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REVIEW

Tooth regeneration: a revolution in stomatology and evolution in regenerative medicine

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A tooth is a complex biological organ and consists of multiple tissues including the enamel, dentin, cementum and pulp. Tooth loss is the most common organ failure. Can a tooth be regenerated? Can adult stem cells be orchestrated to regenerate tooth structures such as the enamel, dentin, cementum and dental pulp, or even an entire tooth? If not, what are the therapeutically viable sources of stem cells for tooth regeneration? Do stem cells necessarily need to be taken out of the body, and manipulated *ex vivo* before they are transplanted for tooth regeneration? How can regenerated teeth be economically competitive with dental implants? Would it be possible to make regenerated teeth affordable by a large segment of the population worldwide? This review article explores existing and visionary approaches that address some of the above-mentioned questions. Tooth regeneration represents a revolution in stomatology as a shift in the paradigm from repair to regeneration: repair is by metal or artificial materials whereas regeneration is by biological restoration. Tooth regeneration is an extension of the concepts in the broad field of regenerative medicine to restore a tissue defect to its original form and function by biological substitutes.

***Keywords*:** stem cells; bioactive cues; biomaterials; cell homing; dental pulp; tooth regeneration; growth factors; scaffold; dental implants; pulp regeneration

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Clinical need

A tooth is a major organ and consists of multiple tissues. The hard tissues of the tooth include the enamel, dentin and cementum. The only vascularized tissue of the tooth is dental pulp that is encased in the mineralized dentin [1]. Life ends for a number of wildlife species after loss of complete dentition [2]. In humans, tooth loss can lead to physical and mental suffering that compromise an individual's self-esteem and quality of life [3]. Americans make about 500 million visits to dentists each year. In 2009, an estimated $102 billion was spent on dental services in the U.S. Dental carries is one of the most common disorders in humans, second only to common cold. Dental caries, also known as tooth decay or cavity, is an infectious disease primarily by bacterial colonies that breakdown hard tissues of the tooth such as enamel and dentin, as well as soft tissue of the tooth known as dental pulp. According to CDC, 1 in 2 Americans are affected by tooth decay by age 15. By age 20, roughly 1 in 4 teeth are decayed or filled in the U.S. By age 60, more than 60% of the teeth and more 90% of the Americans are affected by dental caries.

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Periodontal disease is another major cause for tooth loss. In both children and adults, facial trauma may also lead to tooth loss. Teeth can be congenitally missing, as a phenotype of myriads craniofacial anomalies including cleft palate [4-7]. Resection of orofacial tumors may involve the extraction of teeth. Indeed, tooth loss represents a major challenge for contemporary dentistry or stomatology, and the bulk of daily dental practice.

Contemporary dentistry or stomatology restores missing teeth by dentures or dental implants. Whereas dental implants are becoming favorite choices in developed countries, a large segment of the world population, frequently in developing countries, cannot afford dental implants. Dental implants, despite being the currently preferred treatment modality, can fail and will not adapt with surrounding bone that necessarily remodels throughout life [8]. A comparison of dental implants and regenerated teeth is provided in Table 1. Dental profession has had the longstanding aspiration to regenerate teeth [9-11].

Table 1 Comparison of current dental treatments including dental implants and dentures with tooth regeneration

Items of comparison	Dental implants	Tooth regeneration
Materials	Artificial materials	Regenerated tissues
Bone grafting	Needed in ~50% cases	Stimulates bone regeneration along with tooth regeneration
Remodeling potential	Metal fails to remodel with host bone	Regenerated periodontal bone remodels with existing alveolar bone
Complications	Aseptic loosening or infections, leading to implant failure	Regenerated teeth have native defense in dental pulp and periodontal tissues

Regeneration of teeth can be broadly divided into several areas as listed below. References and review articles are provided for those areas that are not covered in this article.

- Regeneration or *de novo* formation of the entire, anatomically correct teeth (discussed at length below; *c.f.* [12];
- Regeneration of dental pulp (discussed below; *c.f.* [13]);
- Regeneration of dentin based on biological approaches and potentially as biological fillers that may replace current synthetic materials for restorative dentistry [14-16];
- Regeneration of cementum as a part of periodontium regeneration or for loss of cementum and/or dentin resulting from trauma or orthodontic tooth movement [17-18];
- Regeneration of the periodontium including cementum, periodontal ligament and alveolar bone [19-22];
- Regeneration or synthesis of enamel-like structures that may be used as biological substitute for lost enamel [23-25].

Since a tooth is a biological organ, it is unavoidable that regeneration of various components of the tooth is highly inter-connected. Furthermore, successful regeneration of tooth components does not necessarily translate to regeneration of an entire tooth. The overall objective of this review article is to explore therapeutically viable approaches for tooth regeneration by contrasting cell transplantation and cell homing approaches.

Barriers of tooth regeneration towards clinical applications

For the regeneration of the entire tooth or tooth elements, we are ingrained to believe that stem cells and/or other cells must be transplanted. When tissue engineering was initiated as an interdisciplinary approach to heal tissue defects, three key components were proposed: cells, biomaterial scaffolds and signaling factors [26]. There is no question that cells, including stem/progenitor cells, play central roles in tissue regeneration. However, do cells (including stem/progenitor cells) necessarily need to be taken out of the body, manipulated *ex vivo* and transplanted back into the patient?

Tooth regeneration by cell transplantation is a meritorious approach. However, there are hurdles in the translation of cell-delivery-based tooth regeneration into therapeutics. The most important one of these difficulties is inaccessibility of autologous embryonic tooth germ cells for human applications [9, 27-28]. Xenogenic embryonic tooth germ cells (from non-human species) may elicit immunorejection and tooth dysmorphogenesis. Autologous postnatal tooth germ cells (*e.g.* third molars) or autologous dental pulp stem cells are of limited availability and remain uncertain as a cell source to regenerated an entire tooth. Regardless of cell source, cell-delivery approaches for tooth regeneration, similar to cell-based therapies for other tissues, encounter trans-

lational barriers. The costs of commercialization process and difficulties in regulatory approval in association with *ex vivo* cell manipulation have precluded any significant clinical translation effort to date in tooth regeneration (Table 2). As in tissue engineering of other biological structures, regeneration of an entire tooth or various tooth structure, including the enamel, dentin, cementum and dental pulp, by cell transplantation encounters a number of scientific, translational and regulatory difficulties [29].

Table 2 Comparison of cell transplantation *vs*. cell homing approaches for tooth regeneration

Items of comparison	Tooth regeneration by cell transplantation	Tooth regeneration by cell homing
Isolation of cells from patient	Yes - autologous cells	No
Ex vivo cell manipulation	Yes	No
Cell transplantation	Yes	No
Develop into off-the-shelf product	Difficult	Possible
Cost	High	Not as high

Tooth regeneration by cell transplantation

Table 3 provides a summary of various cell sources that have been used for tooth regeneration. Disassociated cells of porcine or rat tooth buds in biomaterials yielded putative dentin and enamel organ [30-31]. Tooth bud cells and bone marrow osteoprogenitor cells in collagen, PLGA or silk-protein scaffolds induced putative tooth-like tissues, alveolar bone and periodontal ligament [32-34]. Embryonic oral epithelium and adult mesenchyme together up-regulate odontogenesis genes upon mutual induction, and yielded dental structures upon transplantation into adult renal capsules or jaw bone [35]. Similarly, implantation of E14.5 rat molar rudiments into adult mouse maxilla produced tooth-like structures with surrounding bone [9, 36]. Multipotent cells of the tooth apical papilla in tricalcium phosphate in minipig incisor extraction sockets generated soft and mineralized tissues of the root [37]. Dental bud cells from unerupted molar tooth of a 1.5-month-old swine were expanded and then seeded in gelatin-chrondroitin-hyaluronan-tri-copolymer scaffold. Cell-seeded scaffolds were implanted autologously in the swine's tooth extraction socket. Thirty-six weeks after implantation, dentin/pulp-like complex structures were identified with odontoblast-like cells and blood vessels in the pulp and appearance of cellular cementum [34]. However, the regenerated teeth were much smaller in size than the normal teeth in the same

Table 3 Cell sources that have been used for tooth regeneration

Cell source	Species	Age	Scaffold	Method	References
Embryonic tooth bud cells	Rat	E14.5		Implantation into adult mouse maxilla	[27]
Embryonic tooth bud cells	Mice	E14.5	Acid-soluble collagen	Transplanted into subrenal capsule of 8-week-old mice	[9, 36]
Adult tooth bud cells	Porcine	1,5 months	Gelatin-chrondroitin-hyaluronan-tri-copolymer	Autotransplantation into swine's original alveolar socket	[34]
Adult tooth bud cells and bone marrow osteoprogenitors	Porcine	6 months	PGA/PLGA	implanted in the omenta of adult rat	[10, 32]
Adult dental pulp cells	Human	18-20 years	HA/TCP	Implantation to incisor tooth extraction sockets in minipigs	[37]
Non-dental mesenchymal cells and + embryonic endothelium	Mice	Non-dental mesenchyme cells: 6-9 weeks Embryonic endothelium: E10	membrane filters	*In vitro* incubation or mice kidney implantation	[35]

host. E14.5 oral epithelium and dental mesenchyme were reconstituted in collagen gels and cultured *ex vivo* [27], and when implanted into the maxillary molar extraction sockets in 5-week-old mice, tooth morphogenesis took place and was followed by eruption into occlusion [28]. Several studies have begun to tackle an obligatory task of scale up towards human tooth size [38-39]. Thus, tooth regeneration by cell transplantation is a meritorious approach. However, there are hurdles in the translation of cell-delivery-based tooth regeneration into therapeutics. Autologous embryonic tooth germ cells are inaccessible for human applications [9, 27-28]. Xenogenic embryonic tooth germ cells (from non-human species) may elicit immunorejection and tooth dysmorphogenesis. Autologous postnatal tooth germ cells (*e.g.* third molars) or autologous dental pulp stem cells are of limited availability. Regardless of cell source, cell delivery for tooth regeneration, similar to cell-based therapies for other tissues, encounters translational barriers. Excessive costs of commercialization and difficulties in regulatory approval have precluded, to date, any significant clinical translation of tooth regeneration.

Dental pulp is a vascularized tissue encapsulated in highly mineralized structures including dentin, enamel and cementum, and maintains homeostasis of the tooth as a viable biological organ [40]. The overall health of the tooth is compromised upon dental pulp trauma or infections, frequently manifested as pulpitis. A typical endodontic treatment or root canal therapy for irreversible pulpitis is pulpectomy, involving pulp extirpation followed by root canal enlargement and obturation of root canal with gutta percha, a bioinert thermoplastic material. Despite reported clinical success, endodontically treated teeth become de-vitalized and brittle, susceptible to post-operative fracture and other complications including re-infections due to coronal leakage or microleakage [41]. A substantial amount of tooth structures including enamel and dentin is removed during endodontic treatment, potentially leading to post-treatment tooth fracture and trauma [41-42]. Endodontically treated teeth have lost pulpal sensation, and are deprived of the ability to detect secondary infections [42-43]. The complications of current endodontic treatment are inevitable because of pulp devitalization or the loss of the tooth's innate homeostasis and defense mechanisms.

Similar to tooth regeneration, existing effort in dental pulp regeneration has focused on cell transplantation [44-46]. Several reports have documented regeneration of dental pulp-like tissue *in vitro* or ectopically by transplantation of dental pulp stem cells [47-50]. Deciduous and adult dental pulp stem cells seeded in a self-assembling peptide-amphiphile hydrogel showed distinctive behavior: greater proliferative rate for deciduous cells but greater osteogenic differentiation potential for adult cells [47-48]. Delivery of collagen scaffolds with dental pulp stem cells and dentin matrix protein-1 in dentin slices in mice led to ectopic formation of pulp-like tissue [50]. Deciduous dental pulp stem/progenitor cells seeded in matrigel in 1.5-mm cross-sectional tooth slices regenerated vascular pulp-like tissue following ectopic implantation in SCID mice [51]. Similarly, stem/progenitor cells from apical papilla and dental pulp in root fragments yielded vascularized pulp-like tissue following ectopic implantation also in SCID mice [50]. Despite its scientific validity, dental pulp regeneration by dental pulp stem cells encounters clinical and commercialization hurdles. Pulpectomy, the most common endodontic treatment, involves extirpation of dental pulp, and therefore leaves no dental pulp stem cells in the same tooth for pulp regeneration. For a patient who requires endodontic treatment in a given tooth but has intact dentition otherwise, no healthy tooth is to be sacrificed for isolation of dental pulp stem cells. Even in patients whose autologous dental pulp stem cells can be harvested, *e.g.* from extracted wisdom teeth, clinical therapy of dental pulp regeneration is difficult to develop due to excessive costs including cell isolation, handling, storage, and shipping, *ex vivo* manipulation, immune rejection (for allogeneic cells), not to mention liabilities of potential contamination, pathogen transmission and tumorigenesis that may be associated with cell transplantation [52]. Regeneration of dental pulp is discussed in detail in Kim *et al.* [13].

A biomaterial tooth scaffold can be fabricated by 3D bioprinting (Figure 1). For a patient who needs to have a tooth extracted, anatomic form can be derived from CT or MRI scans of the contralateral tooth (if it is healthy) or published anatomic norms. Two-dimensional CT or MR images can be reconstructed to yield high resolution 3D shape and dimensions of the patient's tooth to be

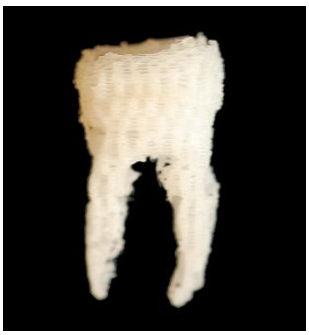

Figure 1 Biomaterial scaffold fabricated from the patient's tooth

extracted. The fabricated 3D tooth scaffold can be sterilized and shipped to the clinic within 2-3 days. Upon tooth extraction, the dentist implants the biomaterial tooth scaffold. In our report [12], a bio-root was regenerated within ~2 months. The advantage of this approach is that no stem cells need to be harvested or *ex vivo* manipulated.

Tooth regeneration by cell homing

As an initial attempt to regenerate teeth, we first fabricated an anatomically shaped and dimensioned scaffold from biomaterials, using our previously reported approach [53-54]. The dimensions of the permanent mandibular first molar were derived from textbook averages and therefore IRB exempt. Scaffolds with the shape of the human mandibular first molar (Figure 2A) were fabricated via 3D layer-by-layer apposition [54-55]. The composite consisted of 80% (*m*/*m*) polycaprolactone (PCL) and 20% (*m*/*m*) of hydroxyapatite (HA) (Sigma, St. Louis, MO). PCL-HA was co-molten at 120 ℃ and dispensed through a 27-gauge metal nozzle to create repeating 3D microstrands (200 μm wall thickness) and interconnecting microchannels (dia: 200 μm) (Figure 2A).

All scaffolds were sterilized in ethylene oxide for 24 h. A blended cocktail of stromal derived factor 1 (SDF1) (100 ng·mL^{-1}) and bone morphogenetic protein 7 (BMP7) (100 ng·mL^{-1}) was adsorbed in 2 mg·mL^{-1} neutralized type Ⅰ collagen solution (all from R&D, Minneapolis, MN). SDF1 was selected for its effects to bind to CXCR4 receptors of multiple cell lineages including mesenchymal stem/progenitor cells [55-56]. BMP7 was selected for its effects on dental pulp cells, fibroblasts and osteoblasts in elaborating mineralization [57-58]. SDF1 and BMP7 doses were chosen from *in vivo* work [56, 59]. SDF1- and BMP7-loaded collagen solution was infused in scaffold's microchannels by micropippeting, and crosslinked at 37 ℃ for 1 h. Control scaffolds were infused with the same collagen gel but without growth-factor delivery.

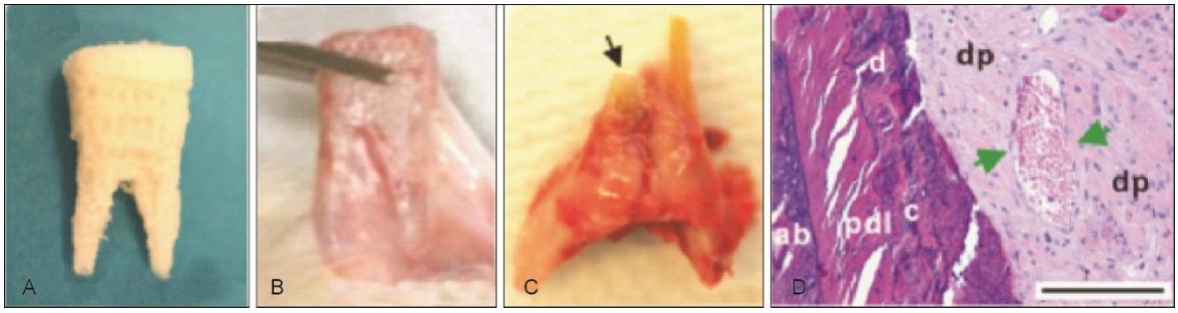

Figure 2 Tooth regeneration by cell homing. (**A**) A 3D biomaterials scaffold was fabricated by layer by layer fabrication *via* bioprinting. In a clinical setting, a patient's missing tooth can be reconstructed by multi-slice imaging using CT or MRI of the contralateral, normal tooth or from anatomic averages. Microchannels are built in the 3D biomaterial human tooth shaped scaffold and serve as conduits for cell recruitment and vascularization. (**B**) Harvest of human shaped tooth scaffold following 9-week *in vivo* implantation. (**C**) A rat shaped tooth scaffold was implanted to replace the rat lower incisor that was freshly extracted. (**D**) Harvest of regenerated tooth scaffold showed the formation of multiple dental tissues including newly formed alveolar bone (ab), periodontal ligament-like tissue (pdl), dentin-like tissue (d) and dental pulp-like tissue (dp) with blood vessels (arrows). Bar: 200 μm.

A total of 22 male Sprague-Dawley rats (12-week-old) were randomly divided equally into treatment and control groups (Charles River, NY). All rats were anesthetized by *i.p*. administration of ketamine (80 mg·kg^{-1}) and xylazine (5 mg·kg^{-1}). A 2-cm incision was made in the dorsum. Human mandibular molar scaffolds were implanted in surgically created subcutaneous pouches followed by wound closure. The rat right mandibular central incisor was extracted with periotome, followed by implantation of the anatomically shaped mandibular incisor scaffold [12] into the extraction socket. The flap was advanced for primary closure around the scaffold.

Nine weeks post-surgery, all rats were euthanized by pentobarbital overdose. The human shaped mandibular first molar scaffold was retrieved from the dorsum (Figure 2B). The rat incisor scaffolds were harvested with surrounding bone and native tooth structures (Figure 2C) (also *c.f.* [12]). All samples were fixed in 10% formalin, embedded in poly(methyl methacrylate) (PMMA), sectioned at 5-μm thickness for hematoxylin

and eosin (H&E) and von-Kossa (VK) staining (HSRL, Jackson, VA). PMMA was used because PCL-HA scaffolds cannot be de-mineralized for paraffin embedding. The average areal cell density and blood vessel numbers were quantified from the coronal, middle, and apical thirds of the rat incisor scaffolds and similarly of the human molar scaffolds by a blinded and calibrated examiner.

Microscopically, host cells populated scaffold's microchannels with growth-factor delivery (Figure 2D). Quantitatively, combined SDF1 and BMP7 delivery homed significantly more cells into the microchannels of the human molar scaffolds than without growth-factor delivery (P<0.01) [12]. Angiogenesis took place in microchannels with growth-factor delivery as exemplified in Figure 2D. Combined SDF1 and BMP7 delivery elaborated significantly more blood vessels than without growth-factor delivery (P<0.05) [12]. Scaffolds in the shape of the rat mandibular incisor integrated with surrounding tissue, showing tissue ingrowth into scaffold's microchannels (Figure 2D). It was not possible to separate the implanted scaffolds without physical damage to surrounding tissue. Microscopically, the scaffolds within the extraction sockets clearly showed multiple tissue phenotypes including the newly formed alveolar bone (ab) and newly formed dentin-like tissue (d) with a fibrous tissue interface that is reminiscent of the periodontal ligament (pdl) in between (Figure 2D). There were areas of irregular cementum-like tissue (c) that did not completely cover dentin-like tissue (Figure 2D). Dental pulp (dp)-like tissue was formed in scaffold's microchannels and was rich with angiogenesis (Figure 2D). Quantitatively, combined SDF1 and BMP7 delivery elaborated significantly more blood vessels than growth-factor-free group (P<0.05) [12].

Anatomically dimensioned tooth scaffolds were designed and 3D bioprinting was performed as follows: anatomic shape and dimensions of the rat mandibular central incisor and human mandibular first molar were derived from multiple slices of 2D laser scanning of extracted rat incisor and mandibular first molar. The anatomical contour of an extracted rat mandibular central incisor and human mandibular first molar was acquired from computed tomography scans and manipulated using computer aided design software (Rhinoceros, McNeel, Seattle, WA) for 3D reconstruction. Engineering parameters were used to fabricate a composite polymer scaffold per our prior methods [53-54]. Scaffolds with the shape of the human mandibular first molar (Figure 2A, 2B) were fabricated *via* 3D layer-by-layer apposition [53-54]. The composite consisted of 80% (*m*/*m*) polycaprolactone (PCL) and 20% (*m*/*m*) of hydroxyapatite (HA) (Sigma, St. Louis, MO). PCL-HA was co-molten at 120 ℃ and dispensed through a 27-gauge metal nozzle to create repeating 3D microstrands (20 μm wall thickness) and interconnecting microchannels (diameter: 200 μm) (Figure 2A).

SDF1 is chemotactic and anti-apoptotic for bone marrow stem/progenitor cells and endothelial cells [55]. SDF1's role to elaborate angiogenesis is likely of paramount importance because stem/progenitor cells usually derive from *via* blood vessels or perivascular cells. Neovascularization in engineered teeth plays an important role in tissue survival, and promotes cell growth and mineralization [60]. SDF1 has effects to bind to CXCR4 receptors of multiple cell lineages including mesenchymal stem/progenitor cells. It binds to CXCR4, a chemokine receptor for endothelial cells and bone marrow stem/progenitor cells [55-56]. As an another cell homing factor BMP7 was chosen because it plays a key role in the differentiation of mesenchymal cells into osteoblasts [61]. BMP7 has many effects on dental pulp cells, fibroblasts and osteoblasts in elaborating mineralization [58-59]. BMP7 plays a key role in the differentiation of mesenchymal cells into osteoblasts [61] triggering the phosphorylation of SMAD1 and SMAD5, which in turn induces the transcription of many osteogenic/odontogenic genes [62].

Along with variety of animal models, clinical trials investigating long bone applications have also provided supportive evidence for the use of BMP7 in the treatment of open many fractures and atrophic nonunions as well as in spinal fusion. BMP7 doses for cell homing approach for tooth regeneration were chosen from the promising therapeutic potential for this molecule from the positive clinical data [59]. SDF1 dose was chosen from an *in vivo* work showing SDF1 is induced in the periosteum of injured bone and promotes endochondral bone repair [56].

These findings represent the first report of the regeneration of tooth-like structures *in vivo* without cell delivery, and may provide a clinically translatable approach. Interconnecting microchannels (diameter: 200 μm) were constructed as conduits within anatomically correct scaffolds to allow the homing of host endogenous cells and angiogenesis. Upon *in vivo* implantation, a putative periodontal ligament and new bone formed at the scaffold's interface with native alveolar bone. Remarkably, cell homing mediated by a cocktail of SDF1 and BMP7 recruited not only more cells, but also elaborated more vasculature throughout the scaffold's microchannels than without growth-factor delivery.

Cell homing offers an alternative, especially regarding clinical translation, to previous meritorious methods of

tooth regeneration by cell transplantation. The omission of cell isolation and *ex vivo* cell manipulation accelerates regulatory, commercialization and clinical processes [63]. The cost of cell-homing-based tooth regeneration is not anticipated to be as robust as cell delivery with regard to both commercialization process and as a treatment cost to the patient. Cell homing is an under-recognized approach in tissue regeneration [52]. Here, all cells in growth-factor delivery or growth-factor-free scaffolds are host derived endogenous cells. Tissue genesis requires condensation of sufficient cells of correct lineages [9, 64]. The observed putative periodontal ligament and adjacent, newly formed bone suggest the potential of combined delivery of SDF1 and BMP7 to recruit multiple cell lineages. Additional growth factors may constitute an optimal conglomerate that is yet to be unveiled for tooth regeneration.

SDF1 is chemotactic and anti-apoptotic for bone marrow stem/progenitor cells and endothelial cells [55]. Our data show not only more homed cells, but also more vasculature upon combined SDF1 and BMP7 delivery. SDF1 binds to CXCR4, a chemokine receptor for endothelial cells and bone marrow stem/progenitor cells [55-56]. Here, SDF1 likely has homed mesenchymal and endothelial stem/progenitor cells in alveolar bone into the porous tooth scaffolds in the rat jaw bone, and connective tissue progenitor cells in the dorsal subcutaneous tissue [65-67]. On the other hand, BMP7 likely is responsible for the ectopic mineralization in the dorsum and, importantly, newly formed bone in scaffold's interface in tooth extraction socket.

The present scaffold design represents the first anatomically dimensioned tooth scaffolds, and a variation from previous approaches in tooth regeneration by relying primarily on soft materials such as collagen gel, silk or PLGA [9, 28, 30]. PCL-HA composite offers mechanical stiffness that is suitable for load-bearing [69]. Among rapid prototyping methods, 3D bioprinting offers the advantage of precise control of pore size, porosity, permeability, stiffness and interconnectivity as well as anatomic dimensions [53, 68]. Clinically, the patient's healthy, contra-lateral tooth form can be imaged by CT or MR, and then fed to a computer-aided design and a bioprinter to generate 3D scaffolds. Anatomically dimensioned scaffolds can either be patient-specific or of generic sizes, and made available as off-the-shelf implants in dental offices.

The present study, being the first of its kind for *de novo* formation of tooth-like tissues by cell homing, is not without limitations. All *in vivo* harvested samples were embedded in PMMA, because PCL-HA cannot be decalcified for paraffin embedding. PMMA embedding disallows immunoblotting by antibodies. Our ongoing work attempts to further characterize regenerated tissues by various imaging modalities. The regenerated mandibular incisor-like structure was mostly the root with a portion of sub-occlusal crown. We suggest that a regenerated tooth is biological primarily because of its root, rather than the crown that can be readily restored with a clinical crown anchorable to a biologically regenerated root.

Tooth regeneration: future directions

The doctrine of cells, biomaterial scaffolds and signaling molecules has been the guiding principle in tissue engineering. Given the vast diversity of tissues that are being regenerated, it is difficult to conceive that one doctrine would govern all. In tooth regeneration, the doctrine of cells, biomaterial scaffolds and signaling molecules is considered below:

Cells

- Embryonic tooth bud cells are not accessible as an autologous cell source for tooth regeneration in human.
- Allogeneic (human) embryonic tooth bud cells are associated with ethic issues and limited availability
- Xenogenic embryonic tooth bud cells may lead to dysmorphogenesis of regenerated teeth, even if it is applicable to humans.

Adult stem/progenitor cells from the third molar (wisdom tooth) or extracted teeth, per current practice, will need to be expanded *ex vivo*, manipulated and then transplanted into the patient, leading to unbearable cost, potential pathogen contamination and tumorigenesis of long-term manipulated cells.

Cells are indeed required for tooth regeneration; however, cells do not necessarily need to be transplanted. Tooth regeneration by cell homing is an under-explored approach and deserves to be further studied [12-13].

Scaffolds

- Biomaterial scaffolds are likely indispensible for tooth regeneration. A tooth is a biological organ but also a structural material that withstand mechanical forces in mastication.
- Ideal scaffolds for tooth regeneration should allow functionality of multiple cell types including odontoblasts, cementoblasts, pulp fibroblasts, vascular cells and/or neural endings, and potentially ameloblasts.
- Ideal scaffolds for tooth regeneration must be clinically viable, *i.e.* easy to handle up to the point

of a turn-key approach, can be readily sterilized and with a reasonably long shelf life.
- Ideal scaffolds for tooth regeneration should be biocompatible, non-toxic and may need to undergo biologically safe degradation.
- Either native or synthetic polymers, or a hybrid, are valid choices as scaffolding materials for tooth regeneration.

Signaling molecules
- If biomaterial scaffolds are sufficient to recruit cells for tooth regeneration, signaling molecules are not needed.
- In our proof of concept study, SDF1 and BMP7 are capable of elaborating mineralization and induce the formation of multiple periodontal tissues including the periodontal ligament and newly formed alveolar bone.
- There is a need to determine the minimally needed signaling molecule(s) that is necessary for regeneration of tooth structures.

In summary, progress has been made to regenerate teeth with both cell transplantation and cell homing approaches (Table 2). One of the pivotal issues in tooth regeneration is to devise clinically translatable approaches that are not cost-prohibitive and can translate into therapies for patients who cannot afford or do not have access to dental implants. Costs for development of cell homing approaches for tooth regeneration are anticipated not as substantial as for tooth regeneration by cell transplantation. Molecular cues can be packaged and made available off-the-shelf in devices for tooth regeneration. In contrast, cell transplantation relies on costly procedures including procurement, *ex vivo* processing, potential cryopreservation, packaging, shipping, handling, and re-implantation into the patient. Thus, tooth regeneration by cell homing may provide tangible pathways towards clinical translation.

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