 on collagen (Hildebrand et al., 1994). Decorin is decreased by as much as 75% in hypertrophic scars and this is thought to account for the unorganized collagen arrangement and increased ECM observed in hypertrophic scars (Sayani et al., 2000).

The differential expression of SPARC and hevin are also thought to modulate wound healing. SPARC and hevin are ECM proteins that regulate ECM deposition and organization during wound healing by suppressing cell migration. Increased expression of these proteins in mice has demonstrated reduced angiogenesis and scar formation (Bradshaw and Sage, 2001; Bradshaw et al., 2002; Barker et al., 2005; Sullivan et al., 2008).

Tenascin-C is an ECM protein produced at the wound edge and is believed to help recruit fibroblasts to the wound. While it is abundant in the wound at day 3, it drops off markedly by day 7. The exact action of tenascin-C is disputable as there are conflicting study results. Overexpression of tenascin-C shows has been associated with both keloid scars and scarless wound healing in human oral mucosa (Whitby et al., 1991; Sible et al., 1995; Chen et al., 2010).

A critical stage in the remodeling phase of wound healing is fibrillogenesis of collagen. This is the transformation of poorly organized, thin fibrils with few intermolecular cross-links into well-organized, thicker fiber bundles with increased intermolecular cross-linking. Fibrillogenesis of type I collagen is mediated by fibroblasts. It is a process that requires fibronectin, type III and V collagen, and expression of fibronectin and collagen binding integrins (Liu et al., 1997; Kadler et
al., 2008). However, excess cross-linking can contribute to scar formation. Levels of cross-linking enzymes are higher in scars and can stay at a high level for many months or years in established scars (van den Bogaerd et al., 2009).

1.6.4 Fibroblast phenotype

Uninjured tissue contains fibroblasts in a quiescent state (Häkkinen et al., 2013) and while in this state, fibroblasts continue to synthesize, degrade, and organize the ECM (Lemons et al., 2010). However, their role is significantly altered upon injury as they are the key cell type responsible for ECM deposition and also for modulation of inflammation, re-epithelialization, and angiogenesis (Häkkinen et al., 2013). Normal connective tissue contains few myofibroblasts, however they are present in greater percentages in hypertrophic scars (Nedelec et al., 2001; Hinz and Gabbiani, 2010).

Hypertrophic scars are hypercellular, when compared to normal tissue, and show a gradual decrease in fibroblast cell density during the remodeling stage of wound healing. This decrease is cellularity is partly due to apoptosis of myofibroblasts (Armour et al., 2007). Apoptotic cells are known to promote the proliferation of mesenchymal progenitor cells (Li et al., 2010), and in the case of the oral palatal mucosa, this would include fibroblast progenitor cells.

1.7 Unique characteristics of oral wound healing

Oral wounds have been shown to heal faster and with less clinical and histological scar formation than similar skin wounds (Sciubba et al., 1978; Szpaderska et al., 2003; Szpaderska et al., 2005; Mak et al., 2009; Wong et al., 2009). The precise reasons behind these differences are
unclear however they may be partly due to the differences in the pericellular ECM of oral mucosa and skin. The two structures show differences in sulfated glycosaminoglycans, heparan, chondroitin, dermatan and keratan sulfate, hyaluron, type III collagen, and tenascin-C (Pedlar, 1984; Bronson et al., 1988).

In addition to the above differences, phenotypical variations in the fibroblasts of oral mucosa and skin may also give rise to the differences in healing between these two tissues (Zhang et al., 2010). Fibroblasts isolated from the buccal mucosa and skin of the same patient exhibit a different gene expression profile (Okazaki et al., 2002). The varied behavior of the fibroblasts may be related to their embryonic origin as those in the skin originate from mesodermal mesenchymal cells while those in the oral mucosa are derived from neural crest cells (Breau et al., 2008).

Oral mucosal wounds are subjected to a milder inflammatory response than skin wounds with reduced recruitment of inflammatory cells such as neutrophils, mast cells, macrophages, and T-cells (Sciubba et al., 1978; Szpaderska et al., 2003; Mak et al., 2009; Chen et al., 2010). In the red Duroc pig model, it has been shown that oral mucosal wounds had significantly less mast cells than skin wounds at 60 days post wounding (Mak et al., 2009). As well, fewer T-lymphocytes at day 7 post-wounding has been found in the oral mucosal wounds of mice when compared to skin wounds (Szpaderska et al., 2003). As part of their overall decreased inflammatory response, oral wounds have decreased levels of cytokine expression including TGF-β1 and TNF-α. There is also an observed increase in the ratio of antifibrotic TGF-β3 relative to pro-fibrotic TGF-β1 in oral mucosal wounds compared to skin wounds (Schrementi et
al., 2008; Eslami et al., 2009). Lastly, oral wounds exist in the presence of saliva, which contains growth factors EGF, TFG-β and IGF (Häkkinen et al., 2000; Loo et al., 2010). A lack of saliva has been shown to delay wound closure of oral wounds (Bodner et al., 1992). Some of the findings in oral wound healing are similar to those in fetal wound healing and thus a short divulgence into the topic shall follow.

1.8 Fetal wound healing

As discussed, the inflammatory response is a critical component in scar formation, and in situations where the inflammatory response is minimal or absent there is an associated decrease in scar formation. One such environment which lacks inflammatory cells such as neutrophils, macrophages, T-cells, and B-cells is the developing fetus (Armstrong and Ferguson, 1995). There are a number of factors that contribute to the decreased immune response observed in the fetus and one of these is an alteration in fetal platelets. Fetal platelets produce less PDGF, TGF-β1 and –β2 and this results in less neutrophil and macrophage recruitment (Olutoye et al., 1996). In addition, fetal wounds have a decreased expression of TGF-β1, a pro-inflammatory cytokine, when compared to adult skin wounds (Cowin et al., 2001). The expression profile of other cytokines in fetal wounds is one that would seem to favor scarless healing as well. Pro-inflammatory cytokines IL-6 and IL-8 are reduced. Anti-inflammatory cytokine IL-10 has been shown to be critical to scarless healing. IL-10 deactivates macrophages and in fetal mice lacking IL-10, significant inflammation and scarring is observed (Liechty et al., 2000). Mast cells have also been implicated in increasing scarring as they are found in fewer numbers in wounds of younger embryonic age than those of increased age (Schwartzfärb and Kirsner, 2012).
As the decreased scar formation seen in the early stages of development could be thought to be due to the intrauterine environment itself, researchers have sought to show otherwise. Armstrong and Ferguson (1995) studied fetal marsupials and found that cutaneous wounds healed without scarring, despite the fetus’s development in a pouch rather than in utero. Thus the ability of fetal wounds to heal without scars appears to be intrinsic and not just a result of the surrounding environment (Armstrong and Ferguson, 1995).

1.9 Wound healing of the cleft lip/palate

The study of fetal wound healing is of particular relevance to cases of cleft lip and palate. Dentofacial defects are quite common, as the prevalence of cleft lip with or without cleft palate at birth varies from 3.4–22.9 per 10000 births (Zhu and Li, 2011). Scar formation following the repair of dentofacial defects such as cleft lip and/or palate is a significant negative factor associated with treatment. Scars impair function (i.e. tight lip), block growth (i.e. midface and dentoalveolar process), and can make the face esthetically unpleasant (Lorenz and Longaker, 2003; Zhu and Li, 2011). At 1 year after surgery, there is a reported prevalence of hypertrophic scar formation of 25% following cleft lip repair (Soltani et al., 2012). Using an animal model, researchers have shown scarless healing of the cleft lip/palate when treated in utero. As the mechanisms of fetal wound healing are more clearly understood, in utero repair of cleft lip/palate may become a viable alternative to post natal surgery with less scarring and a decreased need for secondary procedures which are common in this population (Lorenz and Longaker, 2003).
1.10 Methods of scar assessment

A reliable, valid, and clinically useful tool for scar assessment is an important component for the advancement of research in the impact of therapeutic modalities for scar management (Esselman et al., 2006). There is no standardized means for clinical scar assessment, however the modified Vancouver Scar Scale (mVSS), the Patient and Observer Scar Assessment Scale (POSAS), the Visual Analog Scale (VAS), and the Manchester Scar Scale (MSS) are commonly used tools.

Visual scar analysis tools, such as the above-listed scales, are best used for small linear scars similar to those created in research. They are far less useful for assessing large scars and/or the functional aspects of scars (Fearmonti et al., 2010). Most methods of visual scar analysis produce a scar scale of categorical data where each category is assumed to be of equal importance (Duncan et al., 2006).

The Manchester scar scale was first described in 1998 in an effort to create a systematic rating scale for the assessment of scars using photographs. Beausang used it to score scars both clinically and from photograph panels (Beausang et al., 1998). The scale provides an overall scar score based on a 5-point clinical assessment including color, contour, surface (matte or shiny), distortion, texture. Each scar is also given a global assessment with the use of a visual analog scale. The MSS is more suited to linear scars that are not caused by burns than is the VSS (Duncan et al., 2006). In addition, the MSS uses descriptors that are related to clinical significance rather than physical measurement alone (Fearmonti et al., 2010).

An earlier study comparing the healing of palatal wounds with skin wounds using a modified MSS found a significantly higher score in the skin wounds from days 28 to 49 post wounding
(Wong et al., 2009). The histological findings supported the results obtained using the scar scale. In the palatal mucosa, from days 14 to 49, there was decreasing cellularity and the collagen appeared similar to that found in unwounded mucosa. In contrast, the skin wounds at this time point were still highly cellular, and had thin, loosely organized collagen fiber bundles that were arranged perpendicular to the wound edge. Not only was the modified MSS score significantly higher in skin than mucosa at day 49 after wounding, but so was the total histological scar assessment score (Wong et al., 2009).

There are varying results in the literature when it comes to assessing the correlation of scar scores that are obtained from a clinical exam compared to those obtained from a photographic assessment. Beausang reported an overall mean correlation coefficient (Spearman’s) of 0.9 (range 0.85 to 0.95, \( p < 0.001 \)), but a recent study by Simons questions the level of agreement between clinical and photographic assessment of scars using any of MSS, mVSS, or POSAS (Simons et al., 2013).
Chapter 2: Aim of the Study

Clinicians who have observed oral wounds to heal often anecdotally report that oral wounds in the human palate heal without scars. Based on limited human and animal studies reviewed above, the histological and molecular healing appears to reflect the clinicians’ view with significant normalization of oral wounds in about two months after healing. There are, however, no long-term systematic studies describing scarring (or lack of it) after the two main types of clinical grafting procedures, namely free gingival grafts (FGG) and connective tissue graft (CTG).

Autogenous gingival grafts involve the detachment of gingiva from a donor site and transfer to a recipient bed. The posterior palate is frequently the donor site. The classic FGG harvest is described as a split thickness graft, removing only part of the lamina propria, and with an overall thickness of 0.75-1.25mm (HC Sullivan and Atkins, 1968). In comparison, a CTG typically requires a full thickness harvest (removing all of the lamina propria) (B Langer and L Langer, 1985). As shown in Figure 1, the CTG harvest creates a deeper wound than an FGG harvest.

The aim of the study was, therefore, to compare clinical scar formation after a minimum of six months of healing in CTG harvest sites (deep wounds) and FGG harvest sites (superficial wounds) of the human palatal mucosa.
Hypothesis:

1. Palatal wounds in general show relatively little scarring
2. Deep wounds (CTG) produce more scarring than more superficial wounds (FGG)
Figure 1 Illustration of palatal tissue and FGG and CTG harvest thicknesses.
Chapter 3: Materials and Methods

This was a retrospective clinical study that was approved by the Clinical Research Ethics Board of the University of British Columbia. A database search was completed which identified patients who had attended the UBC Graduate Periodontics Clinic and had at least one of either FGG or CTG procedure from August 1998 to August 2012. This search identified 165 candidates and an initial letter of contact was mailed to these candidates. 41 patients contacted the investigator and consented to participating in the study. They were seen in the UBC Graduate Periodontics Clinic for a single appointment, which entailed taking a series of intraoral photographs. Two patients were excluded because they had a history of each of FGG and CTG harvest from the same donor site on the palate. An additional two patients were excluded because the record of their graft donor site could not be found. Thus, in the end, 37 patients were enrolled in the study. Intraoral photographs of the palate were obtained for every patient. The photographs were taken with a single digital SLR camera with macro lens and ring flash. The camera settings were kept standard for each photograph. Two independent calibrated blinded examiners assessed the photographs using a modified version of the Manchester Scar Proforma (Table 1) (Mak et al., 2009). A value of zero, one, or two was given for each parameter, with no difference from normal tissue scored as zero, and gross mismatch scored as two. The values for each parameter were summed to produce a total scar score for each site. The component parameters were also examined individually so that color, contour, and distortion could be evaluated independently. The photographs were assembled into panels of nine containing a mix of FGGs and CTGs. Each photograph was centered upon the original surgical site, as determined by the patients’ surgical photographs on record. The inter-examiner reliability was 98% and therefore it was decided to
use the results obtained from the first examiner only. Amongst the test sites, the examiners were also shown a total of 18 sites that had not previously been surgerized. Each of these sites scored zero in all three of color, contour, and distortion.

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Perfect match with surrounding tissue</td>
<td>Slight mismatch</td>
<td>Obvious mismatch</td>
</tr>
<tr>
<td>Contour</td>
<td>Flush with surrounding tissue</td>
<td>Slightly proud or indented</td>
<td>Hypertrophic</td>
</tr>
<tr>
<td>Distortion</td>
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<td>Severe</td>
</tr>
<tr>
<td>Total Score</td>
<td>0-6</td>
<td></td>
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</table>

Table 1 Modified Manchester Scar Proforma for clinical scar assessment; score range: 0–6; lower values represent improved healing quality, i.e., less scar (Mak et al., 2009).
Chapter 4: Results

4.1 Demographics

Of the 37 eligible study participants, 32% (n=12) were male and 68% (n=25) were female. The mean age was 55 years. The age distribution was broad (Figure 2) including two subjects ages 15-20 years, three subjects 21-35 years, seven subjects 36-50 years, 15 subjects 51-65 years, and 10 subjects 66-78 years.

Amongst these participants, there were a total of 23 FGGs and 23 CTGs assessed. Nine subjects had two sites assessed, which were located on opposite sides of the palate. Those patients who contributed two sites to the study were quite evenly distributed between the FGG and CTG groups. Three patients had two CTG sites, two patients had two FGG sites, and four patients had one of each CTG and FGG sites.

4.2 Scar formation and graft type

The modified MSS (score 0-6) was determined separately for the FGGs and CTGs. Figures 3-5 are examples of each component scar score (0-2) and the scars that were scored as such. As there were no scars in the sample that scored two for contour, there are no photographic examples of this value.

The FGG and CTG groups, and their overall modified MSS scores were compared first. In the FGG group, the scores were fairly narrowly distributed and two was the most frequently occurring score. Four sites (17.3%) scored zero, three sites (13.0%) scored one, eight sites
(34.8%) scored two, five sites (21.7%) scored three, three sites (13.0%) scored four, zero sites (0%) scored five, and also zero sites (0%) scored six (Figure 6). In the CTG group, the numbers were more evenly distributed. Three sites (13.0%) scored zero, six sites (26.1%) scored one, four sites (15.4%) scored two, six sites (26.1%) scored three, four sites (15.4%) scored four, zero sites (0%) scored five, and also zero sites (0%) scored six (Figure 6).

The individual components of the modified MSS score, including color, contour, and distortion, were examined separately for both FGGs and CTGs. The modified MSS score for color in the two groups was virtually even (Figure 7). The FGG group was as follows: n= 8 (34.8%) scored zero, n= 12 (52.2%) scored one, n=3 (13.0%) scored two. In comparison, the modified MSS component score for color in the CTG group was n=9 (39.1%) scored zero, n=10 (43.5%) scored one, n=4 (17.4%) scored two (Figure 7). The differences were not statistically significant as seen with Fisher’s exact test, \( p=0.854 \).

Not unlike the results for color, the results for contour between the two groups were very similar. The modified MSS component score for contour in the FGG group was as follows: n=9 (39.1%) scored zero, n=14 (60.9%) scored one, n=0 (0%) scored two. In the CTG group, the component score for contour was n=7 (30.4%) scored zero, n=16 (69.6%) scored one, n=0 (0%) scored two (Figure 8). Once again, the differences were not statistically significant, \( p=0.758 \).

The third component of the modified MSS score, distortion, had the same score in each of the FGG and CTG group. For distortion n=10 (43.5%) scored zero, n=12 (52.2%) scored one, n=1 (0.04%) scored two (Figure 9).
Amongst the FGGs, 3/23 (13%) had an overall score zero while 4/23 (17%) of CTGs had an overall score zero.

### 4.3 Scar formation and age

Figure 10 is a comparison of the differences in scar formation amongst subjects who were aged 60 or less (n=26) and those more than 60 years (n=20). In the younger age group six subjects (23%) had an overall modified MSS score of zero. In contrast, the older group had just one subject (5%) with a score of zero (p 0.146). Figures 11, 12, and 13 illustrate that the difference between these two groups of younger and older subjects is due mainly to the distortion scores. Amongst the subjects aged 60 of less, 14 (54%) scored zero for distortion while just six (30%) of those aged more than 60 years scored zero (p 0.115).

### 4.4 Scar formation and elapsed time since wounding

Within the study population, there was a range of times from eight to 155 months between wounding and scar scoring. The mean time was 42 months. The scars were divided into three groups of elapsed times. These were defined as early (8-24 months), mid (25-60 months), and late (> 60 months). The number of subjects in each group were as follows: early = 22, mid = 15, and late = nine. The results of this comparison are shown in Figure 14. Within the early group, two subjects (9%) scored zero. In the mid group, two subjects (13%) scored zero, and in the late group, three subjects (33%) scored zero. Such, there was a greater proportion of subjects in the late group who scored zero than in the early and mid elapsed time groups. Figures 15-17 show the modified MSS component scores. For color (Figure 15), the late elapsed time group had 6 scars (67%) score zero, while there were eight (36%) and three (20%) scars that
scored zero for the early and mid groups respectively. In reference to contour 17 (77%) of the early elapsed time group scored one, and just five (23%) scored zero (Figure 16). The mid group showed a similar trend, nine (60%) scored one and six (40%) scored zero. In comparison to the early and mid groups, the late group showed a reverse trend with four (44%) scoring one, and five (56%) scoring zero.

4.5 Scar formation and gender

The modified MSS component scores were compared between males and females. There were an unequal number of sites in these groups (females n= 32, males n= 14) and therefore the scores are expressed as both a raw score and a percentage of the total in the group.

For color, males had six (43%) score zero, six (43%) score one, and two (14%) score two. The numbers were very similar for females and as follows: 11 (34%) scored zero, 16 (50%) scored one, and five (16%) score two (Figure 18).

With respect to contour, males were evenly distributed between zero and one. Seven (50%) scored zero and seven (50%) scored one. Females, on the other hand scored mainly one, n=24 (75%). The remaining scars, n=9 (25%) scored zero (Figure 19).

The modified MSS component score for distortion was fairly similar between males and females with a p value nearing 1.0. Amongst males eight (57%) scored zero, five (36%) scored one, and one (0.07%) scored two. In females, 12 (38%) scored zero, eight (25%) scored one, and one (3%) scored two (Figure 20).
A sample of scars that were scored is presented in Figure 21. In addition to the scored photograph of each scar in Figure 21, there are surgical and post-operative photographs of each. They show a considerable amount of variation in surgical technique.

Figure 22 is also a sample of scars that were scored. Each scar in Figure 22 is shown alongside a pre-surgical, surgical, and post-operative photograph. The scars in this figure represent the significant variation in individual healing response that was observed.
Figure 2 Age of study population

<table>
<thead>
<tr>
<th>Age Group</th>
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<tr>
<td>15-20 years</td>
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</tr>
<tr>
<td>21-35 years</td>
<td>27%</td>
</tr>
<tr>
<td>36-50 years</td>
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<td>51-65 years</td>
<td>5%</td>
</tr>
<tr>
<td>66-78 years</td>
<td>19%</td>
</tr>
</tbody>
</table>
Figure 3 Sample of sites - color
Figure 4 Sample of sites – contour
Figure 5 Sample of sites – distortion
Figure 6 A comparison of scar formation between FGG and CTG donor sites

Figure 7 A comparison of scar formation (color) between FGG and CTG donor sites. $p = 0.854$
Figure 8 A comparison of scar formation (contour) between FGG and CTG donor sites. *p* 0.758

Figure 9 A comparison of scar formation (distortion) between FGG and CTG donor sites. *p* 1.000
Figure 10 A comparison of scar formation and subject age at time of wounding. *p* 0.146

Figure 11 A comparison of scar formation (color) and subject age at time of wounding. *p* 0.429
Figure 12 A comparison of scar formation (contour) and subject age at time of wounding. $p \ 1.000$

Figure 13 A comparison of scar formation (distortion) and subject age at time of wounding. $p \ 0.115$
Figure 14 A comparison of scar formation and elapsed time since wounding. $p = 0.130$

Figure 15 A comparison of scar formation (color) and elapsed time since wounding. $p = 0.197$
Figure 16 A comparison of scar formation (contour) and elapsed time since wounding, $p = 0.168$

Figure 17 A comparison of scar formation (distortion) and elapsed time since wounding, $p = 0.976$
Figure 18 A comparison of scar formation (color) between males and females. $p = 0.971$

Figure 19 A comparison of scar formation (contour) between females and males. $p = 0.182$
A comparison of scar formation (distortion) between females and males.

Figure 20 A comparison of scar formation (distortion) between females and males. $p \approx 0.999$
Figure 21 Variation in surgical technique amongst FGG (F1 and F2) and CTG (C1 and C2) sites.
Figure 22 Variation in healing response amongst FGG (examples A and B) and CTG (examples C and D) sites
Chapter 5: Discussion

The impression by clinicians is that the palatal mucosa heals virtually without scars. However, this has not been quantified previously. The mechanism of scar formation in the human palatal mucosa is poorly understood, and it was hypothesized that there is increased scar formation of the palatal mucosa following a CTG harvest (deep wound) than a FGG harvest (superficial wound). The results of this study demonstrated that scar formation in CTG harvest sites is not greater than that of FGG sites. These results were contrary to what was expected, but similar to other reports of scarless healing in the palatal mucosa (Wong et al., 2009). While there were some sites with scarless healing (FGG 17%, CTG 13%), the majority of sites exhibited minor scar formation. This study found that scar formation was independent of harvest type and gender. However, there were weak positive correlations between younger age at time of wounding and scarless healing, as well as an increased elapsed time since wounding and scarless healing.

There are very few studies that compare the type of scar formation in the palatal mucosa to the depth of wounding, and this study is one of the first in the field. A critical depth of wounding to induce scar formation is not known (Dunkin et al., 2007). There is some evidence to support the notion that dermal wounds extending beyond the superficial papillary dermis, and into the reticular dermis, result in scarring (Dunkin et al., 2007). An earlier study (Wong et al., 2009) reported scarless healing of the human palatal mucosa. However, upon further review of the study results it was noted that the investigator had found minimal scar formation and not true scarless healing. In the referenced study, subjects had standardized full thickness excisional wounds from the palate that were approximately 12mm long and 2mm wide. The modified MSS
score was reported as a mean value of 0.8 ± 0.2 from the healed sites. Mean scores were not chosen as the most accurate method to report the findings of this study, however it can be noted that the mode modified MSS score in this study was 2 (Figure 6). There was a definite increased score observed in the current study in comparison to Wong’s and this may be due to the generally larger size of the wounds in the current study. While they were not of standardized size, it can be observed from the photographs on Day 1 that most wounds in the current study were greater than 12mm in length. This assumption can be made based on the knowledge that the mesial distal width of a typical maxillary molar is 10.5 mm (Fekonja, 2013) and most of the incisions were far larger than the width of a single molar. A follow-up to Wong’s study, using a similar design, was undertaken (Mak, 2009). Palatal mucosal wounds were created in red Duroc pigs, but with a much larger wound area; 27mm long and 15mm wide. Despite the larger wounds, the modified MSS mean score in Mak’s follow-up study was 1 ± 0.2. It is noteworthy that Mak’s study was carried out in pigs and while pigs are a very good model for wound healing, they have some dissimilar skin characteristics from humans (Sullivan et al., 2001). For example, pig skin has a less developed subepidermal plexus, which supplies adenexal structures (Forbes, 1969).

The virtually scarless healing observed in this study is likely the result of various differences between the palatal mucosa and skin. As mentioned earlier, the palatal mucosa contains fibroblasts derived from neural crest cells (Breau et al., 2008). The phenotype of oral mucosal fibroblasts is similar to that of fetal-type fibroblasts, which enable scarless healing in fetal skin (Enoch et al., 2010). Therefore, it is possible that the palatal mucosa heals in a fetal-like way as a result of its fibroblast composition.
Oral mucosa has a different composition of pericellular ECM than does skin. There is increased hyaluronan and tenasin-C in the oral mucosa. Hyaluronan is thought to play a role in reduction of scar formation by indirectly increasing the production of type III collagen and TGF-β3 (Raoudi and Tranchepain, 2008).

In addition, the inflammatory response observed in oral wounds is milder when compared to that in skin. There are fewer neutrophils, mast cells, macrophages, and T-cells in the inflammatory response within the oral mucosa, and subsequently less cytokine expression (Szpaderska et al., 2003). This allows faster re-epithelialization of the oral mucosa following wounding. It has been found that the basal expression of many genes that affect wound healing is higher even in unwounded oral mucosa compared to skin (Chen et al., 2010). This would imply that the cells of the oral mucosa are actually primed for quicker healing response. There is speculation that fast wound healing in both the skin and mucosa is an evolutionary advantage that has been naturally selected for over time (Ferguson and O'Kane, 2004). The presence of saliva that contains growth factors EGF, TFG-β and IGF (Häkkinen et al., 2000; Loo et al., 2010) is yet another reason why oral palatal mucosa heals with minimal scar formation.

This study was retrospective in design and that proved to be a limitation. There was a significant variation in surgery techniques within the two groups (FGG and CTG). In reference to Figure 21, F1 and F2 show FGG harvest sites. F1 is a relatively invasive harvest technique and heals with scarring and an overall modified MSS score of four. The scar is lighter in color compared to the surrounding tissue (score one), proud (score one), and severely distorted (score two). In comparison, F2 is a more superficial harvest and heals with just a slight color mismatch (score
one) and therefore an overall modified MSS score of one. In an attempt to determine a critical depth of wounding, it would be difficult to extrapolate findings from one individual to another as a similar depth wound in one person may penetrate to a deeper level of the mucosa than in another. The thickness of the palatal mucosa in humans is highly variable and may range from 1.8 to 5.6mm (Kim et al., 2013).

In Figure 21, C1 and C2 are CTG sites with a similar harvest technique as evident from the photographs on Day one. They show remarkably different healing at days seven and eight respectively, however the final scar formation is surprisingly similar. C1 has a small change in contour, being slightly proud (score one). C2 has a lighter color (score one), is slightly indented (score one), and also shows distortion, especially evident in the rugae (score one).

In addition to variation in surgical technique, there was also variation in individual healing response amongst individuals. In Figure 22, examples A and B show deep FGG harvest sites from two different subjects. Example A healed with scarring and changes in color (score two), contour (score one), and distortion (score one). On the other hand, example B healed with scarring, but only a change in color (score one). The site is flush with the surrounding tissue and shows no distortion.

In Figure 22, examples C and D are from the same subject, opposite sides of the palate. Note that the photographs in the study are all displayed as left side in order to improve standardization. This individual had two CTG harvests, which resulted in similar healing outcomes. Example C healed with no scar formation whereas example D shows no change in color (score zero) but is
slightly proud (score one) and has some distortion in the rugae area (score one). This individual’s degree of scar formation was similar at both sites.

Patient age at time of surgery, and the resultant scarring, was compared between a younger and older age group. There was a trend towards increased likelihood of scarless healing (modified MSS score zero) amongst subjects in the younger age category, aged 60 or less. The results of this study are somewhat confounding. While there is scientific evidence to indicate delayed healing in healthy older adults, this is thought to be a temporal delay and not a true impairment of the wound healing process (Keylock et al., 2008). This delay in healing in the aged population is thought to be the result of an altered inflammatory response consisting of delayed T-cell infiltration, altered chemokine expression, and reduced macrophage activity (Swift et al., 2001).

As mentioned earlier, there was also a slight correlation noted between longer elapsed time since wounding (i.e. greater than five years) and scarless healing. As all of the wounds scored in this study were more than seven months post wounding, and were therefore in the remodeling phase of wound healing. At this stage of wound healing, new ECM molecules are being formed as the poorly organized, collagen rich ECM formed during granulation tissue formation is broken down and/or remodeled (Singer and Clark, 1999). Proteolytic degradation mediated by MMPs was originally thought to be the main modifier of ECM reorganization (Toriseva and Kähäri, 2009), however it is now known to be influenced by wound cells such as macrophages and fibroblasts (Dieck and Codriansky, 2009), and even ECM molecules like decorin (Honardoust et al., 2008). While the timeline for this remodeling is not precisely quantified, it would seem from the results of this study that remodeling in scars may continue even after extended healing times.
The selected tools for the assessment of scars in this study, the modified MSS, was not without challenges. The scale was a useful tool for scar assessment, and while noted as one of the better scar scales for small scars (Fearmonti et al., 2010), it was still not ideal for the assessment of sites with minimal scarring. The scale has just three possible values for each parameter and for contour, a value of two was not observed. This would indicate a site with hypertrophic scarring and there were no sites that showed this. It is possible, that more subtle changes in contour were not accounted for because of the rather broad categories within the modified MSS.

The broad categories within the modified MSS made it easy to use and likely contributed to the 98% agreement between evaluators. As many of the scars were small, they were more easily appreciated and compared in an enlarged photograph than in the clinical setting. Figures 3, 4, and 5 are examples of each parameter with photographs from the study of scars of each value. Identifying color change was sometimes difficult from the photographs as there was reflection on the tissue from the flash, however both contour and distortion were easily discernible from the photographs. The accuracy of a clinical scar scale being applied to photographs has been questioned (Simons et al., 2013), but as there is currently no standardized scar scale, the modified MSS seems well-suited for this purpose.
Clinical healing occurred with scar formation in the palatal mucosa of the majority of the individuals in this study. The donor side morbidity was minimal for both types of grafts, FGGs and CTGs and while the scarring was minimal, it was not scarless. The palatal gingiva appears to heal via repair and not regeneration. Individual variation seems to dictate the outcome of palatal scar formation more than any other known factor.

A prospective study with a larger sample size would be a valuable tool in the assessment of scar formation in palatal mucosa. Wounds of a standardized size and depth with accompanying histologic analysis would help to correlate depth of wound (millimeters) with anatomical depth (tissue type). Measuring the palatal mucosa thickness of each subject prior to the graft harvest and also following healing would also be valuable information.

A broader understanding of the wound healing process in human palatal mucosa may prove beneficial in the development of treatment strategies. This increased knowledge may affect both the timing of treatment (i.e. embryonic surgery) and also the modality of treatment. Specific modalities may involve mesenchymal stem cells from gingiva for transfer to other areas of the body, such as the skin, for use as a treatment modality to reduce scarring (Fournier et al., 2013).
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