Oral stem cells, decoding and mapping the resident cells populations

Xuechen Zhang[1], Ana Justo Caetano[1], Paul T. Sharpe[1,2,], Ana Angelova Volponi[1,*]*

Key Words:
dental pulp stem cells; dental stem cells; gingival stem cells; periodontal ligament stem cells; stem cells from apical papilla

From the Contents

Introduction	24
The Tooth: Harbouring Different Dental Stem Cells	25
Decoding Dental Tissues Cell By Cell	27

ABSTRACT

The teeth and their supporting tissues provide an easily accessible source of oral stem cells. These different stem cell populations have been extensively studied for their properties, such as high plasticity and clonogenicity, expressing stem cell markers and potency for multilineage differentiation in vitro. Such cells with stem cell properties have been derived and characterised from the dental pulp tissue, the apical papilla region of roots in development, as well as the supporting tissue of periodontal ligament that anchors the tooth within the alveolar socket and the soft gingival tissue. Studying the dental pulp stem cell populations in a continuously growing mouse incisor model, as a traceable in vivo model, enables the researchers to study the properties, origin and behaviour of mesenchymal stem cells. On the other side, the oral mucosa with its remarkable scarless wound healing phenotype, offers a model to study a well-coordinated system of healing because of coordinated actions between epithelial, mesenchymal and immune cells populations. Although described as homogeneous cell populations following their in vitro expansion, the increasing application of approaches that allow tracing of individual cells over time, along with single-cell RNA-sequencing, reveal that different oral stem cells are indeed diverse populations and there is a highly organised map of cell populations according to their location in resident tissues, elucidating diverse stem cell niches within the oral tissues. This review covers the current knowledge of diverse oral stem cells, focusing on the new approaches in studying these cells. These approaches "decode" and "map" the resident cells populations of diverse oral tissues and contribute to a better understanding of the "stem cells niche architecture and interactions. Considering the high accessibility and simplicity in obtaining these diverse stem cells, the new findings offer potential in development of translational tissue engineering approaches and innovative therapeutic solutions.

http://doi.org/10.12336/biomatertransl.2022.01.004

How to cite this article: Zhang, X.; Caetano, A. J.; Sharpe, P. T.; Volponi, A. A. Oral stem cells, decoding and mapping the resident cells populations. *Biomater Transl.* **2022**, *3*(1), 24-30.

Introduction

A tooth is a complex organ consisting of differently mineralized tissues, such as enamel, dentin and cementum, forming a chamber where the dental pulp (loose connective tissue) is enclosed and provided with blood and nerve supply from apical openings at the tips of the roots. Embedded within the socket of the alveolar bone through an attachment, known as periodontal ligament, teeth form a complex known as periodontium, comprising of cementum (the outer layer of the root), attachment to the bundle bone of the alveolar socket through the periodontal ligament, the alveolar socket, and the soft gingival tissue.

Teeth are ectodermal organs that are formed by reciprocal and sequential interaction between the oral epithelial cells (ectoderm) and cranial neural crest-derived mesenchymal cells, during the embryonic development. The epithelial cells give rise to the enamel-forming ameloblasts, which are highly specialized cells that undergo apoptosis following the production of enamel, and the mesenchymal cells give rise to differentiated cells that contribute to form the majority of dental tissues, such as dentine-forming odontoblasts, other diverse populations of dental pulp cells,

periodontal ligament and cementum.[1]

Cells with stem cells properties have been derived and characterized from different dental/oral tissues,[2-6] making the teeth and the supporting oral tissues attractive and accessible sources of stem cells.[7-10]

The Tooth: Harbouring Different Dental Stem Cells

Dental pulp stem cells

The tooth has an ability of limited repair by production of newly formed layer of dentin, deposited by odontoblast-like cells observed and reported by Smith and Lesot,[11] and Smith et al.[12] suggesting that within the dental pulp, there is a pool of cells with mesenchymal stem characteristics, able to be recruited and activated when the need for repair occurs (**Figure 1A**).

In shallow enamel and enamel/dentinal damage, resident odontoblasts, polarised and highly specialised cells, located on the dentin-pulp border are activated, protecting the dental pulp via formation of reactionary dentine.[13, 14] If the damage advances, resident odontoblasts may not survive and this initiates a cascade of stem cell activation, proliferation, and differentiation into new odontoblast-like cells that engage in reparative dentine secretion.[9, 15]

When addressing the question on the origin of the dental pulp stem cells (DPSCs), Feng et al.[16] by using genetic lineage tracing, reported a dual origin of the dental pulp cells. Pericytes were shown to differentiate into specialized tooth mesenchyme-derived cells, odontoblasts, during tooth growth and in response to damage *in vivo*.[16, 17] Nevertheless, this contribution to odontoblast differentiation does not account for all cell differentiation, and an additional source, displaying

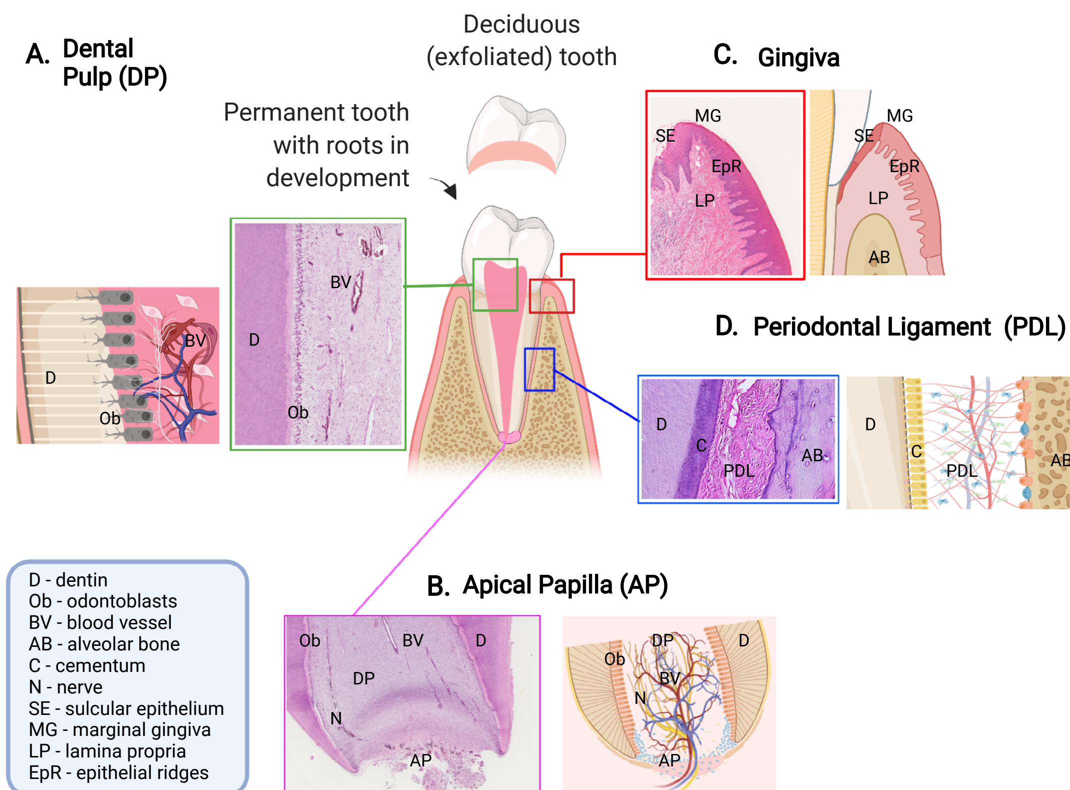

Figure 1. Teeth and their supporting tissues provide an easily accessible source of oral stem cells. These different stem cell populations reside within stem cell niches and can be obtained from: dental pulp tissue of deciduous and permanent teeth (A), apical papilla tissue (B), gingival tissue (C) and periodontal ligament (D). Created with BioRender.com.

***Corresponding authors:**
Ana Angelova Volponi, ana.angelova@kcl.ac.uk; Paul T. Sharpe, paul.sharpe@kcl.ac.uk.

1 Centre for Craniofacial and Regenerative Biology, Faculty of Dentistry, Oral & Craniofacial Sciences, King's College University of London, London, UK;
2 Laboratory of Odontogenesis and Osteogenesis, Institute of Animal Physiology and Genetics, CAS, v.v.i., Brno, Czech Republic

mesenchymal stem cell (MSC)-like properties, was identified within the dental pulp resident cells that migrate toward areas of tissue damage and differentiate into odontoblasts, when stimulated.[16] A significant population of MSCs during development, self-renewal and repair of a tooth were also suggested to be derived from peripheral nerve-associated glial cells that generate multipotent MSCs and further differentiate into pulp cells and highly specialized odontoblasts, shown in a mouse incisor as a model that exhibits continuous growth.[18]

When teeth are damaged, the local odontoblast cells upregulate their activity, but if damage persists these local odontoblasts cannot cope and their death provides a trigger for proliferation of resident pericytes and glial cells located in the vicinity of the damage. These cells have stem cell properties and differentiate into new odontoblast-like cells, capable to engage in reparative processes.[16-18] These studies importantly indicated the different origins of the resident, mesenchyme derived stem cells in the dental pulp.

The isolation of cells that exhibited stem cell properties from adult human dental pulps was reported by Gronthos and colleagues[2, 3] who described population of cells, DPSCs obtained from extracted permanent third molars, showing high proliferation and high colony-forming properties, being able to produce sporadic, densely calcified nodules. These cells, when transplanted *in vivo* into immunocompromised mice, demonstrated ability to generate dentin/pulp-like complexes.[2, 3] Further characterization revealed that DPSCs have multilineage differentiation capacity *in vitro*.[2-9]

Cells with stem cell properties were also isolated from the remnants of human deciduous teeth dental pulp (**Figure 1**), termed "stem cells from human deciduous teeth".[19] These cells can be easily obtained, making the deciduous, physiologically replaced teeth, a highly accessible, otherwise discarded, source for MSCs.[7-10] Stem cells from human deciduous teeth were also shown to be highly proliferative, clonogenic cells that have capacity for multilineage differentiation with higher proliferation rates and increased population doublings, when compared to DPSCs isolated from permanent teeth. These cells show osteo-inductive capacity and were able to generate dentine-like structures when transplanted *in vivo*.[19-24]

Stem cells from human deciduous teeth lack expression of major histocompatibility complex-II and have been shown to express hypo-immunogenic phenotype, inhibiting T-cell function through conserved induction of cellular stress.[25] Moreover, these immunomodulatory properties have been shown to be conserved *in vitro* and follow the common strategy of immunoregulation that takes place *in vivo*, during the process of tissue repair.[26] These important immunomodulatory properties, in addition to their neural crest origin[27] and ability to keep their stem cell properties after a long-term period cryopreservation, as well as their accessibility, (from usually discarded tissues, such as [20] deciduous teeth in our lifetime), make them a particularly attractive source for MSCs and target for a therapeutic strategy.[7-10]

Stem cells from apical papilla

Teeth that are still undergoing development of their roots harbour a unique population of cells located in the apical papilla region that have been shown to exhibit stem cells properties, termed stem cells from apical papilla (SCAP).[28, 29] The apical papilla region plays an important role in root development and formation of different dental tissues during this physiological process[1] (**Figure 1B**).

This apical papilla region tissue and cells can easily be derived from routinely extracted, human immature third molars, by separating the tissue at the tips of the developing roots in the clinics, that exhibit unfinished root development, following tooth extraction.

These cells have been characterized by their plasticity, expression of stem cell markers, such as STRO-1 and CD146 as well as CD73, CD90, and CD105; typical stem cell markers.[28-30] One surface marker for pluripotency, CD24, has been found to be directly correlated to SCAP; where it was found to be exclusively expressed in SCAP.[31] CD24a^{+} cells could be detected in primary dental papilla in mice and humans, and marked as unique multipotent stem cells from the dental pulp with enhanced osteogenic/odontogenic differentiation capabilities to form dentin and neurovascular-like structures.[32]

When isolated, SCAP have been characterised by their capacity for multilineage differentiation, following induction *in vitro*.[5] In comparison to DPSCs, these cells express higher proliferation and cell migration capacity[5, 33] and have a higher expression of antiapoptotic protein, longer telomere length, and greater telomerase activity associated with cellular lifespan and cell proliferation than DPSCs.[34] All different stem cells isolated from dental/oral tissues have shown to be able to undergo osteogenic differentiation and produce minerals with different chemical composition *in vitro*, when analysed with Raman spectroscopy.[35] A possible downregulation of RUNX2 has been suggested when SCAP cells undergo diversion from osteoblastic to odontoblastic differentiation route.[36]

The secretome analysis of SCAP cells indicates that they secrete significantly more chemokines, neurotrophins and proteins involved in metabolic processes and transcription, compared to bone marrow MSCs.[37] Being located at the very tip of the developing root, enables the region of the apical papilla to have accessibility to a collateral circulation,[5, 38] which might affect the diverse dynamic of the cellular components and processes of the region and therefore the SCAP population of cells.

Through a process of transdifferentiation, SCAP cells were successfully converted into endothelial cells by small molecules and growth factors and shown to generate vascular structures using the *in vivo* Matrigel plug angiogenesis assay in an immune-deficient mouse model.[39]

Gingival stem cells

The oral mucosa is the first line of defence against oral diseases. One of the most rapidly dividing tissues in our body, it exhibits remarkable healing properties of foetal-like, scarless wound healing phenotype.[40] The tissue that directly surrounds

the tooth and attaches to the tooth surface by a junctional epithelium, providing a seal, is known as gingiva (**Figure 1C**). Gingiva is part of a complex of tissues, known as periodontium, along with the alveolar socket, periodontal ligament and the cementum, that provide attachment of the tooth and play role in its homeostasis.[1, 41] It is particularly important structure, providing defence against microorganisms, during plaque formation and providing resistance to mechanical forces during mastication.

The gingiva is composed of epithelium, derived from embryonic ectoderm, and underlying connective tissue that originates from neural crest cells and mesoderm.[1] Gingival tissue is easily accessible during routine dental procedures and very often remnants of the tissue are found following tooth extraction, making it a very attractive source for isolation of different stem cells.[7-10] Cells derived from the connective tissue compartment of human gingiva have been characterised by expression of MSC markers, such as Oct-4 (octamer-binding transcription factor 4), SSEA-4 (stage-specific embryonic antigen-4) and STRO-1[42, 43] and undergo multilineage differentiation under *in vitro* conditions.[43] The cells from the epithelial compartment and derived from oral mucosa were also characterised by expression of stem cells markers *in vitro* and used in ocular surface reconstruction when grown as oral mucosal epithelial sheets in rabbit[44] and human,[45] offering promising results for future regenerative therapies.

Periodontal ligament stem cells

The periodontal ligament is a connective tissue that provides the anchorage to the tooth within the alveolar bone socket and takes the mechanical pressure during dental function and can be defined as a fibrous joint (**Figure 1D**).[1] The periodontal ligament develops from the dental follicle, which is a fibrous sac that surrounds the developing tooth bud.[1] *In vivo* lineage-tracing experiments suggested that the dental follicle contains mesenchymal progenitor cells expressing parathyroid hormone-related protein, which give rise to cells forming the periodontal attachment apparatus in a manner regulated by autocrine signalling through the parathyroid hormone/parathyroid hormone-related protein receptor.[46]

Cells with stem cell properties have been isolated and characterised, showing multilineage differentiation and expressing common MSC markers such as STRO-1, STRO-4, CD29, CD73, CD90 (Thy1), CD106 (vascular cell adhesion molecule 1) and CD146 (MUC18).[47, 48] Cultured human periodontal ligament stem cells were also shown to be positive for pericyte markers such as CD146, neural/glial antigen 2 and CD140b,[49] suggesting perivascular origin. Periodontal ligament stem cells, as all other dental stem cells, can be easily acquired from extracted teeth or during clinical procedure when the root surface is exposed. Interestingly, periodontal ligament stem cells retain similar immunophenotypic characteristics, when isolated from healthy and inflamed tissue,[50] pointing at a possible usage of inflamed tissue, regularly exported during routine periodontal surgery, as a source to isolate cells with MSCs properties.

Decoding Dental Tissues Cell By Cell

Mapping dental pulp cells

Mapping the cellular composition of different organs and tissues offers understanding of mechanisms of growth and repair. Recent studies, using cell sequencing approaches shed light on the cellular architecture of different dental tissues, offering a deeper view of the populations of cells and understanding the underlying mechanisms that drive the homeostasis, repair, and regeneration. These studies greatly contribute to the mission stated by the Human Cell Atlas project as "*To create comprehensive reference maps of all human cells—the fundamental units of life—as a basis for both understanding human health and diagnosing, monitoring, and treating disease*".[51]

Lineage tracing using mouse models, have become a well-established and "gold standard" approach in dental stem cell research, allowing permanent marking and tracing of putative stem cells *in vivo*. The model of mouse incisor (as a model of continuous growth and replenishment of stem cell population) is an established, coherent model of cell dynamics. It enables us to study spatially restricted stem, progenitor and specialised populations of cells in the epithelial and mesenchymal compartments, in a setting of continuous growth and high cell turnover.

During feeding, abrasion occurs at the tips of mouse incisor, causing a continuous need for compensation to maintain the sharpness of the teeth. The loss of cells at the tips is compensated for by continuous cell production at the apical end where distinct MSC and epithelial stem cell niches reside.[18, 52, 53]

In vitro studies fail to capture the very important "*in vivo* microenvironment", to fully understand cell clonogenicity and differentiation within the tissue-specific, stem cell niches. These obstacles are overcome with single-cell RNA-sequencing technologies, where robust transcriptome analysis, down to single-cell level enables mapping of specific tissue architecture and cell interactions.

Using the mouse incisor model to compare with human teeth, offers possibility to elucidate similarities and to highlight important differences in the underlying mechanisms that drive growth and proliferation, potentially feeding information on the process of evolution and comparison between species.

Differentiated and progenitor cells were described in stem-cell niches using bulk RNA-sequence, revealing a transcriptional complexity within the dental tissues.[54] In a recent study, using single-cell sequencing, the whole population of ameloblasts in a mouse incisor was mapped, suggesting that epithelial progeny appear in diverse, characteristic patches.[55] They suggested that the progenitor area of these cells, previously noted for their plasticity[56] might rely on functional diversification of different stem cells with a stemness gradation in a continuously growing model, such as the mouse incisor.[52-56]

When analysing human teeth and comparing the apical papilla region of a human tooth with developing roots and comparing this with the dental pulp of a fully developed tooth, it was found that these differ substantially and form at least several transcriptionally distinct subpopulations.[55]

In the same study Krivanek et al, compared the pulp of human nongrowing molars to the mouse nongrowing molars and found that human molars contained a pulp subpopulation that was localized in the peri-odontoblastic layer (cell-free and cell-rich zones) which are absent in mouse molars.[55] Interestingly, the results from the study showed that populations of *Smoc2*⁻ and *Smoc2*⁺ human pulp subtypes expressed maturation hierarchy like that in mouse continuously growing incisor. *Smoc2*⁺ human subtype cells were in the apical papilla region, around the Hertwig epithelial root sheath, region where the SCAP cells are derived from.[55]

Decoding gingival and periodontal ligament tissue

The remarkable way that oral wounds heal with negligible complications compared to cutaneous wounds, has always triggered interest among researchers. Various mechanisms, such as differential proliferative and differentiation cell programs, epithelial remodelling, reduced inflammatory response, and presence of a distinct modulation of adult stem cells were suggested to contribute to this remarkable wound-healing property.[40] The homeostasis of gingiva requires coordinated interactions between different populations of epithelial, mesenchymal, and immune cells.[50]

The cells derived from the underlying mesenchyme that reside in the subepithelial compartment are characterised by high plasticity and respond to signals sent by the overlying epithelial layer.[40] Single-cell RNA-sequencing of the epithelial basal layer, adjacent to the subepithelial population of mesenchymal cells has elucidated the oral mucosa epithelial stem cell niche and suggested their role in the process of healing.[50]

Fully mapping the identity and organisation of the tissue architecture on a single-cell level is essential to understand their roles in homeostasis and disease. A recent study revealed a well-orchestrated remodelling of the gingival tissue, with changes in actions between epithelial, mesenchymal, and immune cells, when transition happens from health and mild to severe disease (periodontitis). At a transcriptomic level, it was shown that there is a corresponding shift in cellular proportions.[50] In health, there were low numbers of follicular and plasma B cells with a marked progressive increase from mild to severe. Memory B cells showed distinctive increase at disease onset with a subsequent decrease in the severe sample.[50] A novel, mesenchymal population characterised by adipocyte enhancer binding protein 1 expression was also identified. This study raised intriguing questions about the role of certain cell types in response to inflammatory processes offering venues for translating this knowledge into clinical strategies for drug development for the wide range of chronic inflammatory diseases.[50]

Similarly to other dental tissues, single-cell RNA sequencing studies revealed fundamental cellular heterogeneity of the mouse periodontium.[46] Three major cell types were identified, including mesenchymal cells, endothelial cells, and immune cells, with some (periodontal ligament) mesenchymal populations, such as Axin2 and CD90/Thy1 that form cementoblasts.[57] Cells expressing Scleraxis (Scx⁺), basic helix-loop-helix transcriptional factor, that is abundantly expressed by tendons and ligaments, have been suggested to give rise to osteoblasts and other fibroblasts.[46] These recent studies map the lineage hierarchy in the periodontium at a single-cell level, largely contributing to understanding of development of periodontium, as well as understanding the intercellular interactions of cells during health and disease.[46, 57]

Author contributions

All authors were involved in drafting the manuscript and revising it. AAV and PS contributed to the conception of the work. AAV created the figures using BioRender.com. All authors have approved the final version and agreed to be accountable for the work.

Financial support

The study was supported by the Czech Science Foundation, project 21-21409S (to PS) and XZ is supported by a China Scholarship Council award.

Acknowledgement

None.

Conflicts of interest statement

All authors declare no conflict of interest.

Open access statement

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

1. Nanci, A. *Ten Cate's oral histology: development, structure, and function.* 8th ed.; Elsevier: St. Louis, 2012.
2. Gronthos, S.; Mankani, M.; Brahim, J.; Robey, P. G.; Shi, S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci U S A.* **2000**, *97*, 13625-13630.
3. Gronthos, S.; Brahim, J.; Li, W.; Fisher, L. W.; Cherman, N.; Boyde, A.; DenBesten, P.; Robey, P. G.; Shi, S. Stem cell properties of human dental pulp stem cells. *J Dent Res.* **2002**, *81*, 531-535.
4. Jo, Y. Y.; Lee, H. J.; Kook, S. Y.; Choung, H. W.; Park, J. Y.; Chung, J. H.; Choung, Y. H.; Kim, E. S.; Yang, H. C.; Choung, P. H. Isolation and characterization of postnatal stem cells from human dental tissues. *Tissue Eng.* **2007**, *13*, 767-773.
5. Huang, G. T.; Gronthos, S.; Shi, S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. *J Dent Res.* **2009**, *88*, 792-806.
6. Balic, A.; Aguila, H. L.; Caimano, M. J.; Francone, V. P.; Mina, M. Characterization of stem and progenitor cells in the dental pulp of erupted and unerupted murine molars. *Bone.* **2010**, *46*, 1639-1651.
7. Volponi, A. A.; Pang, Y.; Sharpe, P. T. Stem cell-based biological tooth repair and regeneration. *Trends Cell Biol.* **2010**, *20*, 715-722.
8. Volponi, A. A.; Sharpe, P. T. The tooth -- a treasure chest of stem cells. *Br Dent J.* **2013**, *215*, 353-358.
9. Angelova Volponi, A.; Zaugg, L. K.; Neves, V.; Liu, Y.; Sharpe, P. T. Tooth repair and regeneration. *Curr Oral Health Rep.* **2018**, *5*, 295-303.
10. Yelick, P. C.; Sharpe, P. T. Tooth bioengineering and regenerative dentistry. *J Dent Res.* **2019**, *98*, 1173-1182.
11. Smith, A. J.; Lesot, H. Induction and regulation of crown dentinogenesis: embryonic events as a template for dental tissue repair? *Crit Rev Oral Biol Med.* **2001**, *12*, 425-437.
12. Smith, J. G.; Smith, A. J.; Shelton, R. M.; Cooper, P. R. Recruitment of

dental pulp cells by dentine and pulp extracellular matrix components. *Exp Cell Res.* **2012**, *318*, 2397-2406.

13. Smith, A. J.; Cassidy, N.; Perry, H.; Bègue-Kirn, C.; Ruch, J. V.; Lesot, H. Reactionary dentinogenesis. *Int J Dev Biol.* **1995**, *39*, 273-280.
14. Couve, E.; Osorio, R.; Schmachtenberg, O. Reactionary dentinogenesis and neuroimmune response in dental caries. *J Dent Res.* **2014**, *93*, 788-793.
15. Teaford, M. F.; Smith, M. M.; Ferguson, M. W. J. *Development, function and evolution of teeth.* Cambridge University Press: Cambridge, 2000.
16. Feng, J.; Mantesso, A.; De Bari, C.; Nishiyama, A.; Sharpe, P. T. Dual origin of mesenchymal stem cells contributing to organ growth and repair. *Proc Natl Acad Sci U S A.* **2011**, *108*, 6503-6508.
17. Vidovic, I.; Banerjee, A.; Fatahi, R.; Matthews, B. G.; Dyment, N. A.; Kalajzic, I.; Mina, M. αSMA-expressing perivascular cells represent dental pulp progenitors in vivo. *J Dent Res.* **2017**, *96*, 323-330.
18. Kaukua, N.; Shahidi, M. K.; Konstantinidou, C.; Dyachuk, V.; Kaucka, M.; Furlan, A.; An, Z.; Wang, L.; Hultman, I.; Ahrlund-Richter, L.; Blom, H.; Brismar, H.; Lopes, N. A.; Pachnis, V.; Suter, U.; Clevers, H.; Thesleff, I.; Sharpe, P.; Ernfors, P.; Fried, K.; Adameyko, I. Glial origin of mesenchymal stem cells in a tooth model system. *Nature.* **2014**, *513*, 551-554.
19. Miura, M.; Gronthos, S.; Zhao, M.; Lu, B.; Fisher, L. W.; Robey, P. G.; Shi, S. SHED: stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci U S A.* **2003**, *100*, 5807-5812.
20. Shi, S.; Bartold, P. M.; Miura, M.; Seo, B. M.; Robey, P. G.; Gronthos, S. The efficacy of mesenchymal stem cells to regenerate and repair dental structures. *Orthod Craniofac Res.* **2005**, *8*, 191-199.
21. Sakai, V. T.; Zhang, Z.; Dong, Z.; Neiva, K. G.; Machado, M. A.; Shi, S.; Santos, C. F.; Nör, J. E. SHED differentiate into functional odontoblasts and endothelium. *J Dent Res.* **2010**, *89*, 791-796.
22. Cordeiro, M. M.; Dong, Z.; Kaneko, T.; Zhang, Z.; Miyazawa, M.; Shi, S.; Smith, A. J.; Nör, J. E. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. *J Endod.* **2008**, *34*, 962-969.
23. Wang, J.; Wang, X.; Sun, Z.; Wang, X.; Yang, H.; Shi, S.; Wang, S. Stem cells from human-exfoliated deciduous teeth can differentiate into dopaminergic neuron-like cells. *Stem Cells Dev.* **2010**, *19*, 1375-1383.
24. Nakamura, S.; Yamada, Y.; Katagiri, W.; Sugito, T.; Ito, K.; Ueda, M. Stem cell proliferation pathways comparison between human exfoliated deciduous teeth and dental pulp stem cells by gene expression profile from promising dental pulp. *J Endod.* **2009**, *35*, 1536-1542.
25. Laing, A. G.; Fanelli, G.; Ramirez-Valdez, A.; Lechler, R. I.; Lombardi, G.; Sharpe, P. T. Mesenchymal stem cells inhibit T-cell function through conserved induction of cellular stress. *PLoS One.* **2019**, *14*, e0213170.
26. Laing, A. G.; Riffo-Vasquez, Y.; Sharif-Paghaleh, E.; Lombardi, G.; Sharpe, P. T. Immune modulation by apoptotic dental pulp stem cells in vivo. *Immunotherapy.* **2018**, *10*, 201-211.
27. Gazarian, K. G.; Ramírez-García, L. R. Human deciduous teeth stem cells (SHED) display neural crest signature characters. *PLoS One.* **2017**, *12*, e0170321.
28. Huang, G. T.; Sonoyama, W.; Liu, Y.; Liu, H.; Wang, S.; Shi, S. The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. *J Endod.* **2008**, *34*, 645-651.
29. Sonoyama, W.; Liu, Y.; Yamaza, T.; Tuan, R. S.; Wang, S.; Shi, S.; Huang, G. T. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. *J Endod.* **2008**, *34*, 166-171.
30. Hilkens, P.; Bronckaers, A.; Ratajczak, J.; Gervois, P.; Wolfs, E.; Lambrichts, I. The angiogenic potential of DPSCs and SCAPs in an in vivo model of dental pulp regeneration. *Stem Cells Int.* **2017**, *2017*, 2582080.
31. Liu, C.; Xiong, H.; Chen, K.; Huang, Y.; Huang, Y.; Yin, X. Long-term exposure to pro-inflammatory cytokines inhibits the osteogenic/dentinogenic differentiation of stem cells from the apical papilla. *Int Endod J.* **2016**, *49*, 950-959.
32. Chen, H.; Fu, H.; Wu, X.; Duan, Y.; Zhang, S.; Hu, H.; Liao, Y.; Wang, T.; Yang, Y.; Chen, G.; Li, Z.; Tian, W. Regeneration of pulpo-dentinal-like complex by a group of unique multipotent CD24a(+) stem cells. *Sci Adv.* **2020**, *6*, eaay1514.
33. Nada, O. A.; El Backly, R. M. Stem cells from the apical papilla (SCAP) as a tool for endogenous tissue regeneration. *Front Bioeng Biotechnol.* **2018**, *6*, 103.
34. Jeon, B. G.; Kang, E. J.; Kumar, B. M.; Maeng, G. H.; Ock, S. A.; Kwack, D. O.; Park, B. W.; Rho, G. J. Comparative analysis of telomere length, telomerase and reverse transcriptase activity in human dental stem cells. *Cell Transplant.* **2011**, *20*, 1693-1705.
35. Volponi, A. A.; Gentleman, E.; Fatscher, R.; Pang, Y. W.; Gentleman, M. M.; Sharpe, P. T. Composition of mineral produced by dental mesenchymal stem cells. *J Dent Res.* **2015**, *94*, 1568-1574.
36. Bakopoulou, A.; Kritis, A.; Andreadis, D.; Papachristou, E.; Leyhausen, G.; Koidis, P.; Geurtsen, W.; Tsiftsoglou, A. Angiogenic potential and secretome of human apical papilla mesenchymal stem cells in various stress microenvironments. *Stem Cells Dev.* **2015**, *24*, 2496-2512.
37. Yu, S.; Zhao, Y.; Ma, Y.; Ge, L. Profiling the secretome of human stem cells from dental apical papilla. *Stem Cells Dev.* **2016**, *25*, 499-508.
38. Diogenes, A.; Hargreaves, K. M. Microbial modulation of stem cells and future directions in regenerative endodontics. *J Endod.* **2017**, *43*, S95-S101.
39. Yi, B.; Ding, T.; Jiang, S.; Gong, T.; Chopra, H.; Sha, O.; Dissanayaka, W. L.; Ge, S.; Zhang, C. Conversion of stem cells from apical papilla into endothelial cells by small molecules and growth factors. *Stem Cell Res Ther.* **2021**, *12*, 266.
40. Pereira, D.; Sequeira, I. A scarless healing tale: comparing homeostasis and wound healing of oral mucosa with skin and oesophagus. *Front Cell Dev Biol.* **2021**, *9*, 682143.
41. Lindhe, J.; Lang, N. P.; Karring, T. *Clinical periodontology and implant dentistry.* 5th ed.; Wiley-Blackwell: 2008.
42. Zhang, Q.; Shi, S.; Liu, Y.; Uyanne, J.; Shi, Y.; Shi, S.; Le, A. D. Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. *J Immunol.* **2009**, *183*, 7787-7798.
43. Yang, H.; Gao, L. N.; An, Y.; Hu, C. H.; Jin, F.; Zhou, J.; Jin, Y.; Chen, F. M. Comparison of mesenchymal stem cells derived from gingival tissue and periodontal ligament in different incubation conditions. *Biomaterials.* **2013**, *34*, 7033-7047.
44. Nakamura, T.; Inatomi, T.; Sotozono, C.; Amemiya, T.; Kanamura, N.; Kinoshita, S. Transplantation of cultivated autologous oral mucosal epithelial cells in patients with severe ocular surface disorders. *Br J Ophthalmol.* **2004**, *88*, 1280-1284.
45. Nakamura, T.; Takeda, K.; Inatomi, T.; Sotozono, C.; Kinoshita, S. Long-term results of autologous cultivated oral mucosal epithelial transplantation in the scar phase of severe ocular surface disorders. *Br J Ophthalmol.* **2011**, *95*, 942-946.

46. Nagata, M.; Chu, A. K. Y.; Ono, N.; Welch, J. D.; Ono, W. Single-cell transcriptomic analysis reveals developmental relationships and specific markers of mouse periodontium cellular subsets. *Front Dent Med.* **2021**, *2*, 679937.
47. Wada, N.; Menicanin, D.; Shi, S.; Bartold, P. M.; Gronthos, S. Immunomodulatory properties of human periodontal ligament stem cells. *J Cell Physiol.* **2009**, *219*, 667-676.
48. Zhao, J.; Volponi, A. A.; Caetano, A.; Sharpe, P. T. Mesenchymal stem cells in teeth. *In Encyclopedia of Bone Biology*, Elsevier: 2020; pp 109-118.
49. Iwasaki, K.; Komaki, M.; Yokoyama, N.; Tanaka, Y.; Taki, A.; Kimura, Y.; Takeda, M.; Oda, S.; Izumi, Y.; Morita, I. Periodontal ligament stem cells possess the characteristics of pericytes. *J Periodontol.* **2013**, *84*, 1425-1433.
50. Caetano, A. J.; Yianni, V.; Volponi, A.; Booth, V.; D'Agostino, E. M.; Sharpe, P. Defining human mesenchymal and epithelial heterogeneity in response to oral inflammatory disease. *eLife.* **2021**, *10*, e62810.
51. Human cell atlas. https://www.humancellatlas.org/.Access March 1.2022.
52. An, Z.; Sabalic, M.; Bloomquist, R. F.; Fowler, T. E.; Streelman, T.; Sharpe, P. T. A quiescent cell population replenishes mesenchymal stem cells to drive accelerated growth in mouse incisors. *Nat Commun.* **2018**, *9*, 378.
53. An, Z.; Akily, B.; Sabalic, M.; Zong, G.; Chai, Y.; Sharpe, P. T. Regulation of mesenchymal stem to transit-amplifying cell transition in the continuously growing mouse incisor. *Cell Rep.* **2018**, *23*, 3102-3111.
54. Seidel, K.; Marangoni, P.; Tang, C.; Houshmand, B.; Du, W.; Maas, R. L.; Murray, S.; Oldham, M. C.; Klein, O. D. Resolving stem and progenitor cells in the adult mouse incisor through gene co-expression analysis. *eLife.* **2017**, *6*, e24712.
55. Krivanek, J.; Soldatov, R. A.; Kastriti, M. E.; Chontorotzea, T.; Herdina, A. N.; Petersen, J.; Szarowska, B.; Landova, M.; Matejova, V. K.; Holla, L. I.; Kuchler, U.; Zdrilic, I. V.; Vijaykumar, A.; Balic, A.; Marangoni, P.; Klein, O. D.; Neves, V. C. M.; Yianni, V.; Sharpe, P. T.; Harkany, T.; Metscher, B. D.; Bajénoff, M.; Mina, M.; Fried, K.; Kharchenko, P. V.; Adameyko, I. Dental cell type atlas reveals stem and differentiated cell types in mouse and human teeth. *Nat Commun.* **2020**, *11*, 4816.
56. Yu, T.; Volponi, A. A.; Babb, R.; An, Z.; Sharpe, P. T. Stem cells in tooth development, growth, repair, and regeneration. *Curr Top Dev Biol.* **2015**, *115*, 187-212.
57. Zhao, J.; Faure, L.; Adameyko, I.; Sharpe, P. T. Stem cell contributions to cementoblast differentiation in healthy periodontal ligament and periodontitis. *Stem Cells.* **2021**, *39*, 92-102.

Received: December 11, 2021
Revised: March 9, 2022
Accepted: March 12, 2022
Available online: March 28, 2022