

# World Journal of *Stem Cells*

*World J Stem Cells* 2020 November 26; 12(11): 1237-1438



**REVIEW**

- 1237 Role of platelets and breast cancer stem cells in metastasis  
*Mendoza-Almanza G, Burciaga-Hernández L, Maldonado V, Melendez-Zajgla J, Olmos J*
- 1255 Understanding cellular and molecular mechanisms of pathogenesis of diabetic tendinopathy  
*Lu PP, Chen MH, Dai GC, Li YJ, Shi L, Rui YF*
- 1276 Multifunctional role of microRNAs in mesenchymal stem cell-derived exosomes in treatment of diseases  
*Xu HK, Chen LJ, Zhou SN, Li YF, Xiang C*
- 1295 Advance in metabolism and target therapy in breast cancer stem cells  
*Gao X, Dong QZ*
- 1307 Stem cell quiescence and its clinical relevance  
*Luo M, Li JF, Yang Q, Zhang K, Wang ZW, Zheng S, Zhou JJ*
- 1327 New insight into dental epithelial stem cells: Identification, regulation, and function in tooth homeostasis and repair  
*Gan L, Liu Y, Cui DX, Pan Y, Wan M*

**MINIREVIEWS**

- 1341 Isolation and characterization of mesenchymal stem cells in orthopaedics and the emergence of compact bone mesenchymal stem cells as a promising surgical adjunct  
*Anastasio A, Gergues M, Lebhar MS, Rameshwar P, Fernandez-Moure J*

**ORIGINAL ARTICLE****Basic Study**

- 1354 AlCl<sub>3</sub> exposure regulates neuronal development by modulating DNA modification  
*Cheng XJ, Guan FL, Li Q, Dai G, Li HF, Li XK*
- 1366 Application of cattle placental stem cells for treating ovarian follicular cyst  
*Peng SY, Wu TH, Lin TY, Hii LY, Chan KS, Fu TY, Chang SC, Shen PC, Liu KY, Shaw SW.*
- 1377 Immunophenotypic characteristics of multipotent mesenchymal stromal cells that affect the efficacy of their use in the prevention of acute graft *vs* host disease  
*Petinati N, Kapranov N, Davydova Y, Bigildeev A, Pshenichnikova O, Karpenko D, Drize N, Kuzmina L, Parovichnikova E, Savchenko V*
- 1396 Effect of conditioned medium from neural stem cells on glioma progression and its protein expression profile analysis  
*Zhang GL, Qian C, Zhang SZ, Tuo YH, Zeng BY, Ji YX, Wang YZ*

- 1410**    Glutathione metabolism is essential for self-renewal and chemoresistance of pancreatic cancer stem cells  
*Jagust P, Alcalá S, Sainz Jr B, Heeschen C, Sancho P*

**LETTER TO THE EDITOR**

- 1429**    Acquired aplastic anemia: Is bystander insult to autologous hematopoiesis driven by immune surveillance against malignant cells?  
*Zhao XC, Sun XY, Ju B, Meng FJ, Zhao HG*

**ABOUT COVER**

Editorial Board Member of *World Journal of Stem Cells*, Dr. Alessandra Pelagalli is a Senior Researcher of veterinary physiology in the Department of Advanced Biomedical Sciences at the University of Naples. Having completed her Pharmacy Degree from the University of Naples in 1991, Dr. Pelagalli continued her postgraduate training and received her PhD in 1996. She became a Young Researcher at the University of Naples in 1999, working in animal platelet physiology and biochemistry. Her current research interests and publications focus on the roles and behavior of bone marrow mesenchymal stem cells in the differentiation processes after stimulation, water channel proteins in cell migration, and aquaporins in various tissues, such as gut and the male reproductive tract. (L-Editor: Filipodia)

**AIMS AND SCOPE**

The primary aim of *World Journal of Stem Cells (WJSC, World J Stem Cells)* is to provide scholars and readers from various fields of stem cells with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. *WJSC* publishes articles reporting research results obtained in the field of stem cell biology and regenerative medicine, related to the wide range of stem cells including embryonic stem cells, germline stem cells, tissue-specific stem cells, adult stem cells, mesenchymal stromal cells, induced pluripotent stem cells, embryonal carcinoma stem cells, hemangioblasts, lymphoid progenitor cells, *etc.*

**INDEXING/ABSTRACTING**

The *WJSC* is now indexed in Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports/Science Edition, Biological Abstracts, BIOSIS Previews, PubMed, and PubMed Central. The 2020 Edition of Journal Citation Reports® cites the 2019 impact factor (IF) for *WJSC* as 3.231; IF without journal self cites: 3.128; Ranking: 18 among 29 journals in cell and tissue engineering; Quartile category: Q3; Ranking: 113 among 195 journals in cell biology; and Quartile category: Q3.

**RESPONSIBLE EDITORS FOR THIS ISSUE**

**Production Editor:** Yan-Xia Xing **Production Department Director:** Yun-Xiaojian Wu, **Editorial Office Director:** Jin-Lai Wang.

**NAME OF JOURNAL**

*World Journal of Stem Cells*

**ISSN**

ISSN 1948-0210 (online)

**LAUNCH DATE**

December 31, 2009

**FREQUENCY**

Monthly

**EDITORS-IN-CHIEF**

Shengwen Calvin Li, PhD, MPhil., FRSM, Tong Cao, Carlo Ventura

**EDITORIAL BOARD MEMBERS**

<https://www.wjgnet.com/1948-0210/editorialboard.htm>

**PUBLICATION DATE**

November 26, 2020

**COPYRIGHT**

© 2020 Baishideng Publishing Group Inc

**INSTRUCTIONS TO AUTHORS**

<https://www.wjgnet.com/bpg/gerinfo/204>

**GUIDELINES FOR ETHICS DOCUMENTS**

<https://www.wjgnet.com/bpg/GerInfo/287>

**GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH**

<https://www.wjgnet.com/bpg/gerinfo/240>

**PUBLICATION ETHICS**

<https://www.wjgnet.com/bpg/GerInfo/288>

**PUBLICATION MISCONDUCT**

<https://www.wjgnet.com/bpg/gerinfo/208>

**ARTICLE PROCESSING CHARGE**

<https://www.wjgnet.com/bpg/gerinfo/242>

**STEPS FOR SUBMITTING MANUSCRIPTS**

<https://www.wjgnet.com/bpg/GerInfo/239>

**ONLINE SUBMISSION**

<https://www.f6publishing.com>

## New insight into dental epithelial stem cells: Identification, regulation, and function in tooth homeostasis and repair

Lu Gan, Ying Liu, Di-Xin Cui, Yue Pan, Mian Wan

**ORCID number:** Lu Gan [0000-0002-8778-8595](https://orcid.org/0000-0002-8778-8595); Ying Liu [0000-0002-1047-8468](https://orcid.org/0000-0002-1047-8468); Di-Xin Cui [0000-0002-8102-9504](https://orcid.org/0000-0002-8102-9504); Yue Pan [0000-0001-8197-869X](https://orcid.org/0000-0001-8197-869X); Mian Wan [0000-0002-3373-2836](https://orcid.org/0000-0002-3373-2836).

**Author contributions:** Gan L and Liu Y contributed equally to this work; Gan L, Liu Y, and Wan M conceived the idea and designed the work; Gan L, Liu Y, Cui DX, and Pan Y integrated the materials; Gan L and Liu Y wrote the paper; Wan M revised the manuscript critically; all authors have read and approved the final manuscript.

**Supported by** National Natural Science Foundation of China, No. 81800929; and Sichuan Science and Technology Program, No. 2019JDR0096.

**Conflict-of-interest statement:** The authors declare that there is no conflict of interests regarding the publication of this paper.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially,

**Lu Gan, Ying Liu, Di-Xin Cui, Yue Pan,** State Key Laboratory of Oral Diseases & National Clinical Research Center for Oral Diseases & Department of Pediatric Dentistry, West China Hospital of Stomatology, Sichuan University, Chengdu 610041, Sichuan Province, China

**Mian Wan,** State Key Laboratory of Oral Diseases & National Clinical Research Center for Oral Diseases & Department of Cariology and Endodontics, West China Hospital of Stomatology, Sichuan University, Chengdu 610041, Sichuan Province, China

**Corresponding author:** Mian Wan, DDS, PhD, Academic Fellow, Academic Research, Attending Doctor, Lecturer, Postdoctoral Fellow, State Key Laboratory of Oral Diseases & National Clinical Research Center for Oral Diseases & Department of Cariology and Endodontics, West China Hospital of Stomatology, Sichuan University, No. 14 3<sup>rd</sup> Section of Renmin South Road, Chengdu 610041, Sichuan Province, China. [mianwan@scu.edu.cn](mailto:mianwan@scu.edu.cn)

### Abstract

Tooth enamel, a highly mineralized tissue covering the outermost area of teeth, is always damaged by dental caries or trauma. Tooth enamel rarely repairs or renews itself, due to the loss of ameloblasts and dental epithelial stem cells (DESCs) once the tooth erupts. Unlike human teeth, mouse incisors grow continuously due to the presence of DESCs that generate enamel-producing ameloblasts and other supporting dental epithelial lineages. The ready accessibility of mouse DESCs and wide availability of related transgenic mouse lines make mouse incisors an excellent model to examine the identity and heterogeneity of dental epithelial stem/progenitor cells; explore the regulatory mechanisms underlying enamel formation; and help answer the open question regarding the therapeutic development of enamel engineering. In the present review, we update the current understanding about the identification of DESCs in mouse incisors and summarize the regulatory mechanisms of enamel formation driven by DESCs. The roles of DESCs during homeostasis and repair are also discussed, which should improve our knowledge regarding enamel tissue engineering.

**Key Words:** Dental epithelial stem cells; Tissue engineering; Label-retaining cells; Lineage tracing; Single-cell sequencing

©The Author(s) 2020. Published by Baishideng Publishing Group Inc. All rights reserved.

and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Specialty type:** Cell biology

**Country/Territory of origin:** China

**Peer-review report's scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0

**Received:** July 10, 2020

**Peer-review started:** July 10, 2020

**First decision:** August 9, 2020

**Revised:** August 21, 2020

**Accepted:** September 15, 2020

**Article in press:** September 15, 2020

**Published online:** November 26, 2020

**P-Reviewer:** Cenciarelli C, Mitsiadis T

**S-Editor:** Gong ZM

**L-Editor:** Wang TQ

**P-Editor:** Wang LL



**Core Tip:** In the present review, we update the current understanding about the identification of dental epithelial stem cells (DESCs) in mouse incisors and summarize the regulatory mechanisms of enamel formation driven by DESCs. The roles of DESCs during homeostasis and repair are also discussed, which should improve our knowledge regarding enamel tissue engineering.

**Citation:** Gan L, Liu Y, Cui DX, Pan Y, Wan M. New insight into dental epithelial stem cells: Identification, regulation, and function in tooth homeostasis and repair. *World J Stem Cells* 2020; 12(11): 1327-1340

**URL:** <https://www.wjgnet.com/1948-0210/full/v12/i11/1327.htm>

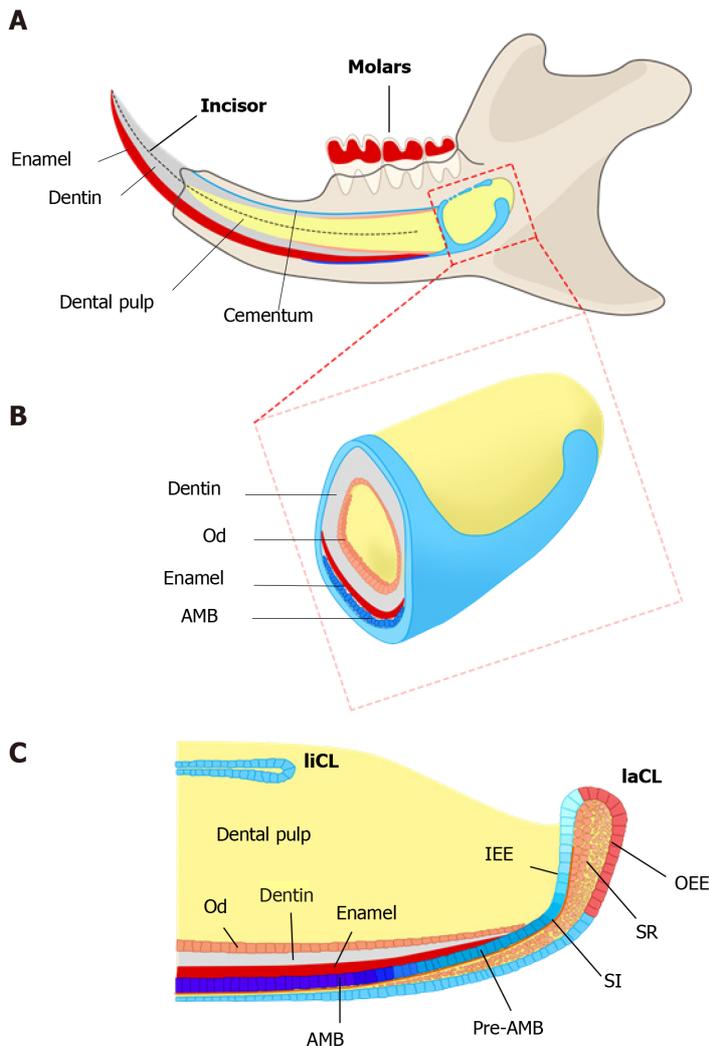
**DOI:** <https://dx.doi.org/10.4252/wjsc.v12.i11.1327>

## INTRODUCTION

Tooth enamel, the most highly mineralized tissue in the human body, consists of hydroxyapatite organized into enamel rods and inter-rods which are interwoven, therefore serving as a protective covering for the tooth crown. During enamel formation, inner enamel epithelial cells in the enamel organ differentiate into enamel-forming ameloblasts, which secrete enamel matrix and create an extracellular environment for mineralization<sup>[1-4]</sup>. These cells commit apoptosis once the enamel formation is accomplished and the tooth erupts into the oral cavity<sup>[5]</sup>. The loss of ameloblasts and neighboring environment renders enamel an acellular and nonvital tissue that, when insulted by dental caries or trauma, is incapable of repair or renewal. To restore the missing enamel tissue, current treatment is limited to using acid-etching techniques and artificial materials such as resin, amalgam, and porcelain, which are not perfect due to frequent microleakage, limited life span, or inherent inability to fully restore its function<sup>[6-8]</sup>.

One potential remedy for this is to construct natural enamel. Enamel-dentin complex structure has been detected on polyglycolic acid fiber mesh using dissociated porcine third molar tooth germ cells, suggesting tissue engineering as an alternative strategy to regenerate enamel<sup>[9]</sup>. The classic tissue engineering relies on three elements, including stem cells, suitable scaffolds, and bioactive molecules to initiate a sequence of events inducing tissue formation<sup>[10,11]</sup>. However, unlike successful implementation of tissue engineering in other dental tissues, such as dentin and pulp regeneration, enamel tissue engineering is hindered since ameloblasts and dental epithelial stem cells (DESCs) are lost when the tooth erupts<sup>[5]</sup>. Consequently, the generation of potent and viable DESCs would be a major step toward promising enamel tissue engineering<sup>[12]</sup>. Interestingly, nature has provided us a good example, the rodent incisor, which grows continuously throughout the animal's life<sup>[13]</sup>. Harada *et al*<sup>[14]</sup> have identified DESCs at the proximal end of mouse incisors, in a structure named the labial cervical loop (laCL). These cells can self-renew and differentiate into enamel-secreting ameloblasts and the other supporting dental epithelial lineages<sup>[15-18]</sup>. Owing to the ready accessibility of mouse DESCs and the wide availability of related transgenic mouse lines, mouse incisors serve as an ideal system to explore the identity and heterogeneity of dental epithelial stem/progenitor cells. The continuous replenishment of enamel tissue is fueled by the DESCs, making mouse incisors an excellent model to uncover the regulatory mechanisms underlying enamel formation<sup>[19]</sup> (Figure 1). Moreover, studying how the homeostasis and repair are maintained by DESCs in mouse incisors can help us answer the open question regarding the therapeutic development of enamel engineering.

In the present review, we update the current understanding about the identification of DESCs in mouse incisors and summarize the regulatory mechanisms of enamel formation driven by DESCs. The roles of DESCs during homeostasis and repair are also discussed, which could improve our knowledge regarding enamel tissue engineering.



**Figure 1 Schematics of adult mouse incisor and cervical loops.** A: Most of adult mouse incisor is embedded in the jawbone. Enamel (red) exists only at the labial side, which is produced by dental epithelial stem cells (DESCs) of the labial cervical loop (laCL). Dental pulp (yellow) is surrounded by dentin (gray), which is produced by dental mesenchymal stem cells. The lingual root analog is covered by cementum; B: Cervical loop is located in the proximal end of mouse incisor. The enamel and dentin are produced by the ameloblasts (AMB) and odontoblasts; C: Diagram of sagittal section of the proximal incisor. In growing adult mouse incisor, lingual cervical loops (liCL) and laCL persist throughout the adult stages. Slow-cycling DESCs are located in the outer enamel epithelium (OEE) and underlying stellate reticulum (SR). Transit-amplifying (TA) cells, also considered as actively cycling progenitors, undergo massive proliferation in the inner enamel epithelium. These cells give rise to pre-ameloblasts (Pre-AMB) and fully differentiated ameloblasts (AMB). Compared to the laCL, liCL is smaller and does not normally give rise to ameloblasts. SI: Stratum intermedium; AMB: Ameloblasts; OEE: Outer enamel epithelium; IEE: Inner enamel epithelium; SR: Stellate reticulum; Od: Odontoblasts; liCL: Lingual cervical loop; laCL: Labial cervical loop.

## IDENTIFICATION OF DESCs

### Classical model

In the classical model of tissue supported by stem cells, rare slow-cycling stem cells, which divide infrequently, contribute to the production of active-cycling transit-amplifying (TA) cells, which then generate all the related lineages<sup>[20-23]</sup>. Therefore, the stem cells in the adult tissue are always identified by their characteristics, for instance, their quiescent feature and their potential of uni- or multilineage differentiation<sup>[21]</sup>.

A credible approach to locate the stem cell niche in adult tissue is to take the benefit of their quiescent property by positioning the distribution of label-retaining cells (LRCs)<sup>[21]</sup>. LRCs are capable of retaining DNA synthesis labels, including tritiated thymidine (3H-thymidine) and 5-bromo-2'-deoxyuridine (BrdU), after a long-term chase<sup>[24,25]</sup>. Active-cycling cells, such as TA cells, remove the DNA label following cell divisions within a short time. On the contrary, the quiescent stem cells retain the label during a long chasing period owing to their infrequent division<sup>[21-23]</sup>. LRCs have been confirmed as stem cells in various tissues and organs, such as the epidermis, intestine, and mammary gland<sup>[26-28]</sup>. With one administration of 3H-thymidine, an early study showed proliferating cells in the adult rat enamel organ, which exited the cell cycle

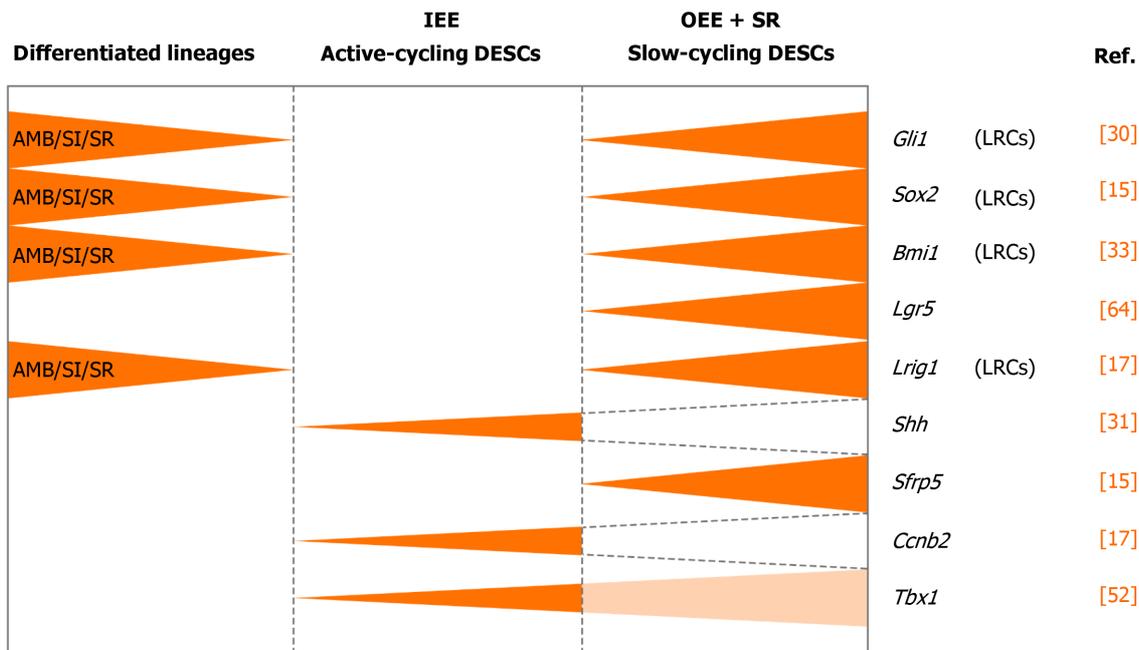
and migrated distally. After 32 d, the cells retaining the label were detected in the outer enamel epithelium (OEE) and underlying stellate reticulum (SR) of laCL, suggesting the existence of DESCs at the proximal end of the laCL<sup>[29,30]</sup>. To detect LRCs *in vivo*, the neonatal mice were pulsed with BrdU, to label the cycling cells in the dental epithelium at the time of tissue expansion and subsequently to identify the LRCs that rarely divide and retain the label till adulthood. The distribution of BrdU<sup>+</sup> LRCs in the OEE and underlying SR confirmed the presence of slow-cycling cells in laCL<sup>[14,31]</sup>. Later, this assumption was demonstrated with *K5Tta; H2B-GFP* mice, in which the expression of doxycycline-repressible *H2B-GFP* is regulated by the keratin 5 promoter<sup>[32]</sup>. All dental epithelial lineages uniformly express green fluorescent protein (GFP) in the absence of doxycycline administration. Following 2 mo administration of doxycycline, H2B-GFP-retaining cells were observed in the same compartments, suggesting that putative DESCs were present in these compartments and might have contributed to the continuous growth of the incisor enamel<sup>[33,34]</sup>.

To further assess the uni- or multilineage differentiation potential of putative stem cells, the cells and their progeny are permanently genetically marked and chased using lineage tracing, an essential strategy in the identification of stem cells in adult mammalian tissue<sup>[21,35]</sup>. Genetic lineage tracing in mice is preferably achieved with the Cre-loxP system. In this system, the Cre specifically activates the reporter in cells upon the control of the tissue- or cell-specific promoter, by excising the *loxP-STOP-loxP* sequence. With inducible recombination (Cre recombinase is fused to estrogen receptor), the Cre recombinase activity can be manipulated temporally and spatially with tamoxifen<sup>[36-38]</sup>. The application of inducible Cre for lineage tracing has provided maximum information about all the progeny of the stem cells in postnatal tissue. With CreER controlled by *Gli1* promoter (*GliCreER*), *Gli1*<sup>+</sup> cells residing in the LRCs region (OEE and underlying SR) have been demonstrated to be capable of generating functional ameloblasts and supporting epithelial lineages, such as stratum intermedium (SI) cells<sup>[31]</sup>. The distribution of *Bmi1*<sup>+</sup> cells in laCL is consistent with that of LRCs and *Gli1*<sup>+</sup> cells. Similarly, *Bmi1*<sup>+</sup> cells also give rise to enamel-producing ameloblasts and neighboring SI cells<sup>[33]</sup>. Unlike *Gli1* and *Bmi1*, *Sox2* has a broader expression domain, which expands both distally and proximally in the laCL. Genetic lineage tracing *in vivo* has revealed that *Sox2*<sup>+</sup> cells could generate all the mouse incisor epithelial lineages<sup>[15]</sup>. Later, *Lrig1* has been proposed as a putative stem cell marker by gene coexpression module analysis. Moreover, *Lrig1*<sup>+</sup> cells have been shown to contribute to dental epithelial lineages *via in vivo* lineage tracing<sup>[17]</sup>. These previous studies have constructed the classical model of enamel renewal, in which slow-cycling DESCs, residing in the OEE and underlying SR of the laCL, regularly move to the inner enamel epithelium (IEE) and generate active-cycling TA cells<sup>[16]</sup>. These active-cycling cells migrate distally, exit the cell cycle, and differentiate into enamel-producing ameloblasts. However, identification of cell types, properties, and cellular relationships remains unknown in this classical model. Furthermore, the latest study has shown that the expression of the putative DESCs markers, including *Bmi1*, *Gli1*, and *Sox2*, are broader than we expected, since they have been detected in the IEE as well, which unravels the limitation of the currently available genetic strategies (Figure 2).

### Updated model

The previous studies mentioned above characterized stem cells based on the known properties, which would inevitably introduce researchers' preconceptions, such as significant signaling pathways certified in the putative stem cell niche<sup>[39]</sup>. Therefore, unbiased technology is desired to identify cells and characterize cells with new clusters of markers. Single-cell sequencing, in particular, mRNA sequencing from single cells (scRNA-seq), contributes to the unbiased profiling of cells from tissues and organs<sup>[40-42]</sup>. Generally, single cells are isolated and assigned to a specific barcode; thus, gathered mRNA can be sequenced and reassigned to its cellular origin. According to the transcriptomes, cells are divided into groups with unsupervised clustering<sup>[43]</sup>. This scRNA-seq shows a series of advantages in uncovering heterogeneity within the population which was considered to be homogeneous, discovering novel and rare cell types, and unraveling the relationships between cell types.

Sharir *et al*<sup>[18]</sup> performed scRNA-seq of sorted dental epithelial cells to resolve the cellular heterogeneity and lineage dynamics of the adult incisor epithelium in an unbiased manner. Single cells from mouse incisors were generated and sequenced. The high-dimensional, whole-transcriptome data were visualized using SPRING<sup>[18]</sup>, which is suitable for analyzing differentiation trajectories as maintaining relationships of cells with the similar transcriptome. Mouse incisor epithelial cells are divided into three groups, including cycling cells (class 1), ameloblast lineages (class 2), and

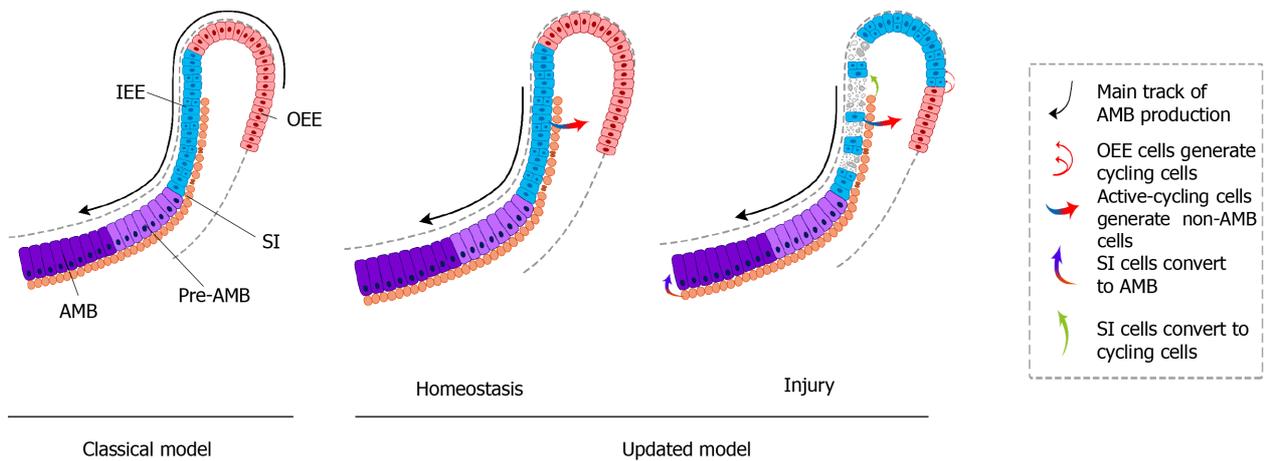


**Figure 2 Identification of dental epithelial stem cells in mouse incisor.** Lateral: Represents different cell compartments of labial cervical loop. Vertical: Represents the stem cell marker genes. AMB: Ameloblasts; SI: Stratum intermedium; IEE: Inner enamel epithelium; OEE: Outer enamel epithelium; SR: Stellate reticulum; LRCs: Label retaining cells; DESCs: Dental epithelial stem cells.

nonameloblast epithelial cells (class 3)<sup>[17]</sup>. With the expression of cell-cycle markers like *Cdc20* and *Ccnb2*, class 1 cells have the majority of dividing and cycling cells, located in the IEE and adjacent SI region. Moreover, class 1 cells maintain active self-renewal as their transcriptomes have shown successive phases of the cell cycle, and several cells return to their original state at the end of the cell cycle. Signatures reflecting classes 2 and 3 populations have also been observed in class 1 cell populations, suggesting that progenitors are cycling with upregulated expression of differentiation genes. Combined with differentiation trajectories and kinetics experiments, class 1 houses progenitor cells considered as the root, which produces cells of classes 2 and 3<sup>[18]</sup>. Therefore, distinct from the classical views, an updated dynamic model of stem cells in mouse incisors reveals that the IEE (class 1) possesses active cycling stem cells that differentiate into both the functional ameloblasts and the surrounding nonameloblast epithelial lineages (classes 2 and 3).

**Difference between the classical and updated models**

The classic model of DESCs in mouse incisors is similar to that of hematopoietic stem cells. The homeostasis of mouse incisor enamel is fueled by the quiescent stem cells in the OEE and underlying SR of the laCL<sup>[13-17,19]</sup>. These slow-cycling cells produce TA cells in the IEE that undergo limited divisions before terminal differentiation (Figure 3A). Even though scientists stick to this concept for a long time, the concept is still incapable of accounting for the great demand of ameloblasts in daily production of enamel. In this model, DESCs were identified with LRCs and lineage tracing of specific stem cell marker candidates, including *Sox2*, *Bmi1*, *Gli1*, and *Lrig1*. On the contrary, the novel model is based on scRNA-seq, an unbiased method. The dental epithelial cells in laCL can be divided into three different groups. The daily production of enamel is supported by a group of actively cycling progenitor cells in the IEE, which are responsible for the production of ameloblasts and nonameloblast epithelial lineages, including the OEE and underlying SR, which are considered as DESCs in the classic model<sup>[18]</sup> (Figure 3B). Previously established stem cell markers, including *Sox2*, *Bmi1*, *Gli1*, and *Lrig1*, are not expressed specifically in any groups of the updated model.



**Figure 3 Classical and updated models of dental epithelial stem cells in mouse incisors.** A: In classical model, slow-cycling dental epithelial stem cells (pink) from the outer enamel epithelium (OEE) and underlying stellate reticulum (SR) of the labial cervical loop, generate active-cycling TA cells in the inner enamel epithelium (IEE) (blue), which undergo several divisions, migrate distally, and differentiate into pre-ameloblasts (Pre-AMB) and AMB (purple); B: In updated model, actively cycling progenitors in the IEE contribute to the formation of both AMB and the adjacent non-ameloblast epithelial cells (SR, stratum intermedium and OEE) during homeostasis; C: After injury induced with 5-fluorouracil, the proliferation region of IEE is impaired and reduced. During recovery stage, additional progenitors from OEE enter the cell cycle and then recover ameloblast differentiation trajectory, and SI cells can also convert to differentiate into active-cycling cells and AMB. AMB: Ameloblasts; SI: Stratum intermedium; IEE: Inner enamel epithelium; OEE: Outer enamel epithelium.

## REGULATORY NETWORK OF DESCs IN MOUSE INCISORS

### Genetic regulation

The proliferation and differentiation of DESCs are regulated by genetic regulatory signals in a temporospatial manner<sup>[39]</sup>. Surrounding dental mesenchymal cells provide essential signals for maintenance and differentiation of DESCs<sup>[44]</sup>. To date, several signaling pathways have been found to be important for the steady state of the DESCs niche and subsequent differentiation in mouse incisors<sup>[16,19]</sup>. Fibroblast growth factor (FGF) signaling was first put forward. *Fgf3*, *Fgf9*, and *Fgf10* are key mesenchymal signals for stimulating the proliferation of DESCs in the developing cervical loops<sup>[30,45-48]</sup>. Transforming growth factor (TGF)- $\beta$  signaling participates in DESC maintenance and TA cell proliferation by regulating the activity of FGF signaling<sup>[47,48]</sup>. The expression of *Fgf3* and *Fgf10* and the number of BrdU<sup>+</sup> LRCs are markedly reduced in the laCL of mice with mutation of the *Alk5* gene, which is responsible for encoding the TGF- $\beta$  type I receptor<sup>[48]</sup>. The reduction of *Fgf3/10* expression by TGF- $\beta$  type II receptor (*Tgfr2*) deletion in mesenchyme-promoted differentiation of DESCs results in wavy mineralized structure formation<sup>[49]</sup>. *Sprouty* genes, which encode the intracellular antagonists of receptor tyrosine kinase signaling, ensure lineage differentiation of DESCs through the FGF signaling pathway<sup>[50]</sup>. Once *Sprouty* genes are deleted, the inhibitory signal is removed, leading to increased sensitivity to *Fgf3/10* expression in both liCL and laCL as well as the adjacent mesenchyme<sup>[51]</sup>. The inhibitory effect of Sprouty protein on FGF signaling functions is mediated by changing the expression of *TBX1* and *BCL11B* indirectly in the *Spry4*<sup>-/-</sup> mice<sup>[52,53]</sup>. The removal of *BCL11B* affects the differentiation and proliferation of DESCs, reduces the size of the laCL, and shortens the zone of ameloblast progenitors<sup>[53]</sup>. Conversely, an allele of *BCL11B* promotes the proliferation of TA cells, thus ensuring a stable number of DESCs<sup>[54]</sup>. Cao *et al*<sup>[55]</sup> have demonstrated that *TBX1* regulates DESCs by suppressing the transcriptional activity of *Pitx2* which is in connection with a cell cycling inhibitor p21. The downstream of FGF signaling has been explored by Goodwin *et al*<sup>[56]</sup>. Ras signaling is activated after FGFs bind to receptor tyrosine kinases (RTKs) and then regulate the proliferative activity of DESCs through the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways. Further evidence about the role of MAPK and PI3K in amelogenesis has been proposed through a mouse incisor model of Ras dysregulation<sup>[57]</sup>.

Another important signaling pathway is Hedgehog (Hh), which is essential for maintaining epithelial cell size, proliferation, and polarization<sup>[58]</sup>. *Runx2* mutation results in downregulated expression of *Shh* in the dental epithelium<sup>[59]</sup>. It has been shown that *Runx* gene and its binding protein core binding factor  $\beta$  gene (*Chfb*) modulate the continuous proliferation and differentiation of DESCs by activating FGF signaling loops and maintaining the expression of *Shh* mRNA<sup>[60]</sup>. The BMP-Smad4

signaling cascade inhibits the activity of Shh-Gli1 signaling to maintain Sox2<sup>+</sup> DESCs in the CL region of mouse molars. Conversely, loss of *Smad4* prolongs maintenance of the CL and affects cell expansion and differentiation<sup>[61]</sup>. *Ptch1* and *Ptch2* are binding receptors of Hh ligands and have distinct functional roles. *Ptch1* transduces Hh signaling to maintain Sox2<sup>+</sup> stem cells, whereas *Ptch2* with Desert hedgehog negatively regulates P-cadherin expression, suggesting that Hh signaling contributes to the maintenance and differentiation of DESCs simultaneously<sup>[62]</sup>. Several studies have revealed that the Notch signaling pathway is required for survival of DESCs and the formation of ameloblasts<sup>[63]</sup>. Multiple genes of this pathway are expressed in the CL, including *Delta-like 1 (Dll1)* and *Jagged 2 (Jag2)* genes encoding the Notch ligands, as well as *lunatic fringe (Lfng)* encoding transferase that modifies Notch receptors<sup>[63-65]</sup>. The notch responsive gene *Hes1* is expressed in the SR. When dissected CL is cocultured with the Notch signaling inhibitor *N*-[*N*-(3,5-difluorophenacetyl)-*L*-alanyl]-*S*-phenylglycine *t*-butyl ester (DAPT), the size of the CL is reduced because of increased apoptosis and reduced proliferation of DESCs<sup>[63]</sup>. *Jag2* and *Lfng* genes are regulated by FGF and bone morphogenetic protein (BMP) signaling. Deletion of *Jag2* results in abnormal ameloblast differentiation<sup>[65]</sup>. The number of SI cells was increased when CL-derived dental epithelial cells were cultured with Jagged 1 protein and overexpressed the *Notch1* internal domain. Differentiation of SI cells was inhibited when *Jagged1* was neutralized with specific antibody, suggesting that Notch signaling regulates SI cells, which function as a reserve progenitor pool<sup>[66]</sup>. Elimination of *Notch1*<sup>+</sup> cells disrupted the repair process of injured epithelium and obstructed the regeneration of damaged dental epithelium<sup>[18]</sup>. The essential role of Hippo signaling has been suggested in coordinating the proliferation and differentiation of DESCs<sup>[67]</sup>. The effectors of the evolutionarily conserved Hippo signaling pathway, Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (*TAZ*), are expressed in TA cells<sup>[67]</sup>. It has been reported that *YAP/TAZ* induce the ITG43-FAK-CDC42 signaling axis in the TA zone and activate mammalian target of rapamycin (mTOR) signaling by controlling *Rheb* expression, maintaining TA cell proliferation and survival, and inhibiting precocious differentiation<sup>[67]</sup>.

Intriguingly, there is neither expression of the Wnt-responsive gene *Axin2* nor expression of Wnt pathway mediators or inhibitors in the region of putative stem cells. These signs indicate that DESCs are not modulated directly by Wnt/ $\beta$ -catenin signaling<sup>[68]</sup>. However, Wnt/ $\beta$ -catenin can inhibit expression of *Fgf10*, a key antiapoptotic signal, to maintain a proper rate of apoptosis in DESCs<sup>[69]</sup>. And *Lgr5*, a Wnt signaling target gene and stem cell marker, is expressed in the putative stem cell region, the SR region underlying the OEE. These *Lgr5*<sup>+</sup> cells are identified as slow-cycling stem cells and a subpopulation of Sox2<sup>+</sup> DESCs<sup>[70,71]</sup>. A recent study reports that *Runx1* regulates the *Lgr5*-expressing epithelial stem cells and differentiation of ameloblast progenitors in the developing incisors. The *Runx1-Lgr5* axis is partially modulated by signal transducer and activator of transcription 3 (STAT3) phosphorylation in the CL of growing incisors. *Runx1* deficiency results in downregulated expression of *Lgr5* and *Sox2* and underdevelopment of the CL<sup>[70]</sup>.

Apart from signaling pathways, stem cell markers, like *Bmi1* and *Sox2*, are functional in the maintenance and differentiation of DESCs. The deletion of *Bmi1* decreased the number of stem cells, disorganized gene expression, and impaired enamel production. Knockdown of *Ink4a/Arf* partially rescued *Bmi1*-null phenotypes. *Ink4a/Arf* is one critical target gene of *Bmi1*, which encodes the cell cycle inhibitors. *Bmi1* also targets *Hox* genes, which maintain the undifferentiated state of stem cells and prevent inappropriate differentiation<sup>[33]</sup>. Conditional removal of *Sox2* during incisor renewal resulted in the morphological change of laCL and slowed down incisor growth<sup>[72]</sup>. Conditional overexpression of lymphoid enhancer-binding factor (*Lef-1*) in the dental epithelium increased cell proliferation, created a new stem cell compartment in the laCL, and rescued tooth arrest resulting from deletion of *Sox2*<sup>[72]</sup>. These results show that *Lef-1* regulates maintenance of DESCs and enamel formation, but the underlying mechanism remains unresolved.

### Epigenetic regulation

In addition to transcription factors, epigenetics also regulates the gene expression in mammalian development without alterations in the DNA sequence<sup>[73-75]</sup>. Epigenetic mechanisms involve DNA methylation, modification of histone tails, and gene regulation by noncoding RNAs and miRNAs<sup>[76]</sup>. Although various studies have shown the epigenetic regulation in tooth development and regeneration, there are only a few reports about the epigenetic effects in DESCs in mouse incisors.

Several studies have demonstrated that miRNAs play a role in enamel formation and the renewal and differentiation of DESCs in mouse incisors. Conditional deletion

of Dicer1, an essential factor for mature miRNA formation, led to the complete loss of miRNA genesis, resulting in ectopic formation of the CL<sup>[77]</sup>. This was the result of increased proliferation of incisor epithelial stem cells and impaired differentiation of ameloblasts. Furthermore, microarray analysis unraveled that the distinct expression pattern of miRNAs in different compartments of the dental epithelium, including the laCL, lingual CL, and ameloblasts, suggests the potential role of miRNAs in the self-renewal and differentiation of DESCs<sup>[77]</sup>. miR-200c, one of the differentially expressed miRNAs, participates in differentiation by repressing *noggin*, an antagonist of BMP signaling. Noggin induces expression of E-cadherin and Amelogenin to maintain cell adhesion and promote ameloblast differentiation, respectively<sup>[74]</sup>. BMP signaling is simultaneously the upstream regulator of miRNA-200c, and a positive-feedback loop has been shown between miR-200c and BMP signaling. Another upstream regulatory pathway has also been identified in which endogenous *Pitx2* interacts with the *miR-200c/141* promoter to activate miR-200c<sup>[75]</sup>. Also, miR-200a-3p is activated by *Pitx2* and targets the BMP antagonist *Bmp6* to further regulate BMP signaling<sup>[74]</sup>. Recently, miR-1 expressed at the CL of the dental epithelium was revealed by an *in situ* hybridization assay and its expression was inversely correlated with its target connexin (Cx) 43. Deletion of miR-1 induced DESCs to express Cx43, which regulated cell proliferation during DESC differentiation, by formation of cell-cell gap junctions and hemichannels at the plasma membrane<sup>[78]</sup>.

### **Regulators from adjacent cells and extracellular matrix**

Interactions among different cell populations and between cells and the extracellular matrix are indispensable for the homeostasis of the stem cell niche in the dental epithelium<sup>[79]</sup>. For instance, integrin  $\beta 3$  is required for the formation and maintenance of the CL and proliferation of DESCs. The knockdown of the CD61 gene results in a reduction of the CL size with downregulated expression of *Lgr5* and *Notch1*<sup>[80]</sup>. E-cadherin, a protein for cell-cell adhesion, regulates proliferation in TA cells and controls differentiation of DESCs<sup>[81]</sup>. Prominin-1 (Prom1/CD133), an essential protein for ciliary kinetics, regulates DESCs to respond appropriately to extracellular signals. Conditional removal of *Prom1* impairs ciliary dynamics and the positive effects of SHH treatment, leading to the destruction of stem cell activation and homeostasis in mouse incisor tooth epithelium<sup>[82]</sup>.

Finally, DESCs are regulated by other factors, such as follistatin, activin, heparin-binding molecule midkine, and heparin-binding growth-associated molecule<sup>[45,83]</sup>. Exogenous retinoic acid (RA) has negative effects on DESCs *via* inhibition of *Fgf10*. It has been demonstrated that supplementation of FGF10 in incisor cultures blocks RA's negative effects to antagonize apoptosis and increase proliferation of DESCs in the laCL<sup>[84]</sup>.

---

## **FUNCTION OF DESCs**

---

### **Role of DESCs during homeostasis**

The renewal and differentiation of DESCs are critical drivers of continuously growing mouse incisors<sup>[16]</sup>. The heterogeneity in DESCs has been discovered, hinting that different stem cell populations play a distinct function in mouse incisors<sup>[19]</sup>. Numerous studies have found that renewal of mouse incisors requires a balance of proliferation and differentiation of DESCs, which is controlled tightly by a complex regulated network, to maintain proper lineage ratios (Figure 3).

The renewal process of incisor enamel has been studied by constantly developing investigation methods. Continuous renewal of mouse incisors was initially observed by cutting the erupted enamel<sup>[85,86]</sup>. Sequential 3H-thymidine tracing showed the growth rate of rat incisors to be approximately 365  $\mu\text{m}/\text{d}$ <sup>[13]</sup>. It was confirmed that proper incisor growth requires proliferative dividing cells in an early experiment in animals treated with mitotic arrest agents<sup>[87]</sup>. The discovery of stem cell marker candidates and genetic lineage tracing helped researchers to propose a classical model of renewal of mouse incisors. It is thought that quiescent stem cells residing in the OEE generate actively proliferating TA cells, which migrate distally and differentiate into ameloblasts that are responsible for enamel formation<sup>[16,19]</sup>. How the slowly cycling DESCs meet the daily requirement of ameloblasts has been an open question for a long time. To address this, Sharir *et al.*<sup>[18]</sup> established a novel model of mouse incisor DESCs, in which actively cycling dental epithelial progenitor cells generate both the functional ameloblasts and the surrounding nonameloblast epithelial cell populations, which are subsequently responsible for the homeostasis of mouse incisor enamel (See session

'Updated model' for details)<sup>[18,19]</sup>. Even though this study identified the DESCs responsible for the homeostasis, the mechanism responsible for the committed differentiation is still absent. More studies are needed to determine the underlying mechanisms by which incisor epithelial self-renewal and cell lineage distribution remain stable under physiological conditions.

### **Role of DESCs during injury repair**

DESCs also support damaged incisor epithelium regeneration after injury (Figure 3). The number of *Sox2* and *Lgr5* transcripts decreased significantly and the spherical shape of the laCL was lost after transient deletion of *Sox2*. Some *Sox2* transcripts could be detected and the laCL shape was restored after 5 d. During recovery, the percentage of EdU<sup>+</sup> cells was significantly increased in the central and proximal sections of the SR. These findings demonstrated that the *Sox2*<sup>+</sup> DESC population could be regenerated quickly from the SR<sup>[87]</sup>.

A recent study has demonstrated the migration and plasticity of DESCs during recovery. In the IEE of mouse incisors, the proliferative cells eliminated by 5-fluorouracil (5-FU) treatment were supplemented by the burgeoning proliferating cell population after 3 d. After 10 d, abnormal ameloblast organization and disorganized enamel matrix resulting from 5-FU treatment both recovered to normal. The function and dynamic changes of DESCs were further analyzed by scRNA-seq during injury repair. After cytotoxic injury, the number of cycling cells was increased with expanded expression domains of *Ccnb1* and *Birc5*, but the numbers of pre-ameloblasts and ameloblasts were significantly decreased with the distal shift. Expression of *Sfrp5* and *Cldn10* was upregulated and expanded towards the proliferating regions, suggesting an increased nonameloblast population. Further study has demonstrated that *Notch1*<sup>+</sup> SI cells are induced to differentiate into ameloblasts and critical for tissue recovery. These findings have shown that dental epithelium regeneration after injury is driven by recruiting more progenitors or nonmitotic pre-ameloblasts to divide, shortening the cell cycle, and delaying onset of ameloblast differentiation. Therefore, DESCs play an essential role in an appropriate and rapid response to tissue damage<sup>[18]</sup>.

### **Potential of DESCs in tooth regeneration and tissue engineering**

Whole tooth regeneration based on epithelial and mesenchymal interaction through simulating tooth development is a promising strategy for replacing lost teeth. The odontogenic potential could be retained in epithelial and mesenchymal cells isolated from the tooth germ of early development. Tooth-like structures can be produced *in vitro* based on individual dental epithelial cells and mesenchymal cells from mouse embryos<sup>[88]</sup>. Bioengineered tooth germs, which are reconstructed by epithelial and mesenchymal cells, have generated functional teeth when they were placed into the alveolar socket of adult mice<sup>[89]</sup>. In addition to the mouse regenerative model, a recent study, using an allogeneic cell reassociation approach, achieved whole-tooth regeneration in minipig jawbone *in situ*<sup>[90]</sup>.

To resolve the problem of a source of DESCs for tooth engineering, DESCs from mouse incisors have been demonstrated as an excellent tool. Besides, several studies have attempted to induce available stem cells to form dental epithelial cells. It has been reported that human tooth germ stem cells can differentiate into epithelial cell types, but not functional ameloblasts<sup>[91]</sup>. Mouse induced pluripotent stem cells (miPSCs) can differentiate into dental epithelial-like cells in serum-free culture conditions, with the addition of neurotrophin-4. These cells derived from miPSCs can express dental epithelial surface marker CD49f and ameloblast-specific markers<sup>[92]</sup>. As possible alternative sources for the human dental epithelium, human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) may be options due to their potency of multilineage differentiation<sup>[93,94]</sup>. Based on the vital role of interactions between dental epithelial and ectodermal mesenchymal cells in dental development, both hESCs and hiPSCs are induced to differentiate into epithelial-like stem cells by the HERS/ERM cell line<sup>[95]</sup>. Newly *ex-vivo*-formed differentiated hESCs express special epithelial stem cell markers including E-cadherin, ABCG2, Bmi-1, p63, and p75<sup>[94]</sup>. Even though rare ameloblasts, enamel, or dentin-enamel tissue were detected in this study, some progress has been made on how to obtain DESCs.

Ameloblast cell lines are indispensable for enamel formation and regeneration, because they secrete amelogenin, which is an essential constituent of enamel<sup>[96,97]</sup>. The generation of ameloblasts is still an obstacle. Although several mouse ameloblast-like cell lines, such as ALC and LS8, have been established, they do not generate enamel<sup>[98,99]</sup>. Human gingival epithelial cells have been a source of ameloblast-like cells induced by BMPs and TGF- $\beta$ . It has been reported that there are 20 ameloblast-specific genes as cell surface markers, which will contribute to the isolation of human

ameloblast-like cells<sup>[100]</sup>.

## CONCLUSION

To replace enamel defects due to caries or trauma, dentists use several artificial materials, which do not completely resemble the mechanical, physical, and esthetic features of the lost enamel<sup>[101]</sup>. Enamel regeneration has been considered as an alternative clinical strategy. Our understanding of identification, regulation, and role of DESCs has been strengthened by studying continuously growing mouse incisor models. By scRNA-seq, the heterogeneity of DESCs in the laCL has been identified and a novel mouse incisor model distinct from early evidence has been established. The updated understanding of the regulation and role of DESCs in tissue homeostasis and repair contributes to the therapeutic development of enamel engineering. Despite all this progress with DESCs in recent years, enamel regeneration still faces various challenges, which have been outlined in two recent conferences<sup>[97,102]</sup>. The difficulties include the acellular structure, high mineralization, essential epigenetic regulation during mineralization, unique migration of ameloblasts during crystal formation, and ultimate organization with prismatic and interprismatic structures of natural enamel. Several issues remain to be addressed before clinical application, such as the combination of regenerated enamel with natural teeth and the control of shape, size, color, and time. Although there are limitations in enamel tissue engineering, the exciting progress with DESCs provides researchers with novel insight into stem cell-based tooth engineering, and consequently, to pave the way for future treatments.

## REFERENCES

- 1 **Bartlett JD.** Dental enamel development: proteinases and their enamel matrix substrates. *ISRN Dent* 2013; **2013**: 684607 [PMID: 24159389 DOI: 10.1155/2013/684607]
- 2 **Honda MJ, Sumita Y, Kagami H, Ueda M.** Histological and immunohistochemical studies of tissue engineered odontogenesis. *Arch Histol Cytol* 2005; **68**: 89-101 [PMID: 16079455 DOI: 10.1679/aohc.68.89]
- 3 **Fincham AG, Moradian-Oldak J, Simmer JP.** The structural biology of the developing dental enamel matrix. *J Struct Biol* 1999; **126**: 270-299 [PMID: 10441532 DOI: 10.1006/jbsi.1999.4130]
- 4 **Fukumoto S, Yamada Y.** Review: extracellular matrix regulates tooth morphogenesis. *Connect Tissue Res* 2005; **46**: 220-226 [PMID: 16546825 DOI: 10.1080/03008200500344017]
- 5 **Kaneko H, Ogiuchi H, Shimono M.** Cell death during tooth eruption in the rat: surrounding tissues of the crown. *Anat Embryol (Berl)* 1997; **195**: 427-434 [PMID: 9176665 DOI: 10.1007/s004290050062]
- 6 **Arrow P.** Dental enamel defects, caries experience and oral health-related quality of life: a cohort study. *Aust Dent J* 2017; **62**: 165-172 [PMID: 27539008 DOI: 10.1111/adj.12449]
- 7 **Tirlet G, Crescenzo H, Crescenzo D, Bazos P.** Ceramic adhesive restorations and biomimetic dentistry: tissue preservation and adhesion. *Int J Esthet Dent* 2014; **9**: 354-369 [PMID: 25126616]
- 8 **BaniHani A, Deery C, Toumba J, Munyombwe T, Duggal M.** The impact of dental caries and its treatment by conventional or biological approaches on the oral health-related quality of life of children and carers. *Int J Paediatr Dent* 2018; **28**: 266-276 [PMID: 29288546 DOI: 10.1111/ipd.12350]
- 9 **Young CS, Terada S, Vacanti JP, Honda M, Bartlett JD, Yelick PC.** Tissue engineering of complex tooth structures on biodegradable polymer scaffolds. *J Dent Res* 2002; **81**: 695-700 [PMID: 12351668 DOI: 10.1177/154405910208101008]
- 10 **Zhang W, Vázquez B, Yelick PC.** Bioengineered post-natal recombinant tooth bud models. *J Tissue Eng Regen Med* 2017; **11**: 658-668 [PMID: 25424341 DOI: 10.1002/term.1962]
- 11 **Bakhshandeh B, Zarrintaj P, Oftadeh MO, Keramati F, Fouladiha H, Sohrabi-Jahromi S, Ziraksaz Z.** Tissue engineering: strategies, tissues, and biomaterials. *Biotechnol Genet Eng Rev* 2017; **33**: 144-172 [PMID: 29385962 DOI: 10.1080/02648725.2018.1430464]
- 12 **Yu T, Volponi AA, Babb R, An Z, Sharpe PT.** Stem Cells in Tooth Development, Growth, Repair, and Regeneration. *Curr Top Dev Biol* 2015; **115**: 187-212 [PMID: 26589926 DOI: 10.1016/bs.ctdb.2015.07.010]
- 13 **Smith CE, Warshawsky H.** Cellular renewal in the enamel organ and the odontoblast layer of the rat incisor as followed by radioautography using 3H-thymidine. *Anat Rec* 1975; **183**: 523-561 [PMID: 1200409 DOI: 10.1002/ar.1091830405]
- 14 **Harada H, Kettunen P, Jung HS, Mustonen T, Wang YA, Thesleff I.** Localization of putative stem cells in dental epithelium and their association with Notch and FGF signaling. *J Cell Biol* 1999; **147**: 105-120 [PMID: 10508859 DOI: 10.1083/jcb.147.1.105]
- 15 **Juuri E, Saito K, Ahtiainen L, Seidel K, Tummers M, Hochedlinger K, Klein OD, Thesleff I, Michon F.** Sox2+ stem cells contribute to all epithelial lineages of the tooth via Sfrp5+ progenitors. *Dev Cell* 2012; **23**: 317-328 [PMID: 22819339 DOI: 10.1016/j.devcel.2012.05.012]
- 16 **Kuang-Hsien Hu J, Mushegyan V, Klein OD.** On the cutting edge of organ renewal: Identification, regulation, and evolution of incisor stem cells. *Genesis* 2014; **52**: 79-92 [PMID: 24307456 DOI: 10.1002/dvg.22732]

- 17 Seidel K, Marangoni P, Tang C, Houshmand B, Du W, Maas RL, Murray S, Oldham MC, Klein OD. Resolving stem and progenitor cells in the adult mouse incisor through gene co-expression analysis. *Elife* 2017; **6** [PMID: 28475038 DOI: 10.7554/eLife.24712]
- 18 Sharir A, Marangoni P, Zilionis R, Wan M, Wald T, Hu JK, Kawaguchi K, Castillo-Azofeifa D, Epstein L, Harrington K, Pagella P, Mitsiadis T, Siebel CW, Klein AM, Klein OD. A large pool of actively cycling progenitors orchestrates self-renewal and injury repair of an ectodermal appendage. *Nat Cell Biol* 2019; **21**: 1102-1112 [PMID: 31481792 DOI: 10.1038/s41556-019-0378-2]
- 19 Yu T, Klein OD. Molecular and cellular mechanisms of tooth development, homeostasis and repair. *Development* 2020; **147** [PMID: 31980484 DOI: 10.1242/dev.184754]
- 20 Greco V, Chen T, Rendl M, Schober M, Pasolli HA, Stokes N, Dela Cruz-Racelis J, Fuchs E. A two-step mechanism for stem cell activation during hair regeneration. *Cell Stem Cell* 2009; **4**: 155-169 [PMID: 19200804 DOI: 10.1016/j.stem.2008.12.009]
- 21 Snippet HJ, Clevers H. Tracking adult stem cells. *EMBO Rep* 2011; **12**: 113-122 [PMID: 21252944 DOI: 10.1038/embor.2010.216]
- 22 Fuchs E. The tortoise and the hair: slow-cycling cells in the stem cell race. *Cell* 2009; **137**: 811-819 [PMID: 19490891 DOI: 10.1016/j.cell.2009.05.002]
- 23 Li L, Clevers H. Coexistence of quiescent and active adult stem cells in mammals. *Science* 2010; **327**: 542-545 [PMID: 20110496 DOI: 10.1126/science.1180794]
- 24 Goodlad RA. Quantification of epithelial cell proliferation, cell dynamics, and cell kinetics in vivo. *Wiley Interdiscip Rev Dev Biol* 2017; **6** [PMID: 28474479 DOI: 10.1002/wdev.274]
- 25 Maurer HR. Potential pitfalls of [3H]thymidine techniques to measure cell proliferation. *Cell Tissue Kinet* 1981; **14**: 111-120 [PMID: 6451296 DOI: 10.1111/j.1365-2184.1981.tb00516.x]
- 26 Sada A, Jacob F, Leung E, Wang S, White BS, Shalloway D, Tumber T. Defining the cellular lineage hierarchy in the interfollicular epidermis of adult skin. *Nat Cell Biol* 2016; **18**: 619-631 [PMID: 27183471 DOI: 10.1038/ncb3359]
- 27 Li N, Nakauka-Ddamba A, Tobias J, Jensen ST, Lengner CJ. Mouse Label-Retaining Cells Are Molecularly and Functionally Distinct From Reserve Intestinal Stem Cells. *Gastroenterology* 2016; **151**: 298-310. e7 [PMID: 27237597 DOI: 10.1053/j.gastro.2016.04.049]
- 28 Boras-Granic K, Dann P, Wysolmerski JJ. Embryonic cells contribute directly to the quiescent stem cell population in the adult mouse mammary gland. *Breast Cancer Res* 2014; **16**: 487 [PMID: 25467960 DOI: 10.1186/s13058-014-0487-6]
- 29 Smith CE. Cell turnover in the odontogenic organ of the rat incisor as visualized by graphic reconstructions following a single injection of 3H-thymidine. *Am J Anat* 1980; **158**: 321-343 [PMID: 7446435 DOI: 10.1002/aja.1001580307]
- 30 Samperiz MM, Blumen G, Merzel J. Effect of vinblastine on the cell cycle and migration of ameloblasts of mouse incisors as shown by autoradiography using 3H-thymidine. *Cell Tissue Kinet* 1985; **18**: 493-503 [PMID: 4028109 DOI: 10.1111/j.1365-2184.1985.tb00691.x]
- 31 Seidel K, Ahn CP, Lyons D, Nee A, Ting K, Brownell I, Cao T, Carano RA, Curran T, Schober M, Fuchs E, Joyner A, Martin GR, de Sauvage FJ, Klein OD. Hedgehog signaling regulates the generation of ameloblast progenitors in the continuously growing mouse incisor. *Development* 2010; **137**: 3753-3761 [PMID: 20978073 DOI: 10.1242/dev.056358]
- 32 Tumber T, Guasch G, Greco V, Blanpain C, Lowry WE, Rendl M, Fuchs E. Defining the epithelial stem cell niche in skin. *Science* 2004; **303**: 359-363 [PMID: 14671312 DOI: 10.1126/science.1092436]
- 33 Biehs B, Hu JK, Strauli NB, Sangiorgi E, Jung H, Heber RP, Ho S, Goodwin AF, Dasen JS, Capecchi MR, Klein OD. BMI1 represses Ink4a/Arf and Hox genes to regulate stem cells in the rodent incisor. *Nat Cell Biol* 2013; **15**: 846-852 [PMID: 23728424 DOI: 10.1038/ncb2766]
- 34 Zhao H, Feng J, Seidel K, Shi S, Klein O, Sharpe P, Chai Y. Secretion of shh by a neurovascular bundle niche supports mesenchymal stem cell homeostasis in the adult mouse incisor. *Cell Stem Cell* 2014; **14**: 160-173 [PMID: 24506883 DOI: 10.1016/j.stem.2013.12.013]
- 35 He S, Nakada D, Morrison SJ. Mechanisms of stem cell self-renewal. *Annu Rev Cell Dev Biol* 2009; **25**: 377-406 [PMID: 19575646 DOI: 10.1146/annurev.cellbio.042308.113248]
- 36 Joyner AL, Zervas M. Genetic inducible fate mapping in mouse: establishing genetic lineages and defining genetic neuroanatomy in the nervous system. *Dev Dyn* 2006; **235**: 2376-2385 [PMID: 16871622 DOI: 10.1002/dvdy.20884]
- 37 Wu SS, Lee JH, Koo BK. Lineage Tracing: Computational Reconstruction Goes Beyond the Limit of Imaging. *Mol Cells* 2019; **42**: 104-112 [PMID: 30764600 DOI: 10.14348/molcells.2019.0006]
- 38 Yang H, Adam RC, Ge Y, Hua ZL, Fuchs E. Epithelial-Mesenchymal Micro-niches Govern Stem Cell Lineage Choices. *Cell* 2017; **169**: 483-496. e13 [PMID: 28413068 DOI: 10.1016/j.cell.2017.03.038]
- 39 Simons BD, Clevers H. Strategies for homeostatic stem cell self-renewal in adult tissues. *Cell* 2011; **145**: 851-862 [PMID: 21663791 DOI: 10.1016/j.cell.2011.05.033]
- 40 Svensson V, Vento-Tormo R, Teichmann SA. Exponential scaling of single-cell RNA-seq in the past decade. *Nat Protoc* 2018; **13**: 599-604 [PMID: 29494575 DOI: 10.1038/nprot.2017.149]
- 41 Haque A, Engel J, Teichmann SA, Lönnerberg T. A practical guide to single-cell RNA-sequencing for biomedical research and clinical applications. *Genome Med* 2017; **9**: 75 [PMID: 28821273 DOI: 10.1186/s13073-017-0467-4]
- 42 Clevers H, Watt FM. Defining Adult Stem Cells by Function, not by Phenotype. *Annu Rev Biochem* 2018; **87**: 1015-1027 [PMID: 29494240 DOI: 10.1146/annurev-biochem-062917-012341]
- 43 Kiselev VY, Andrews TS, Hemberg M. Challenges in unsupervised clustering of single-cell RNA-seq data. *Nat Rev Genet* 2019; **20**: 273-282 [PMID: 30617341 DOI: 10.1038/s41576-018-0088-9]
- 44 Tummers M, Thesleff I. The importance of signal pathway modulation in all aspects of tooth development. *J Exp Zool B Mol Dev Evol* 2009; **312B**: 309-319 [PMID: 19156667 DOI: 10.1002/jez.b.21280]
- 45 Wang XP, Suomalainen M, Jorgez CJ, Matzuk MM, Werner S, Thesleff I. Follistatin regulates enamel patterning in mouse incisors by asymmetrically inhibiting BMP signaling and ameloblast differentiation.

- Dev Cell* 2004; **7**: 719-730 [PMID: 15525533 DOI: 10.1016/j.devcel.2004.09.012]
- 46 **Harada H**, Toyono T, Toyoshima K, Ohuchi H. FGF10 maintains stem cell population during mouse incisor development. *Connect Tissue Res* 2002; **43**: 201-204 [PMID: 12489159 DOI: 10.1080/03008200290000989]
- 47 **Yokohama-Tamaki T**, Ohshima H, Fujiwara N, Takada Y, Ichimori Y, Wakisaka S, Ohuchi H, Harada H. Cessation of Fgf10 signaling, resulting in a defective dental epithelial stem cell compartment, leads to the transition from crown to root formation. *Development* 2006; **133**: 1359-1366 [PMID: 16510502 DOI: 10.1242/dev.02307]
- 48 **Zhao H**, Li S, Han D, Kaartinen V, Chai Y. Alk5-mediated transforming growth factor  $\beta$  signaling acts upstream of fibroblast growth factor 10 to regulate the proliferation and maintenance of dental epithelial stem cells. *Mol Cell Biol* 2011; **31**: 2079-2089 [PMID: 21402782 DOI: 10.1128/MCB.01439-10]
- 49 **Yang G**, Zhou J, Teng Y, Xie J, Lin J, Guo X, Gao Y, He M, Yang X, Wang S. Mesenchymal TGF- $\beta$  signaling orchestrates dental epithelial stem cell homeostasis through Wnt signaling. *Stem Cells* 2014; **32**: 2939-2948 [PMID: 24964772 DOI: 10.1002/stem.1772]
- 50 **Klein OD**, Lyons DB, Balooch G, Marshall GW, Basson MA, Peterka M, Boran T, Peterkova R, Martin GR. An FGF signaling loop sustains the generation of differentiated progeny from stem cells in mouse incisors. *Development* 2008; **135**: 377-385 [PMID: 18077585 DOI: 10.1242/dev.015081]
- 51 **Boran T**, Peterkova R, Lesot H, Lyons DB, Peterka M, Klein OD. Temporal analysis of ectopic enamel production in incisors from sprouty mutant mice. *J Exp Zool B Mol Dev Evol* 2009; **312B**: 473-485 [PMID: 19101957 DOI: 10.1002/jez.b.21254]
- 52 **Catón J**, Luder HU, Zoupa M, Bradman M, Bluteau G, Tucker AS, Klein O, Mitsiadis TA. Enamel-free teeth: Tbx1 deletion affects amelogenesis in rodent incisors. *Dev Biol* 2009; **328**: 493-505 [PMID: 19233155 DOI: 10.1016/j.ydbio.2009.02.014]
- 53 **Kyrylkova K**, Kyryachenko S, Biehs B, Klein O, Kioussi C, Leid M. BCL11B regulates epithelial proliferation and asymmetric development of the mouse mandibular incisor. *PLoS One* 2012; **7**: e37670 [PMID: 22629441 DOI: 10.1371/journal.pone.0037670]
- 54 **Katsuragi Y**, Anraku J, Nakatomi M, Ida-Yonemochi H, Obata M, Mishima Y, Sakuraba Y, Gondo Y, Kodama Y, Nishikawa A, Takagi R, Ohshima H, Kominami R. Bcl11b transcription factor plays a role in the maintenance of the ameloblast-progenitors in mouse adult maxillary incisors. *Mech Dev* 2013; **130**: 482-492 [PMID: 23727454 DOI: 10.1016/j.mod.2013.05.002]
- 55 **Cao H**, Florez S, Amen M, Huynh T, Skobe Z, Baldini A, Amendt BA. Tbx1 regulates progenitor cell proliferation in the dental epithelium by modulating Pitx2 activation of p21. *Dev Biol* 2010; **347**: 289-300 [PMID: 20816801 DOI: 10.1016/j.ydbio.2010.08.031]
- 56 **Goodwin AF**, Tidyman WE, Jheon AH, Sharir A, Zheng X, Charles C, Fagin JA, McMahon M, Diekwisch TG, Ganss B, Rauert KA, Klein OD. Abnormal Ras signaling in Costello syndrome (CS) negatively regulates enamel formation. *Hum Mol Genet* 2014; **23**: 682-692 [PMID: 24057668 DOI: 10.1093/hmg/ddt455]
- 57 **Zheng X**, Goodwin AF, Tian H, Jheon AH, Klein OD. Ras Signaling Regulates Stem Cells and Amelogenesis in the Mouse Incisor. *J Dent Res* 2017; **96**: 1438-1444 [PMID: 28644741 DOI: 10.1177/0022034517717255]
- 58 **Gritli-Linde A**, Bei M, Maas R, Zhang XM, Linde A, McMahon AP. Shh signaling within the dental epithelium is necessary for cell proliferation, growth and polarization. *Development* 2002; **129**: 5323-5337 [PMID: 12403705 DOI: 10.1242/dev.00100]
- 59 **Wang XP**, Aberg T, James MJ, Levanon D, Groner Y, Thesleff I. Runx2 (Cbfa1) inhibits Shh signaling in the lower but not upper molars of mouse embryos and prevents the budding of putative successional teeth. *J Dent Res* 2005; **84**: 138-143 [PMID: 15668330 DOI: 10.1177/154405910508400206]
- 60 **Kurosaka H**, Islam MN, Kuremoto K, Hayano S, Nakamura M, Kawanabe N, Yanagita T, Rice DP, Harada H, Taniuchi I, Yamashiro T. Core binding factor beta functions in the maintenance of stem cells and orchestrates continuous proliferation and differentiation in mouse incisors. *Stem Cells* 2011; **29**: 1792-1803 [PMID: 21898689 DOI: 10.1002/stem.722]
- 61 **Li J**, Feng J, Liu Y, Ho TV, Grimes W, Ho HA, Park S, Wang S, Chai Y. BMP-SHH signaling network controls epithelial stem cell fate via regulation of its niche in the developing tooth. *Dev Cell* 2015; **33**: 125-135 [PMID: 25865348 DOI: 10.1016/j.devcel.2015.02.021]
- 62 **Binder M**, Chmielarz P, McKinnon PJ, Biggs LC, Thesleff I, Balic A. Functionally Distinctive Ptc Receptors Establish Multimodal Hedgehog Signaling in the Tooth Epithelial Stem Cell Niche. *Stem Cells* 2019; **37**: 1238-1248 [PMID: 31145830 DOI: 10.1002/stem.3042]
- 63 **Felszeghy S**, Suomalainen M, Thesleff I. Notch signalling is required for the survival of epithelial stem cells in the continuously growing mouse incisor. *Differentiation* 2010; **80**: 241-248 [PMID: 20692087 DOI: 10.1016/j.diff.2010.06.004]
- 64 **Harada H**, Ichimori Y, Yokohama-Tamaki T, Ohshima H, Kawano S, Katsube K, Wakisaka S. Stratum intermedium lineage diverges from ameloblast lineage via Notch signaling. *Biochem Biophys Res Commun* 2006; **340**: 611-616 [PMID: 16378597 DOI: 10.1016/j.bbrc.2005.12.053]
- 65 **Mitsiadis TA**, Graf D, Luder H, Gridley T, Bluteau G. BMPs and FGFs target Notch signalling via jagged 2 to regulate tooth morphogenesis and cytodifferentiation. *Development* 2010; **137**: 3025-3035 [PMID: 20685737 DOI: 10.1242/dev.049528]
- 66 **Liang Y**, Chen G, Yang Y, Li Z, Chen T, Sun W, Yu M, Pan K, Guo W, Tian W. Effect of canonical NF- $\kappa$ B signaling pathway on the differentiation of rat dental epithelial stem cells. *Stem Cell Res Ther* 2019; **10**: 139 [PMID: 31109359 DOI: 10.1186/s13287-019-1252-7]
- 67 **Hu JK**, Du W, Shelton SJ, Oldham MC, DiPersio CM, Klein OD. An FAK-YAP-mTOR Signaling Axis Regulates Stem Cell-Based Tissue Renewal in Mice. *Cell Stem Cell* 2017; **21**: 91-106. e6 [PMID: 28457749 DOI: 10.1016/j.stem.2017.03.023]
- 68 **Suomalainen M**, Thesleff I. Patterns of Wnt pathway activity in the mouse incisor indicate absence of Wnt/beta-catenin signaling in the epithelial stem cells. *Dev Dyn* 2010; **239**: 364-372 [PMID: 19806668]

- DOI: [10.1002/dvdy.22106](https://doi.org/10.1002/dvdy.22106)]
- 69 **Yang Z**, Balic A, Michon F, Juuri E, Thesleff I. Mesenchymal Wnt/ $\beta$ -Catenin Signaling Controls Epithelial Stem Cell Homeostasis in Teeth by Inhibiting the Antiapoptotic Effect of Fgf10. *Stem Cells* 2015; **33**: 1670-1681 [PMID: [25693510](https://pubmed.ncbi.nlm.nih.gov/25693510/) DOI: [10.1002/stem.1972](https://doi.org/10.1002/stem.1972)]
  - 70 **Sarper SE**, Inubushi T, Kurosaka H, Ono Minagi H, Kuremoto KI, Sakai T, Taniuchi I, Yamashiro T. Runx1-Stat3 signaling regulates the epithelial stem cells in continuously growing incisors. *Sci Rep* 2018; **8**: 10906 [PMID: [30026553](https://pubmed.ncbi.nlm.nih.gov/30026553/) DOI: [10.1038/s41598-018-29317-6](https://doi.org/10.1038/s41598-018-29317-6)]
  - 71 **Chang JY**, Wang C, Jin C, Yang C, Huang Y, Liu J, McKeehan WL, D'Souza RN, Wang F. Self-renewal and multilineage differentiation of mouse dental epithelial stem cells. *Stem Cell Res* 2013; **11**: 990-1002 [PMID: [23906788](https://pubmed.ncbi.nlm.nih.gov/23906788/) DOI: [10.1016/j.scr.2013.06.008](https://doi.org/10.1016/j.scr.2013.06.008)]
  - 72 **Sun Z**, Yu W, Sanz Navarro M, Sweat M, Eliason S, Sharp T, Liu H, Seidel K, Zhang L, Moreno M, Lynch T, Holton NE, Rogers L, Neff T, Goodheart MJ, Michon F, Klein OD, Chai Y, Dupuy A, Engelhardt JF, Chen Z, Amendt BA. Sox2 and Lef-1 interact with Pitx2 to regulate incisor development and stem cell renewal. *Development* 2016; **143**: 4115-4126 [PMID: [27660324](https://pubmed.ncbi.nlm.nih.gov/27660324/) DOI: [10.1242/dev.138883](https://doi.org/10.1242/dev.138883)]
  - 73 **Yin K**, Hacia JG, Zhong Z, Paine ML. Genome-wide analysis of miRNA and mRNA transcriptomes during amelogenesis. *BMC Genomics* 2014; **15**: 998 [PMID: [25406666](https://pubmed.ncbi.nlm.nih.gov/25406666/) DOI: [10.1186/1471-2164-15-998](https://doi.org/10.1186/1471-2164-15-998)]
  - 74 **Sharp T**, Wang J, Li X, Cao H, Gao S, Moreno M, Amendt BA. A pituitary homeobox 2 (Pitx2):microRNA-200a-3p: $\beta$ -catenin pathway converts mesenchymal cells to amelogenin-expressing dental epithelial cells. *J Biol Chem* 2014; **289**: 27327-27341 [PMID: [25122764](https://pubmed.ncbi.nlm.nih.gov/25122764/) DOI: [10.1074/jbc.M114.575654](https://doi.org/10.1074/jbc.M114.575654)]
  - 75 **Cao H**, Jheon A, Li X, Sun Z, Wang J, Florez S, Zhang Z, McManus MT, Klein OD, Amendt BA. The Pitx2:miR-200c/141:noggin pathway regulates Bmp signaling and ameloblast differentiation. *Development* 2013; **140**: 3348-3359 [PMID: [23863486](https://pubmed.ncbi.nlm.nih.gov/23863486/) DOI: [10.1242/dev.089193](https://doi.org/10.1242/dev.089193)]
  - 76 **Lin Y**, Zheng L, Fan L, Kuang W, Guo R, Lin J, Wu J, Tan J. The Epigenetic Regulation in Tooth Development and Regeneration. *Curr Stem Cell Res Ther* 2018; **13**: 4-15 [PMID: [27897123](https://pubmed.ncbi.nlm.nih.gov/27897123/) DOI: [10.2174/1574888X11666161129142525](https://doi.org/10.2174/1574888X11666161129142525)]
  - 77 **Jheon AH**, Li CY, Wen T, Michon F, Klein OD. Expression of microRNAs in the stem cell niche of the adult mouse incisor. *PLoS One* 2011; **6**: e24536 [PMID: [21931743](https://pubmed.ncbi.nlm.nih.gov/21931743/) DOI: [10.1371/journal.pone.0024536](https://doi.org/10.1371/journal.pone.0024536)]
  - 78 **Nakamura T**, Iwamoto T, Nakamura HM, Shindo Y, Saito K, Yamada A, Yamada Y, Fukumoto S, Nakamura T. Regulation of miR-1-Mediated Connexin 43 Expression and Cell Proliferation in Dental Epithelial Cells. *Front Cell Dev Biol* 2020; **8**: 156 [PMID: [32258035](https://pubmed.ncbi.nlm.nih.gov/32258035/) DOI: [10.3389/fcell.2020.00156](https://doi.org/10.3389/fcell.2020.00156)]
  - 79 **Zhang X**, Diekwisch TG, Luan X. Structure and function of ameloblastin as an extracellular matrix protein: adhesion, calcium binding, and CD63 interaction in human and mouse. *Eur J Oral Sci* 2011; **119** Suppl 1: 270-279 [PMID: [22243256](https://pubmed.ncbi.nlm.nih.gov/22243256/) DOI: [10.1111/j.1600-0722.2011.00889.x](https://doi.org/10.1111/j.1600-0722.2011.00889.x)]
  - 80 **Yoshida T**, Iwata T, Umemoto T, Shiratsuchi Y, Kawashima N, Sugiyama T, Yamato M, Okano T. Promotion of mouse ameloblast proliferation by Lgr5 mediated integrin signaling. *J Cell Biochem* 2013; **114**: 2138-2147 [PMID: [23553959](https://pubmed.ncbi.nlm.nih.gov/23553959/) DOI: [10.1002/jcb.24564](https://doi.org/10.1002/jcb.24564)]
  - 81 **Li CY**, Cha W, Luder HU, Charles RP, McMahon M, Mitsiadis TA, Klein OD. E-cadherin regulates the behavior and fate of epithelial stem cells and their progeny in the mouse incisor. *Dev Biol* 2012; **366**: 357-366 [PMID: [22537490](https://pubmed.ncbi.nlm.nih.gov/22537490/) DOI: [10.1016/j.ydbio.2012.03.012](https://doi.org/10.1016/j.ydbio.2012.03.012)]
  - 82 **Singer D**, Thamm K, Zhuang H, Karbanová J, Gao Y, Walker JV, Jin H, Wu X, Coveney CR, Marangoni P, Lu D, Grayson PRC, Gulsen T, Liu KJ, Ardu S, Wann AK, Luo S, Zamboni AC, Jetten AM, Tredwin C, Klein OD, Attanasio M, Carmeliet P, Huttner WB, Corbeil D, Hu B. Prolimin-1 controls stem cell activation by orchestrating ciliary dynamics. *EMBO J* 2019; **38** [PMID: [30523147](https://pubmed.ncbi.nlm.nih.gov/30523147/) DOI: [10.15252/embj.201899845](https://doi.org/10.15252/embj.201899845)]
  - 83 **Sonoda A**, Iwamoto T, Nakamura T, Fukumoto E, Yoshizaki K, Yamada A, Arakaki M, Harada H, Nonaka K, Nakamura S, Yamada Y, Fukumoto S. Critical role of heparin binding domains of ameloblastin for dental epithelium cell adhesion and ameloblastoma proliferation. *J Biol Chem* 2009; **284**: 27176-27184 [PMID: [19648121](https://pubmed.ncbi.nlm.nih.gov/19648121/) DOI: [10.1074/jbc.M109.033464](https://doi.org/10.1074/jbc.M109.033464)]
  - 84 **Xi J**, He S, Wei C, Shen W, Liu J, Li K, Zhang Y, Yue J, Yang Z. Negative effects of retinoic acid on stem cell niche of mouse incisor. *Stem Cell Res* 2016; **17**: 489-497 [PMID: [27771497](https://pubmed.ncbi.nlm.nih.gov/27771497/) DOI: [10.1016/j.scr.2016.09.030](https://doi.org/10.1016/j.scr.2016.09.030)]
  - 85 **Thomson G**. A Method of Crowning Incisor Teeth. *Proc R Soc Med* 1915; **8**: 77-79 [PMID: [19978851](https://pubmed.ncbi.nlm.nih.gov/19978851/) DOI: [10.1177/003591571500801008](https://doi.org/10.1177/003591571500801008)]
  - 86 **Warshawsky H**, Smith CE. Morphological classification of rat incisor ameloblasts. *Anat Rec* 1974; **179**: 423-446 [PMID: [4135484](https://pubmed.ncbi.nlm.nih.gov/4135484/) DOI: [10.1002/ar.1091790403](https://doi.org/10.1002/ar.1091790403)]
  - 87 **Sanz-Navarro M**, Seidel K, Sun Z, Bertonnier-Brouty L, Amendt BA, Klein OD, Michon F. Plasticity within the niche ensures the maintenance of a Sox2<sup>+</sup> stem cell population in the mouse incisor. *Development* 2018; **145** [PMID: [29180573](https://pubmed.ncbi.nlm.nih.gov/29180573/) DOI: [10.1242/dev.155929](https://doi.org/10.1242/dev.155929)]
  - 88 **Hu B**, Nadiiri A, Bopp-Küchler S, Perrin-Schmitt F, Lesot H. Dental Epithelial Histomorphogenesis in vitro. *J Dent Res* 2005; **84**: 521-525 [PMID: [15914588](https://pubmed.ncbi.nlm.nih.gov/15914588/) DOI: [10.1177/154405910508400607](https://doi.org/10.1177/154405910508400607)]
  - 89 **Ikeda E**, Morita R, Nakao K, Ishida K, Nakamura T, Takano-Yamamoto T, Ogawa M, Kasugai S, Tsuji T. Fully functional bioengineered tooth replacement as an organ replacement therapy. *Proc Natl Acad Sci USA* 2009; **106**: 13475-13480 [PMID: [19666587](https://pubmed.ncbi.nlm.nih.gov/19666587/) DOI: [10.1073/pnas.0902944106](https://doi.org/10.1073/pnas.0902944106)]
  - 90 **Wu Z**, Wang F, Fan Z, Wu T, He J, Wang J, Zhang C, Wang S. Whole-Tooth Regeneration by Allogeneic Cell Reassociation in Pig Jawbone. *Tissue Eng Part A* 2019; **25**: 1202-1212 [PMID: [30648470](https://pubmed.ncbi.nlm.nih.gov/30648470/) DOI: [10.1089/ten.TEA.2018.0243](https://doi.org/10.1089/ten.TEA.2018.0243)]
  - 91 **Doğan A**, Demirci S, Şahin F. In vitro differentiation of human tooth germ stem cells into endothelial- and epithelial-like cells. *Cell Biol Int* 2015; **39**: 94-103 [PMID: [25077982](https://pubmed.ncbi.nlm.nih.gov/25077982/) DOI: [10.1002/cbin.10357](https://doi.org/10.1002/cbin.10357)]
  - 92 **Abdullah AN**, Miyauchi S, Onishi A, Tanimoto K, Kato K. Differentiation of mouse-induced pluripotent stem cells into dental epithelial-like cells in the absence of added serum. *In Vitro Cell Dev Biol Anim* 2019; **55**: 130-137 [PMID: [30659476](https://pubmed.ncbi.nlm.nih.gov/30659476/) DOI: [10.1007/s11626-019-00320-z](https://doi.org/10.1007/s11626-019-00320-z)]
  - 93 **Lee CH**, Kim JH, Lee HJ, Jeon K, Lim H, Choi Hy, Lee ER, Park SH, Park JY, Hong S, Kim S, Cho SG.

- The generation of iPS cells using non-viral magnetic nanoparticle based transfection. *Biomaterials* 2011; **32**: 6683-6691 [PMID: 21683440 DOI: 10.1016/j.biomaterials.2011.05.070]
- 94 **Zhu K**, Li J, Lai H, Yang C, Guo C, Wang C. Reprogramming fibroblasts to pluripotency using arginine-terminated polyamidoamine nanoparticles based non-viral gene delivery system. *Int J Nanomedicine* 2014; **9**: 5837-5847 [PMID: 25540584 DOI: 10.2147/IJN.S73961]
- 95 **Kim GH**, Yang J, Jeon DH, Kim JH, Chae GY, Jang M, Lee G. Differentiation and Establishment of Dental Epithelial-Like Stem Cells Derived from Human ESCs and iPSCs. *Int J Mol Sci* 2020; **21** [PMID: 32575634 DOI: 10.3390/ijms21124384]
- 96 **Pandya M**, Diekwisch TGH. Enamel biomimetics-fiction or future of dentistry. *Int J Oral Sci* 2019; **11**: 8 [PMID: 30610185 DOI: 10.1038/s41368-018-0038-6]
- 97 **Kirkham J**, Brookes SJ, Diekwisch TGH, Margolis HC, Berdal A, Hubbard MJ. Enamel Research: Priorities and Future Directions. *Front Physiol* 2017; **8**: 513 [PMID: 28775693 DOI: 10.3389/fphys.2017.00513]
- 98 **Huang Z**, Sargeant TD, Hulvat JF, Mata A, Bringas P Jr, Koh CY, Stupp SI, Snead ML. Bioactive nanofibers instruct cells to proliferate and differentiate during enamel regeneration. *J Bone Miner Res* 2008; **23**: 1995-2006 [PMID: 18665793 DOI: 10.1359/jbmr.080705]
- 99 **Huang Z**, Newcomb CJ, Zhou Y, Lei YP, Bringas P Jr, Stupp SI, Snead ML. The role of bioactive nanofibers in enamel regeneration mediated through integrin signals acting upon C/EBP $\alpha$  and c-Jun. *Biomaterials* 2013; **34**: 3303-3314 [PMID: 23398885 DOI: 10.1016/j.biomaterials.2013.01.054]
- 100 **Hyun SY**, Mun S, Kang KJ, Lim JC, Kim SY, Han K, Jang YJ. Amelogenic transcriptome profiling in ameloblast-like cells derived from adult gingival epithelial cells. *Sci Rep* 2019; **9**: 3736 [PMID: 30842534 DOI: 10.1038/s41598-019-40091-x]
- 101 **Chatzistavrou X**, Papagerakis S, Ma PX, Papagerakis P. Innovative approaches to regenerate enamel and dentin. *Int J Dent* 2012; **2012**: 856470 [PMID: 22666253 DOI: 10.1155/2012/856470]
- 102 **Klein OD**, Duverger O, Shaw W, Lacruz RS, Joester D, Moradian-Oldak J, Pugach MK, Wright JT, Millar SE, Kulkarni AB, Bartlett JD, Diekwisch TG, DenBesten P, Simmer JP. Meeting report: a hard look at the state of enamel research. *Int J Oral Sci* 2017; **9**: e3 [PMID: 29165423 DOI: 10.1038/ijos.2017.40]



Published by **Baishideng Publishing Group Inc**  
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA

**Telephone:** +1-925-3991568

**E-mail:** [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

**Help Desk:** <https://www.f6publishing.com/helpdesk>

<https://www.wjgnet.com>

