Identification of the Potential Biomarkers Involved in the Human Oral Mucosal Wound Healing: A Bioinformatic Study

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Objective. To identify the key genetic and epigenetic mechanisms involved in the wound healing process after injury of the oral mucosa.

Materials and Methods. Gene expression profiling datasets pertaining to rapid wound healing of oral mucosa were identified using the Gene Expression Omnibus (GEO) database. Differential gene expression analysis was performed to identify differentially expressed genes (DEGs) during oral mucosal wound healing. Next, functional enrichment analysis was performed to identify the biological processes (BPs) and signaling pathways relevant to these DEGs. A protein-protein interaction (PPI) network was constructed to identify hub DEGs. Interaction networks were constructed for both miRNA-target DEGs and DEGs-transcription factors. A DEGs-chemical compound interaction network and a miRNA-small molecular interaction network were also constructed.

Results. DEGs were found significantly enriched in several signaling pathways including arachidonic acid metabolism, cell cycle, p53, and ECM-receptor interaction. Hub genes, GABARAPL1, GABARAPL2, HDAC5, MAP1LC3A, AURKA, and PLK1, were identified via PPI network analysis. Two miRNAs, miR-34a-5p and miR-335-5p, were identified as pivotal players in the miRNA-target DEGs network. Four transcription factors FOS, PLAU, BCL6, and RORA were found to play key roles in the TFs-DEGs interaction network. Several chemical compounds including Valproic acid, Doxorubicin, Nickel, and tretinoin and small molecular drugs including atorvastatin, 17β-estradiol, curcumin, and vitamin D3 were noted to influence oral mucosa regeneration by regulating the expression of healing-associated DEGs/miRNAs.

Conclusion. Genetic and epigenetic mechanisms and specific drugs were identified as significant molecular mechanisms and entities relevant to oral mucosal healing. These may be valuable potential targets for experimental research.

1. Introduction

Oral mucosal injury is inherent to several disorders and conditions such as trauma, periodontal disease, cancer, and dental implant surgery [1]. Oral mucosal regeneration is an area of increasing research focus and includes the application of regenerative modalities such as 3D cell sheets consisting of oral mucosal cells (keratinocytes, fibroblasts, and endothelial...
cells) [2, 3]. Genetic modification of such cell-based constructs may potentially further accelerate their healing capacity [4, 5] and provide a novel strategy to promote wound healing in oral mucosa injury. Therefore, investigating the genetic mechanisms involved in the wound healing process of oral mucosa injury could be of significant translational value.

Wound healing of oral mucosa is unique because it is typically faster and shows a low tendency for scarring [6]. The superior healing characteristics of the oral mucosa might be owing to various factors including the specific anatomy of the oral cavity and environmental factors such as microbial flora, saliva secretion, and pH balance. Some other or reasons plausible causes include growth factor production, stem cell levels, cellular proliferation capacity, and genetic and epigenetic factors [7, 8]. As genetic and epigenetic regulation plays a pivotal role in the unique healing characteristics of the oral mucosa, significant research attention [9–12] has focused on the involvement of genetic and epigenetic factors (e.g., genes, signaling pathways, transcription factors, and miRNAs) in the wound healing process of oral mucosa injury. In this context, vascular endothelial growth factor (VEGF) has been found to promote palatal mucosal wound healing by accelerating neovascularization and reepithelialization [9]. In another report, intrinsic apoptosis pathways Casp2 and Trp53 were found activated during oral mucosal wound healing [10]. The transcription factor forkhead box O1 (FOXO1) has been found as increased in the nucleus of keratinocytes and promote reepithelialization during wound healing of oral mucosa [11]. The overexpression of miR-31 has been found to promote oral mucosal wound closure by enhancing the proliferation and migration of keratinocytes [12].

Apart from these experimental studies, high-throughput sequencing investigations have shown alterations of miRNAs or noncoding RNA expression profiles during oral mucosa healing by establishing paired injury models in rats (GSE23006 [7] and GSE121996 [13]) and humans (GSE97615 [14]). To the authors’ knowledge, there is no comprehensive bioinformatic investigation leveraging the publicly available datasets pertaining to genetic and epigenetic mechanisms involved in oral mucosa regeneration. Bioinformatics can enable the interpretation and visualization of large datasets through network construction [15]. Plausibly, the important factors critical to a specific pathophysiological process can be identified through bioinformatic analysis of relevant gene expression datasets. The present study is aimed at analyzing the data in the GSE97615 [14] using multiple bioinformatic techniques in order to identify specific molecular mechanisms that are key to the regulation of the wound healing processes in human oral mucosa injury.

2. Materials and Methods

2.1. Sourcing of Gene Expression Data. Datasets investigating genetic alterations using paired models of rapid wound healing of oral mucosa were searched for in the Gene Expression Omnibus (GEO) database in NCBI [16]. The study design of the included datasets consisted of two groups, the control group where oral mucosal injury was created and the experimental group where wound healing was complete. The model of oral mucosa injury was created by a sterile uniform punch biopsy (McKesson) in the mucosa of the cheek [14]. The inclusion criteria pertaining to this dataset were as follows: [1] all participants being nonsmokers without any systemic diseases; [2] uniform biopsy at less than 5 mm, which was within self-regenerative capacity of the human oral mucosa; and [3] datasets with at least 3 pairs of samples (3 controls and 3 experimental samples).

2.2. Differential Gene Expression Analysis. RNAseq data were processed using TopHat software [17], and RPKM (Reads Per Kilobase Million) was calculated by using Partek software [18]. Differential expression analysis was conducted by using the “DESeq” package in R environment [19]. The differentially expressed mRNAs (genes) with p value < 0.05 and |log FC| ≥ 1 were selected as DEGs. The DEGs with log FC ≥ 1 were considered upregulated DEGs, while DEGs with log FC ≤ −1 were considered downregulated DEGs.

2.3. Functional Enrichment Analysis. Functional enrichment analysis of the DEGs involved in the wound healing process of oral mucosa injury was performed using the “clusterProfiler” package in R [20]. The functions of the DEGs were explored by investigating their enriched Gene Ontology (GO) terms, in particular, biological processes (BPs) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. The GO terms and KEGG pathways with p value < 0.05 were regarded as significant functions. If the number of enriched BPs and pathways was more than 30, the top 30 were chosen to be visualized in the bar plot. If the number of enriched BPs and pathways was less than 30, then all of the BPs and pathways were visualized in the bar plot.

2.4. Construction of Protein-Protein Interaction (PPI) Network. Experimentally validated PPI pairs were downloaded from a variety of databases including HPRD [21], BIOGRID [22], DIP [23], MINT [24], mentha [25], PINA [26], InnateDB [27], and Instruct [28]. PPI pairs of DEGs were extracted from these databases and further used for constructing the PPI network. The constructed PPI network was plotted using Cytoscape Version 3.7.2 [29]. The topological characteristics of the nodes in the PPI network were determined.

2.5. Construction of miRNA-Target DEGs Interaction Network. Experimentally validated miRNA-target interaction pairs were downloaded from three databases, including “miRTarBase” [30], “miRecords” [31], and “TarBase” v.8 [32]. miRNAs targeting DEGs were extracted from these databases and used for constructing a miRNA-target DEGs interaction network using the Cytoscape platform [29]. The topological characteristics of the nodes in the miRNA-target DEGs network were determined.

2.6. Construction of Transcription Factor- (TF-) Target DEGs Interaction Network. The TF-target gene interaction pairs were downloaded from many databases, including “TRANSFAC” [33], “HTRIdb” [34], “TRRUST” [35], “ÖRTT” [36],
and “cGRNB” [37]. The TFs targeting DEGs were extracted and used for constructing the TFs-target DEGs interaction network using Cytoscape [29]. The topological characteristics of the nodes in the TFs-target DEGs network were determined.

2.7. Construction of the DEGs-Chemical Compound Interaction Network. Data pertaining to the interactions between genes and chemical compounds were downloaded from CTD (Comparative Toxicogenomics Database) [38]. Only the data regarding the human species were extracted. 858,086 interaction pairs between genes and chemical compounds were obtained. Among these interaction pairs, chemical compounds that correspond to DEGs were extracted, and thus, 41,634 interaction pairs between DEGs and chemical compounds were derived. Thereafter, Cytoscape [29] was employed to visualize the interaction relationships between DEGs and their interacting chemical compounds. Following the construction of the DEGs-chemical compound interaction network, a topological analysis was performed to calculate the characteristics of the nodes in the network.

2.8. Construction of the miRNA-Small Molecule Drug Interaction Network. The data regarding known miRNAs and small molecular drugs were downloaded from the SM2miR database [39]. Only data associated with human species were extracted, and 2,756 interaction pairs between known miRNAs and small molecular drugs were obtained. Among these pairs, miRNAs that target DEGs were extracted, and the small molecule drugs that impact the expression levels of these miRNAs were further obtained. Therefore, 1,074 interaction pairs between small molecular drugs and miRNAs that target DEGs were studied. In order to visualize these interaction pairs, Cytoscape [29] was used to construct the miRNA-small molecule drug interaction network.

3. Results

3.1. Included Gene Expression Dataset. The dataset GSE97615 was included in this analysis [14]. This dataset examined gene expression profiles using high-throughput sequencing technology on the GPL11154 platform of Illumina HiSeq 2000. In the experimental group, oral buccal mucosal injury was generated on day 1, and wound healing was found to be almost complete on day 3. As nonsignificant differences in gene expression were noted between biopsies obtained on day 1 versus day 6, the oral biopsy samples of day 1 (GSM2573167, GSM2573168, GSM2573169, and GSM2573170) and day 3 (GSM2573171, GSM2573172,
**Table 1: The top 30 DEGs involved in the process of oral mucosa regeneration, ranked by the ascending order of p value.**

| Name         | Base mean | Base meanA | Base meanB | Fold change | log2 fold change | p val       | p adj       |
|--------------|-----------|------------|------------|-------------|------------------|-------------|-------------|
| DEFB4A       | 40.12592  | 2.388343   | 77.8635    | 32.60148    | 5.026865         | 1.05E-25    | 7.32E-22    |
| S100A7       | 310.8578  | 17.06716   | 604.6484   | 35.42758    | 5.146801         | 1.33E-23    | 6.19E-20    |
| DEFB4B       | 13.92444  | 0.57421    | 27.27467   | 47.49947    | 5.56984          | 3.75E-23    | 1.31E-19    |
| FLG          | 21.40743  | 40.1873    | 2.627564   | 0.065383    | -3.93494         | 3.94E-22    | 1.10E-18    |
| KRT76        | 8.60035   | 16.71021   | 0.490492   | 0.029353    | -5.09036         | 4.74E-19    | 1.10E-15    |
| CES1         | 9.660384  | 18.36781   | 0.952953   | 0.051882    | -4.26863         | 7.36E-19    | 1.47E-15    |
| CCL19        | 11.84998  | 1.619644   | 22.08032   | 13.63283    | 3.769013         | 2.64E-18    | 4.61E-15    |
| ALOX12       | 101.6108  | 186.2771   | 16.94444   | 0.090964    | -3.45857         | 5.61E-17    | 8.69E-14    |
| CYP4F35P     | 13.31745  | 24.28438   | 2.350513   | 0.096791    | -3.36898         | 8.10E-17    | 1.03E-13    |
| ETNK2        | 25.88451  | 47.104     | 4.66503    | 0.099037    | -3.33589         | 8.10E-17    | 1.03E-13    |
| TUBB3        | 9.802032  | 1.010702   | 18.59334   | 18.39647    | 4.201357         | 8.86E-17    | 1.03E-13    |
| DPT          | 13.10894  | 23.69376   | 2.524124   | 0.106531    | -3.23065         | 1.35E-16    | 1.45E-13    |
| KPRP         | 12.68058  | 23.2699    | 2.091252   | 0.089869    | -3.47603         | 2.49E-16    | 2.48E-13    |
| CLEC3B       | 16.77294  | 29.85829   | 3.68758    | 0.123503    | -3.01739         | 7.10E-16    | 6.60E-13    |
| ANKRD37      | 55.14818  | 100.8559   | 9.44044    | 0.093603    | -3.4173          | 9.28E-16    | 8.09E-13    |
| CYP4F29P     | 22.09795  | 39.23028   | 4.969215   | 0.126668    | -2.98088         | 2.17E-15    | 1.78E-12    |
| KRT3         | 46.56198  | 82.43067   | 10.6933    | 0.129725    | -2.94647         | 8.72E-15    | 6.76E-12    |
| CRISP3       | 47.9651   | 84.80899   | 11.12121   | 0.131132    | -2.9309          | 6.57E-14    | 4.83E-11    |
| PPP1R3C      | 50.11791  | 89.58462   | 10.65119   | 0.118895    | -3.07224         | 9.76E-14    | 6.80E-11    |
| SOCS3        | 7.850827  | 1.029401   | 14.67225   | 14.2532     | 3.833214         | 7.74E-13    | 5.14E-10    |
| KRT16P2      | 25.30934  | 6.520768   | 44.09791   | 6.762687    | 2.757597         | 8.42E-13    | 5.34E-10    |
| KLK6         | 49.11907  | 12.67357   | 85.56277   | 6.750319    | 2.754956         | 9.84E-13    | 5.97E-10    |
| MAF4         | 14.13919  | 23.95185   | 4.326524   | 0.180634    | -2.46886         | 3.25E-12    | 1.89E-09    |
| CD177        | 6.239805  | 0.532066   | 11.94754   | 22.45498    | 4.488964         | 6.07E-12    | 3.38E-09    |
| CDH3         | 20.64668  | 5.979595   | 35.3174    | 5.909913    | 2.563137         | 7.13E-12    | 3.82E-09    |
| ANKRD20A5P   | 6.083548  | 11.44316   | 0.723938   | 0.063264    | -3.98248         | 4.05E-11    | 2.02E-08    |
| PSCA         | 36.97691  | 63.83741   | 10.11641   | 0.158471    | -2.65771         | 4.88E-11    | 2.35E-08    |
| SFRP2        | 27.36962  | 45.66674   | 9.072498   | 0.198668    | -2.33157         | 5.22E-11    | 2.43E-08    |
| DUSP5        | 32.6366   | 55.26694   | 10.00626   | 0.181053    | -2.46551         | 5.51E-11    | 2.48E-08    |
| CLDN17       | 30.11305  | 49.5288    | 10.69729   | 0.215981    | -2.21102         | 2.50E-10    | 1.09E-07    |

GSM2573173, and GSM2573174) were selected as representatives of injured versus healed oral mucosal tissue for comparative analysis in this study.

**3.2. Analytical Approaches.** A series of bioinformatic analyses were performed and are depicted as a flowchart (Figure 1). Firstly, differential gene expression analysis was performed to identify DEGs during the healing process of oral mucosa injury. Subsequently, functional enrichment analysis was performed to explore the signaling pathways in which DEGs were enriched. In addition, a protein-protein interaction (PPI) network was constructed to identify the hub proteins that are likely to play critical roles in the healing process. Additionally, a transcription factor- (TF-) target DEGs interaction network was constructed to identify the transcription factors that might be key to regulation of the healing process by targeting the greatest number of DEGs. Thereafter, a miRNA-target interaction network was constructed based on DEGs and miRNAs which target these DEGs. Finally, in order to explore the chemical compounds and small molecular drugs that can regulate the healing process of oral mucosa injury, a DEGs-chemical compound interaction network and miRNA-small molecular drug interaction network were each constructed.

**3.3. Identification of DEGs Involved in the Oral Mucosa Regeneration.** By performing differential expression analysis, a total of 650 DEGs consisting of 448 downregulated DEGs and 202 upregulated DEGs were identified as significantly involved in the oral mucosa regeneration (File S1). Table 1 shows the top 30 DEGs with the most significant p values, including defensin beta 4A (DEFB4A), C-C motif chemokine
Figure 2: The enriched functions of DEGs involved in the oral mucosa regeneration process: (a) GO terms-biological processes; (b) signaling pathways. The count represents the number of genes enriched in a BP term, and $-\log_{10}(p\text{ value})$ represents the enrichment score. The bigger size of the dots corresponding to a BP term means more genes were enriched in this term. The coloured dots represent the term enrichment: green indicates low enrichment, and red indicates high enrichment.
ligand 19 (CCL19), filaggrin (FLG), cysteine-rich secretory protein 3 (CRIPT3), and suppressor of cytokine signaling 3 (SOCS3).

3.4. Functional Terms Enriched by DEGs. File S2 and S3 show the significantly enriched biological processes and signaling pathways associated with DEGs, respectively. Figure 2(a) shows that DEGs involved in oral wound healing were implicated in many biological processes such as epidermis development, keratinocyte differentiation, keratinization, epidermal cell differentiation, extracellular matrix organization, biological processes related to cell cycle, and spindle checkpoint. Figure 2(b) shows that DEGs involved in oral wound healing were implicated in arachidonic acid metabolism, ECM-receptor interaction, cell cycle, and the p53 signaling pathway.

3.5. Identification of Hub Genes in the PPI Network. The PPI network (shown in Figure 3) consisted of 7,055 nodes and 16,851 edges (File S4). As shown in Table 2, the top 30 nodes with the highest degree were listed, such as GABA type A receptor-associated protein-like 1 (GABARAPL1), GABA type A receptor-associated protein-like 2 (GABARAPL2), microtubule-associated protein 1 light chain 3 alpha (MAP1LC3A), histone deacetylase 5 (HDAC5), aurora kinase A (AURKA), and polo-like kinase 1 (PLK1). These genes with the highest degree were deemed to be of the highest significance in the PPI network by interacting with the greatest number of genes.

3.6. Identification of the Significant miRNAs Targeting DEGs. The miRNA-target DEGs interaction network shown in Figure 4 consisted of 2,995 nodes and 20,362 edges (File S5). Table 3 shows the topological characteristics of the top 30 nodes in the network. Two miRNAs (miR-34a-5p and miR-335-5p) played the most significant roles in this network by targeting the greatest number of DEGs. By tracking the target relationship between a certain miRNA and its target DEGs within the top 30 nodes, it was determined that miR-34a-5p targets several DEGs including AMOTL2, CCNB1, CCND1, DDX21, FSCN1, HBP1, ID4, KPNA2, MKI67, MSN, NAV1, RRM2, SECISBP2L, SESN2, THBS1, and TXNIP. miR-335-5p was found to target several DEGs including DDX21, HBP1, RMND5A, RORA, SECISBP2L, SESN2, TXNIP, and YOD1.

3.7. Identification of Hub Transcription Factors That Target DEGs. The TFs-target DEGs interaction network shown in Figure 5 consisted of 1,054 nodes and 946 edges (File S6). Table 4 shows the top TFs with the highest degree that are pivotal to this network. Protoonocogene c-Fos (FOS), B-cell lymphoma 5 protein (BCL6), and urokinase-type plasminogen activator (PLAU) were not only TFs but also upregulated DEGs. RAR-related orphan receptor A (RORA) was TF and a downregulated DEG.

3.8. Identification of Chemical Compounds That Regulate the Expression of DEGs. A DEGs-chemical compound interaction network was constructed and is shown in Figure 6. This network is comprised of 3,612 nodes and 27,426 edges (File S7). Specific chemical compounds targeting the DEGs that were dysregulated in the healing process of oral mucosa injury included Valproic acid, Doxorubicin, Nickel, and tretinoin.

3.9. Identification of Small Molecular Drugs That Regulate the Expression of miRNAs. A miRNA-small molecular drug interaction network was constructed and is shown in Figure 7. This network is comprised of 522 nodes and 1,074 edges (File S8). Several small molecular drugs were found to regulate the expression of miRNAs targeting the DEGs dysregulated during healing of oral mucosa injury including atorvastatin, 17β-estradiol, curcumin, and vitamin D3.

4. Discussion

Bioinformatic analysis revealed several genetic and epigenetic regulatory mechanisms implicated in oral mucosa wound healing including specific miRNAs, miRNAs, pathways, TFs, and targeted drugs. Hub genes identified as central to the PPI network that interacted with the greatest number of genes included GABARAPL1, GABARAPL2, HDAC5, MAP1LC3A, AURKA, and PLK1. Gamma-aminobutyric acid receptor-associated protein-like 1 (GABARAPL1) and GABARAPL2 are proteins associated with gamma-aminobutyric acid (GABA), a non-protein amino acid, and the overexpression of GABA has been shown to suppress inflammation by inhibiting inflammatory mediators (iNOS, IL-1beta, and TNF-alpha), promoting fibroblast cell proliferation, and stimulating reepithelialization, suggesting that GABA could accelerate the healing process at the early stage of cutaneous wound healing [40]. Histone deacetylase 5 (HDAC5) belongs to class IIa HDACs and plays a key role in angiogenesis, inflammation, and immunity [41]. HDAC was shown to inhibit fibroblast proliferation by...
repressing the PDGF-receptor (R)-α [42], which aligns with our finding implicating downregulation of HDAC in the oral mucosal wound healing processes. Microtubule-associated protein 1 light chain 3 alpha (MAP1LC3A), also named LC3, encodes a central protein in the autophagy pathway and is therefore widely used as a marker for detecting autophagy and autophagic cell death [43]. As the activation of the autophagy pathway is reported as essential for myofibroblast differentiation and collagen deposition during wound healing [44], the MAP1LC3A gene as an autophagy-related marker is plausible as integral to oral mucosal wound healing. Besides, another family member of microtubule-associated protein—microtubule-associated protein 4 (MAP4) has been shown to regulate the migration and proliferation of epidermal keratinocytes and further influence epidermal wound healing by activating the p38/MAPK pathway. However, the function of the microtubule-associated protein family has not been investigated in the context of oral mucosal injury healing. Aurora kinase A (AURKA) encodes a cell cycle-regulated kinase that is involved in the formation and/or stabilization of microtubules [45]. Targeting microtubules could potentially enhance the wound healing process by promoting cell migration [46]; thus, AURKA might impact oral mucosa wound repair by influencing cell migration. PLK1 is a family member of the polo-like kinase (Plks) family, which has been demonstrated to be a key regulator of the cell cycle [47]. PLK1 plays a role in regulating mitotic entry, spindle assembly, anaphase entry, and cytokinesis in the mitotic phase and DNA checkpoints [48]. Since cell cycle regulatory genes are important for the reepithelialization of oral mucosal wounds by influencing cellular proliferation [49], the overexpression of PLK1 possibly enhances the oral mucosal wound healing by promoting cell proliferation. While existing evidence supports the role of most of the hub genes’ involvement in oral mucosal wound healing, their specific functions have not yet been verified by previous research. Future experimental studies should be designed to validate their roles during the healing of oral mucosal injury.

Table 2: The topological characteristics of the top 30 hub genes in the PPI network.

| Name               | Label | Degree | Average shortest path length | Betweenness centrality | Closeness centrality | Clustering coefficient | Topological coefficient |
|--------------------|-------|--------|-------------------------------|------------------------|---------------------|------------------------|-------------------------|
| GABARAPL2          | Down  | 583    | 2.689935                     | 0.071254               | 0.371756            | 0.003399               | 0.005516               |
| GABARAPL1          | Down  | 559    | 2.711402                     | 0.057754               | 0.368813            | 0.006036               | 0.005542               |
| MAP1LC3A           | Down  | 463    | 2.683537                     | 0.053743               | 0.372643            | 0.007429               | 0.005812               |
| HDAC5              | Down  | 363    | 2.837504                     | 0.045007               | 0.352422            | 0.001614               | 0.007383               |
| AURKA              | Up    | 332    | 2.768126                     | 0.049883               | 0.361255            | 0.003904               | 0.007122               |
| UBC                | Other | 320    | 2.217088                     | 0.270483               | 0.451042            | 0.005258               | 0.007895               |
| PLK1               | Up    | 288    | 2.75462                      | 0.047684               | 0.363026            | 0.006259               | 0.00705               |
| MDFI               | Up    | 277    | 2.965596                     | 0.057747               | 0.3372              | 1.91E – 04             | 0.005234               |
| LNX1               | Down  | 265    | 3.016776                     | 0.047711               | 0.33148             | 1.50E – 04             | 0.006827               |
| RUVBL1             | Up    | 243    | 2.846034                     | 0.034842               | 0.35166             | 0.002092               | 0.010082               |
| KRIT1              | Down  | 235    | 2.960904                     | 0.039526               | 0.337735            | 0.002633               | 0.006649               |
| FOS                | Up    | 232    | 2.932897                     | 0.035432               | 0.34096             | 0.004146               | 0.010324               |
| NINL               | Down  | 226    | 2.914984                     | 0.038519               | 0.343055            | 0.002989               | 0.007416               |
| KPNA2              | Up    | 216    | 2.813260                     | 0.032401               | 0.355199            | 0.003874               | 0.009379               |
| LGALS3             | Down  | 201    | 2.922377                     | 0.033476               | 0.342187            | 0.002459               | 0.007997               |
| ECT2               | Up    | 168    | 2.89238                      | 0.025887               | 0.345736            | 0.003302               | 0.010711               |
| DDX21              | Up    | 161    | 2.89437                      | 0.0152                 | 0.345498            | 0.008063               | 0.014814               |
| LDHA               | Up    | 156    | 2.850299                     | 0.0153                 | 0.35084             | 0.013245               | 0.013064               |
| ACTN1              | Up    | 155    | 2.871908                     | 0.021745               | 0.348201            | 0.003848               | 0.011807               |
| CDC20              | Up    | 155    | 2.823003                     | 0.017314               | 0.354233            | 0.008182               | 0.012271               |
| CCNB1              | Up    | 155    | 2.90816                      | 0.014414               | 0.34386             | 0.017677               | 0.014428               |
| ANLN               | Up    | 153    | 2.993034                     | 0.02056                | 0.334109            | 0.005517               | 0.012216               |
| ATP1A1             | Up    | 144    | 2.873472                     | 0.018625               | 0.348011            | 0.00483                | 0.012658               |
| LDHB               | Up    | 142    | 2.954933                     | 0.010182               | 0.33847             | 0.012589               | 0.016739               |
| MSN                | Up    | 142    | 2.969719                     | 0.015946               | 0.336732            | 0.00402                | 0.015602               |
| BCL6               | Up    | 138    | 2.938442                     | 0.022118               | 0.340316            | 0.004055               | 0.011744               |
| GTSE1              | Up    | 138    | 2.953512                     | 0.017776               | 0.33858             | 0.007634               | 0.012333               |
| KIFC3              | Down  | 136    | 2.949957                     | 0.018258               | 0.338988            | 0.006904               | 0.012187               |
| CCNA2              | Up    | 132    | 2.961189                     | 0.013698               | 0.337702            | 0.011937               | 0.016085               |
| CCND1              | Down  | 128    | 2.925931                     | 0.013883               | 0.341772            | 0.00427                | 0.016102               |
Two miRNAs, miR-34a-5p and miR-335-5p, were identified as pivotal in the miRNA-target DEGs network, by targeting the greatest number of DEGs. miR-34a-5p, derived from 5’ ends of pre-miR-34a, is a member of the miR-34 family, and miR-34a is a well-known tumor suppressor [50], shown to mediate endothelial progenitor cell-mediated angiogenesis [51]. The knockout of miR-34a has been shown to impair wound healing by augmenting inflammatory response and delaying reepithelialization [52], implying that miR-34a upregulation could be a potential novel strategy for enhancing mucosal regeneration. As shown in Figure 4, miR-34a-5p was noted to target several DEGs including AMOTL2, THBS1, and FSCN1. Among these, Angiotatin-Like 2 (AMOTL2) has been previously reported as downregulated during wound healing after oral mucosal injury. The AMOTL2 gene has also been found to regulate the polarity, migration, and proliferation of endothelial cells [53]. Thus, it may be considered a potential target for gene therapy in cell-based regenerative strategies, as its upregulation in oral mucosal endothelial cells could facilitate angiogenesis during wound healing. Thrombospondin 1 (THBS1), also named TSP1, is documented to be a major activator of transforming growth factor-β (TGF-β) [54]. Previous studies have shown that the knockdown of TSP1 could decrease the rate of reepithelialization, wound neovascularization, and migration of fibroblast and endothelial cells, thereby impairing the wound healing process [55–57]. Our results indicated that TSP1 was upregulated during oral mucosal wound healing, which corroborates previous observations. The fascin actin-bundling protein 1 (FSCN1), also called fascin, encodes an actin-bundling protein that regulates the focal adhesion dynamics of cytoskeletal structures during cell migration [58]. The overexpression of FSCN1 is reported to promote the cell migration of dendritic cells [59], which is in agreement with the expression pattern of FSCN1 noted in the present study. These genes may be considered targets of further research focused on gene modulation to enhance oral mucosal wound healing.

miR-335-5p was also identified as an important factor. The overexpression of miR-335 has been reported to promote diabetic wound healing by repressing the Sp1 transcription factor (Sp1) and downregulating matrix metalloproteinase (MMP)-9 [60]. Figure 3 in this study shows the DEGs targeted by miR-335-5p, including YOD1, TXNIP, and HBP1. Their relevance to wound healing is supported by earlier reports. For example, YOD1 (also known as OTUD2) is known to encode a protein that belongs to the ovarian tumor (OUT) family of deubiquitinating enzymes (DUBs) [61]. The overexpression of YOD1 was shown to promote the migration of oral keratinocytes by activating the TGF-β3 signaling pathway [62]. Contradictory to this observation, the present study indicated a downregulation of YOD1 during the mucosal healing process. Another identified gene, thioredoxin interacting protein (TXNIP), also known as vitamin D3 upregulated protein, is a major regulator of redox signaling and endoplasmic reticulum (ER) stress [63]. A recent study showed that the supplementation of vitamin D could significantly accelerate wound healing by suppressing ER stress [64]; however, the present study noted a downregulation of TXNIP in mucosal regeneration. Another gene targeted by miR-335-5p, HMG-box transcription factor 1 (HBP1), is a repressor of the cyclin D1 gene and can inhibit the Wnt-mediated beta-catenin signaling pathway. The activation of Wnt signaling has been shown to promote mucosal repair [65]. Our results indicated the downregulation of HBP1 during oral mucosal repair, suggesting that downregulating HBP1 might promote Wnt signaling and thereby augment oral mucosal repair.

Four signaling pathways (arachidonic acid metabolism, cell cycle, p53, and extracellular matrix (ECM)- receptor interaction) were found significantly enriched during the healing of oral mucosa. Arachidonic acid (AA) is a fatty acid that is abundantly expressed after tissue injury [66]. AA and its metabolites have been demonstrated to facilitate wound healing by inducing the adhesion and migration of endothelial cells, as well as angiogenesis [66]. The effects of induction of AA on cell motility were found regulated by membrane type 3-matrix metalloproteinase (MT3-MMP)-dependent fibronectin degradation [67]. The arrest of the cell cycle has been shown to impair wound healing, which is characterized by a significant number of senescent fibroblasts that are irreversible and refractory to growth factor stimulation, unless genetically modified [68]. In addition, it has been shown that periodontal pathogens (Fusobacterium nucleatum and Porphyromonas gingivalis) reduce wound healing by downregulating the cell cycle genes cyclin1, cyclin-dependent kinase 1 (CDK1), and CDK4 that are critical for G1/S transition and initiating the further process of DNA replication in S phase [69]. Concerning the cell cycle, the tumor suppressor gene p53 is of importance, being a master cell cycle regulator [70] and linked to the intrinsic apoptosis pathway, which is predominantly involved in the wound healing process.
healing of oral mucosa, in contrast to that of skin [10]. The inhibition of p53 was reported to accelerate the early epithelization and neovascularization during wound healing [71], which supports a previous report showing that the expression level of p53 on the 3rd-day postwounding was close to that with prewounding levels and very low [10]. Furthermore, the interaction between ECM and cellular adhesion receptors (e.g., integrin and growth factor receptors) has been shown to regulate cellular activities of progenitor stem cells, including migration, differentiation, proliferation, and survival, during all phases of cutaneous wound healing [72]. In addition, fibronectin (FN) and chondroitin sulphate (CS), two components of ECM, are found as significantly elevated in oral mucosa as compared with skin [73], which could account for the faster healing of oral mucosa wounds than skin wounds [14]. These findings, together with our results, suggest that p53 signaling and ECM (especially FN and CS) interaction signaling can be regarded as specific characteristics of oral mucosa wound healing that are distinct from skin wound healing.

Four transcription factors, FOS, PLAU, BCL6, and RORA, were identified as highly crucial to oral mucosa wound healing and as differentially expressed during the healing process. The transcription factor FOS (also called c-Fos) has been found to stimulate cell proliferation and regulate cell growth by interacting with c-Jun and further activating the activator protein- (AP-) 1 complex [74]. FOS was also found to be rapidly and specifically expressed by epidermal cells at the margin of the wound, thereby acting as an early molecular component during the wound healing process [75]. Another identified factor, plasminogen activator, urokinase (PLAU), encodes a secreted serine protease that can convert plasminogen to plasmin. The plasminogen activator/plasmin system has been shown to modulate cell adhesion and cellular migration by activating MMPs and TGF-β, thereby providing the important link between PLAU

| Name      | Label | Degree | Average shortest path length | Betweenness centrality | Closeness centrality | Topological coefficient |
|-----------|-------|--------|------------------------------|------------------------|---------------------|------------------------|
| CCND1     | Down  | 388    | 2.593186                     | 0.081676               | 0.385626            | 0.035366               |
| SOD2      | Up    | 363    | 2.631931                     | 0.071979               | 0.379949            | 0.032418               |
| TXNIP     | Down  | 322    | 2.646627                     | 0.053959               | 0.377839            | 0.033412               |
| LMRB2     | Up    | 297    | 2.706747                     | 0.049038               | 0.369447            | 0.029849               |
| YOD1      | Down  | 282    | 2.674015                     | 0.046754               | 0.37397             | 0.04023                |
| RORA      | Down  | 231    | 2.725451                     | 0.028034               | 0.366912            | 0.043835               |
| TSC22D2   | Down  | 225    | 2.752171                     | 0.030429               | 0.36353             | 0.04615                |
| THBS1     | Up    | 209    | 2.738811                     | 0.02335                | 0.365122            | 0.047046               |
| ZNF431    | Down  | 203    | 2.790247                     | 0.028431               | 0.358391            | 0.040197               |
| IGFBP5    | Down  | 193    | 2.775551                     | 0.023039               | 0.360289            | 0.042372               |
| SECISBP2L | Down  | 186    | 2.755511                     | 0.022275               | 0.362909            | 0.048889               |
| SESN2     | Down  | 186    | 2.738811                     | 0.023606               | 0.365122            | 0.04734                |
| MIB1      | Up    | 178    | 2.782231                     | 0.016852               | 0.359424            | 0.051973               |
| SLC16A1   | Up    | 176    | 2.834335                     | 0.020942               | 0.352816            | 0.037253               |
| NAV1      | Up    | 168    | 2.784903                     | 0.019746               | 0.359079            | 0.045105               |
| MSN       | Up    | 162    | 2.804275                     | 0.020327               | 0.356598            | 0.042947               |
| CCNB1     | Up    | 159    | 2.798931                     | 0.0135                 | 0.357279            | 0.0552                 |
| GPRC5A    | Down  | 155    | 2.813801                     | 0.018532               | 0.354823            | 0.052165               |
| SIK1      | Down  | 153    | 2.806947                     | 0.012722               | 0.356259            | 0.061204               |
| AMOTL2    | Down  | 149    | 2.815631                     | 0.015657               | 0.35516             | 0.042484               |
| WDR26     | Down  | 148    | 2.814295                     | 0.01461                | 0.353529            | 0.053959               |
| DDX21     | Up    | 147    | 2.765531                     | 0.012253               | 0.361594            | 0.057614               |
| RMND5A    | Down  | 146    | 2.777555                     | 0.012082               | 0.360029            | 0.054446               |
| hsa-miR-34a-5p | Down  | 144  | 2.359051                    | 0.030631               | 0.423899            | 0.028501               |
| ZNF426    | Down  | 142    | 2.831663                     | 0.016397               | 0.353149            | 0.041514               |
| RRM2      | Up    | 139    | 2.812291                     | 0.013288               | 0.355582            | 0.058531               |
| FSCN1     | Up    | 136    | 2.843687                     | 0.015655               | 0.351656            | 0.042006               |
| HBP1      | Down  | 135    | 2.795591                     | 0.01225                | 0.357706            | 0.06161                |
| KPNA2     | Up    | 135    | 2.817635                     | 0.010852               | 0.354908            | 0.062084               |
| hsa-miR-335-5p | Down  | 130  | 2.58016                      | 0.032712               | 0.387573            | 0.022844               |
expression and wound healing [76]. B-cell lymphoma 6 protein (BCL6) (also named zinc finger protein 51 (ZNF51)) has been validated as a target of miR-155 [77]. The downregulation of BCL6 mRNA expression induced by the depletion of miR-155 has been noted to enhance dermal wound healing [77]. Retinoid-related orphan receptor alpha (RORA) is a transcription factor demonstrated to regulate the differentiation/survival of keratinocytes under hypoxic conditions [78]. Since oral mucosa wounds heal under significantly lower hypoxic conditions than skin wounds [79], the overexpression of RORA might be considered a specific feature of oral mucosa wound healing.

Four significant chemical compounds, Valproic acid, Doxorubicin, Nickel, and tretinoin, were identified to regulate the expression of DEGs that were dysregulated during oral mucosal wound healing. Valproic acid (VPA) has been commonly prescribed to treat neurological disorders for several decades owing to its neuroprotective effects [80]. VPA has the potential to enhance cutaneous wound healing as it exerts differential effects on cell proliferation and migration, via activating Wnt/β-catenin, extracellular signal-regulated kinase (ERK), and phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathways [81]. Doxorubicin (DXR) is a typical chemotherapeutic agent that is widely used in cancer treatment and can act as an inhibitor of wound healing processes. Former research suggested that DXR significantly delayed the restoration of tensile strength, resulting in skin thinning and degeneration of skin appendage after surgery [82]. Nickel (Ni), a metallic element routinely utilized for orthodontic and prosthetic device construction, improves chemical stabilization, corrosion resistance, and durability [83]. However, Ni can cause side effects under inflammatory conditions [84] and in turn induce immune responses along with exacerbating inflammation, thus impairing oral wound healing [85]. A high concentration of Ni was noted to promote inflammatory processes and proliferation via toll-like receptor-4/nuclear factor-kappa B (NF-κB) activation in Interleukin-1β-induced human gingival fibroblasts (HGFs) [85]. Tretinoin, also known as all-trans retinoic acid (ATRA), has been applied as a useful treatment for epithelial wound healing and keratinization disorders, because of its role in stimulating angiogenesis and collagen production [86]. However, a previous study demonstrated that tretinoin retarded reepithelialization in open wounds [87]. In contrast, collagen production was found increased by tretinoin [88] in recent experimental research [89]. Therefore, the impact of tretinoin on wound healing remains controversial.

Four small molecules, atorvastatin, 17β-estradiol, curcumin, and vitamin D3, were identified as primary drug candidates relevant to oral mucosa regeneration. Statins are
recognized as therapeutic agents for treating different inflammatory conditions and wound healing and display diverse pleiotropic effects including anti-inflammatory, antioxidant, immunomodulation, antibacterial, and neangiogenesis effects [90]. Both oral and topical administration of atorvastatin produced a significant enhancement in wound healing [91, 92], which accelerated tissue repair by modulating inflammatory cytokines and cell growth-associated proteins.

The female sex hormone 17β-estradiol (estrogen/E2) is found to exert differential effects on females and males during wound healing [93]. 17β-Estradiol treatments are well established to have a beneficial influence on female cutaneous physiology: 17β-estradiol promotes anti-inflammatory (M2) macrophage actions and the migration of keratinocytes and fibroblasts while decreasing the expression of proinflammatory cytokines and wound protease [93]. However, 17β-estradiol is found to delay wound repair in male mice, since it retards reepithelialization and inhibits collagen secretion [94]. Curcumin, a highly pleiotropic molecule, is considered a novel therapeutic for improving wound regeneration and is characterized by its anti-infectious, anti-inflammatory, and antioxidant properties [95]. Different topical formulations of curcumin, including nanoformulations, have been applied for wound healing activities (e.g., inflammation, proliferation, and remodelling) and play a vital role in scavenging free radicals, alleviating proinflammatory activities, improving neovascularization and extracellular matrix (ECM) synthesis, and inhibiting apoptosis and scar maturation [96]. Vitamin D3 is a fat-soluble secosteroidone and exerts various effects on biological activities, including immunomodulation, antiaging, anticarcinogenesis, and anti-infection effects [97]. Previous studies have suggested that vitamin D has a potent anti-inflammatory and immunomodulating effect on dental caries [98] and oral candidiasis [99]. Specifically, vitamin D is shown to promote oral mucosal wound healing in many autoimmuneologic oral diseases such as recurrent aphthous stomatitis, Behcet syndrome, and Sjögren syndrome [97].

Table 4: The topological characteristics of the top 30 nodes in the TF-target interaction network.

| Name      | Label | Degree | Average shortest path length | Betweenness centrality | Closeness centrality | Clustering coefficient | Topological coefficient |
|-----------|-------|--------|-------------------------------|------------------------|----------------------|------------------------|-------------------------|
| GATA2     | TF    | 253    | 2.191833                     | 0.082064               | 0.456239             | 0.001663               | 0.04192                 |
| ETS1      | TF    | 241    | 2.178538                     | 0.08846                | 0.459024             | 0.001729               | 0.040046                |
| AR        | TF    | 227    | 2.194682                     | 0.07873                | 0.455647             | 0.00191                | 0.041171                |
| YBX1      | TF    | 202    | 2.298196                     | 0.068148               | 0.435124             | 7.8E−04                | 0.044113                |
| GATA1     | TF    | 168    | 2.426401                     | 0.030482               | 0.412133             | 0.001853               | 0.055186                |
| SP1       | TF    | 154    | 2.312441                     | 0.036965               | 0.432444             | 0.00365                | 0.050859                |
| FOS       | Up_TF | 139    | 2.025641                     | 0.069117               | 0.493671             | 0.060369               | 0.045021                |
| ARNT      | TF    | 128    | 2.417854                     | 0.018538               | 0.41359              | 0.002338               | 0.06006                 |
| MIA3      | TF    | 127    | 2.48433                      | 0.017272               | 0.402523             | 3.75E−04               | 0.068054                |
| ESR1      | TF    | 124    | 2.458689                     | 0.021245               | 0.406721             | 0.001967               | 0.053796                |
| CREB1     | TF    | 113    | 2.439696                     | 0.016546               | 0.409887             | 0.006637               | 0.059282                |
| FOXP3     | TF    | 113    | 2.603039                     | 0.021352               | 0.384166             | 6.32E−04               | 0.065569                |
| AHR       | TF    | 109    | 2.504274                     | 0.012008               | 0.399317             | 0.003058               | 0.068831                |
| MYC       | TF    | 108    | 2.5717                       | 0.018731               | 0.388848             | 6.92E−04               | 0.068219                |
| PAX5      | TF    | 105    | 2.623932                     | 0.011361               | 0.381107             | 1.83E−04               | 0.080365                |
| E2F4      | TF    | 104    | 2.518519                     | 0.024369               | 0.397059             | 0.002801               | 0.052057                |
| Pax-5     | TF    | 102    | 2.683761                     | 0.009227               | 0.372611             | 0               | 0.091831                |
| E2F1      | TF    | 99     | 2.477683                     | 0.015085               | 0.403603             | 0.00268               | 0.062549                |
| DAND5     | TF    | 98     | 2.622982                     | 0.007305               | 0.381245             | 0.002525               | 0.078717                |
| PSG1      | TF    | 97     | 2.645774                     | 0.006395               | 0.377961             | 0.002577               | 0.081277                |
| JUN       | TF    | 91     | 2.509972                     | 0.014222               | 0.398411             | 0.016361               | 0.063318                |
| CCND1     | Down  | 91     | 2.195632                     | 0.057784               | 0.45545              | 0.01514                | 0.05193                 |
| STAT1     | TF    | 89     | 2.51377                      | 0.012112               | 0.397809             | 0.006639               | 0.062582                |
| GATA3     | TF    | 87     | 2.593542                     | 0.009757               | 0.385573             | 0.002673               | 0.062089                |
| TP53      | TF    | 87     | 2.60019                      | 0.015924               | 0.384587             | 0.001069               | 0.059128                |
| Delta-CREB| TF    | 85     | 2.717949                     | 0.005681               | 0.367925             | 0               | 0.096063                |
| TCF3      | TF    | 82     | 2.630579                     | 0.009266               | 0.380144             | 0.002409               | 0.071966                |
| PRDM14    | TF    | 73     | 2.65812                      | 0.006654               | 0.376206             | 0.003044               | 0.059795                |
| PLAU      | Up_TF | 73     | 2.189934                     | 0.029773               | 0.456635             | 0.082572               | 0.056671                |
| TBP       | TF    | 72     | 2.595442                     | 0.016121               | 0.385291             | 0.003912               | 0.058185                |
This bioinformatic study provides an overall perspective of the genetic and epigenetic mechanisms underpinning oral mucosa wound healing, deduced from the comprehensive analyses performed, including functional enrichment analysis, PPI network construction, miRNA-target network construction, and DEG-TF network construction. However, the study is subject to several limitations. First, results from the bioinformatic analysis were not validated in clinical experiments, and these should be performed in further research. Only one dataset was found related to oral mucosa wound healing and thus included for the analysis. The availability of more datasets with similar study designs and with
larger sample sizes is essential to replicate these preliminary results. Furthermore, datasets examining the noncoding RNA (e.g., miRNAs, lncRNAs, and circRNAs) expression profiles were not found in public databases; therefore, the identification of the miRNAs in this study was through prediction based on the targeting interactions between DEGs and miRNAs. Studies investigating the epigenetic mechanisms involved in oral mucosa wound healing by examining noncoding RNA expression profiles would be valuable in this context.

Despite these limitations, the findings of our study may serve as the theoretical basis directing future experiments concerning oral wound healing models and related target genes. They also provide insights into potential therapeutic targets and strategies for treating chronic and nonhealing oral mucosa wounds. These results could also plausibly be applied to genetic and epigenetic modification of stem cells utilized for tissue regeneration and optimize the design of the tissue-engineered 3-dimensional (3D) culture models of oral mucosa regeneration. Further, these findings could advance the development of topical drugs targeting relevant gene targets for treating oral mucosa lesions. Challenges in clinical translation include that of synergistic application with conventionally used topical drugs accruing anti-inflammatory, analgesic, anesthetic, and antimicrobial effects. As such, these results should be considered preliminary and a directive for future research design.

5. Conclusion

This study identified several genes (GABARAPL1, GABARAPL2, HDAC5, MAP1LC3A, AURKA, and PLK1), two miRNAs (miR-34a-5p and miR-335-5p), four transcription factors (FOs, PLAU, BCL6, and RORA), and signaling pathways (arachidonic acid metabolism, cell cycle, p53, and extracellular matrix- (ECM-) receptor interaction) to be the most significant biomarkers in the wound repair process of oral mucosa injury. This study also identified significant chemical compounds (Valproic acid, Doxorubicin, Nickel, and tretinoin), as well as small molecular drugs (atorvastatin, 17β-estradiol, curcumin, and vitamin D3), which could influence the oral mucosal wound healing by regulating the expression of healing-associated DEGs/miRNAs. These entities could be important targets for genetic and epigenetic modification of oral mucosa-derived cells for tissue engineering and other treatment approaches for promoting oral mucosal wound healing.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

As this study only applied bioinformatic techniques based on computational analyses, all of the data from oral buccal mucosa biopsies were obtained from the public datasets, and original samples were not obtained. Therefore, this study did not require ethical approval.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Wanchen Ning (Email: wanchen.ning@campus.lmu.de) is the first author and senior author. Dr. Shaonan Hu (Email: shaonan.hu@medizin.uni-leipzig.de), Prof. Dr. Xianda Hu (Email: hellocean@hotmail.com), and Dr.rer.med. Simin Li (Email: simin.li.dentist@gmail.com) are equal to the corresponding author.

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Supplementary Materials

Supplementary 1. File S1. The DEGs dysregulated in the self-healing process of human oral mucosa injury.
Supplementary 2. File S2. The GO terms enriched by DEGs.
Supplementary 3. File S3. The KEGG pathways enriched by DEGs.
Supplementary 4. File S4. The topological characteristics of the nodes in the PPI network.
Supplementary 5. File S5. The topological characteristics of the nodes in the miRNA-target network.
Supplementary 6. File S6. The topological characteristics of the nodes in the TF-target network.
Supplementary 7. File S7. The topological characteristics of the nodes in the DEGs-chemical compound interaction network.
Supplementary 8. File S8. The topological characteristics of the nodes in the miRNA-small molecular drug interaction network.

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