Editorial

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Regenerative Medicine

Regenerated teeth: the future of tooth replacement. An update

"Nanotechnology offers a plethora of exciting perspectives to regenerative dentistry, and combined with stem cell biology might provide new, noninvasive, techniques for tooth regeneration."

Keywords: ameloblasts • dentin • enamel • nanotechnology • regeneration • stem cells • tooth

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A plethora of dental materials are successfully used for partial dental tissue repair, while innovative dental implants are used for tooth replacement. Cell-based tooth regeneration is an attractive approach that complements traditional restorative or surgical techniques for replacement of damaged dental tissues. Recent efforts focus mostly on partial tooth regeneration for the treatment of diseases that commonly affect dental tissues. However, several attempts have been also made for the regeneration of entire teeth.

Tooth formation & regeneration

Teeth form as a result of sequential and reciprocal interactions between cells of the oral epithelium and neural crest-derived mesenchyme [1]. Cells of the dental mesenchyme that face the dental epithelium differentiate into the dentin-secreting odontoblasts. The presence of dentin is essential for the differentiation of dental epithelial cells into ameloblasts that synthesize the enamel. The beginning of tooth eruption into the oral cavity is accompanied by root growth, cementum matrix deposition and periodontium formation.

Stem cells, which are characterized by their potential to self-replicate and differentiate into a vast variety of cell populations [2], are found in human teeth. Dental mesenchymal stem cells (DMSCs) have been isolated from different locations within deciduous and permanent teeth, and tested for their potential applications in regenerative dentistry (e.g., pulp and periodontium regeneration) [3,4]. In contrast to DMSCs, identifying dental epithelial stem cells (DESCs) that are able to form enamel constitutes a major challenge, since most of dental epithelial cell populations disappear shortly after tooth eruption [1,5]. It is thus obvious that any attempt to build a whole new tooth is extremely laborious, since tooth regeneration requires the association of both DMSCs and DESCs [5–7]. The current knowledge on DESCs has been obtained mainly from rodents, in which DESCs constitute the main source of cells for the renewal of the epithelium in their continuously growing incisors [2,8].

Dental mesenchymal stem cells

Stem cells were first isolated from dental pulp of human permanent and exfoliated deciduous teeth. Thereafter, DMSCs have been also identified in the apical part of dental papilla, the dental follicle and the periodontal ligament [7,9]. Due to the lack of specific DMSC markers, generic mesenchymal stem cell markers such as STRO-1, CD146 and CD44 are commonly used for the identification of DMSC populations. Additional identification procedures that rely on the morphology, selective adherence properties, proliferation and differentiation potential and tissue repair abilities of these cell populations are in use [7,10].

Dental pulp stem cells (DPSCs) and stem cells from human exfoliated deciduous teeth are able to differentiate into odontogenic, osteogenic, chondrogenic, adipogenic, myogenic and neurogenic cells *in vitro* and *in vivo* [7,9,10]. Pulp–dentin tissues have been generated after ectopic transplantation of DPSCs

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mixed with hydroxyapatite/tricalcium phosphate [11]. Furthermore, clinical trials using autologous human DPSCs combined with collagen scaffolds for alveolar bone reconstruction have been successfully performed several years ago [12]. Stem cells from human exfoliated deciduous teeth proliferate very fast, but they have a limited capacity to form dentin–pulp complexes *in vivo* [7,10]. Mesenchymal stem cells located at the apex of the developing tooth root (stem cells from the apical papilla) are highly proliferative, and exhibit increased migratory and regenerative potentials [7,10]. Stem cells from the apical papilla have the potential to generate dentin *in vivo*, when transplanted on hydroxyapatite/tricalcium phosphate carriers. Periodontal ligament stem cells and stem cells from the dental follicle are able to form cementum and periodontal ligament tissues *in vivo* [7,10,13].

Dental epithelial stem cells

One possible source of human DESCs is the third molar that develops late after birth. Another source of DESCs is the epithelium of the root that disintegrates into strands of epithelial cells (i.e., epithelial rests of Malassez) [14]. Given the limited knowledge on human DESCs, many studies on DESCs have been performed in the continuously growing rodent incisors [1,2]. DESCs reside in a stem cell niche localized at the apical part of the incisor (i.e., cervical loop). Key regulatory molecules such as Sox2, p75 and Notch1 are involved in both maintenance and proliferation of DESCs.

Induced pluripotent stem cells

Because the source of human DESCs has not been solved, new technologies are required to obtain these cells. Induced pluripotent stem (iPS) cells have the capacity to differentiate into various cell lineages, and can be technically produced from patient's cells [15]. iPS technology can be progressively applied for the regeneration of dental tissues [16]. iPS cells are able to differentiate into ameloblast-like cells in the presence of ameloblastin expressing cells [17]. Also, iPS cells are capable of differentiating into mesenchymal odontogenic cells. iPS cells can form neurospheres in floating culture conditions using nonadherent Petri dishes. Further culture of neurospheres on fibronectin-coated dishes allows their differentiation into neural crest cells. In presence of serum or dental epithelial cell medium, the neural crest cells start to express Pax9 and dentin sialophosphoprotein, which are markers of odontogenic mesenchymal cells and odontoblasts [18].

Nanotechnology

The combination of stem cells with novel nanotechnology platforms holds great promise for applications in regenerative dentistry [10]. The development of nanomaterials could be useful in manipulating and tracking stem cells, gene and protein delivery and formation of artificial niches. Transplanted stem cells can be tracked *in vivo* for long-term periods with noninvasive imaging techniques [10,19]. Furthermore, magnetic nanoparticles might bring information about stem cell kinetics and fate during dental tissue (e.g., periodontium, pulp) regeneration. Light-emitting nanocrystals (i.e., quantum dots) can be also used to monitor the dynamics of dental stem cell niches in real time. Nanotechnology could also create artificial particular dental microenvironments (e.g., apex of the root, pulp chamber) that will direct dental stem cells toward a precise fate and function [20,21], or could improve their survival, and 3D organization [22,23]. These approaches are necessary to evaluate the therapeutic effects of the various dental stem cell populations when exposed to specific microenvironments before any clinical application.

Entire tooth regeneration

Regeneration of the entire tooth would be the ideal therapeutic approach after tooth loss. There are two main approaches in constructing an entire new tooth [2,6,24,25]. The first consists in associating DESCs and DMSCs *in vitro*, where they can form a tooth germ that could then be transplanted into the alveolar bone, where the tooth germ will develop, erupt and become a functional tooth. The second approach consists in implanting into the jaw tooth-shaped polymeric biodegradable scaffolds that are filled with both DESCs and DMSCs that will finally give rise to a functional tooth. These approaches are mainly based on previous pioneer studies in mice, where recombination of embryonic dental epithelium and mesenchyme has been performed for *in vitro* or *ex vivo* tooth development [9].

> "...a major issue in entire tooth regeneration is the unavailability of embryonic stem cells with odontogenic potential."

Recent experiments in mice have shown that it is possible to obtain functional teeth with roots and periodontal structures with these bioengineered approaches [25–28]. Indeed, tooth germs formed by dental epithelial and mesenchymal cells seeded into collagen drops, which served as scaffolds, have been implanted in the mandible of adult mice and gave rise to new functional teeth. Formation of all dental tissues allows the eruption and full integration of the bioengineered teeth into the recipient alveolar bone.

The re-aggregation of iPS-derived neural crest cells and mouse odontogenic epithelial tissues is also able to generate entire teeth *ex vivo* [29]. Although the further

technical improvements for enhancing iPS cell differentiation into odontogenic stem cells may be needed, the iPS technology is expected to open new horizons in regenerative dentistry. Hopefully, it will be possible in the near future to regenerate entire teeth using merely iPS-derived cells.

"A great challenge in regenerative dentistry is the *de novo* formation of enamel in humans."

Such results have not yet been obtained with human cells, since a major issue in entire tooth regeneration is the unavailability of embryonic stem cells with odontogenic potential. Bioengineered teeth formed with human cells have been produced so far in ectopic sites and are still missing some essential elements such as a complete root and periodontal tissues.

Challenges of dental tissue regeneration

Important issues concerning timing of tooth development and eruption, dental aesthetics and tooth dimension have to be clearly addressed before transplantation of bioengineered tooth germs in patients. A great challenge in regenerative dentistry is the *de novo* formation of enamel in humans. DESCs are rare in adult human teeth, making thus necessary the identification of ESCs of nondental origin that will be able to differentiate into ameloblasts. Timing in tooth formation represents an additional major issue [5,10], since in humans the whole process of odontogenesis takes more than 10 years. Such a span of time is inappropriate in clinical practice and thus the entire process should be accelerated before it could be applied on humans. The tooth shape and size are additional essential parameters. It is extremely important to understand deeply how reciprocal signals between the various components of the teeth determine their type and shape, in order to obtain the desired tooth morphology.

Conclusion

Stem cell-based therapies are not yet applicable in dental clinics. These therapies necessitate thorough testing first in animals and then in humans. Nanotechnology offers a plethora of exciting perspectives to regenerative dentistry, and combined with stem cell biology might provide new, noninvasive, techniques for tooth regeneration.

Financial & competing interests disclosure

This work was supported by funds from the University of Zurich (TA Mitsiadis). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

1 Mitsiadis TA, Graf D. Cell fate determination during tooth development and regeneration. *Birth Defects Res. C Embryo Today* 87(3), 199–211 (2009).

2 Mitsiadis TA, Barrandon O, Rochat A, Barrandon Y, De Bari C. Stem cell niches in mammals. *Exp. Cell Res.* 313(16), 3377–3385 (2007).

3 Graziano A, D'Aquino R, Laino G, Papaccio G. Dental pulp stem cells: a promising tool for bone regeneration. *Stem Cell Rev.* 4(1), 21–26 (2008).

4 Wang L, Shen H, Zheng W *et al.* Characterization of stem cells from alveolar periodontal ligament. *Tissue Eng. Part A* 17(7–8), 1015–1026 (2011).

5 Mitsiadis TA, Papagerakis P. Regenerated teeth: the future of tooth replacement? *Regen. Med.* 6(2), 135–139 (2011).

6 Volponi AA, Pang Y, Sharpe PT. Stem cell-based biological tooth repair and regeneration. *Trends Cell Biol.* 20(12), 715–722 (2010).

7 Catón J, Bostanci N, Remboutsika E, De Bari C, Mitsiadis TA. Future dentistry: cell therapy meets tooth and periodontal repair and regeneration. *J. Cell. Mol. Med.* 15(5), 1054–1065 (2011).

8 Jiménez-Rojo L, Granchi Z, Graf D, Mitsiadis TA. Stem cell fate determination during development and regeneration of ectodermal organs. *Front. Physiol.* 3, 107 (2012).

9 Bluteau G, Luder HU, De Bari C, Mitsiadis TA. Stem cells for tooth engineering. *Eur. Cell. Mater.* 16, 1–9 (2008).

10 Mitsiadis TA, Woloszyk A, Jiménez-Rojo L. Nanodentistry: combining nanostructured materials and stem cells for dental tissue regeneration. *Nanomedicine (Lond.)* 7(11), 1743–1753 (2012).

11 Huang GT-J, Yamaza T, Shea LD *et al.* Stem/progenitor cell-mediated *de novo* regeneration of dental pulp with newly deposited continuous layer of dentin in an *in vivo* model. *Tissue Eng. Part A* 16(2), 605–615 (2010).

12 D'Aquino R, De Rosa A, Lanza V *et al.* Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. *Eur. Cell. Mater.* 18, 75–83 (2009).

13 Yang B, Chen G, Li J *et al.* Tooth root regeneration using dental follicle cell sheets in combination with a dentin matrix-based scaffold. *Biomaterials* 33(8), 2449–2461 (2012).

14 Shinmura Y, Tsuchiya S, Hata K-I, Honda MJ. Quiescent epithelial cell rests of Malassez can differentiate into ameloblast-like cells. *J. Cell. Physiol.* 217(3), 728–738 (2008).

15 Takahashi K, Tanabe K, Ohnuki M *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131(5), 861–872 (2007).

16 Arakaki M, Egusa H, Otsu K, Saitoh I, Miura T, Harada H. Frontier dental research on iPS cells. *J. Oral Biosci.* 55(4), 191–199 (2013).

17 Arakaki M, Ishikawa M, Nakamura T *et al.* Role of epithelial-stem cell interactions during dental cell differentiation. *J. Biol. Chem.* 287(13), 10590–10601 (2012).

18 Otsu K, Kishigami R, Oikawa-Sasaki A *et al.* Differentiation of induced pluripotent stem cells into dental mesenchymal cells. *Stem Cells Dev.* 21(7), 1156–1164 (2012).

19 Ferreira L, Karp JM, Nobre L, Langer R. New opportunities: the use of nanotechnologies to manipulate and track stem cells. *Cell Stem Cell* 3(2), 136–146 (2008).

20 Kraehenbuehl TP, Langer R, Ferreira LS. Three-dimensional biomaterials for the study of human pluripotent stem cells. *Nat. Methods* 8(9), 731–736 (2011).

21 Dickinson LE, Kusuma S, Gerecht S. Reconstructing the differentiation niche of embryonic stem cells using biomaterials. *Macromol. Biosci.* 11(1), 36–49 (2011).

22 Mooney DJ, Vandenburgh H. Cell delivery mechanisms for tissue repair. *Cell Stem Cell* 2(3), 205–213 (2008).

23 Sun H, Jin T, Yu Q, Chen F. Biological approaches toward dental pulp regeneration by tissue engineering. *J. Tissue Eng. Regen. Med.* 5(4), e1–e16 (2011).

24 Ikeda E, Morita R, Nakao K *et al.* Fully functional bioengineered tooth replacement as an organ replacement therapy. *Proc. Natl Acad. Sci. USA* 106(32), 13475–13480 (2009).

25 Oshima M, Mizuno M, Imamura A *et al.* Functional tooth regeneration using a bioengineered tooth unit as a mature organ replacement regenerative therapy. *PLoS ONE* 6(7), e21531 (2011).

26 Oshima M, Inoue K, Nakajima K *et al.* Functional tooth restoration by next-generation bio-hybrid implant as a bio-hybrid artificial organ replacement therapy. *Sci. Rep.* 4, 6044 (2014).

27 Oshima M, Tsuji T. Functional tooth regenerative therapy: tooth tissue regeneration and whole-tooth replacement. *Odontology* 102(2), 123–136 (2014).

28 Oshima M, Ogawa M, Yasukawa M, Tsuji T. Generation of a bioengineered tooth by using a three-dimensional cell manipulation method (organ germ method). *Methods Mol. Biol.* 887, 149–165 (2012).

29 Otsu K, Kumakami-Sakano M, Fujiwara N *et al.* Stem cell sources for tooth regeneration: current status and future prospects. *Front. Physiol.* 5, 36 (2014).