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Review

The next generation of regenerative dentistry: From tooth development biology to periodontal tissue, dental pulp, and whole tooth reconstruction in the clinical setting

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ABSTRACT

In modern dentistry, prosthetic approaches such as implants and dentures have been developed as symptomatic solutions for tooth loss. However, the complete regeneration of teeth and periodontal tissue, an ultimate aspiration of humanity, remains unachieved. Recent advancements in fundamental scientific technologies, including single-cell RNA sequencing and spatial transcriptomics, have significantly advanced our molecular understanding of tooth development, paving the way toward achieving this goal. This review summarizes the fundamental processes of tooth development in humans and mice, recent findings from basic research, and current clinical applications in dental regenerative medicine, including periodontal, alveolar bone, and dental pulp regeneration using cellular approaches.

Building on accumulated scientific knowledge, the complete regeneration of teeth and periodontal tissues may be achievable in the near future. We discuss the potential of emerging approaches, such as organoids derived from pluripotent stem cells and xenotransplantation using genetically modified animals, to transform dental medicine. These innovative concepts and integrated technologies hold the promise of enabling the regeneration of fully functional teeth and periodontal tissues.

Contents

Abbreviations: BMP, bone morphogenic protein; CAL, clinical attachment level; DPSCs, dental pulp stem cells; EMA, European Medicines Agency; EMD, enamel matrix derivative; ERM, epithelial cell rests of Malassez; ESCs, embryonic stem cells; FDA, Food and Drug Administration; FGF, fibroblast growth factor; GBR, guided bone regeneration; G-CSF, granulocyte-colony stimulating factor; iPSCs, induced pluripotent stem cells; PCBM, particulate cancellous bone and marrow; PCR, polymerase chain reaction; PDL cells, periodontal ligament derived cells; PMDA, Pharmaceuticals and Medical Devices Agency; PRP, platelet-rich plasma; MDPSCs, mobilized dental pulp stem cells; PDLs, periodontal ligament-derived cells; scRNA-seq, single-cell RNA sequencing; WHO, World Health Organization.

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1. Introduction

The oral cavity plays a crucial role not only in feeding and mastication but also in speech articulation and facial aesthetics, all of which are essential for maintaining and forming human social interactions. Teeth are among the most important organs that shape the function of the oral cavity. However, conditions such as dental caries and periodontitis often necessitate tooth extraction, while congenital tooth agenesis leads to cases where patients are born without teeth. Currently, the only available treatments for missing teeth are symptomatic replacement solutions such as dentures or dental implants. Furthermore, for alveolar bone loss associated with tooth defects, transplantation-based therapies utilizing autologous bone tissues and inorganic materials provide strong support for symptomatic replacement solutions [1,2]. Nevertheless, complete tooth regeneration, as it offers a natural and fundamental solution for the loss of teeth and their supporting tissues, remains the ultimate goal for both dentists and patients.

Regenerative medicine is an emerging field that aims to regenerate or replace lost or dysfunctional structures using cellular approaches [3]. In this paper, we define regenerative medicine as medical technologies involving the use of cells and clearly distinguish it from transplantation-based therapies, which predominantly rely on autologous grafting supported by advancements in surgical techniques. With recent technological advancements, particularly in embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), research and clinical applications in regenerative medicine have rapidly progressed. Regenerative medicine has the potential to develop new treatments for untreatable diseases and injuries, and its application in dentistry is no exception.

In this review, we focused on tooth regeneration and discussed the fundamental research on tooth development necessary for achieving regeneration, the current state of clinical research, and future perspectives for realizing the goal of complete tooth regeneration.

2. Tooth development and previous basic research

In regenerative medicine, a technology aimed at artificially generating tissues and organs by controlling cell differentiation, the knowledge of ontogeny accumulated through developmental biology research provides the strictest model for cell differentiation as guided by nature [4,5]. Therefore, to achieve tooth regeneration, an accurate understanding of tooth development is essential. Research on tooth development has primarily been conducted using rodents, such as mice and rats, because of their biological similarity to humans and the unique characteristics of incisors that continue to grow throughout life, maintaining their stemness [6]. This section presents the processes of tooth development in mice and humans, clearly distinguishing between the two, as they are often confused.

2.1. Tooth development and research advances through comprehensive gene expression analysis

Teeth develop in regions that later become the jaw through interactions and continuous signaling between the mesenchyme and the ectodermal epithelium. Teeth are formed from primordial structures, called tooth germs, and then begin maturing. This maturation requires epithelial–mesenchymal interactions between the oral epithelium and the dental mesenchyme, which are derived from the cranial neural crest. The process of tooth development closely resembles the development of hair and mammary glands and is driven by the same paracrine factors [7,8].

Similar to other ectodermal appendages, tooth development begins with the thickening of the dental lamina (dental placode). Signals such as bone morphogenic protein (BMP) and fibroblast growth factor (FGF) are transmitted from the thickened dental lamina to the mesenchyme, initiating mesenchymal condensation (bud stage). The oral epithelium gradually invaginates into the mesenchyme to form the enamel organ (cap stage). A signaling center called the enamel knot, appears in the enamel organ, and the cervical loop of the enamel organ elongates, shaping the tooth crown and root (bell stage). The enamel is eventually formed from the inner enamel epithelium of the enamel organ derived from the oral ectoderm, dentin and dental pulp from the dental papilla derived from the dental mesenchyme, and the periodontal ligament and alveolar bone from the dental follicle, which are also derived from the dental mesenchyme. The stages of tooth development in mice and humans are summarized in Fig. 1.

Before the era of comprehensive genetic analysis, painstaking experiments using methods such as in situ hybridization were conducted, particularly in mice, to analyze the gene expression specific to each developmental stage of the tooth germ. During tooth development, certain signaling proteins, such as BMP and FGF, are repeatedly expressed while changing their localization [9]. Recently, technologies such as single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics have become widely used, and these techniques have started being applied to the study of tooth germs as well [10]. Through scRNA-seq analysis, it is possible to comprehensively characterize the gene expression of individual cells that make up the tooth germ, allowing the identification of distinct cell populations. Furthermore, the development of various methods such as dimensionality reduction, clustering, trajectory analysis using pseudotime, RNA velocity, and gene interaction network prediction has significantly advanced our molecular understanding of development and epithelial–mesenchymal interactions [11,12].

Spatial transcriptomics, originating from MERFISH [13], differs from scRNA-seq in that it allows gene expression analysis while

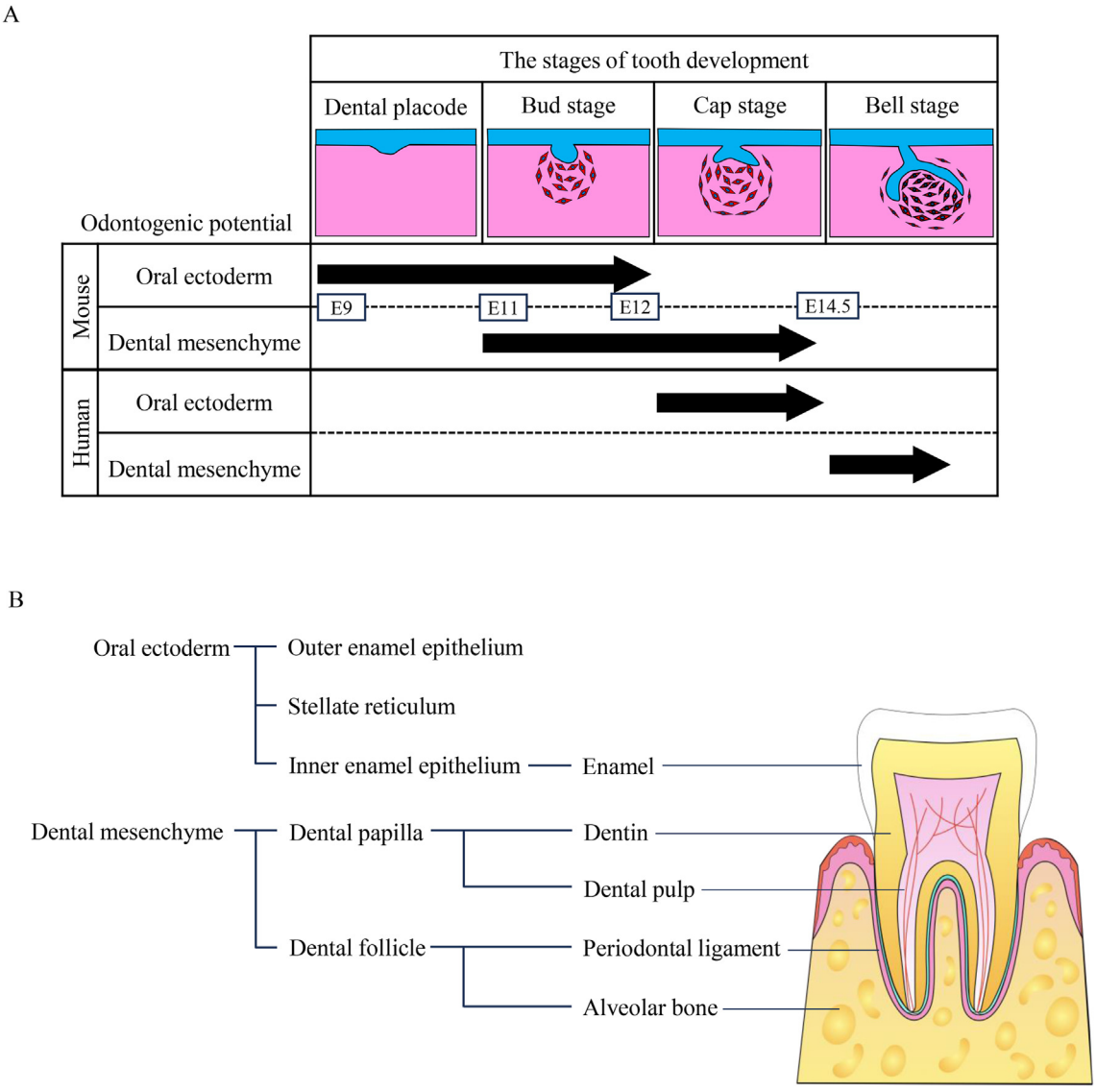

Fig. 1. Schematic diagrams of the period with odontogenic potential during the tooth development process and the tooth structure. (A) Stages of the tooth development and the period with odontogenic potential in mice and humans. (B) Simplified diagram of the tooth structure and its embryological origin.

maintaining spatial information. Although various methods of spatial transcriptomics have been developed [14], commercial products such as Visium by 10X Genomics, which positions histological sections on arrayed reverse transcription primers with unique positional barcodes [15], and GeoMx by NanoString, which uses digital spatial profiling [16], are now available. The photoisolation chemistry method developed by Honda et al. is particularly well suited for studying tooth development, as the region of interest (ROI) can be easily determined histologically, making it an appealing and cost-effective method for deep sequencing [17].

Jing et al. investigated gene expression in mouse teeth and surrounding tissues from embryonic day (E) 13.5 to postnatal day (P) 7.5 using single-cell transcriptomics [18]. They provided valuable insights into tooth development by carefully verifying gene expression at each developmental stage using in situ hybridization. In the mouse molar tooth germ at E14.5, the dental mesenchyme differentiates into the dental follicle, characterized by Epha3+/Foxf1+/Fxyd7+, and the dental papilla, characterized by Egr3+/Crym+/Fgf3+. Subsequently, at E16.5, the dental follicle further differentiates into a lateral region expressing Lepr+/Foxf1+/Bmp3+ and an apical region characterized by Aldh1a2+/Rasl11a+/Sgk1+. From the perspective of gene expression, the dental follicle can be classified into the coronal domain, characterized by Lmo1+/Smpd3+/Fgf3+, and the apical domain, characterized by Lhx6+/Fst+/Gldn+. Furthermore, using genetically modified mice capable of lineage tracing, they discovered that Lepr + lateral follicle cells contribute to the formation of the periodontal ligament and alveolar bone, demonstrating that Lepr + cells play a role in periodontal tissue formation.

Shi et al. collected human tooth germ tissues from aborted fetuses (17, 20, and 24-week embryos) and investigated gene expression using scRNA-seq and spatial transcriptomics [19]. The tooth germs investigated in this study corresponded to the late bell stage to the early crown formation stage of primary teeth. Although the study's limitations include the small sample size (five tooth germ samples in total) and incomplete histological analysis in spatial transcriptomics, the fact that both single-cell transcriptome analysis and 10X Genomics Visium were applied to human tooth germs is noteworthy. If the analysis of the earlier stages (cap and early bell stages) of human tooth germ development becomes

possible in the future, it could more effectively complement the knowledge gained from mouse tooth germ research.

By combining scRNA-seq and spatial transcriptomics to comprehensively study the spatial and temporal gene expression of tooth development, significant advances in our understanding of early tooth development, which had previously been painstakingly analyzed using techniques such as real-time PCR and in situ hybridization, have been made. Although much of the research on early tooth development has focused on rodents, improvements in analytical efficiency have made it possible to conduct basic research using human tooth germs, provided that ethical issues are addressed. This foundational information from developmental biology serves as a powerful guide for studies on cell differentiation aimed at tooth regeneration using pluripotent or somatic stem cells.

2.2. Odontogenic potential

In the early 1900s, Wilson et al. demonstrated that cells from disaggregated sponges could reassemble while maintaining a certain level of organization, proving that cells possess the ability to self-organize [20]. To achieve tooth regeneration, it is essential to harness the self-organizing ability of cells. In the context of tooth development, understanding the capability of cells involved in the developmental process to form tooth germs—referred to as odontogenesis—could enable the recreation of tooth development through the self-organization of cells.

Odontogenesis refers to the ability of cells or tissues to initiate tooth formation and has long been known through transplantation experiments in model organisms. Early-stage tooth germs when transplanted into the anterior chamber of the eye in mice, mature ectopically in a manner nearly identical to how they would mature within the jawbone. Lumsden demonstrated that tooth formation occurs by combining the mandibular epithelium with cranial neural crest cells from mice at E9, which are destined to form teeth. Furthermore, they showed that replacing the cranial neural crest with limb mesenchymal cells did not result in tooth formation. Additionally, the mandibular mesenchyme from a tooth-forming region of E11 mice was combined with the limb epithelia, resulting in tooth formation, whereas the earlier-stage mandibular mesenchyme from embryos did not exhibit odontogenic potential, as confirmed by recombination experiments [21].

Mina and Kollar conducted recombination experiments using the first and second branchial arches from mice on E9–13 and discovered that the mandibular arch epithelia prior to E12 have odontogenic potential [22]. Ohazama et al. investigated the responsiveness of non-dental cells to the odontogenic epithelium by combining the mandibular epithelium from E10 mice with feeder-independent mouse ESCs, neural stem cells isolated from E14 mouse embryos, bone marrow cells collected from the tibiae and femora of 6- to 9-week-old female mice, and C3H10T1/2 and NIH3T3 cells. After 1–2 days of organ culture, the cells were transplanted into the renal capsules of the mice. The experiment demonstrated that mouse ESCs, neural stem cells, and bone marrow cells, believed to have stem cell properties, expressed molecular markers related to tooth development, such as Msx1, Lhx7, and Pax9. Moreover, tooth formation was observed when the mandibular epithelium was combined with bone marrow cells [23]. These experimental studies show that the cells constituting the tooth germ exhibit odontogenic potential in a stage- and region-specific manner. Additionally, when either epithelial or mesenchymal cells possess odontogenic potential, the cells with which they combine must also have certain stem cell characteristics and the ability to respond to odontogenic factors.

Recombination experiments have been conducted in both mice and humans. Hu et al. experimentally investigated the odontogenic potential of human tooth germs by reconstructing them with various tissues derived from humans or mice, using human fetal tooth germs from 10 to 16 weeks of gestation [24]. A key finding was that, unlike mouse tooth germs, human tooth germs showed that the human dental mesenchyme at the bell stage and human dental epithelium at the cap stage possess odontogenic potential, whereas the human dental mesenchyme at the cap stage and human dental epithelium at the bell stage do not. In other words, compared with mouse tooth germs, human tooth germs appear to acquire odontogenic potential at slightly later stages, with the epithelium and mesenchyme exhibiting this potential at different times. The timing of odontogenic potential acquisition in both mouse and human tooth germs is summarized in Fig. 1.

2.3. Tooth regeneration in humans and fundamental technologies

Humans exhibit diphyodonty, which means that they replace their teeth only once in their lifetime, and heterodonty, which means that the shapes of their teeth vary. In one quadrant of the jaw, humans have 20 primary teeth, with five types, and 32 permanent teeth, with eight types. The timeline for tooth development and eruption in humans has been well studied, and the tooth germ formation, calcification initiation, crown completion, eruption, and root completion for each tooth type are summarized in Table 1.

Tooth development can span several years. Therefore, a significant challenge in the implementation of regenerated teeth in clinical practice is determining how to mature artificially created tooth germs. Although methods for maturing teeth using the anterior chamber of the eye or under the renal capsule in mice have been established, as demonstrated by experimental tooth germ formation in studies by Lumsden [21] and Mina and Kollar [22], these *in vivo* maturation methods have the disadvantage of relying on the vascular systems of different species, and experimental intervention with the tooth germ is difficult. Ideally, the long-term culture of tooth germs using chemically defined artificial media and the development of methods to mature teeth *in vitro* is necessary. Organ culture of tooth germs *in vitro* is already a widely used experimental technique, and it has been demonstrated that early tooth germs mature outside the body [25]. For example, Tabata et al. cultured E14 mouse molar germs using BGJb medium (Fitton-Jackson's modified BGJ) supplemented with 100 μg/mL ascorbic acid and 100 U/mL penicillin-streptomycin for up to 14 days. They experimentally demonstrated that inhibiting the expression of hepatocyte growth factor mRNA using antisense phosphorothioate oligodeoxynucleotides hindered the development of the dental mesenchyme [26]. Similarly, Baba et al. separated and reconstituted the epithelium and mesenchyme of E16.5 mouse molars and cultured them for at least 18 days. By measuring the enamel proteins released into the culture medium, the authors confirmed that reconstituted tooth germs maintained their biological properties [27].

From a dental perspective, controlling tooth shape and number and, exploring the mechanisms of tooth eruption, are highly intriguing areas of research. Studies have used mathematical models to predict the factors determining tooth shape [28,29] and scRNA-seq to identify factors that inhibit tooth eruption [30]. Insights gained from elucidating these mechanisms are expected to be important for controlling tooth regeneration and advancing future research in this field.

Finally, we introduce a study that demonstrated the potential for tooth regeneration by utilizing the self-organization ability of somatic stem cells and odontogenesis to reconstitute tooth germs. Nakao et al. successfully induced tooth germ formation both *in vitro* and *in vivo* by enzymatically dissociating the incisor tooth germs of

Table 1
Summary of the tooth development process categorized by tooth type.

		Tooth germ formation initiates at	Calcification begins at	Crown completes at	Eruption		Root completes at
					Maxillary	Mandibular	
Primary teeth	Central incisors	6-8th fetal we	4th fetal mo	1.5–2.5 mo	6–10 mo	5–8 mo	18–24 mo
	Lateral incisors		4th fetal mo	2.5–3 mo	8–12 mo	7–10 mo	18–24 mo
	Canines		4th fetal mo	9 mo	16–20 mo	16–20 mo	30–39 mo
	First molars		4th fetal mo	5.5–6 mo	11–18 mo	11–18 mo	24–30 mo
	Second molars		4th fetal mo	10–11 mo	20–30 mo	20–30 mo	36 mo
Permanent teeth	Central incisors	20th fetal we	3–4 mo	4-5 y	7-8 y	6-7 y	9-10 y
	Lateral incisors	–10 mo	Maxilla: 10–12 mo Mandible: 3–4 mo	4-5 y	8-9 y	7-8 y	Maxilla: 11y Mandible: 10y
	Canines		4–5 mo	6-7 y	11-12 y	9-11 y	12-15 y
	First premolars		18–24 mo	5-6 y	10-11 y	10-12 y	12-13 y
	Second premolars		24–30 mo	6-7 y	10-12 y	11- 13 y	12-14 y
	First molars	20th fetal we	Birth	30–36 mo	5.5–7 y	5.5–7 y	9-10 y
	Second molars	–5 y	30–36 mo	7-8 y	12-14 y	12-14 y	14-16 y
	Third molars		Maxilla: 7–9 y Mandible: 8–10 y		17-30 y	17-30 y	

we, weeks; mo, months; ye, years.
This table is based on Nanci (2024) and Schour et al. (1940) [73,74].

E14.5 mice into epithelial and mesenchymal cells, followed by reconstitution. Unlike previous studies that reconstituted tooth germs with epithelial and mesenchymal tissues while preserving their original structures, Nakao et al. dispersed cells into single cells before reconstituting them. Tooth germs were not formed from tissue fragments reconstituted from epithelial or mesenchymal cells alone or from tissue fragments in which epithelial and mesenchymal cells were evenly mixed. However, when high-density epithelial and mesenchymal cells (5×10^{8} cells/mL) were compartmentalized in the collagen gel, 100 % of the reconstituted tissue fragments developed into tooth germs. Furthermore, tissue fragments reconstituted from E16.5 mouse incisor tooth germs did not form tooth germs. These results were groundbreaking, because they proved that tooth germs could be artificially induced if odontogenic cells were present [31]. Tsuji and colleagues later developed this method, which they named the organ germ method, and succeeded in functionally occluding teeth produced from reconstituted tooth germs in vivo, as well as inducing the formation of teeth, including the surrounding alveolar bone, from reconstituted tooth germs [32,33]. However, because this method uses tooth germ as the raw material, there are limitations to the source of cells, and human tooth regeneration is yet to be realized. If cells with odontogenic potential can be induced to differentiate from pluripotent stem cells, it may be possible to apply the organ germ method to achieve tooth regeneration in humans.

Thus, we believe that the foundational technology necessary to achieve tooth regeneration is already in place based on past research on teeth. By combining accumulated knowledge with new scientific technologies such as bioinformatics, we expect that tooth regeneration, a long-held dream of humanity, will be realized in the near future.

2.4. Current state: clinical research of regenerative dentistry

Historically, dental medicine has progressed along with advancements in materials science. Revolutionary advancements in adhesive materials have profoundly transformed prosthetic dentistry [34,35]. Adhesive materials and composite resin for dental restorations have enabled treatments that closely mimic the regeneration of enamel and dentin, which are predominantly inorganic materials [36]. Furthermore, the explosive growth of dental implants following the discovery of titanium osseointegration [37] has marked significant turning points in dentistry. To ensure the reliable placement of dental implants, various inorganic materials, including artificial bone substitutes and membranes, have been developed for use in guided bone regeneration (GBR) and ridge preservation techniques [38,39]. One reason why dentistry has advanced in parallel with materials science is likely because many treatment needs for oral diseases involve the restoration of hard tissues, which can be replaced with materials. However, conditions that are difficult to treat with conventional prosthetic treatments and reconstructive surgeries, such as severe periodontitis and congenital tooth agenesis, have unmet medical needs.

As illustrated in Fig. 1, teeth consist of five main elements: enamel, dentin, dental pulp, periodontal ligament, and alveolar bone. The periodontal ligament, alveolar bone, and, dental pulp are key targets in dental regenerative medicine, as they benefit more from cellular replacement therapies than from inorganic materials. Therefore, this section examines the current state of clinical research in regenerative medicine focusing on periodontal tissues, including the periodontal ligament, and alveolar bone, as well as dental pulp.

2.5. Regenerative medicine for periodontal diseases

Periodontal disease is a chronic inflammation caused by bacteria in the oral cavity and is one of the most common dental diseases. When a person is affected by periodontal disease, the alveolar bone that supports the teeth is irreversibly destroyed, eventually leading to tooth loss. The incidence of periodontal disease increases sharply in middle aged individuals and continues to increase with age. According to the World Health Organization (WHO), severe periodontal diseases are estimated to affect approximately 19 % of the global adult population, accounting for more than 1 billion cases worldwide (https://www.who.int/news-room/fact-sheets/detail/oral-health). Moreover, periodontal disease is linked to systemic diseases, including diabetes, cardiovascular diseases, nonalcoholic fatty liver disease (NAFLD), pregnancy complications, and gut microbiota dysbiosis, affecting areas far beyond the oral cavity [40].

Since the 1960s, when it was experimentally demonstrated that gingival inflammation was caused by bacteria in the oral cavity [41], periodontal treatment has primarily focused on controlling oral bacteria through thorough tooth brushing and physical removal of risk factors like dental calculus. On the other hand, lost periodontal tissue does not regenerate naturally even with optimal oral hygiene, although advancements in surgical techniques have enabled the regeneration of limited areas of periodontal tissue. In recent

years, periodontal tissue regeneration therapy, which combines periodontal surgical techniques with biologics such as enamel matrix derivative (EMD) and basic fibroblast growth factor (bFGF), has become common practice. These therapies hold the potential to regenerate periodontal tissues; however, their effectiveness remains limited, yielding a clinical attachment level (CAL) gain of only 1–2 mm compared to conventional flap operations [42,43]. Moreover, periodontal regeneration strategies that combine EMD with bone substitutes, such as autogenous bone, bovine-derived natural bone mineral, or biphasic calcium phosphate, provide few additional benefits [42]. Therefore, treatments for extensive periodontal tissue defects necessary to prevent tooth loss remain unmet [44]. Cell-based regenerative medicine has significant potential for addressing such unmet needs in periodontal treatment and is expected to become practical.

Iwata et al. conducted a clinical trial aimed at regenerating periodontal tissues in patients with severe defects, including one-wall intrabony and horizontal defects, by transplanting periodontal ligament-derived cells (PDL cells) [45]. They harvested PDL cells from non-functional teeth, such as wisdom teeth that do not participate in occlusion, and processed them into cell sheets using thermo-reactive culture dishes before transplanting them into bone defect areas. Although this study did not compare the effectiveness of PDL cells against traditional periodontal surgery, the results showed reductions in mean periodontal probing depth (mean ± SD, 3.2 ± 1.9 mm) and, mean clinical attachment gain (2.5 ± 2.6 mm) and an increase in mean radiographic bone height (2.3 ± 1.8 mm), even in patients with severe defects not eligible for conventional periodontal surgery. This study demonstrates the safety and efficacy of autologous PDL cells in regenerating severe periodontal defects.

Ferrarotti et al. focused on a single deep intrabony defect confined to the approximal surface and evaluated the effects of dental pulp stem cells (DPSCs) on periodontal tissue regeneration. Periodontal surgery was performed using a minimally invasive surgical technique, and the intrabony defect was simultaneously filled t with a collagen sponge containing crushed dental pulp from another tooth of the same patient. The control group was administered a collagen sponge without DPSCs. In the experimental group, the results showed greater probing depth reduction (4.9 mm versus 3.4 mm), clinical attachment level gain (4.5 mm versus 2.9 mm), and bone defect fill (3.9 mm versus 1.6 mm) than in the control group. This study demonstrates the potential of DPSCs, in addition to PDL cells, to regenerate periodontal tissues [46].

2.6. Alveolar bone regeneration

Block bone grafts and particulate cancellous bone and marrow (PCBM) grafts are highly effective methods for reconstructing lost alveolar bone in edentulous areas. However, the technical complexity of these procedures and the significant surgical invasiveness remain major challenges [47,48]. In this paper, we clearly distinguish between transplantation-based therapies aimed at tissue reconstruction and regenerative medicine focused on promoting tissue regeneration. Platelet-rich plasma (PRP) facilitates alveolar bone regeneration and represents one of the most common, minimally invasive, and user-friendly approaches in regenerative medicine. Several studies have investigated the use of PRP to promote the regeneration of alveolar bone and periodontal tissue. PRP is a substance rich in platelets and various cytokines, obtained through centrifugation of blood, and it is widely used in various medical fields due to its ease of preparation [49,50]. According to systematic reviews, combining PRP with open flap debridement significantly improves clinical attachment levels and radiographic bone fill compared with open flap debridement alone [51]. PRP alone is not sufficient for extensive alveolar bone regeneration. However, combining PRP with other cells shows significant potential for addressing large-scale alveolar bone defects. To regenerate alveolar bone, clinical studies have explored the combination of PRP with cultured cells, such as periosteal cells and adipose-derived mesenchymal stem cells, to further enhance its therapeutic effects [52,53].

2.7. Dental pulp regeneration

Dental caries is a disease caused by acids produced by oral bacteria, leading to the destruction of hard tissues, such as enamel and dentin. As caries progresses, the nerve located in the root canal of the tooth is stimulated, causing intense pain. According to the WHO, an estimated 2 billion people suffer from caries of permanent teeth, and 514 million children suffer from caries of the primary teeth, making dental caries the most common dental disease worldwide (https://www.who.int/news-room/fact-sheets/detail/oral-health). In the treatment of caries, the part of decayed tooth is physically removed and replaced with dental materials. However, in cases of advanced caries, preserving healthy dental pulp becomes difficult, necessitating the removal of the dental pulp, followed by mechanical and chemical cleaning of the root canal. The root canal is then sealed using a filling material. However, when the dental pulp is removed, the tooth loses its biological and defensive mechanisms, including immune responses, the formation of reparative dentin as a physical defense against external stimuli, and the ability to feel pain. To address this issue, research has focused on regenerating dental pulp to preserve these functions and contribute to tooth preservation.

Nakashima et al. conducted a study in which they attempted to regenerate dental pulp from five single-root teeth of five patients diagnosed with irreversible pulpitis [54]. The procedure involved transplanting mobilized dental pulp stem cells (MDPSCs) obtained by culturing the patient's autologous dental pulp under granulocyte-colony stimulating factor (G-CSF) stimulation, along with G-CSF and an atelocollagen scaffold, into the root canal. The affected teeth developed pulpitis because of caries or crown fractures, and conventional root canal treatment was performed before autologous MDPSCs were transplanted into the root canal instead of using traditional filling material. Although the pulp sensitivity test using an electric pulp tester was negative prior to stem cell transplantation, four of the five patients' teeth showed positive responses after four weeks. Additionally, 24 weeks after transplantation, the relative signal intensity of the root canal, as measured by magnetic resonance imaging, was comparable to that of normal pulp in the same oral cavity. Moreover, cone beam computed tomography scans taken 28 weeks post-transplantation showed a reduction in pulp cavity volume compared with scans taken 16 weeks post-transplantation. These results suggest that soft tissue capable of sensing pain filled the pulp cavity after MDPSC transplantation, and the reduction in pulp cavity volume indirectly indicates the formation of new hard tissue. This study is a significant step forward in dental pulp regeneration, as it provides evidence supporting the regeneration of dental pulp.

Xuan et al. attempted to regenerate the dental pulp in teeth with traumatic pulp necrosis of the incisors by culturing and transplanting autologous DPSCs derived from the patient's primary canines [55]. Autologous DPSCs were transplanted into 26 patients aged 7–12 years. In the group in which DPSCs were transplanted, laser Doppler flowmetry and electrical pulp testing demonstrated blood flow and pain sensitivity in the pulp cavity. One limitation of this study is that the affected teeth were immature permanent teeth; therefore, it is not possible to completely rule out the possibility of natural dental pulp revascularization. However, the use of

primary tooth pulp for human dental pulp regeneration is essential for scaling up future dental pulp regeneration therapies and is a highly important concept.

As these studies suggest, the concept of dental pulp regeneration has been demonstrated, and it is now in a state where it can be applied in clinical practice. Moving forward, to scale up these therapies to industrial levels and make them more widely available as general treatments, it will be crucial to utilize naturally shed primary tooth pulp or allogeneic cells; further research in this area is anticipated.

2.8. Development status of regenerative medical products in the dental field

We reviewed the approval status of pharmaceuticals for cell and gene therapy in the field of dentistry by regulatory authorities including the Pharmaceuticals and Medical Devices Agency (PMDA) in Japan, the Food and Drug Administration (FDA) in the USA, and the European Medicines Agency (EMA) in the European Union. The results are summarized in Table 2. Currently, there is only one regenerative medical product available in the dental field. The FDA has approved GINTUIT, a product consisting of allogeneic cultured keratinocytes and fibroblasts in bovine collagen, for the treatment of mucogingival conditions in adults by application to surgically created vascular wound beds. However, no products are available for the treatment of common dental diseases, such as periodontitis and dental caries. Regenerative medical products tend to have high manufacturing costs. In the field of dental diseases, it is difficult to raise drug prices compared to treatments for life-threatening conditions, which is considered one of the reasons for the slow progress in developing dental regenerative medical products. Consequently, in dental applications, the development of products with dramatic therapeutic effects, such as complete tooth regeneration, or those targeting aesthetic areas, such as gingival regeneration, is likely to be a more practical and viable option.

Clinical research on regenerative medicine in the field of dentistry is progressing gradually [56]. Particularly in Japan, clinical research on regenerative medicine can be conducted under procedures different from those in the USA and European countries, as permitted by the Act on the Safety of Regenerative Medicine [57]. By effectively leveraging such regulatory frameworks, it is hoped that the number of regenerative medical products in the dental field will continue to grow.

3. Future perspectives of tooth regeneration

The development of dental implants is arguably the greatest advancement of the 20th century. Implant treatments strike a good balance between medical and industrial applications because of their standardized nature as medical devices and systematic surgical procedures that can be performed by many general practitioners. However, compared to natural teeth, dental implants still have limitations such as the loss of sensory feedback due to the absence of the periodontal ligament, widespread occurrence of peri-implantitis, and inability to maintain occlusal stability through periodontal tissue remodeling. In terms of long-term occlusal stability, nothing surpasses that of natural teeth; thus complete tooth regeneration remains one of the ultimate goals in the field of dentistry. As discussed in the previous sections, current clinical research has begun to demonstrate the feasibility of regenerating certain tooth structures, such as periodontal tissue and dental pulp.

Fundamental techniques required to achieve complete tooth regeneration already exist; however, based on past research, the limited availability of cell sources remains a bottleneck. Therefore, the use of ESCs and iPSCs, which possess unlimited proliferative capacity, is crucial. Two feasible approaches to achieve complete tooth regeneration are creating artificial tooth germs directly from pluripotent stem cells (tooth organoids) and inducing the differentiation of stem cells with odontogenic potential from pluripotent stem cells and combining them to reconstitute tooth germs.

Organoids, a term combining “organ” and the suffix “-oid,” are three-dimensional structures composed of multiple types of cells arranged in an organized manner [58]. Organoids can be divided into two types: those derived from pluripotent stem cells, which are created by utilizing the stemness of pluripotent stem cells and continuously stimulating them with growth factors, and those derived from somatic stem cells, which are created by combining somatic stem cells harvested from individuals using tissue engineering techniques [59]. There are numerous reports of somatic stem cell-derived organoids used to create tooth germs, including those developed using the organ germ method by Nakao et al., which are considered somatic stem cell-derived organoids [31]. Additionally, Hemeryck et al. reported the successful creation of epithelial tissue organoids capable of long-term culture and passage, derived from stem cells found in the human third molar (wisdom teeth) [60]. Prior to eruption, teeth are surrounded by dental follicle, which contains remnants of epithelial cells from the enamel organ (epithelial cell rests of Malassez, [ERM]). They used the epithelial stem cells remaining in the ERM to create somatic stem cell-derived organoids. This report is significant in that it demonstrates the concept of organoids using human-derived cells in the field of dentistry. However, common bottlenecks in creating somatic stem cell-derived organoids from human tooth germs include limited availability at particular developmental stages of human tooth germs.

ESCs and iPSCs are pluripotent stem cells with unlimited proliferative capacity [61–63]. Therefore, they have the potential to overcome the bottleneck of material limitations in human tooth germs. Research is already being conducted to induce the differentiation of epithelial and mesenchymal cells that make up teeth from pluripotent stem cells. Arakaki et al. demonstrated that iPSCs could differentiate into ameloblast-like cells expressing ameloblastin, a marker gene for enamel-forming cells, by co-culturing rat dental epithelial cells with mouse iPSCs [64]. Hamano et al. reported that human iPSCs differentiated into dental mesenchyme-like periodontal ligament cells when cultured on an extracellular matrix derived from human primary periodontal ligament cells [65]. Wang et al. showed that stimulating iPSC-derived neural crest cells with FGF2, FGF8b, and BMP4 induces differentiation into periodontal ligament-like cells [66]. These studies demonstrated progress in the differentiation of cells that make up the tooth germ from pluripotent stem cells, which holds promise for addressing the cell source problem in somatic stem cell-derived organoids.

However, no reports have been published regarding the direct production of tooth organoids from pluripotent stem cells. Nevertheless, research on hair follicles, which also begin with the invagination of epithelium and condensation of mesenchymal cells, has shown that human iPSCs can be induced to form epithelium by sequentially regulating transforming growth factor β, BMP, and FGF signaling, followed by the induction of cranial neural crest cells [67]. Additionally, pituitary organoids have been created from human iPSCs by inducing the oral ectoderm through the activation of the BMP4 and Sonic hedgehog pathways [68,69]. These findings suggest that if pluripotent stem cells are given the correct developmental cues to mimic the process of natural development, the creation of tooth germ organoids from pluripotent stem cells is highly feasible.

Finally, if the outcomes of regenerative medicine are viewed as organ replacement, xenotransplantation of teeth from other species could contribute to human tooth regeneration.

Table 2
Approval status of pharmaceuticals for cell therapy and gene therapy by PMDA, FDA, and EMA.

	Brand Name	Non-proprietary Name	Modality	Indications	Approval date			Notes
					PMDA	FDA	EMA	
1	Abecma	Idecabtagene vicleucel	ex vivo gene therapy	Multiple myeloma	06/12/2021	03/26/2021	08/18/2021	
2	Adstiladrin	Nadofaragene firadenovec-vncg	in vivo gene therapy	High-risk bacillus Calmette-Guérin–unresponsive non-muscle invasive bladder cancer		12/16/2022		
3	Akuugo	Vandefitemcel	Cell Therapy	Chronic motor paralysis associated with traumatic brain injury	07/31/2024[a]			
4	Allocord	HPC, cord blood	Cell therapy	Hematopoietic progenitor cell transplantation		05/30/2013		
5	Alofisel	Darvadstrocel	Cell therapy	Complex anal fistula associated with Crohn disease	09/27/2021		03/23/2018	
6	Amtagvi	Lifileucel	Cell therapy	Melanoma		02/16/2024[b]		
7	Beqvez	Fidanacogene elaparvovec-dzkt	in vivo gene therapy	Hemophilia B (congenital factor IX deficiency)		04/25/2024		
8	Breyanzi	Lisocabtagene maraleucel	ex vivo gene therapy	Large B-cell lymphoma, chronic lymphocytic leukemia	03/22/2021	02/05/2021	04/04/2022	
9	Carvykti	Ciltacabtagene autoleucel	ex vivo gene therapy	Multiple myeloma	09/26/2022	02/28/2022	05/25/2022[c]	
10	Casgevy	Exagamglogene autotemcel (exa-cel)	ex vivo gene therapy	β Thalassemia, sickle cell disease		12/08/2023	02/09/2024[c]	
11	Chondrocelect	Characterized viable autologous cartilage cells expanded ex vivo expressing specific marker proteins	Cell therapy	Cartilage defects of the femoral condyle of the knee			10/05/2009	MA withdrawn July 2016
12	Clevecord	HPC, cord blood	Cell therapy	Hematopoietic progenitor cell transplantation		09/01/2016		
13	Collategene	Beperminogene perplasmid	in vivo gene therapy	Chronic arterial occlusive disease	03/26/2019[a]			Withdraral of application (06/27/2024)
14	Delytact	Teserpaturev	in vivo gene therapy	Malignant glioma	06/11/2021[a]			
15	Ducord	HPC, cord blood	Cell therapy	Hematopoietic progenitor cell transplantation		10/04/2012		
16	Ebvallo	Tabelecleucel	Cell therapy	Epstein-Barr virus–positive post-transplant lymphoproliferative disease			12/16/2022[d]	
17	Elevidys	Delandistrogene moxeparvovec-rokl	in vivo gene therapy	Duchenne muscular dystrophy		01/10/2023[b]		
18	Gintuit	Allogeneic cultured keratinocytes and fibroblasts in bovine collagen	Cell therapy	A surgically created vascular wound bed in the treatment of mucogingival conditions		03/09/2012		
19	Glybera	Alipogene tiparvovec	in vivo gene therapy	Familial lipoprotein lipase deficiency			10/25/2012[d]	MA not renewed (MA ended in October 2017)
20	Heartsheet	Human skeletal myoblast cell sheet	Cell therapy	Severe heart failure due to chronic ischemic heart disease	09/18/2015[a]			The government expressed the opinion that it was not appropriate to approve.(06/19/2024)
21	Hemacord	HPC, cord blood	Cell therapy	Hematopoietic progenitor cell transplantation		04/03/2013		
22	Hemgenix	Etranacogene dezaparvovec-drlb	in vivo gene therapy	Hemophilia B (congenital factor IX deficiency)		11/22/2022	02/20/2023[c]	
23	Holoclar	ex vivo expanded autologous human corneal epithelial cells containing stem cells	Cell therapy	Limbal stem cell deficiency			02/17/2015[c]	
24	Imlygic	Talimogene laherparepvec	in vivo gene therapy	Melanoma		12/08/2021	12/16/2015	
25	Jacc	Autologous cultured cartilage	Cell therapy	Traumatic cartilage damage or osteochondritis dissecans in the knee joint	07/27/2012			
26	Jace	Human epidermal cell sheet	Cell therapy	Severe burns, giant congenital melanocytic nevus, epidermolysis bullosa	10/29/2007			
27	Jacemin	Melanocyte-containing human (autologous)	Cell therapy	Vitiligo	03/17/2023			

Table 2 (*continued*)

	Brand Name	Non-proprietary Name	Modality	Indications	Approval date			Notes
					PMDA	FDA	EMA	
		epidermis-derived cell sheet						
28	Kymriah	Tisagenlecleucel	ex vivo gene therapy	Lymphoma	03/26/2019	08/20/2017	08/23/2018	
29	Lantidra	Donislecel-jujn	Cell therapy	Type 1 diabetes		06/28/2023		
30	Laviv	Aazficel-T	Cell therapy	Nasolabial fold wrinkles		06/20/2011		
31	Lenmeldy/Libmeldy	Atidarsagene autotemcel	ex vivo gene therapy	Metachromatic leukodystrophy		03/18/2024	11/17/2020	
32	Luxturna	Voretigene neparvovec	in vivo gene therapy	Biallelic RPE65 mutation −associated inherited retinal dystrophy	06/26/2023	12/19/2017	11/22/2022	
33	Lyfgenia	Lovotibeglogene autotemcel	ex vivo gene therapy	Sickle cell disease		12/08/2023		
34	Maci	Autologous cultured chondrocytes on porcine collagen membrane	Cell therapy	Cartilage defects of the knee		12/13/2016	06/27/2013	MA not renewed (MA ended June 2018)
35	Nepic	Human (autologous) corneal limbus-derived corneal epithelial cell sheet	Cell therapy	Limbal stem cell deficiency	03/19/2020			
36	None	HPC, cord blood	Cell therapy	Hematopoietic progenitor cell transplantation		05/04/2012		
37	None	HPC, cord blood - MD Anderson Cord Blood Bank	Cell therapy	Hematopoietic progenitor cell transplantation		06/21/2018		
38	None	HPC, cord blood - LifeSouth	Cell therapy	Hematopoietic progenitor cell transplantation		06/13/2013		
39	None	HPC, cord blood - Blldworks	Cell therapy	Hematopoietic progenitor cell transplantation		01/29/2016		
40	Ocural	Cultivated autologous oral mucosal epithelial cell sheet	Cell therapy	Limbal stem cell deficiency	06/11/2021			
41	Omisirge	Omidubicel-onlv	Cell therapy	To reduce the time to neutrophil recovery and the incidence of infection after umbilical cord blood transplantation		04/17/2023		
42	Provenge	Sipuleucel-T	Cell therapy	Castrate-resistant prostate cancer		04/29/2010	09/06/2013	MA withdrawn May 2015
43	Rethymic	Allogeneic processed thymus tissue−agdc	Cell therapy	Immune reconstitution in pediatric patients with congenital athymia		10/08/2121		
44	Roctavian	Valoctocogene roxaparvovec-rvox	in vivo gene therapy	Hemophilia A (congenital factor VIII deficiency)		06/29/2023	08/08/2022[c]	
45	Sakracy	Human (autologous) oral mucosa-derived epithelial cell sheet using human amniotic membrane substrate	Cell therapy	Reduction of ocular surface adhesion in limbal stem cell deficiency	01/20/2022			
46	Skysona	Elivaldogene autotemcel	ex vivo gene therapy	Cerebral adrenoleukodystrophy		09/16/2022[b]	07/16/2021	MA withdrawn November 2021
47	Spherox	Spheroids of human autologous matrix-associated chondrocytes	Cell therapy	Articular cartilage defects of the femoral condyle and the patella of the knee			07/10/2017	
48	Stemirac	Human bone marrow −derived mesenchymal stem cell	Cell therapy	Traumatic spinal cord injury	12/28/2018[a]			
49	Stratagraft	Allogeneic cultured keratinocytes and dermal fibroblasts in murine collagen-dsat	Cell therapy	To promote durable wound closure and regenerative healing in the treatment for debrided thermal burns		06/15/2021		
50	Strimvelis	Autologous CD34+-enriched cell fraction that contains $CD34^+$ cells transduced with retroviral vector that encodes for the human ADA cDNA sequence	ex vivo gene therapy	Severe combined immunodeficiency due to adenosine deaminase deficiency			05/26/2015	
51	Tecartus	Brexucabtagene autoleucel	ex vivo gene therapy	Mantle cell lymphoma, acute lymphoblastic leukemia		07/24/2020	11/14/2020[c]	
52	Tecelra	Afamitresgene autoleucel	ex vivo gene therapy	Synovial sarcoma		08/01/2024		
53	Temcell	Human bone marrow mesenchymal stem cells	Cell therapy	Acute graft-versus-host disease	09/18/2015			

(*continued on next page*)

Table 2 (*continued*)

	Brand Name	Non-proprietary Name	Modality	Indications	Approval date			Notes
					PMDA	FDA	EMA	
54	Upstaza	Eladocagene exuparvovec	in vivo gene therapy	Aromatic L-amino acid decarboxylase deficiency			07/18/2022[d]	
55	Vyjuvek	Beremagene geperpavec	in vivo gene therapy	Dystrophic epidermolysis bullosa		05/19/2024		
56	Vyznova	Neltependocel	Cell therapy	Bullous keratopathy	03/17/2023			
57	Yescarta	Axicabtagene cioleuce	ex vivo gene therapy	Large B-cell lymphoma	01/22/2021	10/18/2017	08/23/2018	
58	Zalmoxis	Allogeneic T cells genetically modified with a retroviral vector encoding for a truncated form of the human low-affinity nerve growth factor receptor and the herpes simplex I virus thymidine kinase	ex vivo gene therapy	Adjunctive treatment in haploidentical hematopoietic stem cell transplantation			08/18/2016[c]	MA withdrawn October 2019
59	Zolgensma	Onasemnogene abeparvovec	in vivo gene therapy	Spinal muscular atrophy	03/19/2020	05/24/2019	05/29/2019[c]	
60	Zynteglo	Betibeglogene autotemcel	ex vivo gene therapy	β Thalassemia		08/17/2022	05/29/2019[c]	MA withdrawn March 2022
Total products number					21	38	26	

PMDA, Pharmaceuticals and Medical Devices Agency; FDA, Food and Drug Administration; EMA; European Medicines Agency; MA, marketing authorization of the EMA. All data were reviewed on September 21, 2024.

[a] Conditional and limited approval by the PMDA.
[b] Accelerated approval by the FDA.
[c] Conditional marketing authorization by the EMA.
[d] Authorized under exceptional circumstances by the EMA.

Autotransplantation of human teeth is widely accepted as a standard dental treatment worldwide, including Japan, where it is covered by national insurance. However, indications for autotransplantation are limited. Tooth allotransplantation has a long history, with records showing that it was a common practice in late 18th-century London. However, this practice is rarely performed because of the risk of infectious disease transmission and the long-term need for immunosuppressants to ensure the survival of transplanted teeth [70]. However, recent advancements in genetic engineering, such as the discovery of the CRISPR-Cas9 system, have led to the transplantation of genetically modified pig organs, such as heart and kidney, into humans [71,72]. These genetically modified pigs have been engineered to knock out genes like glycoprotein α-galactosyltransferase 1, which triggers hyperacute rejection in xenotransplantation, and to introduce human complement regulatory proteins such as CD46, significantly reducing immune responses during transplantation. As cases of organ transplantation using genetically modified animals accumulate and protocols for addressing infectious disease transmission risks and immunosuppressant use are established, it may become possible to transplant pig teeth or tooth germs into humans, offering a future option for tooth regeneration through xenotransplantation.

4. Conclusion

Regenerative dentistry aims to overcome the limitations of prosthetic and implant treatments by restoring the function and structure of the natural teeth. In particular, advancements in scRNA-seq and spatial transcriptomics and their widespread use provide the foundational developmental biology knowledge necessary for tooth regeneration. Clinical studies and trials on human periodontal ligament and dental pulp regeneration have already been conducted, and regenerative medicine targeting various cell types that make up teeth has become a reality. Moving forward, the development of tooth organoids and the accumulation of knowledge regarding stem cell properties related to odontogenesis will likely make complete tooth regeneration technically feasible. In the future, technologies that utilize ESCs and iPSCs, organoid technologies, and xenotransplantation using genetically modified animals will play critical roles. Once these technologies are established, it will be possible to regenerate teeth with functional periodontal ligaments and the ability to remodel periodontal tissue to maintain occlusal stability similar to that of natural teeth. This will pave the way for further innovations in regenerative dentistry.

Author contributions

Conceptualization: KM; Project administration: KM; Supervision: TI; Writing—original draft: KM; Writing—review and editing: KO, JW, and TI. All authors approved the final version of the manuscript.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT 4o in order to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

None.

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