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Transcriptional signature primes human oral mucosa for rapid wound healing

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Abstract

Oral mucosal wound healing has long been regarded as an ideal system of wound resolution. However, the intrinsic characteristics that mediate optimal healing at mucosal surfaces are poorly understood, particularly in humans. Here we present a unique comparative analysis between human oral and cutaneous wound healing using paired and sequential biopsies during the repair process. Utilizing molecular profiling, we determine that wound-activated transcriptional networks are present at basal state in the oral mucosa, priming the epithelium for wound repair. We show that oral mucosa wound-related networks control epithelial cell differentiation and regulate inflammatory responses, highlighting fundamental global mechanisms of repair and inflammatory responses in humans. The paired comparative analysis allowed for the identification of differentially expressed SOX2 and PITX1 transcriptional regulators in oral versus skin keratinocytes that confer a unique identity to oral keratinocytes. We show that SOX2 and PITX1 transcriptional function has the potential of reprogramming skin keratinocytes to acquire increased

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cell migration capability and improve wound resolution *in vivo*. Our data establishes a novel understanding of the biology of healing in human mucosal and cutaneous environments, and provides insights into therapeutic targeting of chronic and non-healing wounds.

INTRODUCTION

Improving wound healing resolution is becoming a major medical and social priority due to the drastic increase in traumatic injury, chronic wounds and scarring (1). While several studies characterize in detail the mechanisms and pathways altered in these deficient wounds, a different approach that defines factors involved in accelerated wound healing would allow for the identification of novel therapeutic targets to improve tissue repair. In this regard, oral wound healing, along with embryonic wound healing, have long been considered models of optimal wound resolution characterized by rapid and scarless wound healing (1–4). Dissecting the different molecular events that drive wound healing resolution in oral mucosa compared with those of the skin will help us define why these specific lesions heal more efficiently and translate those findings to treat deficient healing processes.

Cutaneous wound healing is well-documented, with the overall classic interpretation for the repair pathway having four systematic phases: hemostasis, inflammation, proliferation and remodeling (5). The molecular circuitries that drive these different phases of cutaneous repair have been characterized, while the unique environment of the oral cavity represents a different wound-healing paradigm that is still poorly understood. Oral wounds heal at an accelerated rate compared to cutaneous wounds, and *in vitro* or animal model studies have attributed this to a variety of mechanisms including differential inflammatory response, distinct modulation of stem cell, proliferative and differentiation programs, and more efficient epithelial remodeling (1, 3, 6). Despite this progress, the lack of detailed clinical studies in humans comparing oral and cutaneous wound healing *in vivo* has limited the advancement of our knowledge on the mechanisms mediating accelerated wound healing. To close this gap, we characterized the molecular and histological aspects of wound healing in paired samples of oral mucosa and the skin in healthy human subjects. Wounds were introduced in the oral buccal mucosa and the skin at the same time and sequentially biopsied for comparison at progressive time points. Our results emphasize how biologically the oral buccal mucosa constitutes a unique environment that is characterized by a radically different wound-healing program. We show that the oral cavity is primed for wound repair and that oral mucosa accelerated wound healing might be attributed to the ability of oral keratinocytes to limit epithelial cell differentiation and pro-inflammatory responses. We also present evidence that transcriptional networks established by functionally active transcription factors such as SOX2 and PITX1 mediate this phenotype and can be exploited to reprogram cutaneous keratinocytes to present oral keratinocyte features, including accelerated wound closure.

RESULTS

Wound-activated transcriptional networks present at basal state prime the oral mucosa for wound repair.

Utilizing paired and sequential biopsy samples during the healing process, we contrast oral mucosal with cutaneous healing to determine the differential regulation of these processes in the human setting (Fig. 1; Clinicaltrials.gov #NCT01078467, see Methods section for details on clinical study). Clinical screening was performed (Day 0) followed by baseline biopsies to create paired identical wounds in the oral buccal mucosa and skin (Day 1). Importantly, day 1 biopsies allowed for evaluation of homeostatic transcriptional profiles in human mucosal versus cutaneous surfaces. Follow-up biopsies of the wound area(s) were collected at day 3 (48 hours after first biopsy) and day 6 (120 hours after first biopsy) of healing in two different groups. This approach allowed for the evaluation of distinct phases during the physiologic process of human wound healing (Fig. 1).

Analysis of the healing time course revealed that oral wounds resolve significantly faster than skin wounds (Fig. 1C). This was observed following the first wound-inducing biopsy (3 mm wound, Fig. 1C, upper panel) and after the secondary biopsy of the wound area (5 mm wound, Fig. 1C, lower panel) (typical examples are shown in fig. S1, B and C). Oral wounds shrink within minutes of completing the biopsy, probably due to muscle contraction. This is nearly absent in the 5 mm biopsy, and yet both wounds showed similarly high healing rates (Fig. 1D). This suggested that the initial contraction of small wounds may not explain the accelerated healing rate of oral wounds. Histological analysis of the wound sections shows re-epithelialization of the oral mucosa wound at earlier time points when compared to the skin (Fig. 1E). Remarkably, as early as day 3, oral wounds are almost completely covered by squamous epithelium, even in the absence of stromal healing.

To molecularly characterize the human repair process, oral and skin biopsies were subjected to RNA-sequencing (RNA-Seq) (Fig. 2A). Unsupervised clustering analysis of the gene expression data provided three major observations: First, we found that the oral and skin samples cluster separately indicating distinct transcriptional identities, consistent with unique tissue microenvironments. Interestingly, even though there is a significant amount of differentially regulated genes between oral mucosa and skin during wound healing, most of these differences are already evident at starting basal conditions (day 1) (fig. S1). Secondly, we observe that within the skin samples there is clear separation between baseline biopsies and wound biopsies at days 3 and 6. In fact, an overall upregulation of gene activity in skin is evident during the healing process, indicating non-resolution of the skin wounds at the time points evaluated (Fig. 2A, skin). Finally, and in contrast to the skin, the oral day 3 biopsies separate from day 1 and 6, indicating wound healing activity at day 3 that resolves by day 6, signifying healing and return of gene expression to basal conditions by day 6 after wounding (Fig. 2A, oral).

Consistent with these observations, ANOVA analysis of the differential gene expression during wound healing in oral and skin revealed few significant gene expression changes during oral healing at day 3 (410) and none at day 6, while a large number of genes are differentially regulated during skin wound healing at both time points (Skin Day 3 / Day 1

(1473); Skin Day 6 / Day 1 (1836) (Fig. 2B). These results demonstrate a significantly enhanced transcriptional activity during skin wound healing but minimal differential transcriptional regulation in oral wounds, raising the possibility that the transcriptional regulatory networks responsible for the accelerated healing in oral mucosa are already present in the unwounded state.

To explore the intrinsic differences between oral mucosa and skin at baseline, we determined the significant differentially expressed genes between unwounded (day 1) oral mucosa and unwounded skin (table S1). Transcripts upregulated in the oral environment were consistent with increased keratinocyte activation and with heightened anti-microbial defenses (Day 1, Fig. 2C). Among the upregulated transcripts in the oral samples, we found genes described in wound- activated keratinocytes (7), including keratins 6 (K6) and 16 (K16), small proline rich (SPRR) and S100 proteins, defensins, serpins and annexins, among others (Fig. 2C). Strikingly, Ingenuity Pathway Analysis (IPA) showed that the top processes represented in the transcriptome of the oral mucosa were related to inflammatory skin disorders such as psoriasis, dermatitis and skin hyperplasia (Fig. 2D), conditions in which transcriptional networks resemble those of the wound-activated skin (8–11). This indicated that gene networks related to increased proliferation, migration and wound resolution were potentially already present in the oral mucosa at basal state. Since psoriasis has been shown to present a particular expression signature of genes, inflammatory cytokines and proteins related to wound healing, we utilized the gene list from the Psoriasis gene signature in IPA (Fig 2D) to perform an unsupervised clustering of our samples (Fig. 2E). Results showed that skin wound samples (days 3 and 6) cluster more closely with oral samples at all days, away from unwounded skin (Fig. 2E). These results provide supporting evidence that wound-activated networks are present in the normal oral epithelium. Interestingly, psoriasis patients have accelerated wound healing with reduced scarring (11, 12) suggesting that the presence of gene networks related to wound healing at basal state might be key for the accelerated wound healing observed in psoriatic skin and oral mucosa.

Analysis of the transcriptome of the oral mucosa also revealed that gene networks related to cell movement and migration are highly activated in the unwounded oral mucosa (fig. S2A), showing upregulation at the basal state of numerous genes linked to epithelial and immune cell migration (fig. S2B). Taken together, these results indicate that wound-activated transcriptional networks are present at basal state in the oral mucosa, priming the epithelium for wound repair.

Oral mucosa shows reduced differentiation and more actively sustained inflammatory response during wound healing.

Consistent with the possibility that the transcriptional regulatory networks responsible for the accelerated healing in oral mucosa are already present in the unwounded state, we identified 250 genes (table S2) that are present at high levels in the unwounded oral mucosa but are only upregulated in the skin during wound healing (Fig. 3A, black line in CIRCOS plot). While there is ample evidence of the importance of the extracellular matrix and underlying stroma during re-epithelialization (1), the top most significant gene ontologies (GOs) represented in this gene list where processes related to keratinization, epidermal cell

differentiation and responses to biotic stimulus and bacterium (Fig. 3A and table S3). Terms related to epithelial and immune cell migration where also represented (table S3). These results highlight specific gene networks both, intrinsic to keratinocyte biology and related to immune responses, as critical elements mediating the priming of the oral mucosa to wound repair.

Indeed, distinct keratin and structural protein expression profiles between oral mucosa and skin are indicative of inherent differences in the epithelial compartments of these tissues (Fig. 3B). Correspondingly, we found that characteristic signature keratins for each epithelia (13) are present: K4 and K13 in the oral mucosa and K1 and K10 in the cornified skin (Fig. 3C). We also examined reciprocal staining for each keratin (K4 and K10) in skin and oral mucosa (fig. S3A), and confirmed the tissue-specific expression for each keratin.

Importantly, stress and wound-activated keratins, including the keratin 6 family (K6A, K6B and K6C) and K16, are uniquely active in unwounded oral epithelium and maintained highly upregulated during wound healing, while they are only expressed in the skin during the wound healing process (Fig. 3B and 3D). These keratins are essential for the *in vivo* migratory and structural capacity of keratinocytes in murine oral epithelia and during wound healing (14, 15). An added powerful result from analyzing these human datasets is that it has allowed the identification of distinct expression profiles for genes clustered in the epidermal differentiation complex (EDC) (16). The majority of the EDC genes (S100s, SPRRs and cell envelope precursors) that are upregulated in skin during wounding (Fig. 3B), are already expressed at heightened levels at baseline day 1 in the oral mucosa.

Differentiation markers such as involucrin (IVL) usually present in both epithelia, were downregulated in the oral mucosa during healing, while were present in the migratory tongue of the wounded skin (Fig. 3B and fig. S3B). Interestingly, even though the oral mucosa has a more extensive total area of proliferating cells during wound healing (Fig. 3E), there is no significant difference in the number of proliferating cells when this is corrected for surface area involved (Fig. 3E). In aggregate, these results indicate that oral wounds do not engage differentiation pathways during wound healing, maintaining a larger pool of regenerative epithelial keratinocytes that aid in accelerated wound closure, while the activated keratin expression corroborates that the oral mucosa is primed for wound repair.

The differential expression of structural keratins observed between oral and cutaneous samples, reflects the unique characteristics of these distinct epithelia and highlight the fact that the oral buccal mucosa is non-cornified and therefore more exposed to environmental signals (17). As such, the oral epithelium is increasingly exposed to the commensal microbiota that inhabit barrier surfaces. In fact, the oral environment is home to some of the most rich and diverse microbial communities harbored on human body surfaces (18) and is an environment of constant mechanical stimulation during mastication, shown to induce heightened immune responsiveness (19). In accordance to this concept, our data demonstrate that oral epithelia have increased anti-microbial defenses and immune responses at steady state, but minimally up-regulate inflammatory pathways during the healing process (Fig. 4A and figs. S4A–B). In contrast, in the cutaneous microenvironment, inflammatory responses are less active at steady state but become upregulated throughout the healing process and do

not resolve by day 6, suggesting of a chronic inflammatory response when compared to the oral mucosa (Fig. 4A–C and fig. S4A). Chronic inflammation is a hallmark of non-healing wounds and over-activation of immune processes during healing has been shown to have detrimental effects on wound resolution, delaying closure and increasing fibrosis and scarring (20). Importantly, several immune mediators including proinflammatory cytokines, chemokines and cyclooxygenases showed higher expression in the skin at basal state and are upregulated in skin continuously through day 6 (Fig. 4B). Additionally, markers of fibrosis, including TGF-β (21) are upregulated in skin wounds compared to oral wounds (fig. S4C). Analysis of the gene expression changes in skin wound healing from day 3 to day 6 revealed that although gene networks related to keratinocyte differentiation (peptide cross-linking, keratinization) where upregulated by day 6, additional networks related to immune response are still active at this time (fig. S4D). This further corroborates the non-resolution of the inflammatory response in the skin wounds.

Histological and quantitative evaluation of the cellular infiltrate during healing reflected an acute response in the oral environment with complete resolution by day 6, consistent with transcriptional responses that revert to baseline by day 6 in the oral environment but continue to amplify in the skin (Fig. 4D). Associated with this rapid and controlled inflammatory response in the oral environment, we observe constitutive activation of several pro-resolution molecules (annexin, SLPI, lipoxygenase, IL1RN (22)) in the oral mucosa (Fig. 4C). In particular, we confirmed that Annexin-A1 and SLPI, two proteins that have been shown to ameliorate wound healing by moderating chronic inflammation (23–25), are detected at significantly higher protein levels in the oral mucosa and oral wounds compared to skin (Fig. 4E). Altogether, these results show that oral mucosa is primed for wound repair by a series of wound-signature networks that help control epithelial cell differentiation and regulate pro-inflammatory responses.

Transcriptional networks in oral mucosa contribute to rapid wound resolution.

Rapid oral versus skin wound healing has previously been identified in mouse and pig models (3, 6, 26) and replicated *in vitro* in oral keratinocyte models (4), suggesting a conserved mechanism in oral keratinocytes that allows for faster wound resolution.

To identify conserved factors/pathways in oral keratinocytes which may be physiologically relevant for improved healing, we searched databases for common overexpressed transcripts that are present in our human oral mucosa dataset (Fig. 2F), and are overexpressed in mouse oral mucosa (26) *in vivo*, and in human (27) and mouse oral keratinocytes (3) (fig. S5). This analysis identified eight genes that are consistently upregulated in oral mucosa and oral keratinocytes (Fig. 5A), four of which encode for transcriptional regulators (*PAX9*, *PITX1*, *PITX2*, *SOX2*), one that encodes the LIM-domain only 7 emerin-binding factor (*LMO7*) (28) and three that encode factors involved in various oral mucosa biological processes (*ALDH3A1, ATP1B1* and *IGFBP2*) (4, 29, 30).

Transcription factors have the potential to reprogram cells to specific developmental states (31). Within the group of oral upregulated transcription factors we found the paired-like homeodomain PITX factors (*PITX1* and *PITX2*), the paired-box homeodomain factor PAX9 and the HMG-domain SOX2, which is part of the SOX family of transcriptional drivers of

somatic cell reprogramming (32) (33, 34). We confirmed the differential expression of the PAX9, PITX1, PITX2 and SOX2 transcriptional regulators in primary human oral (NOK) and skin (NHEK) keratinocytes *in vitro* and in human biopsies, at baseline and during healing (Fig. 5B and C). We determined that PITX1 and SOX2 are expressed in NOK cells and unwounded oral mucosa while their expression is almost undetectable in NHEK cells and skin. Interestingly, the expression of PITX1 and SOX2 increased upon wounding in the epithelial layer of the oral mucosa. PAX9 shows increased expression in epithelia and dermis after wounding in both oral mucosa and skin, and is also expressed at lower levels in NHEK cells.

To functionally validate the role of these factors in controlling a transcription-regulated wound healing program, we analyzed the gene expression changes in NOK cells treated with siRNAs for PAX9, PITX1, PITX2 and SOX2 (Fig. 6 and fig. S6A). We found that knockdown of these factors results in alterations of gene networks related to cell movement and migration, particularly after PITX1 and SOX2 knockdown (Fig. 6B and fig. S6A–C). Indeed, siRNA for PITX1 and SOX2 significantly reduced the migration capacity of NOK cells (Fig. 6C). Both factors achieve this effect by acting on different processes, since SOX2 affects gene networks related to the immune and defense response while PITX1 expression results in the alteration of epidermal developmental and differentiation and keratinization pathways (fig. S6B and C).

We then evaluated the functional effects of overexpressing PITX1 and SOX2 in NHEK cells (Fig. 7 and fig. S7), which do not express these factors under normal conditions (Fig. 5B and C). Transcriptomic analysis of NHEK cells transduced with PITX1 and SOX2 revealed a similar pattern of GO biological process as oral keratinocytes, with SOX2 affecting pathways related to the immune response, including cytokines and antibacterial peptides, and PITX1 expression resulting in the alteration of developmental and differentiation pathways (Fig. 7B). Interestingly, both PITX1 and SOX2 expression in skin keratinocytes resulted in reduced expression of the skin differentiation marker *KRT1* and overexpression of the oral epithelial marker cornulin (*CRNN*) (Fig. 7C and fig. S7B). PITX1 also resulted in increased expression of several markers enriched in the oral epithelium, including keratins (*KRT13*, *KRT78*), *SPRRs* (*SPRR2A*, *SPRR2E*, *SPRR3*), *LCE3D*, *IGFBP2* and *ALDH3A1* (Fig. 7C). Remarkably, transduction with PITX1 and SOX2 significantly increased the migratory capacity of NHEK cells (Fig. 7D and fig. S7C). These results show that the differential expression of transcriptional regulators in oral versus skin keratinocytes confers a unique identity to oral keratinocytes and determines the potential of reprogramming skin keratinocytes to acquire increased cell migration capability and improve wound resolution.

To investigate the effect of overexpressing SOX2 in epidermal keratinocyte on cutaneous wound healing *in vivo*, we generated epidermal-specific SOX2 overexpressing mice (35). Mice expressing a tamoxifen-inducible Cre driven by the keratin 14 promotor (K14CreERTM) were crossed with LSL-SOX2 mice to generate K14CreERTM/LSL-SOX2 mice. Next, we compared the kinetics of cutaneous wound healing in K14CreERTM/LSL-SOX2 mice activated or not by tamoxifen to express SOX2 in basal keratinocytes (Fig. 8A). Immunohistochemical analysis showed that tamoxifen treatment induced SOX2 expression in epidermal keratinocytes in K14CreERTM/LSL-SOX2 mice (Fig. 8B). Interestingly, short

term SOX2 expression in the skin resulted in an amplification of the K5 stem cell compartment (Fig. 8B).

Wound healing was significantly promoted in SOX2 overexpressing skin from 3 to 9 days after wounding compared to mice treated with vehicle (Fig. 8C–D). SOX2 overexpression in epithelial keratinocytes lead to skin acanthosis when compared to control skin (Fig. 8E). Histological analysis in SOX2 overexpressing mice Day 5 after wounding showed a larger migratory tongue than control mice (Fig. 8E). Furthermore, SOX2 overexpression significantly increased PCNA positive proliferating epithelial cells (Fig. 8F). These findings demonstrated that genetic or pharmacological approaches to increase the expression or activity of the SOX2 transcription factor in the skin is an efficacious approach to positively regulate cutaneous wound healing.

DISCUSSION

Wound healing is a major medical and social priority and broadening our understanding of the mechanisms involved in wound repair is needed to improve wound care. While oral wounds, in distinction to cutaneous wounds, are able to heal quicker with far less complications, the lack of detailed comparative analysis in humans prevented the advancement of our knowledge in this area. Buccal mucosa, gingiva and palate show similar accelerated wound healing when compared to cutaneous wounds (3) (21), despite all having different keratinization and terminal differentiation profiles. This indicates that the oral cavity in general is endowed with mechanisms to increase wound resolution that go beyond local differences in epithelial structure.

In this study, by analyzing paired samples of human oral buccal mucosa and skin at basal conditions and during wound healing, we identify the physiological and molecular determinants for this repair paradigm. Our findings could have widespread implications for wound healing study and interventions. Pathways and/or molecules characterized in this study may facilitate rapid, scar- less healing that can be considered for application to non-oral mucosal sites to promote healing.

To identify and explain the mechanisms that define accelerated wound healing, we analyzed the gene expression signature changes during oral mucosal and skin wound resolution in healthy human subjects. Even before wounding, homeostatic oral mucosa is equipped with transcriptional networks that prime the epithelium for wound repair. In addition, our data indicates that the most significant processes driving the repair in acute wounds of healthy individuals are keratinocyte-driven. These networks are determined in part by the differential expression of a set of transcriptional regulators in oral vs skin keratinocytes, indicating that pathways established during development are responsible for the differential wound resolution capacity of these cells.

This priming allows the oral mucosa to rapidly control and limit inflammatory responses, leading to fast inflammatory resolution. In addition, oral keratinocyte activation and reduced differentiation allows for a rapid re-epithelialization of the wound area. Indeed, it has been recently shown that re-epithelialization in mice is dependent on tissue-scale coordination of

proliferation, differentiation and migration (36), with acquisition of stem cell properties in de-differentiated epidermal cells (37). In the long term of human wound repair, the reduced expression of cytokines and fibrosis mediators such as annexin 1 and SLPI potentially contributes to the scarless wound healing observed in the oral mucosa.

At the core of oral wound healing are transcriptional regulators that hold the key to the activation of the molecular events responsible for accelerated wound resolution. In this study we identify eight factors that define oral keratinocytes and demonstrate that two of them, the transcription factors SOX2 and PITX1, regulate networks involved in wound closure. The SOX and PITX family of transcription factors have important roles in development, ranging from regulation of cell fate to axis and pattern formation (32,34). Our results support that these transcription factors are responsible in establishing an oral mucosa-specific network that primes this epithelia for rapid and efficient wound healing. Furthermore, we show that SOX2 and PITX1 can be exploited to reprogram skin keratinocytes to present oral keratinocyte features, including accelerated wound resolution both *in vitro* and *in vivo*. SOX2 in particular is a key factor in adult stem cell maintenance for a myriad of epithelial tissues (38) and has been shown to induce amplification of stem cell in the skin (39). Confirming these results, we show that SOX2 induces an expansion of the K5+ basal/stem cell compartment of the skin in mice, indicating that the wound regenerative capacity of SOX2 might be linked to its stem cell regulatory functions. On the other hand, our data suggests that PITX1 regulates a different set of processes related to the expression of structural proteins, including keratins, LCEs and SPRRs. It is worth noting that PITX2, a member of the PITX family, is an essential component of the genetic network activated by tissue damage during heart repair (40). More studies are needed to identify the specific pathways activated by these transcriptional regulators and their potential for wound repair and tissue regeneration.

Overall, we present a unique combination of human clinical data and histological and gene expression analysis that provides a comprehensive comparative analysis of the molecular and cellular mechanisms underlying the different wound healing processes in oral and skin epithelia. Our data indicates that the unique environment of the oral cavity represents a radically different wound-healing program geared towards rapid wound-resolution at every step (fig. S9). Ultimately, this human transcriptomic dataset highlights fundamental global mechanisms of inflammation and repair in humans that will serve as an invaluable resource, providing insights into therapeutic targeting of chronic and non-healing wounds.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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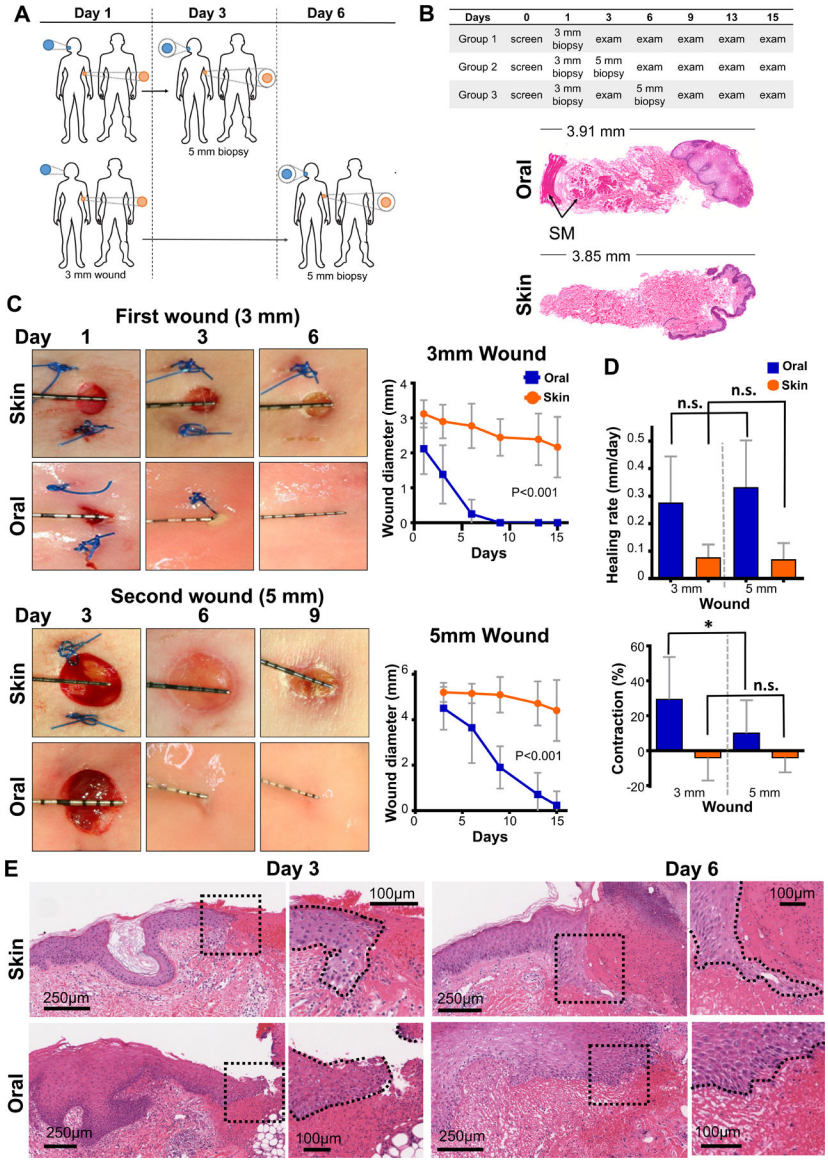

Figure 1. Comparison of paired oral and skin wounds in human subjects.

(A) Schematic of spatiotemporal human biopsy sample collection; Clinicaltrials.gov #NCT01078467. Baseline biopsies were performed to create paired identical wounds in the oral mucosa and skin (day 1). Follow-up biopsies of the wound area(s) were collected at day 3 and day 6 of healing in two different groups. **(B)** Table showing the time-course of the clinical study and biopsy sampling, and representative H&E pictures of longitudinal sections of biopsies taken at day 1. Thirty healthy subjects were randomized in three groups (with 10 subjects on each group). SM: smooth muscle. See Methods section for more information. **(C)** Representative pictures and healing time course of oral wounds and skin wounds in group 1 (after 3 mm primary biopsy), and group 2 (after 5 mm secondary biopsy). Markings in millimeters. Error bars represent SDs. Number of samples for each group as follows: 3 mm wounds, day 1 n=29, day 3 n=30, day 6 n=20, day 9 n=9, day 13 n=9, day 15 n=9; 5mm wounds, day 1 n=11, day 3 n=20, day 9 n=21, day 13 n=21, day 15 n=21. **(D)** Graphs show

healing rate of 3 mm wounds in group 1 and 5 mm wounds in group 2 as well as the contraction percentage immediately after 3 mm biopsy in group 1 and 5 mm biopsy in group 2. n.s.: not significant, *p<0.05. Error bars represent SDs. **(E)** Representative H&E pictures of oral and skin wounds at day 3 and 6. At day 3 there is almost complete epithelization of the wound, with migratory tongue growing on top of blood clot. In the skin, migratory epithelial tongue is growing under the scab that covers the wound. At day 6, oral wounds are completely resolved, while skin wounds are still undergoing re-epithelialization. Magnification of dotted box is shown on the right. In magnification, epithelium is marked with a dotted line.

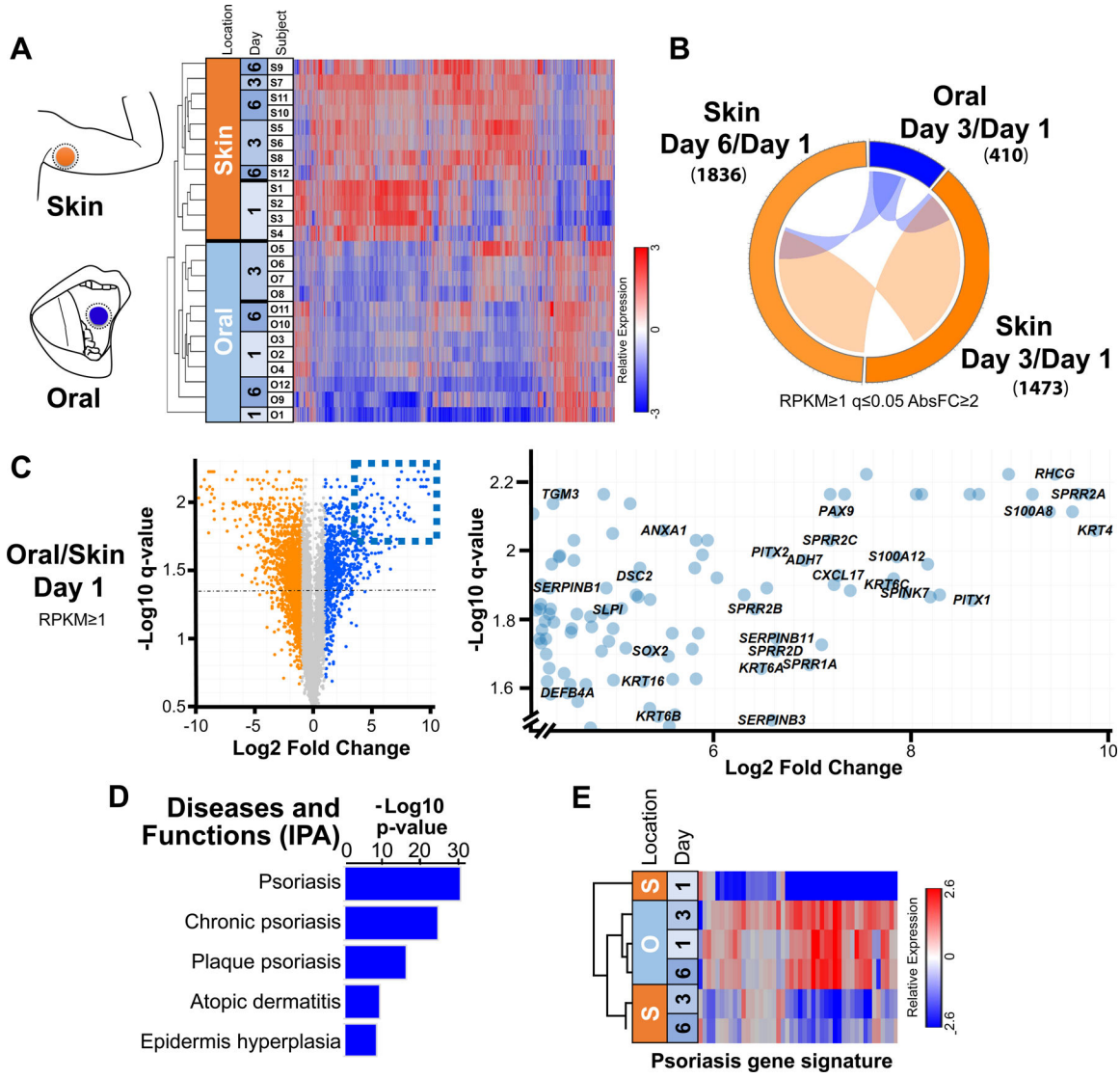

Figure 2. Wound-activated transcriptional networks are present in the unwounded oral mucosa.

(A) Schematic representation of biopsy site in the mucosa of the cheek and posterior axillary region of the arm (left) and unsupervised clustering analysis of RNA-Seq gene expression data of the 24 paired samples at Day 1, 3 and 6. O: oral, S: skin. Numbers indicate matching subject. Paired oral and skin samples were chosen randomly from four subjects for each day (24 total samples from 12 individual subjects) and were a mix of males and females. **(B)** CIRCOS plot of the differential gene expression during wound healing (ANOVA). No significant differences were found on Oral Day 6 versus Day 1 (Day 6/1). Ribbon connectors indicate the same genes present in different datasets. Number of genes with differential expression in each comparison: Oral Day 3 versus Day 1 (Day 3/1): 410 genes, Skin Day 3 versus Day 1 (Day 3/1): 1473 genes, Skin Day 6 versus Day 1 (Day 6/1): 1836 genes. See fig. S1a for explanation on CIRCOS plot. **(C)** Volcano plot indicating differential gene expression between unwounded (Day 1) oral mucosa and skin. Magnification on right panel, highlights the some of the most significantly upregulated genes in the oral mucosa over the skin. **(D)** IPA analysis results showing Diseases and Functions terms found in upregulated

genes in the oral mucosa over the skin. **(E)** Unsupervised hierarchical clustering using a psoriasis gene signature with the gene expression of oral mucosa and skin data at baseline (Day 1) and during wound healing (Day 3 and 6).

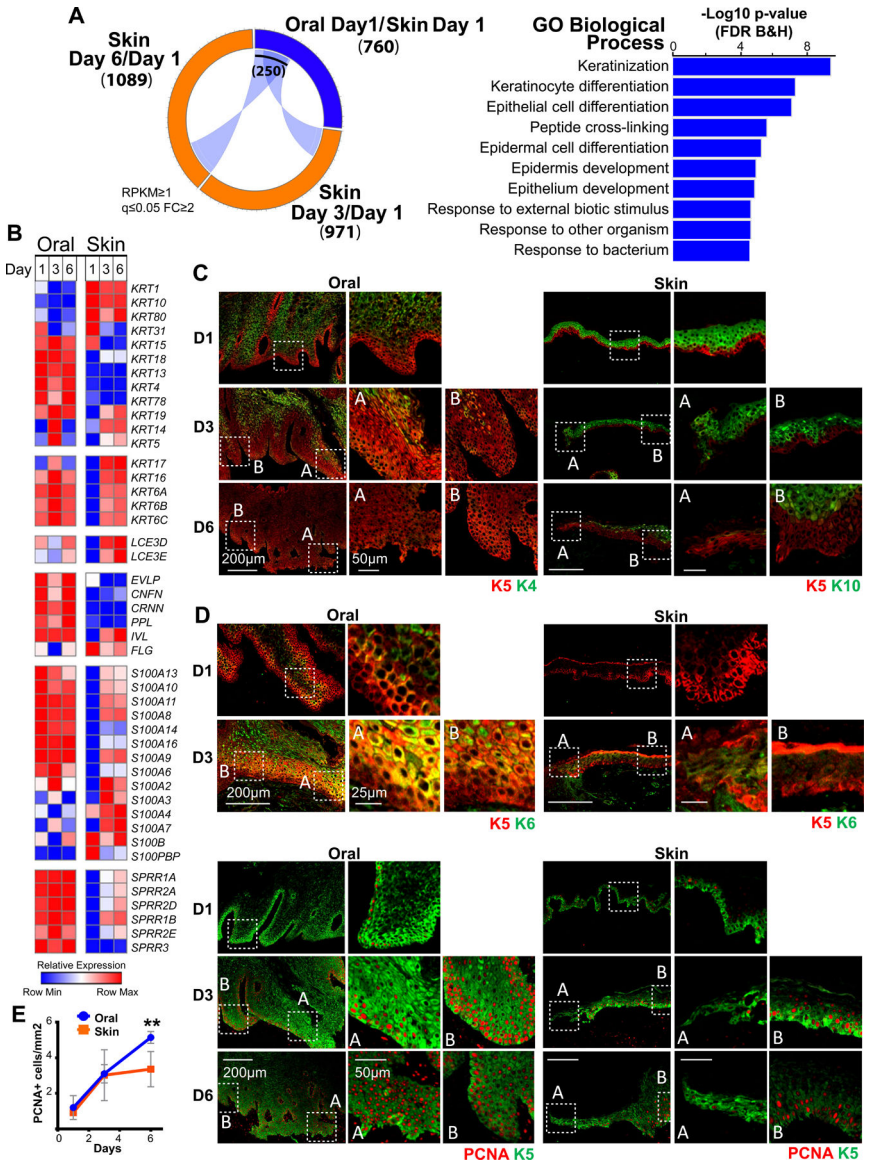

Figure 3. Oral keratinocytes show reduced differentiation during wound healing.

(A) CIRCOS plot summarizing cross-reference of transcripts upregulated during skin wound healing (Skin D3/D1, Skin D6/D1, D=day) with those upregulated in the oral mucosa with respect to the skin at basal conditions (Oral D1/Skin D1). Black line indicates genes upregulated in the unwounded oral mucosa that are upregulated during skin wound healing (250 unique genes; see Table S2). Right panel, graph indicates GO biological process terms enriched in Oral D1/Skin D1 dataset. Ribbon connectors indicate that the same genes are present in different datasets. Oral D1/Skin D1: 760 genes, Skin D3/D1: 971 genes, Skin D3/D1: 1089 genes. **(B)** Relative mRNA expression levels of keratinization and epidermal cell differentiation markers throughout the wound healing process. **(C)** Representative pictures of unwounded (D1) and wounded (D3 and D6) oral mucosa and skin stained to show expression of the basal marker keratin 5 (K5, red), and differentiation markers keratin 4 (K4) for the oral mucosa and keratin 10 (K10) for the skin (green).**(D)** Representative

pictures of unwounded (D1) and wounded (D3) oral mucosa and skin stained to show expression of the basal marker cytokeratin 5 (K5, red), and activated epithelium keratin 6 (K6, green). **(E)** Representative pictures of unwounded (D1) and wounded (D3 and D6) oral mucosa and skin stained to show expression of the basal marker keratin 5 (K5, green), and proliferation marker PCNA (red). Quantification of number of cells positive for the proliferation marker PCNA per area (mm2). **p<0.01 oral vs skin day 6, no asterisk means not statistically different between oral and skin. Error bars represent SDs of three independent samples. Magnification of the dotted box is shown on the right of each picture. For D1 magnification shows the basal, unwounded expression of corresponding marker. For D3 and D6 magnification shows the migratory tongue or wound area, A, or an adjacent epithelial area to the wound, B.

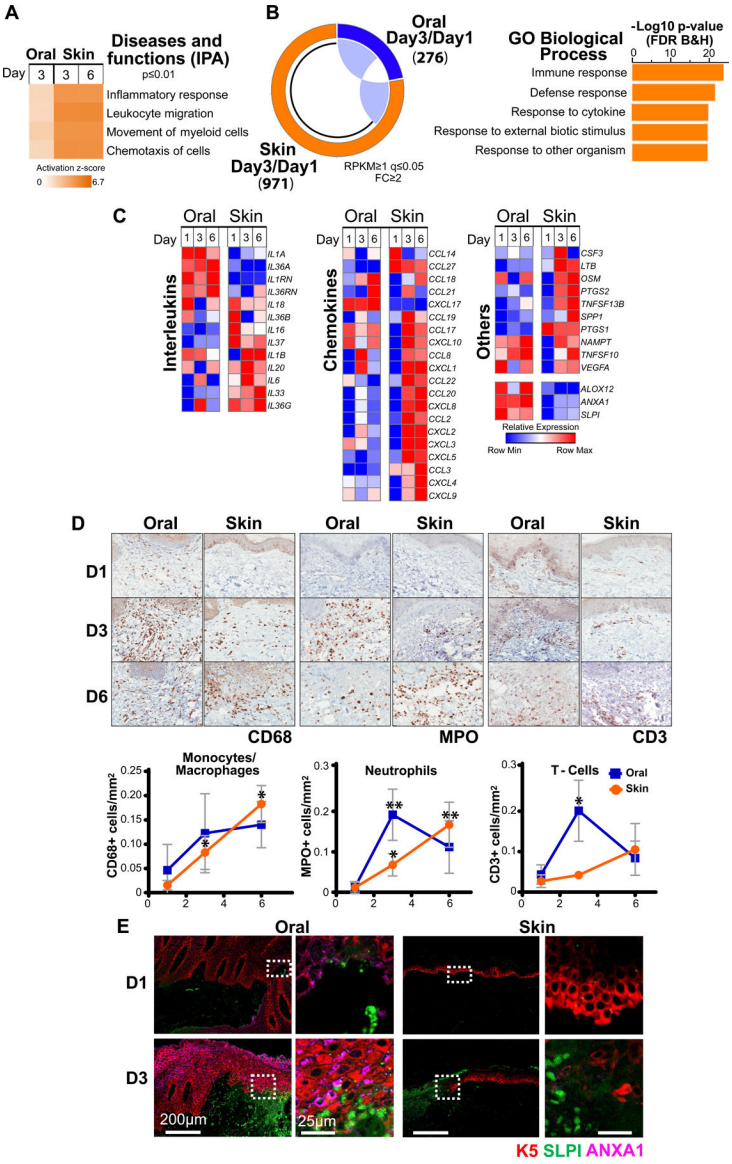

Figure 4. Inflammatory pathways are more active and sustained in skin wounds that in oral wounds.

(A) IPA analysis results showing diseases and functions terms found in differentially regulated genes during the wound healing process included terms related to inflammatory processes. **(B)** CIRCOS plot showing the genes exclusively upregulated during skin wound healing (black line) and GO biological process terms enriched in this dataset. Ribbon connectors indicate that the same genes are present in different datasets. Oral Day 3/Day 1: 276 genes, Skin Day 3/Day 1: 971 genes. D=day. **(C)** Relative mRNA expression levels of interleukins, chemokines and other inflammatory regulators during wound healing. **(D)** Representative pictures of recruitment of immune cells during the wound healing process in the oral mucosa and the skin. Bottom panels show quantification of recruitment of specific immune cell types during the wound healing process in the oral mucosa and the skin. * $p<0.05$, ** $p<0.01$, no asterisk means not statistically different, comparisons between day 3 and day 6 vs day 1 oral or skin respectively. Error bars represent SDs of three independent

samples. **(E)** Representative images of unwounded (D1) and wounded (D3) oral mucosa and skin stained to detect expression of the basal marker keratin 5 (K5, red), and the immune-modulators SLPI (green) and AnnexinA1 (ANXA1, magenta). Magnification of dotted box is shown on the right of each picture.

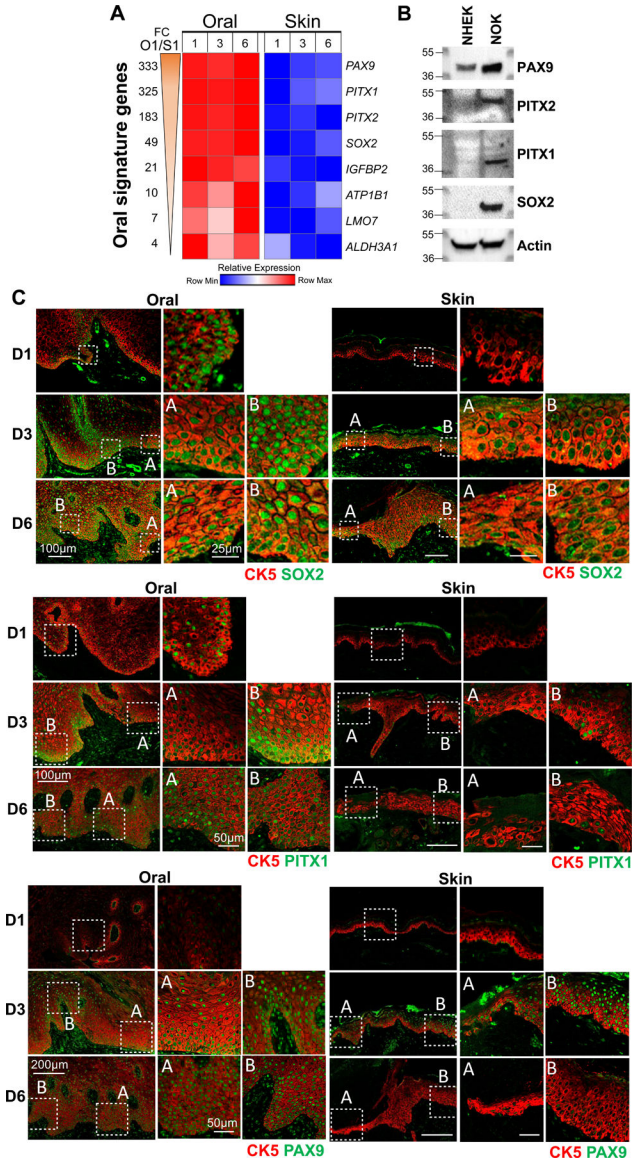

Figure 5. Transcriptional networks in oral keratinocytes contributing to rapid wound resolution.

(A) Relative mRNA expression levels in the wound healing dataset of genes consistently upregulated in oral mucosa and oral keratinocytes, presented by fold change (FC) of the unwounded oral mucosa with respect to the unwounded skin (O1/S1). (**B)** Protein levels of PAX9, PITX2, PITX1 and SOX2 in primary cultures of human oral (NOK) and skin (NHEK) keratinocytes. **(C)** Representative pictures of unwounded (D1) and wounded (D3 and D6) oral mucosa and skin stained to show expression of the basal marker keratin 5 (K5, red), and the indicated transcription factor (green). Magnification of the dotted box is shown on the right of each picture. For D1 magnification shows the basal, unwounded expression of corresponding marker. For D3 and D6 magnification shows the migratory tongue or wound area, A, or an adjacent epithelial area to the wound, B.

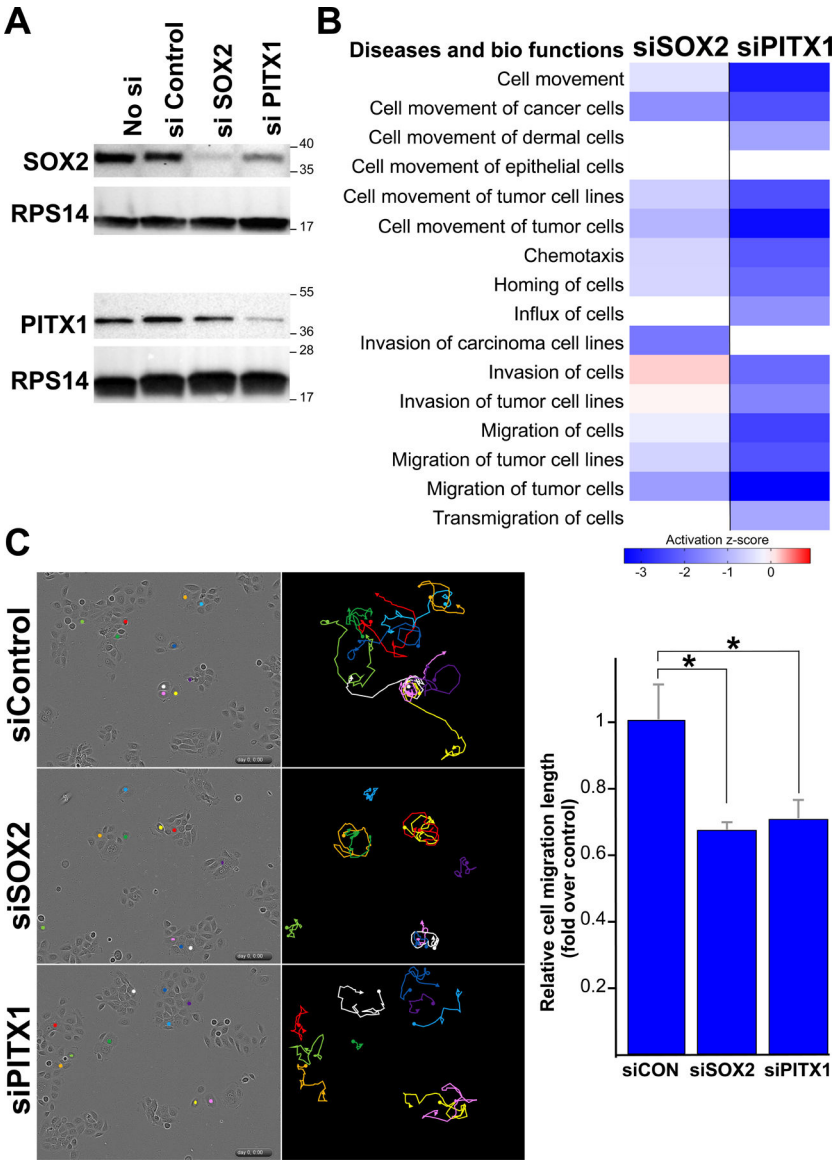

Figure 6. Knockdown of oral signature genes in primary oral keratinocytes.

(**A**) Protein level of SOX2, PITX1 and total protein (RPS14) after transfection with respective siRNAs in NOK cells. (**B**) IPA analysis of RNA-seq data from NOK cells treated with siRNAs for SOX2 and PITX1: Diseases and functions terms related to migration and cell movement found in differentially regulated genes. *p≤0.05 **(C)** Relative cell migration length of NOK cells transduced with siRNA for SOX2, PITX1 and siControl. Values were determined by 3 microscopic fields in n=3 per group. *p < 0.05 siSOX2 and siPITX1 versus control. Error bars represent SDs of three independent experiments.

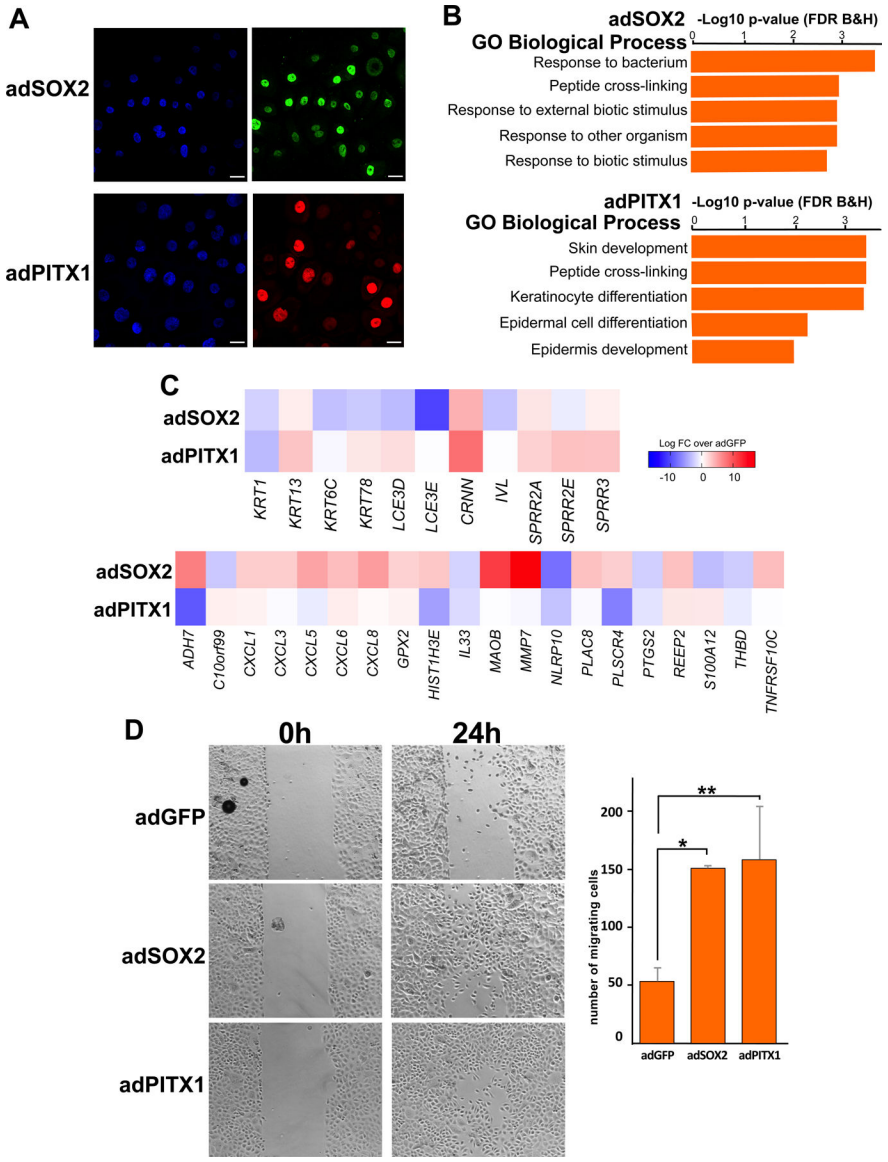

Figure 7. Overexpression of SOX2 and PITX1 in primary skin keratinocytes.

(**A**) Representative pictures of NHEK cells transduced with indicated adenoviruses and stained to show overexpression of corresponding proteins. No expression of PITX1 or SOX2 was observed in non-transduced cells (not shown). Bar= 20μm. (**B**) GO biological process terms enriched in datasets of genes differentially regulated by PITX1 and SOX2 overexpression in NHEK cells. (**C**) Fold change of the expression levels of genes related to differentiation and response to biotic stimulus in NHEK cells transduced with PITX1 and SOX2, presented as log2 fold change (log FC) over GFP expression. (**D**) Migrating NHEK cells transduced with SOX2, PITX1 and GFP (control) by adenoviral delivery. Images were taken at 0 and 24hs after removal of silicone insert. Values were determined by counting the number of migrating cells at 24hs in 6 microscopic fields in n=3 per group. **p < 0.01, *p < 0.05 adSOX2 and adPITX1 versus control. Error bars represent SDs of three independent experiments.

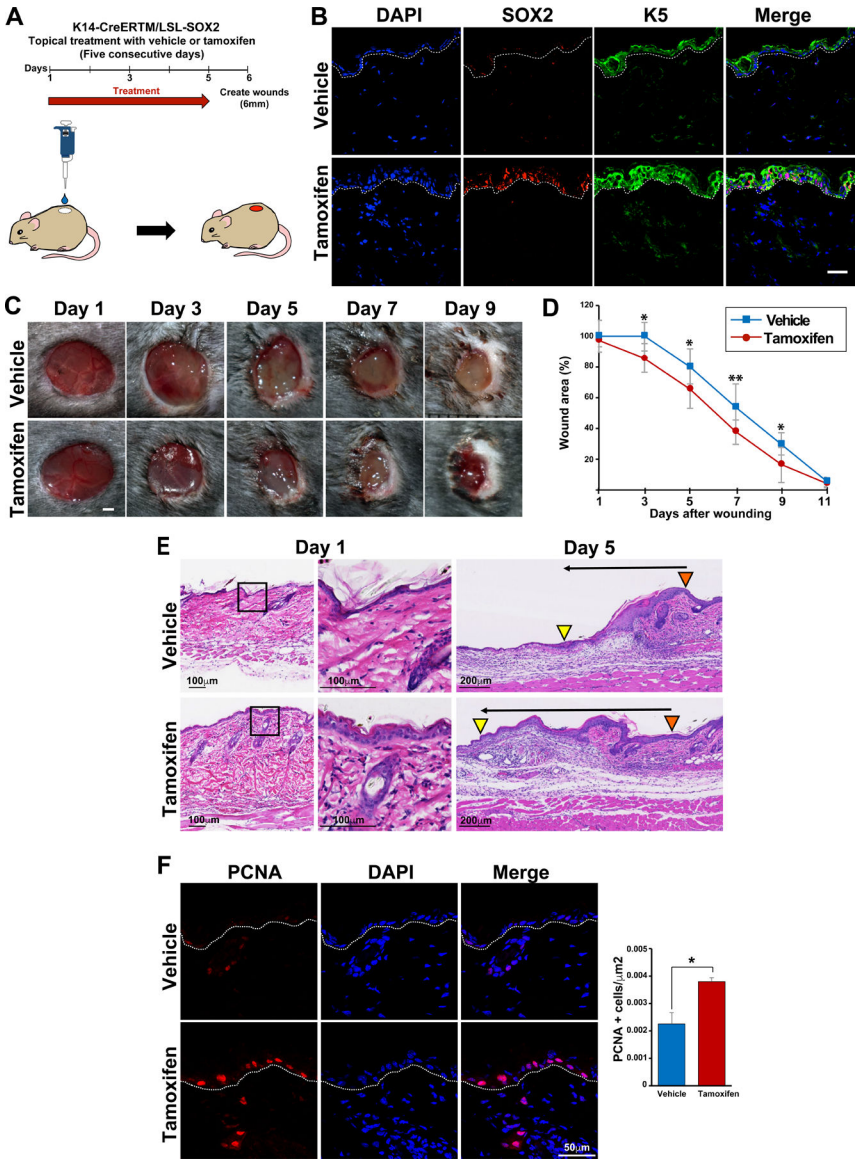

Figure 8. Conditional overexpression of SOX2 contributed to the promotion of cutaneous wound healing.

(A) Schematic representation of the experimental design used for K14CreERTM/LSL-SOX2 mice. Mice are treated with vehicle or tamoxifen five consecutive days by topical application on dorsal skin (Day 1-Day 5). The wounds were created as a 6 mm full-thickness excisional dorsal skin wound by biopsy punch (Day 6). (**B**) Representative images of unwounded skin stained to show expression of SOX2 (red), and the basal marker keratin 5 (K5, green) in K14CreERTM/LSL-SOX2 mice treated with ethanol as vehicle or tamoxifen. Bar = 50 um. (**C**) Photographs of the wound areas after topical treatment with vehicle or tamoxifen in K14CreERTM/SOX2 mice at 1, 3, 5, 7, and 9 days after wounding. Bar=1mm. **(D**) Percent wound area at each time point relative to the original wound area in K14CreERTM/LSL-SOX2 mice treated with vehicle or tamoxifen. Quantification of the wound areas in n = 7 wounds per groups was performed using Image J software. *p < 0.05, **p < 0.01 tamoxifen versus vehicle at each day. Error bars represent SDs of seven wounds. **(E)** Representative

H&E-stained section Day 1(un-wounded) and Day5 (during wound healing, wound edges including epithelial tongue). Skin sections were from K14CreERTM/LSL-SOX2 mice after treatment with tamoxifen or vehicle.