

Expert Opinion

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Cell- & Tissue-based Therapy

Growing bioengineered teeth from single cells: potential for dental regenerative medicine

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Background: The ultimate goal of regenerative therapy is to develop fully functioning bioengineered organs that can replace organs lost or damaged due to disease, injury or aging. Dental regenerative medicine has made the most progress and is the most useful model for the consideration of strategies in future organ replacement therapies. **Objective:** This review describes strategies that have been pursued to date and experiments currently being conducted to bioengineer teeth in anticipation of the production of fully functional organs. **Methods:** To realize the practical application of 'bioengineered tooth' transplantation therapy, four major hurdles must be overcome. The present status of the hurdles to this therapy are described and discussed in this review. **Results/conclusion:** The bioengineering techniques developed for tooth regeneration will in the future make substantial contributions to the ability to grow primordial organs *in vitro* and also to grow fully functioning organs, such as the liver, kidney and heart.

Keywords: bioengineered tooth, regenerative therapy, stem cells, tooth germ

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1. Introduction

Human organs and tissues are made up of about 200 kinds of cells that develop from stem cells. Stem cells are undifferentiated cells with the ability to differentiate into any tissue type in order to carry out specific tasks. Recently, stem-cell-transplantation therapy has attracted attention as an alternative to organ transplantation due to stem cells' abilities to proliferate and differentiate at an engrafted site [1,2]. Human stem cells, including embryonic stem cells (ES cells) and adult tissue stem cells, are excellent candidates for transplantation therapy [3,4].

Human ES cells are enormously promising because of their ability to differentiate into specialized embryonic tissues from all germ layers, including the ectoderm, mesoderm and endoderm [5,6]. Adult stem cells and progenitor cells act as self-repair systems for the body by replication and development into specialized cells [7-11]. Recently, stem-cell-based therapies, including stem cell transplantation to repair injured tissues, have passed significant milestones with the successful treatment of various refractory conditions, such as Parkinson's disease, leukemia, spinal injury, cardiac infarction, diabetes and liver diseases [12-15].

The ultimate goal of regenerative medicine is to develop fully functioning, bioengineered organs to replace damaged organs. However, bioengineering technologies have not yet achieved three-dimensional reconstructions of fully functioning organs, which would result if various types of stem/progenitor cells

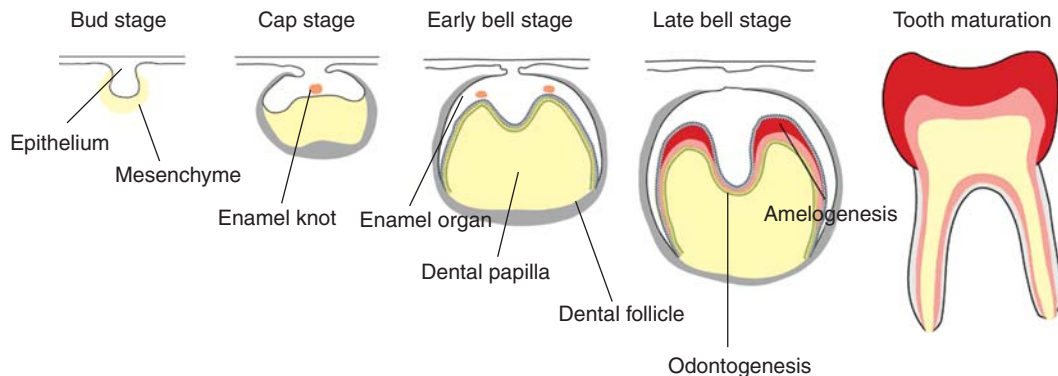


Figure 1. Tooth development. Bud stage: proliferation of oral epithelium and mesenchyme. Cap stage: epithelial bud enlarges, mesenchymal cells gather to form dental mesenchyme, a transient signal center (enamel knot) forms in the epithelium. Early bell stage: tooth germ consists of all three components (enamel organ, dental papilla and dental follicle). Late bell stage: amelogenesis and odontogenesis. Tooth maturation: a mature tooth is a complex of enamel, dentin and dental pulp with periodontum.

could be instructed to become complex, specialized arrangements of differentiated cells. One of the most promising techniques for organ reconstruction is a cell culturing method that uses biodegradable scaffolds into which dissociated cells are seeded, reaggregate and adopt the shape of the scaffold. But, it is still experimental.

During embryonic development, almost all organs, including the teeth, arise from various types of organ germ. These are induced by reciprocal interactions between epithelial and mesenchymal cells. An alternative to the scaffold strategy is to develop fully functioning organs from organ germ by manipulating developmental programs involving epithelial-mesenchymal interactions. In tissue bioengineering, much research is currently directed to the reconstruction of teeth, as the tooth is a convenient model for various organs of ectodermal origin. This review describes strategies that have been pursued, and current experiments designed to bioengineer teeth with the aim of producing fully functional organs.

2. Concepts for tooth regeneration

After the loss of a tooth through injury, caries or diseases, therapy is needed to prevent movement of the neighboring teeth and to supplement their functions. In the dental field, there are established therapies for missing teeth [16,17]. One is the bridge, which is an artificial tooth crown that is put in place by using neighboring teeth. However, this therapy requires that the neighboring healthy teeth must be sharpened. Another strategy is implant therapy. After the installation of a screw-type implant into alveolar bone, an artificial tooth is placed upon it. Although implant therapy is very effective for lost teeth, this therapy needs improvements on several points, such as tooth movement and inhibition of bone development in childhood. Therefore, transplantation of a natural tooth, such as a third molar, has been tried [18]. This biological therapy derives from one of the concepts in organ transplantation.

In the next generation, this therapy will be performed by the transplantation of a bioengineered tooth, which will be constructed by the manipulation of single stem/progenitor cells to become a whole, functional tooth unit, including the periodontal tissues, for the replacement of the missing tooth. Teeth are located close to the body's surface, and are therefore easily accessible. Additionally, the molecular mechanisms of epithelial-mesenchymal interactions during embryonic development are well known [19]. Thus, it is thought that the tooth provides a good feasibility study model for the development of future organ replacement technologies, as the therapies for organ loss will have had their trials in the dental field.

During the 20-day gestation period of mice, tooth development begins at embryonic day 10 (ED10; Figure 1). The tooth germ begins to form from the interactions between the oral mucosal epithelium and mesenchyme at the site of the future tooth. During these processes, expression of homeobox genes is induced by the coordinated actions of growth factors, cytokines and adhesion molecules. Activation of these genes guides each tooth bud along a pathway so as to grow a tooth of a given size and shape that is predetermined by its position [20-24]. Among these molecules are members of the TGF- β super family, including bone morphogenic proteins (BMPs), activin and TGF- β , fibroblast growth factors (FGFs), sonic hedgehog (Shh) and the wingless-related MMTV integration site gene product (Wnt). These play important roles in the regulation of tooth development and morphogenesis [25].

On ED11, the epithelium thickens and invaginates into the underlying mesenchyme, which then condenses around the developing epithelial bud for dental lamina formation. On ED14, the epithelium grows to surround the mesenchyme to form a cap-stage tooth germ. In this stage, a transient epithelial signaling center called an 'enamel knot' is thought to regulate individual cell fates and epithelium-mesenchyme interactions [26,27]. Enamel knot formation is also regulated

Table 1. Concepts in tooth regeneration.

| Concept | Technology |
|--|--|
| Single cell manipulation <i>in vitro</i> | Tissue engineering method using scaffolds Cell aggregation method |
| <i>In vivo</i> transplantation | Transplantation of the bioengineered tooth or tooth germ in adult oral environment |
| Identification of cell sources | Identification of tooth-inductive cells from dental tissues and adult tissues |
| Control of tooth morphology | Control of signaling cascades that regulate tooth morphology Tissue engineering using scaffolds |

by Shh, BMP-4, FGF and Wnt [19,25]. The spatial delimitation of the enamel knot is known to be regulated by the expressions of both ectodin, which belongs to the differential screening-selected gene aberrant in neuroblastoma (Dan)/Cerberus family of secreted BMP antagonists, and the ectodysplasin receptor, Edar [28,29].

The ED15-ED18 tooth germ is in the 'bell stage', where epithelium and mesenchyme differentiate into ameloblasts, which later become enamel, and odontoblasts that will form dentin. The mesenchyme also differentiates into dental pulp and into periodontal tissues, which will become cementum, alveolar bone and periodontal ligament. As mentioned above, tooth organogenesis involves epithelial-mesenchymal interactions that result in the generation of various types of cells and arrangements of cells and hard tissues.

To realize the practical application of 'bioengineered teeth' for transplantation therapy, four major hurdles must be overcome as described in Table 1. The present status and the obstacles arising from these hurdles for bioengineered tooth transplantation therapy will be described in subsequent sections of this review.

3. Tooth regeneration by single cell manipulation

Reconstitution of bioengineered tooth germ, like that for other organs, requires that single cells are completely dissociated from both the epithelium and the mesenchyme, and the correct placement of cells is a major issue. The question of how to reconstitute organs from completely dissociated single cells has focused attention upon basic techniques, such as those used in other fields for the *ex vivo* generation of organs using cell engineering methods, as well as for organ replacement therapy. Bioengineering techniques have been awaited that facilitate high frequencies of reconstitution, such that effective replacement with functional cells occurs in a manner that generally mimics embryonic development. Currently, two approaches are being investigated for reconstructing teeth using cell culture procedures. One approach is to seed tooth germ cells into tooth-shaped scaffolds made of biodegradable materials. The other approach is to reconstitute teeth by reaggregating tooth germ from dissociated single epithelial cells and mesenchymal cells.

3.1 Bioengineered tooth germ by tissue engineering method using scaffolds

One of the three-dimensional tissue engineering methods is scaffolding, in which a complex cell mixture and a prefabricated biodegradable scaffold are employed to grow a tissue of a desired form. This has been used for clinical repairs of various complex, reconstituted tissues, such as cartilage, and bone [30-32]. Feasibility studies for the production of bioengineered tooth tissues using a cell-scaffold composite have been made [33-40].

Yelick's group examined explants containing dissociated cells isolated from porcine unerupted third molars or rat molar tooth buds. These contained both epithelial cells and mesenchymal cells that were plated onto a tooth-shaped scaffold made of polyglycolate (PGA) and poly-L-lactate-co-glycolate (PLGA). The explants were capable of generating a tooth crown containing both dentin and enamel [36,37]. This suggests that a tooth-tissue engineering method, with a prefabricated biodegradable scaffold, could be used to produce a bioengineered tooth using tooth tissue-derived cells. Honda *et al.* reported the effectiveness of using a collagen sponge as a scaffold, and sequentially seeding epithelial cells and mesenchymal cells [39,40]. However, it remains to be seen whether tooth formation will be sufficiently efficient and whether the structure of the regenerated teeth will be satisfactory using this method.

3.2 Bioengineered tooth germ by cell aggregation

The other approach for the reconstitution of a bioengineered tooth germ is the cell reaggregation method. The first step in multi-cellular aggregation of epithelial cells and mesenchymal cells is multi-cellular assembly by self-reorganization of each cell type. This occurs through cell migration and selective cell adhesion until cells reach an equilibrium arrangement [41-47]. Next, reciprocal interactions among epithelial cell layers and mesenchymal cell layers initiate organogenesis that regulates differentiation and morphogenesis [19,48]. The potentials of cells to self-reorganize and to induce organogenesis are thought to differ greatly among the various cell types of different organs.

To create a bioengineered tooth germ, dental epithelium is reassociated with a cell pellet of dissociated mesenchymal cells; the resulting artificial germ is then used to grow a

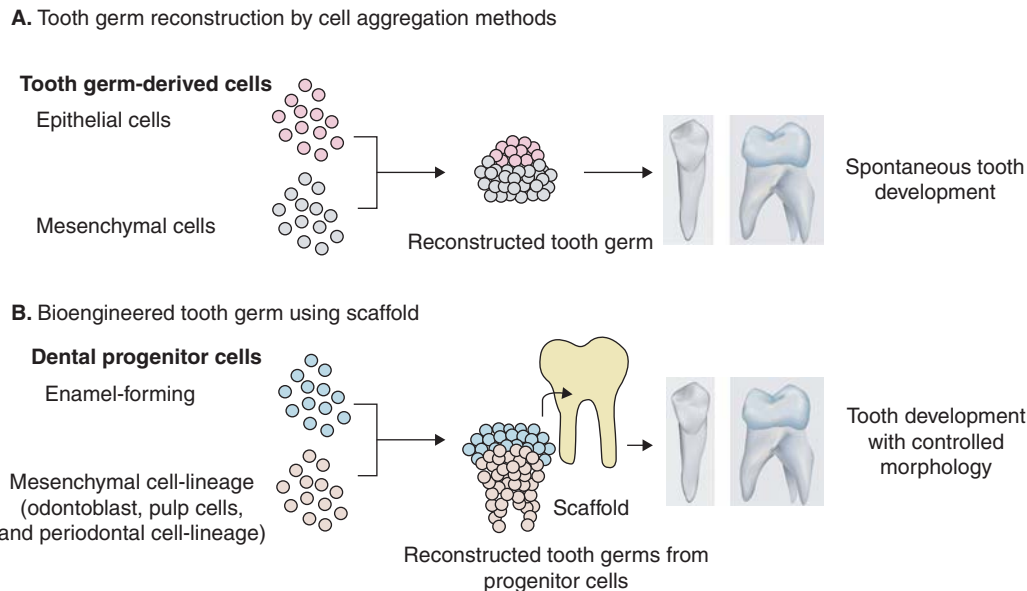


Figure 2. Tooth regeneration by manipulation of individual cells. **A.** Single epithelial and mesenchymal cells are dissociated from tooth germ at an early developmental stage. Dissociated cells reaggregate to regenerate bioengineered tooth germ. Regenerated tooth germ spontaneously develops into a tooth. **B.** Enamel-forming progenitor cells and dentin-forming progenitor cells are arranged in their proper anatomical relationships on a scaffold. The reconstructed tooth germ from the progenitor cells leads to tooth development with controlled morphology.

tooth with a proper structure by transplantation *in vivo* [49]. A bioengineered tooth germ reconstituted from pellets of dissociated dental epithelium cells and mesenchymal cells, isolated from ED14.5 mouse tooth germ, successfully yielded a complete tooth surrounded by periodontal-like tissue [50,51]. It has also been reported that a cell aggregate of mixed epithelial and mesenchymal cells, isolated from ED13.5 mouse-derived molar tooth germ, was capable of generating a complete tooth without cell compartmentalization at high cell density [52].

Interestingly, auto-cell sorting and inductive potentials were found in molar-derived epithelial cells. This agrees with Steinberg's theory concerning early organogenesis [41-45]. In contrast, incisor epithelial cells did not exhibit inductive potential after autologous cell sorting. This suggests that auto-cell sorting and inductive potentials to organize into organ germs differ among the types of organ germs. Although these reports indicated that the cell aggregation method is useful for creating bio-engineered tooth germ for dental regenerative therapy, a bioengineering method that can replicate the organogenesis of the embryo and that is adaptable to a wide variety of organ germ types is desired.

3.3 Comparison of cell processing methods

During tooth development, the properties of cells vary, depending upon the developmental stage. Different cell manipulation techniques are thus required according to the target stage of the tooth germ. In the early developmental stages of the tooth germ, in which tissues have not yet

mineralized, it is essential that reciprocal interactions between epithelial cells and mesenchymal cells regulate the differentiation of odonto-forming or enamel-forming progenitor cells through direct cell-to-cell interactions and cytokine production, and that they promote spontaneous organ development in which cells are properly arranged so as to function normally. In later stages of development such as the late bell stage, in which tissues have mineralized, tooth-specific patterning of the epithelial–mesenchymal junction subsequently forms a dentin–enamel junction. Ameloblasts and odontoblasts, which are differentiated from epithelial cells and mesenchymal cells, respectively, each have a cell polarity of cell-to-mineralized tissue, and a gradient during the differentiation stage of each cell lineage from the tip of the cusp to the apical part.

The cell aggregation method and the tissue engineering method using scaffolds may be useful for regeneration of tooth germ in the early developmental stages (induction phase) and in the late developmental stages (differentiation phase), respectively (Figure 2). Using the cell reaggregation method, epithelial cells and mesenchymal cells isolated from tooth germ at an early developmental stage but not at a late developmental stage, could be induced to reproduce in such a manner so as to direct cell-to-cell interactions between each of the cells. The reaggregated cells may self-renew or proliferate, and promote spatial sorting and self-organization resulting in proper cell arrangements.

The ideal placement of cells may be realized by the scaffolding technique, resulting in full polarization and

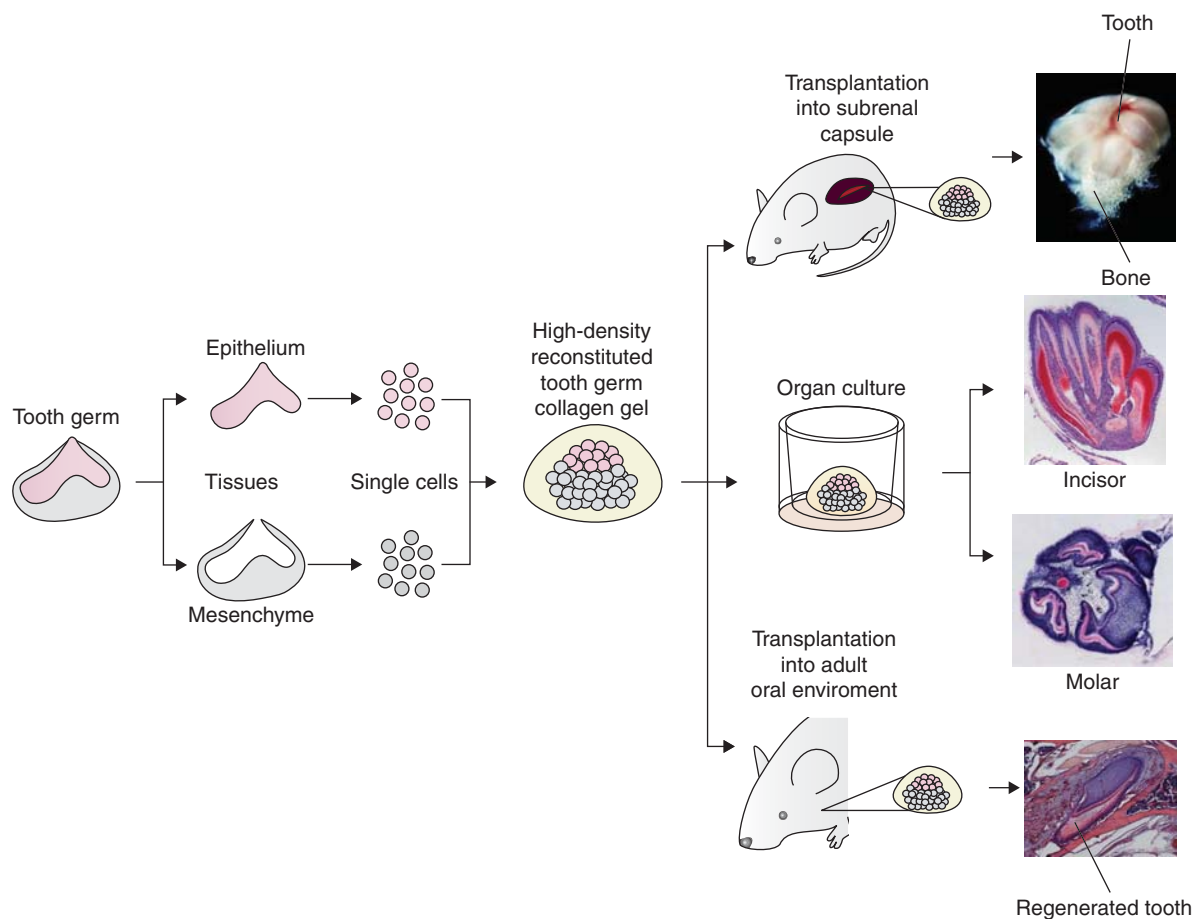


Figure 3. Generation of whole tooth using bioengineered tooth germ. The epithelial and mesenchymal tissues isolated from tooth germ were completely dissociated into single cells. The bioengineered incisor tooth germ was then reconstituted using these dissociated cells and showed cell compartmentalization at a high cell density. The explants were either transplanted into subrenal capsules or were continuously cultured. Bioengineered teeth developed in the subrenal capsules. Bioengineered incisors and molars that had been cultured for 14 days were analyzed using hematoxylin-eosin staining. A bioengineered tooth germ was transplanted into a tooth cavity generated by the extraction of a mandibular incisor. Images show the explants at 14 days after transplantation into a tooth cavity.

proper cell morphology. Using scaffolds and seeding the proper anatomical arrangement of both immature cells and committed progenitor cells for each cell lineage maintains both spontaneous cell polarization toward the enamel-dentin junction and the gradient for each cell lineage. Furthermore, using the scaffolding technique, tooth germ in more advanced developmental stages than the induction stage can be reconstituted, and the total time required to develop into a functional tooth from reconstituted tooth germ engrafted into the oral cavity may be shortened.

3.4 Development of a novel bioengineering method

Recently, we developed a bioengineering method for forming a three-dimensional organ germ in the early developmental stages, termed the 'bioengineered organ germ method' [53]. To precisely replicate tooth organogenesis in the early developmental stages, a cell aggregation method using cap-stage tooth-germ-derived epithelial cells and mesenchymal

cells was chosen (Figure 3). This method has the distinctive feature that the bioengineered tooth germ is reconstituted between epithelial cells and mesenchymal cells using cell compartmentalization at a high cell density in a collagen gel solution. Both incisor and molar bioengineered tooth germs successfully developed into teeth with the correct tooth structures with a high rate of success *in vitro* and *in vivo*; this tooth germ model reproduces the interactions between epithelial cells and mesenchymal cells in early tooth development (Figure 3). Direct cell-to-cell interactions induced by high cell density and cell compartmentalization are essential in tooth organogenesis, and possibly for organogenesis of other organs. Indeed, this method could be applicable to the reconstitution of whisker follicles [53]. Thus, cell compartmentalization, which mimics multicellular assembly and the equilibrium configuration between epithelial cells and mesenchymal cells, is effective for initiating organogenesis in a bio-engineered organ primordium.

Table 2. Stem cells in tooth tissues.

| Cells | Differentiation | Ref. |
|---------------------------------|---|---------|
| DPSCs | Odontogenesis, adipogenesis, neurogenesis and dentin/pulp-like structure-formation | [55,56] |
| SHED | Dentin-like tissues, adipogenesis, neurogenesis, bone and odontoblast-formation | [57] |
| PDLSCs | Cementoblastic/osteoblastic differentiation, adipogenesis and cementum/PDL-like structure-formation | [59] |
| SCAP | Odontoblastic/osteoblastic differentiation, adipogenesis and dentin formation | [60] |
| SP cell from porcine tooth germ | Adipogenesis, neurogenesis, chondrogenesis, dentinogenesis and osteodentin formation | [58] |
| TGPC | Osteogenesis, neurogenesis and hepatogenesis | [61] |

DPSCs: Dental pulp stem cells; PDL: Periodontal ligament; PDLSCs: Periodontal ligament stem cells; SCAP: Stem cells from apical papilla; SHED: Stem cells from human exfoliated deciduous teeth; SP: Side population; TGPCs: Tooth germ progenitor cell.

4. Development of a bio-engineered tooth in the adult oral environment

The adult oral environment differs significantly from the embryonic environment in which induction and early development of tooth germ normally occur. It has been reported that murine embryonic tooth primordium can develop in a toothless oral region (diastema) of an adult mouse [54]. This suggests the potential for a dissociated tooth primordium to develop in an adult oral environment. It is also important to determine if a bio-engineered tooth germ can develop in an adult oral environment into a fully functioning tooth that has both a correct tooth structure and a complex network of blood vessels and nerve fibers. Recently, our group reported that a bio-engineered tooth primordium, which was isolated from bio-engineered tooth germ, could develop in a tooth cavity formed by the extraction of a mandibular incisor into a tooth with correct tooth structure comprising enamel, dentin, root, dental pulp, periodontal ligament, blood vessels and alveolar bone (Figure 3) [53]. This strongly suggested that the replacement of biologically functional teeth is possible by reconstitution in the tooth cavity in cooperation with the surrounding tissues of an adult. Studies of the long-term efficacy of bio-engineered tooth germ transplantation should now be undertaken.

5. Identification of cell sources for tooth regeneration

Recent studies have demonstrated the potential for adult-tissue stem cells to differentiate into cells arising from all three germ layers, and that stem cells may be candidate sources for tissue engineering, including tooth regeneration [7-11]. Stem cells that can differentiate into dental cell lineages will be useful for transplantation therapy in tooth decay, bone remodeling and periodontal diseases. The practical application of dental regenerative medicine may be optimized using the patient's own cells but not embryo-derived cells.

Candidate stem/progenitor cells isolated from various dental tissues have been reported (Table 2). Human dental pulp tissues contain stem cells called 'dental pulp stem cells' (DPSC) [55,56]. Stem cells from human exfoliated deciduous teeth (SHED) and side-population cells isolated from porcine dental pulp cells have also been reported [57,58]. These stem cells differentiate into odontoblasts and secrete dentin *in vivo*. One of the first reports showed that periodontal ligament stem cells (PDLSC), isolated from human periodontal tissue, could regenerate the cementum and periodontal ligament [59]. Recently, a unique approach for tooth root regeneration used the combination of PDLSC and human stem cells from the apical papilla (SCAP) [60]. Using a minipig model, a root-shaped hydroxyapatite/tricalcium phosphate (HA/TCP) carrier, which was loaded with SCAP that covered gelfoam/PDLSC, formed a root-like structure to which a porcelain crown was attached, resulting in normal tooth function [60]. Our group reported that tooth germ progenitor cells (TGPCs), which were isolated from human unerupted third molars and single cell-derived clones, were multi-potent and differentiated into cells of the three germ layers, including bone, neural cells and hepatocytes [61]. The dental stem/progenitor cells may also contribute to advances in a wide variety of dental regenerative therapies.

Investigations of dental differentiation potentials for various non-dental tissue-derived cells should also offer opportunities to advance tooth regeneration for clinical applications. Cultured, non-dental tissue-derived cells, such as embryonic stem cells, neural stem cells or bone marrow stromal cells, have indicators for early odontogenesis, as measured by gene expression during initiation of odontogenesis in an animal model [54]. Also, a reported cell aggregation method showed that bone marrow cells differentiated into ameloblast-like cells and odontoblast-like cells [62]. Tomooka's group, using an organ-germ method, demonstrated that an oral mucosa-derived epithelial cell line differentiated into ameloblasts and formed a regenerated tooth in combination with tooth-germ-derived mesenchyme [63]. Although these reports have not yet shown that regeneration of a whole tooth is

imminently achievable, these findings strongly suggest the presence of cell sources in humans that may be of use for dental regenerative therapy.

6. Regulation of tooth size and morphology

Regulations of tooth sizes and shapes for the crown and the root are important matters to consider in generating an entirely bio-engineered tooth. Teeth have unique morphological features for incisors, canines, premolars and molars. These features are programmed at predetermined sites in the oral cavity during natural tooth development, and the resulting morphology is controlled so that functional occlusion, or bite, is maintained that involves the teeth, muscles and the temporo-mandibular joints [64]. Tooth morphology is also important for dental esthetics to achieve the beautiful smile, one purpose of dental treatment.

To date, many researchers have conducted numerous trials to clarify the molecular mechanisms involved in the regulation of tooth morphology [65-67]. Spatial distributions of gene expressions provide coordinated signals for positional and morphological control, such as for crowns and roots [29,68-70]. The dental mesenchyme controls crown morphologies and epithelial histogenesis, including enamel knots, and regulates the patterning of the cusps and the shapes of tooth crowns [19,71,72]. In experiments on reassociations between dental epithelial and mesenchymal cells, the patterning, size, number and cusps of teeth were analyzed [71]. In order to develop a system to control tooth size and morphology of bio-engineered teeth, it is necessary to identify the key molecules regulating tooth patterning and morphogenesis. In the near future, this will be achieved by identifying regulatory systems of gene expression in combination with cell processing methods.

7. Conclusion

Dental regenerative medicine has made the most progress and is currently the most useful model for planning strategies for future organ-replacement therapies. Recent breakthroughs in single-cell manipulation methods for artificial organ germ and *in vivo* development of bio-engineered tooth germ have been reported. As adult-tissue-derived cell sources are anticipated to be useful in clinical applications for tooth regeneration, several stem/progenitor cells have been found, which were isolated from adult dental/non-dental tissues. Research to identify human cell sources and the development of techniques to control the sizes and morphogenesis of organs will be essential for the bioengineering of artificial organs. The bioengineering techniques developed for tooth regeneration will, in future, make substantial contributions for growing primordial organs *in vitro* and fully functioning organs, such as for liver, kidney and heart. We hope that these

developments also encourage the development of organ replacement using regenerative therapies.

8. Expert opinion

As noted in this review, adult stem cell transplantation has thus far achieved successful therapeutic outcomes in areas such as bone marrow transplantation and bone regeneration, and much broader clinical applications are anticipated. In the future, the development of improved basic techniques and preparations will be necessary in order to establish the next-generation of regenerative medical technologies and organ replacement therapies. For feasibility studies, tooth regeneration is likely to be the most useful model for the development of bioengineered organs. In tooth regeneration, a partial tissue engineering process will be used initially for the treatment of dental caries and periodontal disease, and more advanced treatments will allow regeneration of whole teeth.

In current research on whole-tooth regenerative medicine, a basic strategy is being pursued in which a bioengineered germ is induced to develop into a fully functional tooth; a substantial number of trials have been conducted to develop bioengineering techniques for the production of tooth germs from dissociated single cells. It is essential to use the appropriate cell manipulation technique and cells from the appropriate developmental stage for successful reconstitution of each stage of the tooth germ. Using the cell aggregation method, layers of epithelial and mesenchymal tissues can be reproduced in a manner similar to the initial stages of the tooth germ, such as from the bud to the cap stages. Tissue engineering using scaffolds reproduces the proper anatomical arrangements for both undifferentiated cells and committed odonto-forming or enamel-forming progenitor cells based upon maturation events.

Recently, we developed a three-dimensional organ-germ culture method in which tooth germs could be grown to the early developmental stages (induction phases). We then showed that transplanting a tooth germ into a tooth cavity would grow a tooth having the appropriate structures, including blood vessels and nerves. Furthermore, the bioengineered tooth formed a periodontal ligament like that of a normal tooth. The regeneration of tooth and periodontal tissues into a functional tooth unit is a critical issue for achieving proper oral function, including mastication. These results showed that three-dimensional organ germs, grown from completely dissociated cells, could be fully functional *in vivo* after implantation. They also suggested that a bioengineered organ replacement strategy using an artificially constructed organ germ might be feasible.

One of the major research challenges hindering the clinical application of tooth regeneration is the identification of appropriate cell sources. The bioengineered tooth may be optimized by using the patient's own cells so as to avoid

immunological rejection. In the future, somatic stem cells isolated from patients, including dental-tissue-derived stem cells, will be thoroughly researched to determine whether these might be usable for the regeneration of whole teeth. Candidate cell sources also include multi-potent ES cells, which are capable of differentiating into endoderm, ectoderm and mesoderm, as well as TGPCs. In addition, non-dental-tissue-derived stem cells such as bone marrow-derived cells and oral mucosa-derived epithelial cells, which have been reported to have a tooth-forming ability, would be candidate sources for clinical applications. One or more of these cell types may be induced to grow into dental epithelium and mesenchyme, and their respective precursor cell lines, by tissue engineering techniques.

Techniques for the control of tooth shape and dimensions will also be important for dental functional occlusion and esthetics. It is also crucial to shorten the period for regenerated tooth preparation as human teeth take years to form. For patients, it might be better to transplant a regenerated tooth prepared from a functional tooth with a desired shape, rather than transplant a regenerated tooth germ, which would take a long period of time to develop. However, no technology yet exists that enables control of the morphology of cultured organs *in vitro*. Further research is required to determine the optimal numbers of cells for use in tissue

engineering and methods to achieve control, such as for tissue engineering using scaffolds or gene expression control during the course of morphogenesis. As described above, several cytokines, such as BMPs and FGFs, play important roles in tooth development, morphogenesis and mineralization, and are being applied to regeneration for partial loss of tooth function, such as the repair of pulp and periodontal tissues [25,73,74]. The proper combinations of these factors for *in vitro* culture systems could effectively shorten the culture period and provide controls of morphogenesis.

It is hoped that research into the bioengineering of replacement teeth will provide basic techniques and models that will contribute substantially to the understanding necessary to regenerate other organs. The three-dimensional organ germ method that we recently developed has been shown to apply to the production of germs not only for teeth but also for other organs of ectodermal origin, such as whiskers. Our results also suggest that the method may be useful for replacement and regeneration therapies of other organs. Progress in the replacement and regeneration of a wide variety of organs is anticipated through further basic research in tissue engineering techniques, such as organ germ methods, the identification of sources of stem cells and control of the shapes and dimensions of bioengineered organs.

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