Special Issue: Mesenchymal Stem Cells

Mini Review

Tooth tissue and organ regeneration using stem cells

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Tooth loss or damage, such as that caused by dental caries and periodontal disease, can cause fundamental problems with oral functions. The development of regenerative therapy for tooth tissue repair and whole-tooth replacement is currently considered a novel treatment with the potential to fully recover tooth function. Several mesenchymal stem cell-like cell types have been identified in oral tissues. These cells are thought to be good candidate cell sources for tooth tissue regeneration therapies because they exhibit the ability to differentiate into tooth tissues *in vitro* and *in vivo*. Whole-tooth replacement therapy is regarded as an important model system for the development of the concept of organ regeneration. A novel three-dimensional *in vitro* cell manipulation method, designated as an organ germ method, has been developed to recapitulate organogenesis. This method involves cell compartmentalization between epithelial and mesenchymal cells at a high cell density to mimic the multicellular assembly and epithelial-mesenchymal interactions. The bioengineered tooth germ generates a structurally correct tooth *in vitro*, and erupted successfully with correct tooth structure when transplanted into a tooth socket in the oral cavity. We could also generate a size-controlled bioengineered mature tooth unit composed of periodontal ligament and alveolar bone. The bioengineered tooth unit was successfully engrafted into an adult jaw through bone integration. These bioengineered teeth were able to perform physiological tooth functions such as mastication, periodontal ligament function and response to noxious stimuli. Here, we review recent studies of tooth tissue-derived mesenchymal stem cells and the technologies underpinning tooth regenerative therapy.

Rec.11/14/2012, Acc.12/10/2012, pp29-37

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Key words organ germ method, bioengineered tooth germ, epithelial-mesenchymal interaction, tooth regeneration, organ replacement regenerative therapy

Introduction to tooth regeneration

Organs are maintained by homeostatic mechanisms that regulate the supply and differentiation of distinct tissue stem/progenitor cells. Recent advances in the development of regenerative therapies have been influenced by a large body of previous research in embryonic development, stem cell biology, and tissue engineering[1, 2]. One attractive concept in regenerative therapy is stem cell transplantation into various tissues and organs to restore the partial loss of organ function and to repair damaged tissues: for example, replacing hematopoietic stem cells in cases of hematopoietic malignancy, neural stem cells in cases of Parkinson's disease, mesenchymal stem cells in cases of myocardial infarction, and hepatic stem cells in cases of hepatic insufficiency[3]. Cytokine therapy is considered to have the potential to induce the activation and differentiation of stem/progenitor cells in various tissues[4]. The ultimate goal of regenerative therapy is to develop fully functional bioengineered organs that can replace organs that have been lost or damaged by disease, injury or aging[5].

In dentistry, tooth tissue stem cells and the cytokine network that regulates tooth development have been well characterized and can likely be applied in the future to the repair of dental pulp and periodontal tissues[6-8] (Fig.1). Tooth diseases such as dental caries and periodontal disease cause fundamental problems for oral function and are associated with a number of health issues[9]. Conventionally, the restoration of tooth functions under these circumstances involves replacement with dentures or dental implants. Although these artificial therapies are very effective, it is thought that the proper restoration of tooth physiological functions, such as bone remodeling regulated by the periodontal tissue and a proper responsiveness to noxious stimulations, will be required[10]. Tooth tissue-derived stem cells have recently been used to repair the tissues affected in these diseases by regenerating the dentin, pulp, and periodontal tissues[11] (Fig.1). It is expected that regenerative tooth replacement therapy will be established in the near future as a biological treatment that will allow the essential functional recovery of lost teeth and satisfy aesthetic and physiological requirements[12].

In this review, we describe the various dental tissue-derived mesenchymal stem cells that have been considered as sources for tooth tissue regeneration therapy and the novel technologies that may be used for whole-tooth replacement.

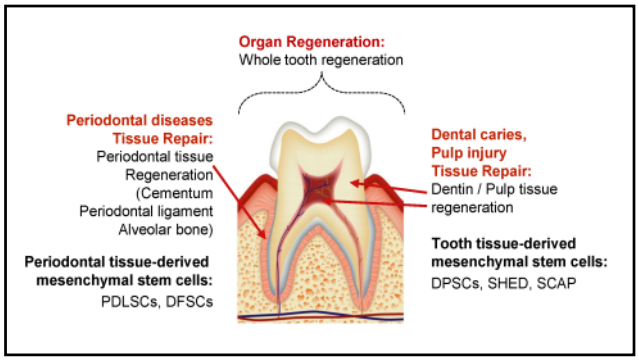

Fig.1 Concepts in tooth regenerative therapy, dental tissue repair and engineering

Recent approaches to developing technologies for tooth regenerative therapy have included tissue repair and whole-tooth organ replacement. Tooth regenerative therapy and stem cell transplantation therapies are regarded as attractive approaches for repairing tissue that has been damaged by dental caries or periodontal disease. For dental caries and pulp injury, the transplantation of dental stem cells, including DPSCs, SHED and SCAP, which can differentiate into odontogenic progenitors and pulp cells, has been examined. In periodontal tissue repair, the transplantation of PDLSCs and DFSCs has the potential to regenerate periodontal tissue.

Tooth development

Ectodermal organs, such as the teeth, hair, and mammary glands, arise from their respective organ germs through reciprocal epithelial-mesenchymal interactions. This interaction is the principal mechanism that regulates almost all organogenesis via signaling molecules and transcription factors[5, 13, 14]. During early craniofacial development in mice, tooth-forming fields are specified at embryonic day (ED) 10.5 by the expression of homeobox genes such as *Lhx8, Msx1, Msx2,* and *Barx1* and secretory molecules, including bone morphogenetic proteins (BMPs) and fibroblast growth factors (FGFs), in the embryonic jaw[15]. The tooth bud is formed from the dental lamina, which consists of an invaginated epithelium that is derived from the oral epithelium and condensed mesenchyme tissue that is derived from neural crest cells, at ED11.5[5, 15]. At ED13.5-14.5, the first enamel knot, which acts as a signaling center to orchestrate tooth development by controlling the gene expression of various signaling molecules and transcription factors, forms in the dental epithelium[5, 13, 15]. At ED16.5, the secondary enamel knots are formed; these tissues play an important role in regulating the position and number of the dental cusps[5, 13-15]. After ED18.5, the epithelial and mesenchymal cells in the tooth germ differentiate into the

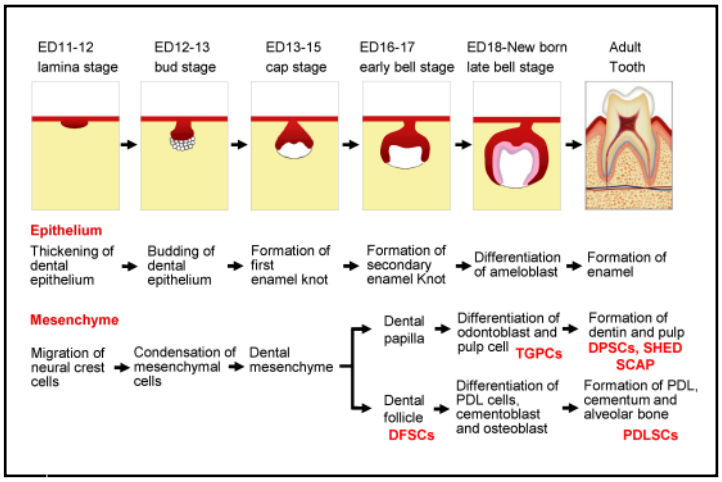

Fig.2 Schematic of tooth germ development
The development of tooth germ, which is formed from dental epithelial bud and neural crest-derived mesenchymal cells, begins at the lamina stage and proceeds to the bud stage. Subsequent morphogenesis occurs at the cap stage during the development of the dental epithelium and dental mesenchyme, which can later diverge into the dental papilla and dental follicle. Tooth crown is formed during the early bell stage and late bell stage. During tooth eruption, the root is developed, and dental follicle cells differentiate into periodontal tissue to attach the tooth root and jawbone (adult tooth). Various dental tissue-derived MSC-like cells were identified in the mesenchymal tissues of developing and mature teeth.

Table 1 Characteristics of dental tissue-derived mesenchymal stem cells

Stem cells	Representative MSCs markers	Differentiation capacity *in vitro*	Differentiation into dental tissues *in vivo*
DPSCs	CD13, CD29, CD44, CD73, CD90, CD105, CD166, STRO-1	odontoblast, osteoblast, adipocyte, chondrocyte, myoblast, neuronal cell	dentin-pulp complex by transplantation into mice
SHED	CD13, CD44, CD73, CD90, CD105, STRO-1	odontoblast, osteoblast, adipocyte, chondrocyte, myoblast, endothelial cell, neuronal cell	dentin-pulp complex by transplantation into mice
TGPCs	CD29, CD44, CD73, CD90, CD105, CD166, STRO-1	osteoblast, adipocyte, endothelial cell, neuronal cell, hepatocyte	not determined
SCAP	CD73, CD90, CD105, CD166, STRO-1	adipocyte, neuronal cell	dentin-matrix by transplantation into mice/ tooth root-like structure by a scaffold complex with PDLSCs-covered SCAP
PDLSCs	CD13, CD29, CD44, CD73, CD90, CD105, CD166, STRO-1	cementoblast, osteoblast, adipocyte, chondrocyte, neuronal cell	cementum and periodontal ligament like structure by transplantation into mice/ tooth root-like structure by a scaffold complex with PDLSCs-covered SCAP
DFSCs	CD13, CD29, CD44, CD73, CD90, CD105, CD166, STRO-1	cementoblast, osteoblast, adipocyte, chondrocyte, neuronal cell	not determined

tooth tissue-forming cells such as ameloblasts, odontoblasts, and dental follicle cells. Ameloblasts and odontoblasts secrete enamel and dentin, respectively, at the boundary surface between the epithelium and mesenchyme, while dental follicle cells differentiate into periodontal tissues including periodontal ligaments, cementum, and alveolar bone. Tooth root formation is initiated after tooth crown formation, and the mature teeth erupt into the oral cavity[5] (Fig.2).

Tissue repair using dental tissue-derived stem cells

Adult somatic stem cells, such as hematopoietic stem cells, neural stem cells, skin stem cells, and mesenchymal stem cells, undergo self-renewal and differentiation to maintain healthy tissues and to repair injured tissues. Recent studies of tooth tissue-derived stem/progenitor cells, which can differentiate into various dental cell lineages such as odontoblasts, pulp cells, periodontal ligament, cementum and alveolar bone[10], have identified many adult mesenchymal stem cell (MSC)-like cells[16] (Fig.2, Table 1). The transplantation of dental stem cells is a promising concept in dental regenerative therapy to restore the partial loss of tooth function (Fig.1).

1) Tissue regeneration using stem/progenitor cells derived from dental pulp

Dental pulp is composed of connective tissue, blood vessels, nerves, fibroblasts and odontoblasts, and it develops from the dental papilla after being encased by dentin tissue[10]. Dental pulp stem cells (DPSCs), which have properties similar to those of bone marrow-derived stem cells

(BMSCs), have been isolated from the dental pulp of human permanent third molars[17]. More recently, stem cells from human exfoliated deciduous teeth (SHED) were identified as bone marrow-derived MSC-like cells in the dental pulp of human deciduous teeth[18]. DPSCs and SHED possess definitive stem cell properties, such as self-renewal and multipotency. These cells express MSC markers including CD73, CD90, CD105, CD146, and STRO-1, and they can differentiate *in vitro* into multiple cell lineages, including odontoblasts, osteoblasts, adipocytes, chondrocytes, myocytes, and neural-like cells. Importantly, these cells can develop into dentin-pulp complex structures upon transplantation into immunocompromised mice[17, 18]. DPSCs and pulp stem cell subfractions that can generate pulp tissue, such as $CD31^-/CD146^-$ SP cells and $CD105^+$ cells, may also be useful for tooth tissue repair and dental pulp regeneration. It is very likely that growth factors and tooth tissue-derived stem cells will be applied clinically to repair damaged dentin and dental pulp tissue in near future[19] (Fig.2, Table 1).

Tooth germ progenitor cells (TGPCs) were identified as novel dental mesenchyme-derived stem cells from discarded human late bell stage third molars (commonly referred to as wisdom teeth)[20]. TGPCs have been shown to have high proliferation activity and the ability to differentiate into cells of all three germ layers, such as osteoblasts, neural cells, and hepatocytes, *in vitro*[20]. Furthermore, TGPCs can prevent progression and restore liver function in a liver fibrosis model[20]. Therefore, these stem cells may be a good resource for stem cell-mediated tissue repair, including dentin, pulp or liver regeneration (Fig.2, Table 1).

The dental papilla, which is the site of origin of root and pulp development, is apical to the developing pulp and is thus known as the apical papilla. This structure is less vascular than the pulp and contains cellular, gelatinous soft tissue. The apical papilla contains stem cells from apical papilla (SCAP), which have a high proliferative potential that is reflected by their high levels of telomerase activity and ability to differentiate into odontoblasts or adipocytes[21]. SCAP can also generate typical dentin structures after transplantation *in vivo* and may offer a promising avenue for cell-based tissue repair and tissue engineering therapies[21]. SCAP also demonstrated superior *in vitro* proliferation and dentin matrix regeneration *in vivo* compared with DPSCs[21]. A unique approach for tooth root regeneration employing a root-shaped hydroxyapatite/tricalcium phosphate (HA/TCP) carrier loaded with gelfoam/PDLSC-covered SCAP has been reported to produce a root-like structure that can be attached to a porcelain crown, resulting in normal tooth function[21]. This report suggests that immature mesenchymal stem cells, which are suitable as sources of regenerative cells, will be found in developing dental tissues rather than mature tissues[16] (Fig.2, Table 1).

2) Periodontal tissue-derived and dental follicle stem cells and their application to periodontal tissue regeneration

Periodontal tissue is composed of cementum, alveolar bone, and periodontal ligaments and serves as a tooth-supporting connective tissue between cementum and alveolar bone and as a shock absorber for occlusal force. The periodontal components are derived from dental follicle cells, which differentiate from the dental papilla into the developing tooth germ[5]. Periodontal tissue structure can be irreversibly damaged by periodontitis, a chronic inflammatory disease, and effective treatment for regenerating the periodontal tissue has not been established completely (Fig.2, Table 1).

Periodontal ligament-derived mesenchymal stem cells (PDLSCs) have been identified in adult human periodontal ligaments from extracted teeth[22]. PDLSCs exhibit rapid growth, similar to that of DPSCs, and express MSC markers such as STRO-1 and CD146. PDLSCs can differentiate into multiple cell lineages, including cementoblast-like cells, adipocytes, and collagen-forming cells, *in vitro* (Fig.2, Table 1). Upon *in vivo* transplantation into an immunocompromised animal, PDLSCs were able to generate a cementum and a periodontal ligament-like structure and contribute to periodontal tissue repair[22]. Dental follicle stem cells (DFSCs) were first identified as mesenchymal stem/progenitor cells in the first mandibular molars of postnatal rat pups, and they have been shown to be able to differentiate into osteoblasts, cementoblasts, adipocytes, and neural cells[23]. These cells are thought to be good candidate cell types for the repair of damaged periodontal tissues (Fig.2, Table 1).

Whole-tooth regeneration as a future organ replacement regenerative therapy

The current approach to generating ectodermal organs such as teeth, hair follicles and salivary glands is to recapitulate organogenesis by mimicking the epithelial-mesenchymal interactions that occur in the developing embryo,

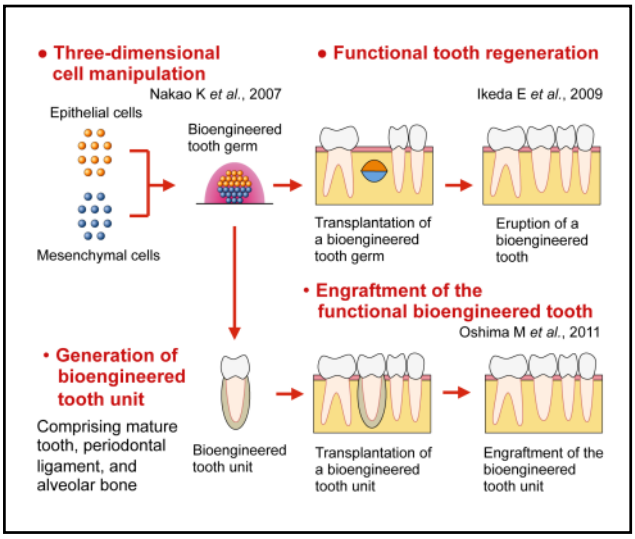

Fig.3 Strategies for whole-tooth replacement via regenerative therapies

In mice, functioning teeth can be regenerated from bioengineered tooth germs reconstituted from embryonic tooth germ-derived epithelial and mesenchymal cells. The bioengineered tooth germs can be transplanted into the jaw or developed into tooth units composed of mature tooth, periodontal ligament, and alveolar bone before transplantation.

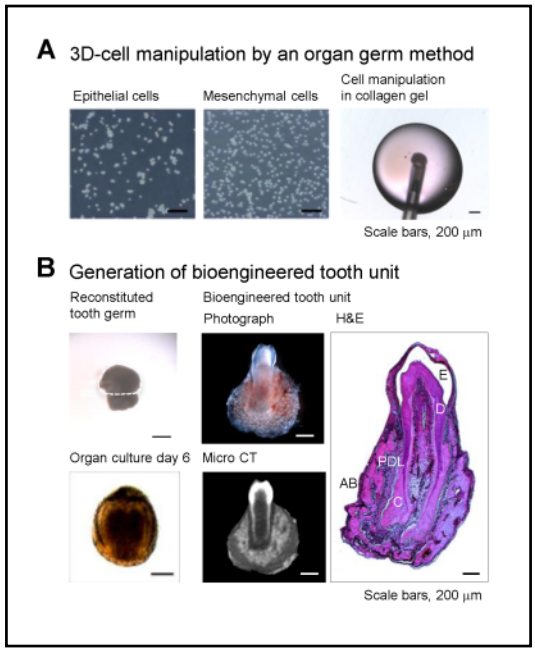

Fig.4 The organ germ method: a three-dimensional cell processing system

Dental epithelial and mesenchymal tissues isolated from tooth germ were completely dissociated into single cells (left and middle panel). The bioengineered tooth germ was reconstituted using these cells and exhibited cell compartmentalization at a high cell density in the collagen gel (right panel) (A). To generate a size-controlled bioengineered tooth unit, reconstituted tooth germs (upper left panel) were organ cultured for 6 days (lower left panel), transplanted into a subrenal capsule with a size-control device, and further cultured for 30 days (middle panel). The resulting bioengineered tooth unit was composed of mature tooth and periodontal tissues with the appropriate structural components, including enamel (E), dentin (D), periodontal ligament (PDL), and alveolar bone (AB) (right panel) (B). Scale bars, 200 μm.

thereby developing fully functioning bioengineered organs from bioengineered organ germ generated from immature stem cells via three-dimensional cell manipulation *in vitro*[8, 12, 24]. For tooth regeneration, it has been proposed that bioengineered tooth germ may be transplanted into a recipient jaw and develop into a functional mature tooth. An alternate possibility is the transplantation of a bioengineered tooth unit that includes mature tooth, periodontal ligament and alveolar bone; this unit will achieve engraftment through physiological bone integration into the recipient's jaw (Fig.3). To achieve whole-tooth replacement, the first major goal is to develop a three-dimensional cell manipulation technology using completely dissociated epithelial and mesenchymal cells *in vitro*. Several novel cell manipulation methods that are currently being investigated for the purpose of generating bioengineered tooth germ or mature teeth are discussed below.

1)A novel three-dimensional cell manipulation method for bioengineered tooth germ: the "organ germ method"

Recently, we developed an *in vitro* three-dimensional novel cell manipulation method, designated as the organ germ method[25]. This method involves cell compartmentalization of epithelial and mesenchymal cells from mouse embryonic cap-stage tooth germs at a high-cell density in a type I collagen gel to mimic multicellular assembly and epithelial-mesenchymal interactions as well as natural tooth development (Fig.4A). The bioengineered tooth germ generates a structurally correct tooth both *in vitro* in organ culture and *in vivo* after transplantation (Fig.4B). Direct cell-to-cell interactions induced by high cell density and cell compartmentalization are essential in tooth organogenesis, and most likely in the organogenesis of other organs. The organ germ method, which regulates crown width by limiting the contact area between the epithelial and mesenchymal cell layers, was designed to address the need for a

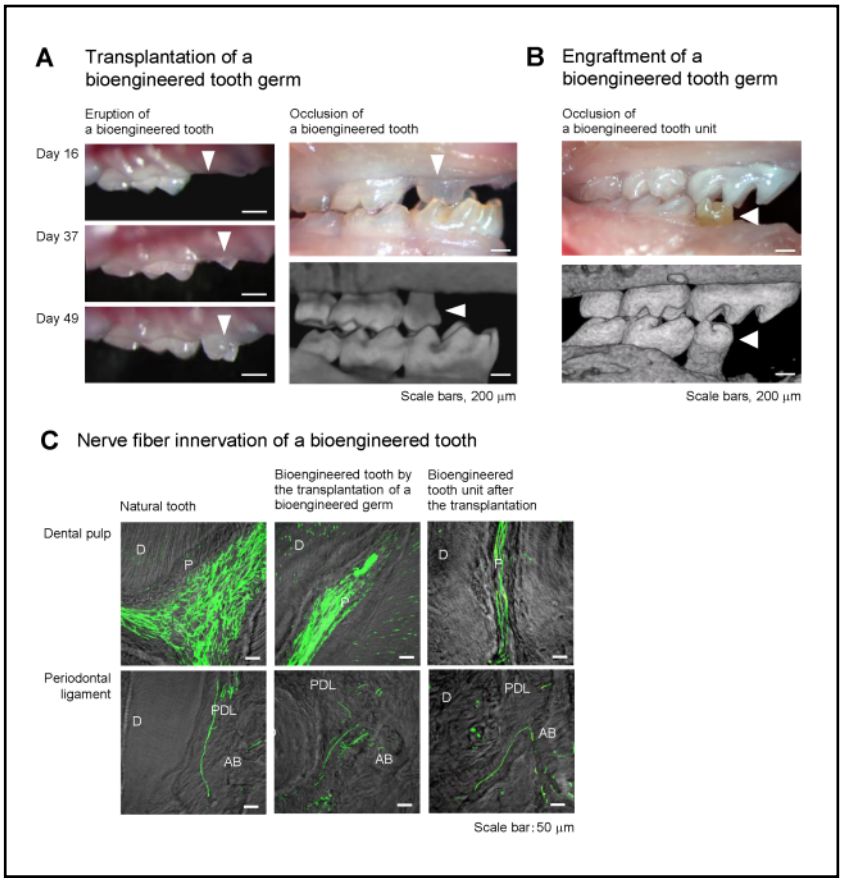

Fig.5 Functional tooth organ replacement *in vivo*

A transplanted bioengineered tooth germ erupted (A) and reached the occlusal plane with the opposing tooth 49 days after transplantation (B). Scale bars, 200 μm. Nerve fiber innervation of the dental pulp (P) and periodontal ligament (PDL) of natural and bioengineered teeth are shown by immunostaining for neurofilament-H. D, dentin; AB, alveolar bone (C). Scale bars, 50 μm.

method to reproducibly induce epithelial-mesenchymal interactions[26]. Thus, cell compartmentalization and strong association between epithelial and mesenchymal cells are essential for initiating organogenesis in a bioengineered organ germ[5]. Another unique technology that can successfully generate a size-controlled bioengineered mature tooth unit comprising bioengineered tooth, periodontal ligament and alveolar bone has also been described[27] (Fig.4B). A bioengineered tooth unit, the size of which was controlled using a specific device, was generated in subrenal capsule. A unit of multiple bioengineered teeth, which can function as a denture and become surrounded by alveolar bone, could also be generated by the transplantation of several tooth germs into this device[27].

2)Functional whole-tooth regeneration *in vivo*

Critical issues in tooth regenerative therapy include whether the bioengineered tooth germ, which will be transplanted into the lost tooth region, can erupt and occlude properly with the opposing tooth in an adult jawbone. It has been shown previously that teeth can erupt in the toothless diastema region of the mouse[28] and that a bioengineered tooth germ can develop the correct structure in a tooth socket[25]. Transplanted bioengineered tooth germ can successfully erupt, reach the occlusal plane, and achieve and maintain occlusion with the opposing tooth[29] (Fig.5A). Furthermore, a bioengineered tooth unit transplanted at a position reaching the occlusal plane with the opposing upper first molar was successfully engrafted and subsequently maintained the periodontal ligament derived from the bioengineered tooth unit through successful bone integration[27] (Fig.5B). The enamel and dentin hardness of bioengineered tooth components were in the normal range when analyzed by the Knoop hardness test[27, 29].

Bioengineered tooth has successfully replicated bone remodeling via the proper localization of osteoclasts and osteoblasts in response to mechanical stress such as the orthodontic force. They can replicate critical dental functions through the restoration and re-establishment of cooperation with the surrounding jawbone[27, 29]. These bioengineered teeth display appropriate perceptive potentials for nociceptive pain stimulation, such as pulp stimulation

and orthodontic treatment, and they can properly transduce these events to the central nervous system through c-Fos immunoreactive neurons[27, 29] (Fig.5C). Therefore, bioengineered teeth can indeed restore the perceptive potential for noxious stimuli in cooperation with the maxillofacial region. These technologies have the potential to be adapted for successful functional tooth replacement *in vivo* and are expected to represent a substantial advance in bioengineered organ replacement regenerative therapies.

Future perspectives for tooth regeneration and conclusion

To achieve the practical clinical application of tooth regeneration therapies, suitable cell sources must be identified. Tooth regenerative therapy should employ the patient's own cells to avoid immunological rejection[8]. Recent studies of stem cells and organogenesis have led to considerable advances in our knowledge of potential cell sources for tissue repair and organ reconstitution, including tooth regenerative therapy. Adult tooth tissue-derived stem cells such as DPSCs, SHED, SCAP, PDLSCs and dental follicle stem cells can differentiate into dental cell lineages and contribute to the turnover and supply of various cell populations (Fig.1, Table 1). Root regeneration using adult tissue-derived MSCs also has the potential for advanced clinical applications, because it might be simpler compared to whole-tooth regeneration.

The most recent whole-tooth regenerative therapy research has aimed to induce bioengineered tooth germ to develop a fully functioning tooth using embryonic tooth germ-derived epithelial and mesenchymal cells via the organ germ method[25, 27, 29] (Fig.4, 5). In the future, it will be important to identify sources of cells with tooth-forming ability from patient-derived somatic dental and non-dental tissue-derived stem cell populations[5]. Candidate cell sources for whole-tooth regeneration include embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, which are both capable of differentiating into the three germ layer lineages[30]. Recently, iPS cells have been established from various oral tissues and tested for their ability to differentiate into dental epithelial and mesenchymal cells[31-33]. Another important task for future tooth regenerative therapy research is the identification of specific combinations of factors capable of reprogramming non-dental cells into dental epithelium and mesenchyme[5] (Fig.6). It will also be important to identify inductive master genes with the potential to initiate tooth organogenesis of bioengineered tooth germ, as well as tooth developmental genes that promote the expression of dental epithelial and mesenchymal genes[5]. Recent studies have reported the *in vitro* self-organization of various tissues, such as the optic cup, adenohypophysis, gut, cerebral cortex, and hair follicle, in culture[34-39]. A three-dimensional *in vitro* organogenesis system using appropriately processed stem/progenitor cells will also be indispensable for the regeneration and replacement of whole teeth and other organs.

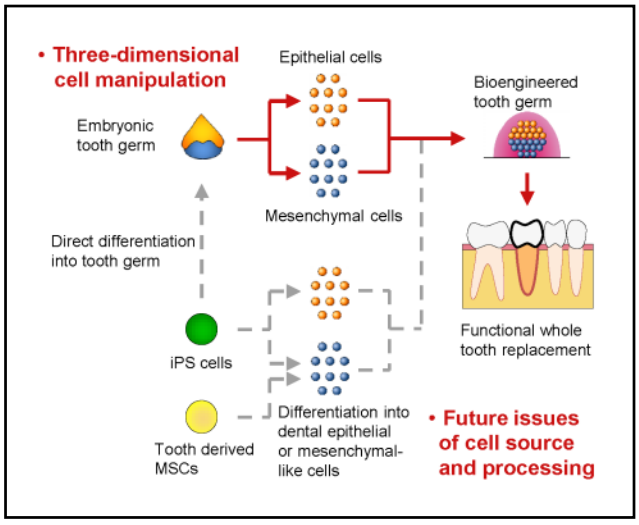

Fig.6 Strategies for whole-tooth replacement via regenerative therapies

Functioning teeth can be regenerated *in vivo* by transplanting bioengineered tooth germ reconstituted from epithelial and mesenchymal cells via the organ germ method or by transplanting bioengineered tooth units with periodontal ligament and alveolar bone developed from bioengineered tooth germ. Further studies of the mechanisms of differentiation into dental epithelial or mesenchymal cells from adult cell sources, including iPS cells and dental tissue-derived MSCs, will contribute to the realization of whole-tooth replacement.

Sources of funding

This work was partially supported by Health and Labour Sciences Research Grants from the Ministry of Health, Labour and Welfare (No. 21040101) to T.T., a Grant-in Aid for Scientific Research (A) to T.T. and a Grant-in-Aid for Young Scientists (B) to M.O. from the Ministry of Education, Culture, Sports and Technology, Japan. Our works was also supported by Organ Technologies Inc.

Conflict of interests

The authors declare that no competing interests exist.

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