Research Article

Long Noncoding RNA Expression Profiles of Periodontal Ligament Stem Cells from the Periodontitis Microenvironment in Response to Static Mechanical Strain

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During the period of orthodontic tooth movement, periodontal ligament stem cells (PDLSCs) play an important role in transducing mechanical stimulation and tissue remodeling. However, our previous studies verified that the periodontitis microenvironment causes damage to the biological functions of PDLSCs and abnormal mechanical sensitivity. Long noncoding RNAs (lncRNAs) participate in the inflammatory pathogenesis and development of many diseases. Whether lncRNAs are abnormally expressed in PDLSCs obtained from periodontal tissues of periodontitis patients (PPDLSCs) and whether putative lncRNAs participate in the mechanotransductive process in PDLSCs remain poorly understood. First, we subjected PDLSCs obtained from healthy periodontal tissues (HPDLSCs) and PPDLSCs to static mechanical strain (SMS) with 12% elongation at 0.1 Hz frequency using an FX-4000T system and screened overall lncRNA profiles in both cell types by microarray. Among lncRNAs with a fold change (FC) > 2.0, 27 lncRNAs were upregulated in strained HPDLSCs, and 16 lncRNAs (9 upregulated and 7 downregulated) were detected in strained PPDLSCs. For mRNAs with FC > 2.0, we detected 25 upregulated mRNAs and one downregulated mRNA in strained HPDLSCs and 7 upregulated and 5 downregulated mRNAs in strained PPDLSCs. Further enrichment analysis showed that, unlike HPDLSCs with annotations principally involving transduction-associated signaling pathways, dysregulated mRNAs in PPDLSCs are mainly responsible for pathological conditions. Moreover, coexpressed lncRNA-mRNA networks confirmed the pathological state and exacerbated inflammatory conditions in strained PPDLSCs. Taken together, when compared with strained HPDLSCs, various lncRNAs and mRNAs were dysregulated in PPDLSCs under mechanical forces, implicating the response of lncRNAs in PPDLSCs to mechanical stress. Moreover, we provide potential lncRNA targets, which may contribute to future intervention strategies for orthodontic treatment in periodontitis patients.

1. Introduction

Periodontitis is a chronic inflammatory disease that causes irreversible periodontal attachment damage [1]. During the pathological process, osteoblasts are distinctly suppressed, whereas osteoclastogenesis becomes hyperactive [2]. Because of the typically high morbidity rate and obvious clinical manifestation of tooth extrusion, space, and labial drifting, many adult patients with periodontitis seek orthodontic treatment to achieve both esthetic restoration and functional...
2. Materials and Methods

2.1. Cell Culture. Primary PPDLSCs were obtained from premolar and/or third molar extractions of 8 donors (38.9 ± 7.9 years old) for therapeutic reasons who were diagnosed with chronic periodontitis. Primary HPDLSCs were isolated from 10 orthodontic patients (37.9 ± 7.2 years old) who underwent routine premolar and/or third molar extractions. All samples were collected at the Department of Orthodontics, School of Stomatology, the Fourth Military Medical University. Periodontitis patients were collected according to the following criteria made by the same periodontal specialist: bleeding on probe; periodontal pocket < 6 mm with 3-4 mm attachment loss; and/or alveolar bone absorption up to 1/3-1/2 root length horizontally on X-ray images. None of these subjects were selected with any clinical evidence of systemic disease or an acute infection in the past 6 months, and no one had a smoking history, drug utilization, or ever received maxillofacial radiotherapy and chemotherapy [6, 15]. All subjects provided written informed consent in accordance with the Declaration of Helsinki, and the study reached a consensus with the Ethics Committee of the Fourth Military Medical University (Approval Number: 2017(026)). The primary cells were cultured in α-MEM (Gibco BRL, Gaithersburg, MD, USA) with 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA, USA) with 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA, USA) in a humidified environment at 37°C with 5% CO₂ [14]. Cell colonies were established by the limiting dilution technique [16].

2.2. SMS Loading. All cells were seeded into collagen I-coated 6-well BioFlex plates (Flexcell International, Burlington, NC, USA), and cells were serum starved for 24 h after achieving 95% confluence. Experimental cells were subjected to SMS for 12 h utilizing a Flexcell Tension Plus system (FX-4000T, Flexcell International) with 12% elongation at 0.1 Hz [6]. Static groups were cultured under the same conditions without SMS exposure.

2.3. RNA Extraction. Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and quantified using a NanoDrop ND-1000 (Thermo Fisher Scientific, Boston, MA, USA). RNA integrity was evaluated by standard denaturing agarose gel electrophoresis.

2.4. Microarray Analysis. Sample labeling and array hybridization were conducted using the Agilent One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technologies, Santa Clara, CA, USA) [13]. In brief, mRNA was purified from total RNA after removing rRNA (mRNA-ONLY Eukaryotic mRNA Isolation Kit; Epicenter, Madison, WI, USA) with 5% CO₂ [14]. Cell colonies were established by the limiting dilution technique [16].

Although many IncRNAs have been identified to be associated with inflammation-induced functional changes, the regulatory effects of IncRNAs on PPDLSCs in response to mechanical forces and the underlying mechanisms remain unclear [12, 14]. Therefore, this study was aimed at determining the IncRNA profiles of PPDLSCs and PPDLSCs and at exploring potential IncRNAs involved in the process of mechanotransduction in an inflammatory microenvironment.
can be detected by collecting data sources from GENCODE, UCSC, Ensembl, ReSeq, and other related sources.

2.5. Real-Time qPCR Confirmation. Total RNA was assembled and reverse-converted to cDNA using a SuperScript First-Strand Synthesis Kit (Invitrogen). An Applied Biosystems Via 7 Real-Time PCR System was used for qPCR. The reaction system included incubation for 10 min at 95°C, followed by 40 cycles at 95°C for 10 s and 60°C for 1 min. Relative expression levels of transcripts were calculated by using the 2−ΔΔCT method and normalized to GAPDH [17]. All experiments were carried out in triplicate. The specific primers (Genscript, China) used are shown in Table 1.

2.6. Bioinformatic Analysis of Differentially Expressed (DE) mRNAs. Gene Ontology (GO) analysis was employed to map DEmRNAs to GO terms annotated by molecular function, biological process, and cellular components (http://www.geneontology.org). Significant pathways of the DE genes were determined using Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/), as previously described [18, 19].

2.7. Coexpression Network Construction (CNC). CNC was conducted based on the top 10 DElncRNAs between strained HPDLSCs and PPDLSCs with coexpressed DEmRNAs [20]. Pearson’s correlation coefficients (PCCs) no less than 0.99 were used to identify coding genes. CNCs were accomplished using Cytoscape software version 3.0.1 (The Cytoscape Consortium, San Diego, CA, USA).

2.8. Lentivirus Transfection. The design and construction of lentiviruses were performed by GeneChem (GeneChem, Shanghai, China). The lentivirus Ubi-MCS-SV40-EGFP-IRES-puromycin was used for lncRNA-XIST overexpression, and hU6-MCS-CBh-gFP-IRES-puromycin was used for lncRNA-XIST interference. The sequences of primers for amplifying lncRNA-XIST were as follows: shlncRNA-XIST (79428-3): 5′-AGAGTGCCAGGTGTCAAGA-3′ and hU6-MCS-SV40-EGFP-IRES-puromycin was used for lncRNA-XIST overexpression, and hU6-MCS-CBh-gFP-IRES-puromycin was used for lncRNA-XIST interference. The sequences of primers for amplifying lncRNA-XIST were F: 5′−ACCCCTACAGACCATACAAAGAG3′ and R: 5′−AGCCGACTACGCCACCCACT3′.

2.9. Osteogenic Differentiation Assays. For osteogenesis assays, HPDLSCs and PPDLSCs were exposed to SMS with FC > 2.0 in each group are provided in Tables 2 and 3. Of those, ENST00000517505 was the most upregulated lncRNA in strained PPDLSCs; the most upregulated and downregulated lncRNAs in strained PPDLSCs were lncRNA-XIST and ENST00000411904 was the most upregulated lncRNA in strained HPDLSCs, and 2,549 were only expressed in strained HPDLSCs. DElncRNAs with FC ≥ 2.0 and PCCs ≥ 0.9 and/or P < 0.05.

3. Results

3.1. Expression Profiles of DElncRNAs and DEmRNAs with SMS. According to the principles of FC ≥ 2.0 and PCCs ≥ 0.9, we screened 8,847 and 9,772 DElncRNAs in strained HPDLSCs and PPDLSCs relative to static controls, respectively (Figure 1(a)). In addition, 1,624 DElncRNAs were only expressed in strained HPDLSCs, and 2,549 were only expressed in strained PPDLSCs. DElncRNAs with FC > 2.0 in each group are provided in Tables 2 and 3. Of those, ENST00000411904 was the most upregulated lncRNA in strained HPDLSCs; the most upregulated and downregulated lncRNAs in strained PPDLSCs were lncRNA-XIST and ENST00000517505, respectively. Volcano and scatter plots as well as hierarchical clustering were examined to assess the lncRNA expression differences between HPDLSCs and PPDLSCs exposed to the strain (Figures 1(b)–1(d)).

Thousands of DEmRNAs were identified (Figure 1(e)). In total, 11,937 and 12,410 DEmRNAs were significantly altered in strained HPDLSCs and PPDLSCs, respectively. In particular, 2,170 specific DEmRNAs were detected in strained HPDLSCs and 2,643 in strained PPDLSCs. The most upregulated and downregulated mRNAs in strained HPDLSCs were ASHGA5P006667 and ASHGA5P003418, and the most upregulated and downregulated mRNAs in strained PPDLSCs were ASHGA5P009176 and ASHGA5P052412 (KIF20A) (Tables 4 and 5). The volcano and scatter plots depicted in Figures 1(f) and 1(g) demonstrate the variation in lncRNA expression between strain-induced HPDLSCs and PPDLSCs.
Figure 1: Continued.
Figure 1: DElncRNAs and DEmRNAs between HPDLSCs and PPDLSCs after SMS exposure. Venn diagrams of DElncRNAs (a) and DEmRNAs (e). Volcano plot of expression profiles of lncRNAs (b) and mRNAs (f). Scatter plot of expression variations of lncRNAs (c) and mRNAs (g). Dots above the top and below the bottom green lines represent FC > 4.0. (d) Hierarchical clustering of DElncRNAs. Red represents relatively high expression, and green represents relatively low expression.
3.2. Confirmation of DElncRNAs Using Real-Time qPCR. To validate the microarray results, we randomly selected four lncRNAs (TCONS_00008604, ENST00000428781, uc004arq.1, and XIST) from the top 10 DElncRNAs between strained HPDLSCs and PPDLSCs and evaluated their expression by qPCR assay (Table 6). All lncRNAs were downregulated in strained PPDLSCs compared to HPDLSCs, which was consistent with the microarray analysis results (Figure 2).

3.3. Preliminary Analysis of DEmRNAs with SMS. To further explore the putative functions of lncRNAs, bioinformatic analysis was applied based on GO and KEGG pathway analyses. According to the results, DEmRNAs in strained HPDLSCs were mainly enriched in the regulation of stress response, signal transduction, and response to stimulus (Figures 3(a), 3(c), and 3(e)). In contrast, pathological processes such as cell-type apoptotic processes and neuronal apoptotic processes were enriched in strained PPDLSCs (Figures 3(b), 3(d), and 3(f)). Enrichment scores revealed prominent assignments for cellular function and metabolism, such as fatty acid degradation and metabolism, in strained HPDLSCs (Figure 3(g)). In strained PPDLSCs, pathological states were largely notable, including Huntington’s disease, bladder cancer, and non-small-cell lung cancer (Figure 3(h)).

3.4. Constructions of the CNC Network. By combining the top 10 DElncRNAs with coexpressed DEmRNAs, an integrated coexpression network containing 1,250 lncRNA-mRNA interactions was established (Figure 4(a)). Notably, RP11-597D13.9 (ENST00000505532) was associated with the maximum number of DEmRNAs, up to 160. XIST, the most downregulated lncRNA in strained PPDLSCs, was coexpressed with 47 DEmRNAs (Table 6). In addition, GO annotations and KEGG analyses showed that the DElncRNAs in the key module are related to chondrocyte development, fibroblast apoptotic process regulation, and cell adhesion as well as leukocyte transendothelial migration, which likely participate in tissue...
regeneration. Taken together, dysregulated lncRNAs are involved in the pathological modification of gene expression in PPDLSCs under mechanical conditions (Figures 4(b)–4(e)).

### Table 4: DEmRNAs with FC > 20.0 in strained HPDLSCs compared to static controls.

| Sequence name     | Source  | Fold change | Regulation | P value (×10^−5) |
|-------------------|---------|-------------|------------|------------------|
| ASHGA5P006667     | RefSeq  | 413.31      | Up         | 4.315            |
| ASHGA5P008770     | RefSeq  | 147.59      | Up         | 0.0003           |
| ASHGA5P021973     | RefSeq  | 125.83      | Up         | 150.671          |
| ASHGA5P013772     | GENCODE | 70.35       | Up         | 1512.671         |
| ASHGA5P03418      | RefSeq  | 63.94       | Down       | 74.253           |
| ASHGA5P011737     | RefSeq  | 42.60       | Up         | 494.192          |
| ASHGA5P07165      | GENCODE | 41.99       | Up         | 302.842          |
| ASHGA5P037277     | RefSeq  | 41.81       | Up         | 1.06.612         |
| ASHGA5P05733      | GENCODE | 38.72       | Up         | 46.774           |
| ASHGA5P02830      | GENCODE | 37.77       | Up         | 15.020           |
| ASHGA5P02962      | RefSeq  | 36.22       | Up         | 5.402            |
| ASHGA5P013771     | RefSeq  | 33.74       | Up         | 46.840           |
| ASHGA5P042689     | RefSeq  | 32.07       | Up         | 92.410           |
| ASHGA5P02150      | RefSeq  | 31.42       | Up         | 0.128            |
| ASHGA5P007745     | RefSeq  | 30.45       | Up         | 6.915            |
| ASHGA5P03294      | GENCODE | 28.31       | Up         | 0.254            |
| ASHGA5P017399     | RefSeq  | 28.24       | Up         | 7.840            |
| ASHGA5P08733      | GENCODE | 25.41       | Up         | 64.712           |
| ASHGA5P05403      | RefSeq  | 23.76       | Up         | 1.361            |
| ASHGA5P054134     | RefSeq  | 23.62       | Up         | 245.163          |
| ASHGA5P017179     | RefSeq  | 23.04       | Up         | 14.825           |
| ASHGA5P051262     | RefSeq  | 22.99       | Up         | 858.448          |
| ASHGA5P045442     | RefSeq  | 22.91       | Up         | 200.313          |
| ASHGA5P050260     | RefSeq  | 22.54       | Up         | 0.001            |
| ASHGA5P004313     | RefSeq  | 21.00       | Up         | 4.695            |
| ASHGA5P001728     | RefSeq  | 20.64       | Up         | 84.501           |

### Table 5: DEmRNAs with FC > 20.0 in strained PPDLSCs compared to static controls.

| Sequence name     | Source  | Fold change | Regulation | P value (×10^−5) |
|-------------------|---------|-------------|------------|------------------|
| ASHGA5P009176     | RefSeq  | 111.34      | Up         | 179.785          |
| ASHGA5P013422     | RefSeq  | 59.61       | Up         | 6.424            |
| ASHGA5P010424     | GENCODE | 56.12       | Up         | 0.032            |
| ASHGA5P012978     | RefSeq  | 42.19       | Up         | 104.171          |
| ASHGA5P052412     | RefSeq  | 42.10       | Down       | 0.001            |
| ASHGA5P003780     | RefSeq  | 34.88       | Up         | 2427.963         |
| ASHGA5P005903     | RefSeq  | 31.08       | Down       | 0.919            |
| ASHGA5P017401     | RefSeq  | 27.31       | Down       | 0.304            |
| ASHGA5P001619     | RefSeq  | 25.51       | Up         | 784.373          |
| ASHGA5P004428     | RefSeq  | 24.18       | Down       | 26.041           |
| ASHGA5P004064     | RefSeq  | 21.49       | Down       | 4.546            |
| ASHGA5P034395     | GENCODE | 20.98       | Up         | 55.833           |

### Table 6: Top 10 DElncRNAs between strained HPDLSCs and PPDLSCs with coexpressed mRNAs.

| Sequence name     | Total mRNA |
|-------------------|------------|
| ENST00000505532   | 160        |
| ENST00000532307   | 156        |
| ENST00000428781   | 126        |
| uc0021q1         | 130        |
| TCONS_00008604    | 93         |
| uc004arq1        | 51         |
| ENST00000423727   | 89         |
| TCONS_00013636   | 65         |
| XIST             | 47         |
| ENST00000340196   | 12         |

**Figure 2:** Real-time qPCR conformation of DE lncRNAs between strained HPDLSCs and PPDLSCs. All experiments were performed in triplicate, and the data are presented as the mean ± S.D. *P < 0.05.

### 3.5. Functional Investigation of DElncRNAs during Osteogenic Differentiation.**

We randomly evaluated the expression of four lncRNAs (TCONS_00008604, ENST00000428781, uc004arq1, and XIST) from the top 10 DElncRNAs between strained HPDLSCs and PPDLSCs after osteogenic induction for 7 days and observed that the expression level of lncRNA-XIST significantly increased in HPDLSCs at day 7 after osteogenic differentiation. Although osteogenic induction upregulated the level of lncRNA-XIST in PPDLSCs, it was still lower than that in HPDLSCs (P < 0.05, Figure 5(a)). We also found that IncRNA-XIST was significantly increased in HPDLSCs after 12 h of SMS elongation (P < 0.05); however, strain-induced IncRNA-XIST expression was not obviously increased in PPDLSCs (Figure 5(b)). We also examined the relationship between IncRNA-XIST and the osteogenic gene Runx2 after 12% SMS loading by lentivirus transfection. We found that Runx2
expression in strained HPDLSCs infected with shlncRNA-XIST was decreased almost 2 times compared with strained HPDLSCs in the negative control group (NC) ($P < 0.05$, Figure 5(c)). In contrast, Runx2 expression in strained PPDLSCs increased after lncRNA-XIST overexpression ($P < 0.05$, Figure 5(d)). Similarly, alizarin red staining and calcium quantification also sustained that shlncRNA-XIST inhibited SMS-induced osteogenic differentiation in HPDLSCs and that overexpression of IncRNA-XIST rescued the osteogenic ability of PPDLSCs ($P < 0.05$, Figures 5(e) and 5(f)).

4. Discussion

Many lncRNAs play critical roles in multiple pathological processes of periodontitis, such as proliferation, differentiation, cell migration, and immune regulation [21, 22].
Cellular response to iron ion
Insemination
Chondrocyte development
Negative regulation of necroptotic process
Embryonic viscerocranium morphogenesis
Positive regulation of osteoblast proliferation
Negative regulation of protein autophosphorylation
Peptidyl-histidine modification
Negative regulation of heart rate
Cardiac ventricle formation
Regulation of germinal center formation
Regulation of fibroblast apoptotic process
Venous blood vessel development
Positive regulation of protein oligomerization

(b)

Figure 4: Continued.
CXCR chemokine receptor binding
Transforming growth factor beta receptor, 
Transforming growth factor beta-activated receptor, 
Protein tyrosine kinase activator activity
Chemoattractant activity
RAGE receptor binding
G-protein alpha-subunit binding
Cyclin-dependent protein serine/threonine kinase inhibitor 
 Arylsulfatase activity
HMG box domain binding

Fold enrichment ((count/pop.hits)/(list.total/pop.total))

Banded collagen fibril
Fibrillar collagen trimer
Sperm fibrous sheath
Paranode region of axon
Axolemma
Dendrite cytoplasm
Complex of collagen trimers
Dendrite shaft
Chloride channel complex
Cortical actin cytoskeleton

Fold enrichment ((count/pop.hits)/(list.total/pop.total))

Figure 4: Continued.
Excessive mechanical stimuli can cause irreversible damage to PDLSCs, especially to those in an inflammatory state [23]. However, studies on the expression of lncRNAs involved in strain-induced PDLSCs and their potential effects on cellular functions are limited. In this study, we identified thousands of DElncRNAs and DEMRNAs in HPDLSCs and PPDLSs after SMS application, and various lncRNAs and mRNAs were found to be solely expressed in strained HPDLSCs or PPDLSs, indicating that different mechanisms may be involved in the mecanotransductive responses of PDLSCs derived from different contexts.

Using microarray analysis, we observed that DElncRNAs in strained HPDLSCs were mainly enriched in mecanococonductive processes; pathological pathways such as cell-type apoptotic process and regulation of neuron apoptotic process were associated with dysregulated lncRNAs in strained PPDLSs. In addition, based on KEGG pathway analysis, the DElncRNAs are largely related to pathological conditions such as Huntington’s disease [24], bladder cancer [25], and non-small-cell lung cancer [26]. Therefore, cell functions regulated by lncRNAs have a potential role in PDLSCs, and aberrant lncRNA transcripts are associated with periodontitis progression [27].

Cytoskeletal dynamics and integrity are of vital importance for cell differentiation commitment, by which the bone loss occurring in periodontitis can be alleviated [28]. By altering the expression of eukaryotic cytoskeleton proteins, which play important roles in cancer progression and cytoskeleton modulation, KIF20A is sensitive to alterations along with mechanical loadings [29]. In this study, KIF20A was most downregulated in strained PPDLSs, suggesting that dysregulated mRNAs possibly modulate expression through interactions with intracellular cytoskeleton mechanisms. Therefore, further functional validation of these dysregulated transcripts in periodontitis is warranted.

Furthermore, to identify potential lncRNAs associated with strained PDLSCs, we integrated the coexpression networks of lncRNAs and mRNAs. A total of 1,250 pairs based on the top 10 dysregulated lncRNAs between strained PPDLSs and HPDLSCs were established. Of those, RP11-597D13.9, an antisense lncRNA, correlated with up to 160 mRNAs, and it may affect a nearby coding gene: FAM198B [30]. FAM198B has been implicated as a tumor inhibitor, attenuating lung cancer cell invasion and thus improving the overall survival of patients with lung adenocarcinoma [31]. In our study, RP11-597D13.9 displayed an inverse trend of downregulation in strained PPDLSs, indicating a possible pathological state for these cells. Moreover, lncRNA XIST has long been recognized as an oncogenic gene and is preferentially expressed in cancers [32, 33]. LPS-induced inflammation can increase the levels of XIST expression, which in turn suppresses acute inflammation via MAPK signaling [34]. Contrary to these results, there was a significant decrease in XIST in strained PPDLSs together with CH1CI (Brx), one of the major downstream target genes for XIST [35]. In our study, we first found that lncRNA-XIST expression decreased in PDLSCs derived from an inflammatory microenvironment. Additionally, although SMS elongation significantly decreased the expression of lncRNA-XIST in PPDLSs compared with HPDLSCs, the upregulation of lncRNA-XIST in strained PPDLSs increased the process of osteogenic differentiation, indicating that lncRNA-XIST may be one of the nonnegligible reasons for the impaired osteogenesis in strained PPDLSs.

5. Conclusions

In summary, differentially expressed lncRNA profiles between HPDLSCs and PDLSCs under mechanical exposure were first identified in this study, and many were specifically expressed. By functional analysis, we confirmed that DE transcripts in PPDLSs participate in many pathological processes and might be involved in regulating periodontitis progression under tension. In our study, we found that the expression of lncRNA-XIST obviously decreased in PPDLSs, and the osteogenic ability of PPDLSs under tension loading was significantly upregulated after upregulation of lncRNA-XIST by lentivirus. These results hint us that lncRNAs could regulate the osteogenic ability of PDLSCs under tension loading. Although some lncRNAs are predicted, comprehensive analyses are still needed to elucidate the details of the relevant molecular mechanisms.
Figure 5: The expression levels of DElncRNAs in HPDLSCs and PPDLSCs during osteogenic differentiation: (a) the expression levels of DElncRNAs in HPDLSCs and PPDLSCs after osteogenic differentiation for 7 days; (b) the expression levels of lncRNA-XIST in strained HPDLSCs and PPDLSCs; (c) detection of the osteogenic gene Runx2 in strained HPDLSCs infected with shlncRNA-XIST; (d) detection of the osteogenic gene Runx2 in strained PPDLSCs infected with lncRNA-XIST; (e) alizarin red staining for HPDLSCs and PPDLSCs; (f) calcium quantification for HPDLSCs and PPDLSCs. All experiments were performed in triplicate, and the data are presented as the mean ± S.D. Scale bar = 100 μm.
Data Availability

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

Conflicts of Interest

The authors declare no potential competing interests.

Authors’ Contributions

Jia Liu, Yan Zhao, and Qiannan Niu contributed equally to this work.

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