Aberrant mucosal wound repair in the absence of secretory leukocyte protease inhibitor

Nikola Angelov1*, Niki Moutsopoulos1*, Moon-Jin Jeong2, Salvador Nares1, Gillian Ashcroft3, Sharon M. Wahl1

1Oral Infection and Immunity Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland, USA
2Current address: Department of Oral Histology, College of Dentistry, Chosun University, Gwang-ju, Korea
3Current address: School of Biological Sciences, University of Manchester, Manchester, England

Summary
Secretory leukocyte protease inhibitor (SLPI) is a cationic serine protease inhibitor with anti-microbial and anti-inflammatory properties found in large quantities in mucosal fluids, including saliva. SLPI is expressed during cutaneous wound healing, however, its role in oral wound repair is unknown. We have used a novel approach involving a murine buccal mucosal acute wound model to investigate the role of SLPI in oral healing. In parallel to the observed cutaneous healing phenotype, an absence of SLPI results in markedly impaired oral wound healing associated with increased inflammation and raised elastase activity. Moreover, matrix deposition was decreased, while MMP activity was enhanced in the oral SLPI null wounds suggesting deregulated proteolysis. Intriguingly, regardless of genotype, reduced collagen deposition was observed in oral compared to dermal wounds, associated with reduced TGF-β expression and decreased fibroblast collagen expression in vitro. We propose that SLPI is a pivotal endogenous factor necessary for optimal tissue repair including intra-oral wound healing. In addition, our model provides a unique opportunity to delineate the cellular and molecular mechanisms underlying the differences between dermal scarring and oral scar-free healing.

Keywords
Wound healing, oral, mucosa, cutaneous, inflammation, SLPI

Introduction
Secretory leukocyte protease inhibitor (SLPI) is a small, highly basic, acid stable protein, found in a variety of mucosal fluids, including the saliva (1). SLPI is produced by various epithelia, and possibly by neutrophils and mast cells (2), as well as by macrophages in rodents (3-6). SLPI inhibits serine proteinases, including proteinases from neutrophils, pancreatic acinar cells and mast cells (2). This 11.7 kDa non-glycosylated serine proteinase inhibitor is composed of two highly homologous cysteine-rich domains, and the C-terminal domain contains the elastase-inhibitory activity (7). In addition to its anti-elastase activity, SLPI exhibits antimicrobial activities that, in conjunction with other well-characterized proteins such as lysozyme and lactoferrin, are thought to play a critical role in mucosal defense (8, 9). Physiologic concentrations of SLPI protect adherent monocytes and activated peripheral blood mononuclear cells against HIV-1 infection (10, 11). Moreover, SLPI possesses anti-bacterial and anti-fungal properties (8, 9), which come into play in the context of an open healing wound. During acute inflammatory reactions, in which tissue is degraded and remodeled, endogenous generation of SLPI becomes an impor-
tant regulator of the inflammatory response by suppressing NFκB activation with resultant inhibition of peptide mediators/cytokines and chemokines (3, 5, 12).

Potentially the most essential role of SLPI, in the context of wound repair, relates to its ability to inhibit serine proteases. The role of excess neutrophil elastase in degradation of functional and structural proteins, such as collagen and fibronectin (13-15), as seen in impaired cutaneous wounds, led to the investigation of SLPI’s importance during the healing process. Mice deficient in SLPI have delayed healing of cutaneous wounds, characterized by an increased and prolonged inflammatory response, enhanced elastase activity, and reduced matrix accumulation (3, 6). This previously demonstrated role of SLPI in dermal wound healing prompted us to evaluate the role of SLPI in the healing of the oral mucosa where this proteinase inhibitor is indigenous.

The oral mucosa generally heals at a rapid rate and with minimal scar formation compared to skin (16, 17). Additionally, adverse healing sequelae such as keloid formation or chronic wounds are very rare in the oral epithelium (16). This increased healing potential of the oral mucosa has been well established, but the mechanisms guiding it are not fully understood. We hypothesize that the presence of high levels of SLPI in saliva may contribute to the superior healing seen in the oral cavity. Identification of unique aspects of oral epithelial wound repair may provide insight into developing approaches to accelerate healing and/or minimize scarring in other tissues. To this end, we have created a model to study oral wound healing events, and investigate the role of SLPI in this process.

**Materials and methods**

**Oral and dermal wounding protocol**

Six- to eight-week-old male wildtype and SLPI null mice (3) were anesthetized with inhaled Metofane-methoxylurane (Schering-Plough Animal Health Corp, Kenilworth, NJ). Oral wounds were created in the buccal mucosa with a 1.5 mm dermal biopsy punch (Miltex Instrument Company, Inc. Bethpage, NY) without perforation of the dermis, and allowed to heal by secondary intention. Standardization of wound depth was achieved by insertion of the dermal biopsy punch approximately 2 mm, as measured by a dental probe (Hu-Friedy, Chicago, IL). The wounds were marked by local application of a black tissue dye (nontoxic-Black Margin Marker; StatLab Medical Products, Inc., Lewisville, TX), unless the wounds were to be used for immunohistochemical analysis. Tissue samples were harvested on days 1-7 post-wounding with application of a 3-4 mm biopsy punch incorporating the oral wound area and consecutive excision through the dermis (Fig. 1A). Wound tissues were bisected and samples were placed in Z-fix (Anatech Ltd, Battle Creek, MI) for histology, in 6 well plates with fibroblast special media for fibroblast isolation (10% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, 0.25 µg/mL Fungizone, 2 mM L-glutamine, 5 mg/mL Gentamycin in Dulbeco’s Modified Eagles Medium (DMEM) (BioWhittaker, Walkersville, MD), or snap-frozen on dry ice for RNA isolation and protein extraction.

In parallel, in some experiments, the dorsum was shaved and cleaned with alcohol. Four equidistant 1 cm full-thickness incisional wounds were created through the skin and panniculus carnosus muscle, and allowed to heal by secondary intention (3); wounds were subsequently harvested on days 1, 2, 3 and 5 postwounding. Harvested tissue was divided for histology, RNA extraction or fibroblast isolation.

**Histology, immunohistochemistry and wound quantitation**

Tissue samples were fixed in Z-Fix for 48 hours, placed into cassettes, washed in running tap water for 20 minutes and processed in an Automatic Tissue Processor (Sakura Finetek USA Inc., Torrance, CA). Tissues were then embedded in paraffin, sectioned at 5 microns, placed on positively charged slides and dried overnight at 37°C. Sections were stained with hematoxylin and eosin (H&E), Masson’s-Trichrome or Combined Eosinophil/Mast (CEM) Cell Stain (American Master Tech Scientific, Inc., Lodi, CA).

For characterization of cellular infiltrates, deparaffinized tissue sections were incubated overnight with a 1:30 dilution of rat anti-mouse Mac-3 (macrophage) monoclonal antibody (BD PharMingen, San Diego, CA) or rat anti-mouse Ly-6G (neutrophil) (BD PharMingen) at 4°C. Additional sections were stained with a 1:50 dilution of rabbit polyclonal antibody to the active form of TGF-β1 (Promega, Madison, WI). The sections were then washed and incubated for 30 minutes with biotin labeled rabbit anti-rat secondary antibody (1:300; Vector Lab, Burlingame, CA) or with biotin labeled goat anti-rabbit secondary antibody (Zymed, San Francisco, CA). All sections were treated with streptavidin peroxidase conjugate (Histostain Plus, Zymed) for 15 minutes, and then washed. Subsequently, Liquid DAB Substrate (Zymed) was applied for 15 minutes, the sections washed and coverslipped.

Image analysis and quantification of oral wound areas were performed using Metamorph 4.6 imaging system (Universal Imaging Corporation, Downingtown, PA) as described (3). Briefly, the imaging program determined wound areas following manual drawing of the wound margins below the neo-epithelium or clot, down the edge of the wound margin and around the perimeter of the wound which included the granulation tissue and infiltrate.

Quantification of the inflammatory cell infiltrate was performed on CEM stained sections and analyzed using the Metamorph 4.6 imaging system (Universal Imaging Corporation) as described (3). Five nonoverlapping images of each wound (n=4) were taken with a digital camera (DXM 1200,
After collecting the wound tissue under sterile conditions, wound explants were placed in 6-well culture dishes (Costar, Corning Inc., Corning, NY) to enable fibroblast outgrowth. Fibroblasts were cultured until confluent, then washed and trypsinized. Cells were re-suspended in fibroblast media (10^4/ml), cultured in 4-chamber slides (Nalge Nunc, Lab-Tek, Naperville, IL) for 72 hours and stained with DiffQuick (Baxter Healthcare Corp, Miami, FL) for cell morphology and Picro-Sirius Red (Aldrich Chem. Co, St Louis, MO) to characterize matrix synthesis (18). Five images of each chamber were taken and Picro-Sirius Red positive cells were counted.

**RNase protection assays**

Total tissue RNA was extracted using the standard TRIzol (Invitrogen, Carlsbad, CA) procedure (19). Total RNA (3µg) was used with the mCK-3b template of the Riboquant Multi-Probe RPA system (BD PharMingen). Band densities were normalized to the GAPDH housekeeping gene using ImageQuant (Molecular Dynamics, Amersham Biosciences, Piscataway, NJ).

**Real-time PCR**

To monitor expression of matrix proteins using real-time reverse transcription/polymerase chain reaction (RT-PCR), oral wound tissue RNA was extracted using TRIzol. Primers were designed for collagen I and fibronectin using Ensemble Genome Browser (www.ensemble.org) and for GAPDH (20). Forward and reverse oligonucleotide primers are listed in Table 1.

RNA was digested with RNase-free DNase (Clontech, Palo Alto, CA), quantified using SybrGreen Reagent (Molecular Probes, Eugene, OR) and visualized by gel electrophoresis and SybrGold (Molecular Probes) to assess integrity. DNase treated RNA (200ng/sample) was reverse transcribed into cDNA using 250 ng oligo-dT (Invitrogen) and 200 U MMLV reverse transcriptase (Promega). Real-time PCR reactions were performed simultaneously for all samples using the Smart Cycler II (Cepheid, Sunnyvale, CA) and a reaction volume of 25 µl containing Omnimix HS beads (Cepheid), 0.25X SybrGreen, 3 µM of each primer and 2 µl of cDNA. Initial denaturation was carried out at 95°C for 150 sec, followed by 45 cycles of denaturation at 95°C for 15 sec, annealing at 55°C for 15 sec, and elongation at 72°C for 10 sec followed by melt curve analysis from 60-95°C at 0.2°C/second with continuous fluorescence detection.

**Matrix metalloproteinase (MMP) zymography**

Gelatin zymography was used to assess the pattern of MMP2 and MMP9 activity. Protein extraction was carried out on fresh-frozen samples from days 0 to 5 post-wounding. Homogenization of samples in 1 ml homogenization buffer (HB: 6 M urea, 100 mM Tris/HCl pH 7.5, 15 mM CaCl₂, 0.25% Triton X-100) was followed by centrifugation at 14,000 rpm for 20 min at 4°C and the supernatant protein determined (21). Pooled samples (n=4) were applied to zymogram gels (Novex, Invitrogen, Carlsbad, CA) and each lane was loaded with a volume equivalent to 10 µg protein. Separate lanes were loaded with conditioned media from HT1080 human fibrosarcoma cells, as a positive control for latent MMPs (22). Following electrophoresis, gels were incubated in zymogram renaturing buffer at room temperature for 30 min and then incubated in zymogram developing buffer (Novex) to equilibrate for 30 min. Subsequently, the gels were incubated overnight at 37°C in developing buffer, stained with 0.5% Coomassie brilliant blue for 1 hr and destained for 30 min in gel fix (10% acetic acid, 40% methanol, in distilled water). Areas of proteinase activity appeared as clear zones against a dark background, at approximately 84 kDa for the active form of MMP9 (23) and 62 kDa for the active form of MMP2 (24). Image analysis was performed using the Alpha Imager 2200 (Alpha Inotech Corporation, San Leandro, CA).

**Elastase activity**

Frozen tissue was extracted with homogenization buffer (HB). For detection of elastase activity in the wound tissue, total protein (30 µg) in 140 µL assay buffer (100 mM Tris pH 8.3, 0.96 M NaCl) was incubated with substrate (0.5mM methoxy succinyl-ala-ala-pro-val-p-nitroanilide, Sigma Aldrich) for up to 2 hours. The reaction was stopped by addition of 1M HCl, and the absorbance was measured at 412 nm. The data were expressed as units of elastase activity per gram of tissue, based on the standard curve of elastase activity.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen I</td>
<td>5'-GAAGAATACGTATCCACCAA-3'</td>
<td>5'-CATGGGCGCATCATAGCCAT-3'</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>5'-CTTTTCAGATAATGGTGC-3'</td>
<td>5'-AACACTGTCAGGGAGCAAAG-3'</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5'-TCACCAACCATGGAGAAGGC-3'</td>
<td>5'-GCTAAGCATTGCGTGTC-3'</td>
</tr>
</tbody>
</table>

Table 1: Primer sequences.
to 2 hours at 37°C, with neutrophil elastase (15 nM, Calbiochem, La Jolla, CA) as a positive control. Substrate degradation was determined by measuring OD$_{405}$ (Dynatech Lab. MRX, Nashville, TN). A standard curve for degradation was prepared from the elastase positive control. Results are expressed as nM of elastase activity in 30 µg protein extract.

**Statistical analysis**
Statistical differences were determined by use of a t-test or ANOVA. A P-value of <0.05 was considered significant.

**Results**

**Effect of SLPI depletion on oral mucosal wound healing**

We established a buccal mucosal punch biopsy model (Fig. 1A) to investigate and define oral wound healing events and determine whether SLPI is critical in this process. Following the initial tissue injury in the oral mucosa, we observed an impaired rate of healing in the SLPI null mouse, compared to the wild-type littermates monitored in parallel (Fig. 1B). Macroscopic differences were apparent on days 1 and 3 in the oral tissues, but beyond day 3 in the WT and day 5 in the null animals, the lesions could not be located nor identified without the use of a tissue dye, due to rapid re-epithelialization. Although this pattern of delayed healing was reflective of aberrant cutaneous healing in SLPI deficient animals (3), the healing process in the oral mucosa, particularly re-surfacing of the epithelium, occurs more rapidly with less scarring than evident in the skin.

Microscopic examination confirmed the delayed healing response in the oral null wounds. The absorption of the tissue dye at the oral wound edges by macrophages and other cells ensured accurate microscopic localization and measurement of wound size (Fig. 1B). As early as day 1, SLPI null oral wounds appear to be larger and less mature compared to those of the WT littermates (Fig. 1B). The most pronounced differences were seen on day 3 when WT wounds appeared completely re-epithelialized (Fig. 1B), whereas the process remained incomplete in the null mice. By histomorphometric analysis, significantly larger oral wounds were present in the null animals on days 1, 3 and 5 post-wounding (Fig. 2A).

By quantifying cells in the wound bed, the number of recruited inflammatory cells was significantly increased in the null wounds, coinciding with the most pronounced differences in overall healing (Fig. 2B). In the wildtype mucosa, the number of inflammatory cells was maximal within the first 24 hours, declining ~70% by 5 days after injury, with statistically significant elevations in the number of recruited cells in the null mucosa from 2-5 days after injury (Fig. 2B). By immunohistochemical staining, the cellular infiltrates were characterized as predominantly neutrophils (Ly6G⁺) (Fig. 2C, D) and macrophages (Mac3⁺) (Fig. 2E, F). As evident in Figure 2, both populations were augmented in the null mucosa compared to the wildtype oral wounds within 24 hours after injury, implicating involvement of SLPI in inflammatory cell recruitment and/or accumulation. Although recruitment of inflammatory cells is key in the process of healing, excess or perpetuation of inflammation may lead to delayed or impaired healing resulting from degradation of newly forming matrix essential for restoration of

![Figure 1: Impaired healing in SLPI null mice oral wounds.](https://example.com/figure1.png)

A). Oral wounding protocol: 1. Use of a 1.5mm punch biopsy for initial wounding. 2. Oral buccal mucosal wound site immediately after wounding. 3. Wound area marked with tissue dye; day 3. 4. Excised wound using a 3 mm punch through the dermis. B). Hematoxylin-Eosin (original magnification 10X) staining of oral wound tissue sections, wildtype (WT) and SLPI null, harvested at different time points after wounding (day 1, 3 and 5). Arrows indicate the wound margins. Black tissue dye used for marking the oral wounds is observed in the wounded tissue.
Reduced matrix deposition due to increased proteolytic activity in the null wounds

Increased numbers of inflammatory cells are often associated with increased proteolytic activity, reduced matrix accumulation, and delayed healing (3). In concordance with the enhanced inflammatory cell infiltrate, the serine protease, elastase, previously associated with matrix degradation and impaired healing (3, 25, 26), was significantly increased in injured null tissues within 3 days after wounding (Fig. 3A). Because enhanced MMP activity has also been connected with delayed cutaneous healing, we investigated MMP activity in oral wounds using zymography (Fig. 3B). Precursor/inactive MMP9 was minimally detected in unwounded mucosa, but substantially increased in both wildtype and null oral wounds within 24 hr after injury. The active form of the enzyme (84 kDa) was significantly elevated in wounds of the null mice compared to the wildtype, as shown for day 1 and 3 (Fig. 3B, C). Pro- and active MMP2 were constitutively expressed at low levels in the oral mucosa, and limited upregulation was observed (Fig. 3B, D) in the wildtype littermates. In the SLPI null mice, MMP2 levels increased significantly in day 3 and 5 wounds.

Matrix deposition in mucosal wounds

The increased inflammation and attendant proteolytic activity in the wound sites were associated with reduced matrix and delayed healing. Based on intensity of Trichrome staining which reflects deposition of collagen (27), a decrease in matrix is most apparent on day 3 in SLPI null wounds (Fig. 4A, B), coinciding with delayed re-epithelialization, augmented wound area, and inflammation. To determine whether the delayed accumulation of matrix was the result of increased degradation and/or decreased matrix synthesis, we obtained mRNA from the oral wound tissues and assessed collagen type I and fibronectin expression by real time PCR (Fig. 4C). As shown for day 3 post-wounding, collagen type 1 mRNA in the SLPI null mucosal wounds was decreased more than two fold compared to parallel tissues from the wildtype mice. Intriguingly, SLPI null wounds exhibited enhanced expression of fibronectin mRNA, suggesting an earlier (i.e. delayed) stage of matrix generation and the healing process (28).

Increased matrix production by dermal fibroblasts

To further dissect cellular mechanisms responsible for impaired healing in the null wounds as well as differences between oral and cutaneous wound healing, we examined matrix production by cultured fibroblasts obtained from control, day 1, 3 and 5 skin and mucosal wound tissue as assessed by Picro-Sirius Red staining (18). Significantly increased numbers of dermal fibro-
blasts were Picro-Sirius Red positive (Fig. 4D), compared to the oral fibroblast populations (p<0.05), consistent with increased fibrosis observed in the skin after wounding. Oral and dermal fibroblasts from uninjured tissue were comparable in staining, revealing differences only in response to injury. The failure to detect differences in matrix between wildtype and null oral fibroblasts suggests that the reduced matrix in the null wound site may result from enhanced inflammation, proteolytic activity and/or delayed influx of matrix-generating fibroblasts.

Expression of TGF-β isoforms in oral and cutaneous wounds

The presence of an increased inflammatory cell infiltrate in the null wounds prompted us to determine whether increased expression of chemoattractants, such as TGF-β (29, 30) might be involved. Furthermore, TGF-β regulates matrix production in vivo and in vitro (31) and increased expression of active TGF-β during wound healing has been documented in the SLPI deficient dermal model (3).

No significant differences in mRNA expression of any of the three TGF-β isoforms were observed between the null and
wildtype oral wounds (Fig. 5A). This lack of difference in mRNA expression was also observed in SLPI-deficient cutaneous wounds in which an increase in post-translationally activated TGF-β was identified (3). Immunohistochemical staining for active TGF-β1 in the oral mucosal lesions revealed evidence of an increase in active TGF-β1 in mice lacking SLPI (Fig. 5B), implicating TGF-β1 as a contributor to the local accumulation of inflammatory cells. Further comparison between the oral and skin wounds revealed a difference in TGF-β1 mRNA expression between the two tissue sites (Fig. 5C). Whereas the levels of TGF-β1 mRNA expression were low and remained relatively unchanged during 7 days of evaluation after wounding in the oral site, an increase occurred during this same interval in the cutaneous tissues. Importantly, the levels achieved in the dermis were several fold higher than in the mucosa, consistent with the minimal scarring evident in mucosal healing. This observation that TGF-β1 mRNA is not up-regulated during oral wound healing may partially explain the reduced inflammatory response and accelerated healing present in the oral environment in contrast to the skin.

**Discussion**

Our results demonstrate the importance of SLPI in the wound healing process of the oral mucosa. Using a buccal mucosal punch biopsy model of oral wound healing, we were able to demonstrate a nonredundant role for SLPI in mucosal tissue repair. Absence of SLPI leads to delayed oral wound healing characterized by impaired re-epithelialization, delayed matrix deposition, increased and prolonged inflammatory response and enhanced elastase and MMP activity. Compared to the traditionally used palatal excisional wound (32), this model does not involve the underlying bone that may be exposed during palatal wounding leading to superficial bone necrosis and tertiary rather than secondary intention healing. Additionally, exposing the periosteum may cause increased discomfort and reduced nutritional intake. Mucosal wounds can be more accurately compared to dermal wounds in that wound contraction, a critical event of dermal healing occurs in the buccal mucosa, but not in the attached palatal tissue. Occasionally, investigators have used a tongue biopsy model (33), due to the accessibility of this...
site and the ability of the wound to contract in this area. Despite these features, tongue wounds involve a specialized epithelium, as well as the increased possibility of bleeding sequelae that may complicate the healing process.

The features of impaired healing in the SLPI null mucosal wounds reflect the abnormalities demonstrated in the cutaneous SLPI null wounds (3), but become of increased interest in the oral cavity where numerous factors could potentially compensate for the absence of SLPI. The increased vascularity of the oral mucosa, the anti-microbial factors present in saliva (9-11, 34), the presence of distinct fibroblast populations (35) as well as the differential expression of pro-inflammatory mediators and growth factors have been associated with the improved healing of the oral mucosa. Intraoral fibroblasts exhibit a “fetal phenotype” in three-dimensional extracellular lattices (36, 37), characterized by their preferential ability to reorganize type I collagen lattices (16) and repopulate in vitro wounds (38), when compared with extraoral wounds. Oral mucosal fibroblasts have selective differences in cellular behavior and in their responses to growth factors compared to dermal fibroblasts, showing higher proliferation rate, earlier collagen gel contraction with or without TGF-β1 stimulation, and increased collagen synthesis when stimulated with TGF-β1, which may contribute to the differences in wound healing (39). Certain factors, like IL-1 have been proven to have a critical role in oral versus dermal healing, by protecting the open wound from bacterial insult (40). In a hamster oral wound healing model, salivary EGF was shown to regulate eosinophil-derived TGF-α expression, leading to rapid healing of the oral wounds in comparison to their cutaneous counterparts (41). Recently, differential expression of FiRE (fibroblast-growth factor inducible response element) (35) was associated with oral wound healing. The fact that the multiple

Figure 5: Expression of TGF-β isoforms in oral and cutaneous wounds. Total tissue RNA from oral and dermal wounds was used to determine TGF-β mRNA expression by RPA. Band densities were normalized to the GAPDH housekeeping gene. A) TGF-β isoform expression in wildtype and SLPI null oral wounds on day 1 post-wounding (representative experiment from pooled RNA). B) Tissues were stained with an antibody which recognizes the active form of TGF-β1 (day 1 post wounding shown, original magnification 10×). C) TGF-β1 mRNA expression in wildtype and SLPI null mucosa and skin harvested at different time points after wounding. TGF-β1 expression increased in skin wound samples, compared to the relatively stable TGF-β1 mRNA expression in oral wounds.
factors present in the oral environment were not capable of counteracting the absence of SLPI indicates a central role for this molecule in the healing events of the oral mucosa.

In addition to the well-documented factors present in the oral cavity that may contribute to the improved healing, we identified differences in the expression of TGF-β1. TGF-β1 is a potent chemoattractant for inflammatory cells and fibroblasts and has been shown to stimulate collagen formation and inhibit MMP production (29, 31). Although limited increases in TGF-β1 mRNA were evident, increased expression of active TGF-β1 locally, in the absence of SLPI, may contribute to the early and sustained influx of inflammatory cells (3). The pool of available latent TGF-β, rapidly released at the site of injury by degranulating platelets and inflammatory cells, may also be increased secondarily to the elastase-mediated release of latent TGF-β binding protein from elastin fibers (42, 43). Inflammatory processes during oral wound healing are additionally exacerbated in the absence of SLPI, likely due in part to elastolytic release of elastin-derived peptides with chemoattractant properties for inflammatory cells, thus perpetuating the proteolytic milieu (44).

Delayed healing in the dermal wounds of SLPI null mice has been partially attributed to the increased inflammation elicited by the upregulation of TGF-β1 (3, 29). Conversely, the scarless repair observed in the oral wounds may be related to their relatively low expression of TGF-β1 compared to their cutaneous counterparts. This reduced expression of TGF-β1 is also consistent with the subsequent reduced production of matrix from oral fibroblasts. Scarless healing in fetal wounds has also been associated with minimal TGF-β1 in fetal compared to adult wounds (45), which remains unchanged during the healing process in fetal lesions (46), as we observe in the oral mucosa. Furthermore, the addition of TGF-β1 to fetal wounds has been shown to result in scarring (47), whereas the treatment of adult rat wounds with TGF-β1 neutralizing antibodies was able to reduce scar formation (48). Consistent with these observations, deletion of SMAD3, a key TGF-β signaling molecule, results in more rapid healing of dermal wounds (49).

Despite the accelerated and scarless healing of the oral mucosa, the mechanisms leading to impaired healing of the null wounds are shared between oral and cutaneous environments. The decreased matrix deposition and perpetuation of inflammation present in the SLPI null wounds is associated with increased elastase activity. Delayed wound-healing states have previously been associated with raised elastase levels and prolonged inflammation in chronic human wounds and acute wounds in elderly (25, 26). The absence of SLPI during the wound healing process led to raised elastase levels, associated with consecutive degradation of proteoglycans, collagen and fibronectin, structural and functional proteins normally deposited in the wounds (13, 15). Reduced matrix deposition as a result of enhanced elastase activity, as well as the additional activation of matrix metalloproteinases (MMPs) by elastase, or other pathways, contributes locally to amplified matrix cleavage (5).

Collectively, our observations support the critical nature of SLPI in the orchestration of inflammation and repair in the oral mucosa, reflecting previous studies in which SLPI was shown to be a dominant mediator of the healing process in the dermis (3, 6). Additionally, we demonstrate the crosstalk between SLPI and TGF-β, which may be instrumental in maintaining the balance necessary between inflammation and matrix deposition to restore tissue integrity. Based on our development and initial exploration of the oral buccal mucosal model, it will become possible to identify additional critical differences between mucosal and cutaneous healing parameters responsible for reduced scarring in mucosal sites, which may reveal potential therapeutic strategies in pathologic wound healing.

References
18. Murphy GI, Bicknell GR, Nicholson ML. The effect of combined rapamycin/cyclosporine on the changes in pro-fibrotic gene expression that occur during the development of allograft vasculopathy in rats, compared with cyclosporine or rapamycin in isolation. Transpl Int 2003; 16(5): 347-53.