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Regeneration of complex oral organs using 3D cell organization technology

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The development of organoid techniques for regenerative therapy has progressed remarkably with the use of tissue-derived stem cells and pluripotent stem cells based on stem cell biology and tissue engineering technology. To realize whole-organ replacement therapy as next-generation regenerative medicine, it is expected that fully functional bioengineered organs can be reconstructed using an *in vitro* three-dimensional (3D) bioengineered organ germ and organoids by stem cell manipulation and self-organization. In this mini-review, we focused on substantial advances of 3D bioengineering technologies for the regeneration of complex oral organs with the reconstruction of 3D bioengineered organ germ using organ-inductive potential embryo-derived epithelial and mesenchymal cells. These bioengineering technologies have the potential for realization of future organ replacement therapy.

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Introduction

Organogenesis is achieved by an autonomous developmental process *via* the self-organization of complex tissues that includes cell-to-cell interactions, spatiotemporal expression of molecules and cell growth/movement [1,2]. Almost all organs arise from their respective organ germs through reciprocal epithelial–mesenchymal interactions in both epithelial tissue and mesenchymal tissue during embryonic development [3,4]. Ectodermal oral organs, including teeth and salivary glands, develop from the respective germ layers based on those reciprocal interactions, and the principal interactions in ectodermal organ development allow for the organization of a three-dimensional (3D) tissue structure to achieve the respective physiological organ functions [3,5].

Current biotechnology in regenerative medicine has advanced dramatically based on new findings in embryonic development, stem cell biology and tissue engineering technology [2,6]. In particular, stem cell research has focused on tissue-derived stem cells, embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, the use of which is considered an attractive regenerative concept, and these stem cells have been attempted to be used regenerate damaged tissues/organs with structural and functional disorders [2]. Many attempts to create bioengineered tissues/organs that can replace damaged organs have been reported [7]. Cell-sheet-based technology, which allows tissue reconstruction from stem cells grown on a sheet, can regenerate a broad range of tissues damaged by burns and cardiac dysfunction through cell-sheet transplantation [8]. Organoids, which can be derived from tissue-specific stem cells or pluripotent stem cells, have functional 3D tissue structures that resemble parts of organs and have the potential to provide an alternative approach to organ transplantation in the clinic [9•,10•]. Ideally, organ replacement regenerative therapy offers enormous potential for the replacement of dysfunctional organs with functional regenerated organs using bioengineering technology [7]. However, ectodermal oral organs, including teeth and salivary glands, cannot be sufficiently reproduced from tissue-derived stem cells or pluripotent stem cells [2]. Thus, it is desired to develop a next-generation regenerative approach in which fully functional bioengineered organs can be reconstructed using *in vitro* 3D stem cell manipulation and organization technology [2,7].

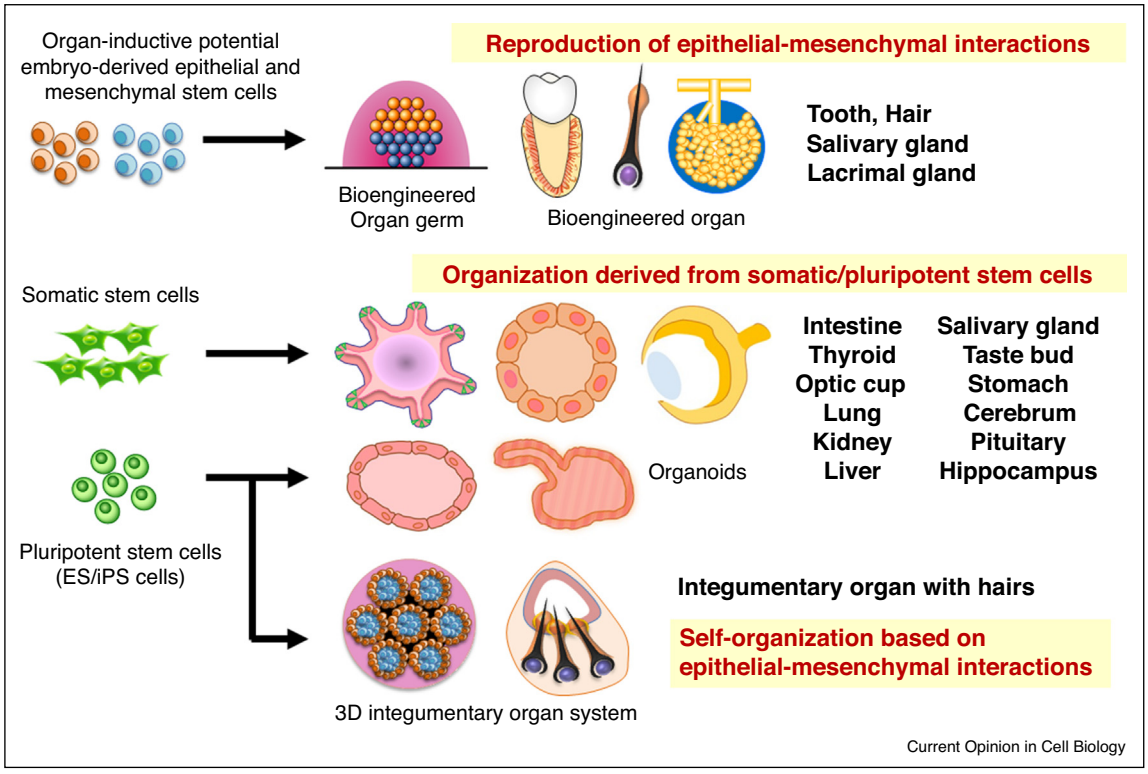
In this mini-review, we focused on the bioengineering technologies for fully functional regeneration of complex oral organs with the reconstruction of the 3D organ germ using completely dissociated organ-inductive potential embryo-derived-epithelial and mesenchymal stem cells. These bioengineering technologies can provide substantial advances in future organ replacement therapy.

3D tissue organization and therapeutic potential by organoid technology

An organoid model, which refers to 3D tissue structures containing various functionally differentiated cells

through self-organization from immature stem cells and isolated tissue fragments, is available for regenerative therapies through the replication of its tissue-specific stem cell niches [11,12]. Organoid studies are divided into two major approaches, reconstitution of bioengineered organ germ using organ-inductive potential embryo-derived and/or adult-derived epithelial and mesenchymal stem cells and organoid induction, which is repeated by organ induction processes during embryogenesis using pluripotent stem cells, including embryonic stem cells (ES cells) and induced pluripotent stem cells (iPS cells). We have developed an organ germ method to reconstitute bioengineered organ germs [13] and demonstrated proof of concepts for functional organ regeneration by orthotopic transplantation of the bioengineered germs, including the tooth [14,15,16••], salivary gland [17,18•], lachrymal gland [19] and hair follicles [20] (Figure 1). Recently, many researchers have reported organoid technologies that could generate 3D tissue structures such as the optic cup [2,21], pituitary epithelium [22,23], intestine [24,25], cerebrum [26,27], inner ear [28], lung [29••], and kidney [30] as mini-organs (Figure 1). Unique technologies to produce liver or other organoids using tissue engineering have also been reported, and this bioengineered organ-bud has a 3D multicellular structure with a microvascular network [31,32]. These reports revealed the important involvement of an intrinsic self-organization mechanism in the distinctive patterning of epithelial tissue architecture using signaling molecules to induce an organ-forming field and organogenesis according to a body plan. These organoid technologies have been suggested to have therapeutic potential and are assumed to be available as a disease model, for drug testing and as organ replacement therapy. As a disease model, gut organoids have already been used to examine infectious diseases [33], tumor biology [34], and genetic diversity [35]. Organoids also contribute to providing a regenerative approach as a source of autologous tissue for transplantation. Several studies have already succeeded in transplanting kidney organoids for renal dysfunction that could represent vascularization and functional substitution [36]. Although these technologies, including organoids, are considered an effective strategy for organ regeneration, problems exist regarding how these mini-organs will grow whole organs that are of sufficient size and have full functionalities for organ regenerative therapy.

Figure 1



3D bioengineering technology for organ regeneration. Current approaches in 3D organ regenerative technologies, such as organoid models, the bioengineered 3D integumentary organ system and the bioengineered organ germ method, are remarkable for their potential in future organ replacement therapy. These technologies attempt to use the available stem cell source, including somatic stem cells, organ inducible stem cells from embryonal tissue and pluripotent stem cells.

Functional whole-organ replacement technology in oral organs

3D bioengineering technology focused on the organoid model is considered an effective regenerative approach to restore partial organ function at local damaged sites. However, the organoid model reconstituting partial components of an organ has not yet achieved the ideal goal of regenerating complex organs that can recover from extensive organ injury or severe organ dysfunction. The ultimate goal of regenerative therapy is to develop organ replacement therapy that can replace lost or damaged organs through the orthotopic transplantation of fully functioning bioengineered organs [7]. The bioengineering technology to regenerate 3D complex organs has been established as an *in vitro* 3D cell manipulation method designated the 'Organ Germ Method' [13]. The most important breakthrough in this cell manipulation method is the achievement of 3D cell compartmentalization of immature epithelial and mesenchymal cells at a high cell density in collagen gel. This unique technology could achieve the precise replication of the developmental processes in organogenesis and organ-size regulation, adjusted by the cell-to-cell contact length between the epithelial and mesenchymal cell layers, thereby enabling the development of many types of bioengineered organ germs such as teeth, hair, salivary glands and lacrimal glands [13–15,17,19,20,37] (Figure 2a).

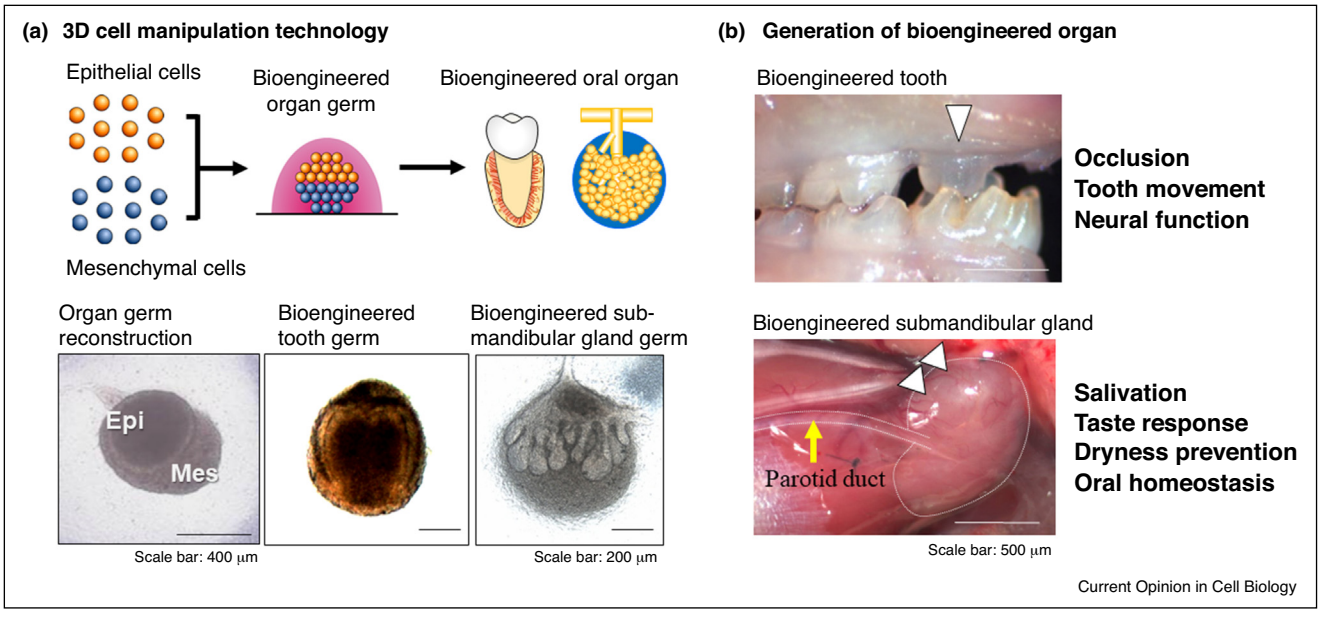
Functional tooth organ regeneration

Teeth have 3D multicellular structures involving distinctive hard tissues and soft connective tissues that can establish functional cooperation with the maxillofacial region and central nervous system [38]. In particular, teeth are strongly related to biological oral functions, including occlusion, pronunciation and facial aesthetics, supporting local/general health [7]. Conventional dental treatments using artificial materials, such as fixed dental bridges, removable dentures and dental implants, have been widely performed to restore oral function after tooth loss. Although these artificial therapies are mainly effective for occlusal restoration, further improvements based on biological requirements are expected to recover tooth physiological functions [7].

Challenges for tooth organ regeneration in the dental field

For the realization of tooth organ regeneration, many researchers have considered developing an *in vitro* 3D cell manipulation technology using immature epithelial and mesenchymal stem cells derived from tooth germs [7]. Tissue-engineering technology using scaffolds has contributed to a broad range of 3D tissue regeneration by seeding stem cells onto the biodegradable materials [39]. Previous reports, which used collagen/gelatin sponges or PLA/PLGA copolymers as scaffolds, have demonstrated

Figure 2



Functional oral organ replacement using the bioengineered organ germ method. **(a)** Bioengineered organ germs are reconstructed using appropriate cell compartmentalization and high-cell-density conditions in collagen gel using tooth germ or submandibular gland germ-derived epithelial and mesenchymal cells (upper panels). At 3–5 days of organ culture, bioengineered organ germ can develop similarly to natural organs *via* the reciprocal epithelial–mesenchymal interactions (lower panels). **(b)** The transplanted bioengineered tooth germ could be developed and reached the occlusal plane with the opposing lower first molar at 49 days after transplantation (arrowheads, upper panels). The transplanted bioengineered submandibular gland germ could be developed and engrafted into the natural parotid gland area at 30 days after transplantation (arrowheads, lower panels). These bioengineered oral organs could completely restore the respective organ functions.

the partial generation of tooth tissue structures through seeding epithelial and mesenchymal single cells isolated from porcine tooth germ [40,41]. The cell aggregation method is also known to be a typical 3D bioengineering technology for the reconstitution of bioengineered organ germ [42]. In previous studies, bioengineered cell aggregates mixed with epithelial and mesenchymal tooth germ cells could generate the correct tooth structure through self-rearrangement of epithelial and mesenchymal cells [43,44]. These technologies are considered useful methods for tooth organ regeneration; however, further improvements are required concerning the regulation of tooth structure and the frequency of tooth formation.

Functional tooth organ regeneration

To achieve functional tooth organ regeneration, the bioengineered tooth must be developed into a tooth loss region after orthotopic transplantation of the bioengineered tooth germ and restore the physiological tooth functions, including occlusal performance and biological cooperation with the periodontal ligament and afferent responsiveness to noxious stimulation [14]. Our bioengineered tooth has not only demonstrated successful engraftment into the tooth loss region but also performed an occlusal function with the opposing natural tooth. The bioengineered tooth had appropriate tooth hardness of the enamel and dentin tissue and had the potential to successfully restore masticatory performance [14,15]. Furthermore, the engrafted bioengineered tooth exerted physiological tooth functions, including tooth movement and neural function, in cooperation with the maxillofacial region in a mouse model [14,15] (Figure 2b). Recently, we demonstrated functional tooth replacement through the orthotopic transplantation of bioengineered tooth germ that was reconstructed using postnatal tooth germ cells in a large animal model. These studies represented a substantial advancement in functional tooth organ regeneration through the transplantation of bioengineered tooth germ as a practical model for future clinical regenerative medicine [16••].

Functional salivary gland organ regeneration

The salivary gland has characteristic 3D multicellular structures that are organized in the acini, myoepithelial cells and ducts for saliva emission. Salivary glands play essential roles in the protection of appendage oral organs and the maintenance of upper gastrointestinal tract function *via* serous and mucous saliva production [5]. Salivary gland impairment leads to xerostomia, which is a dysfunction of saliva secretion caused by aging, injury, Sjögren's syndrome and radiation therapy. Xerostomia involves fundamental oral/general problems, including dental decay, periodontal disease and swallowing dysfunction [5]. Although conventional treatments for xerostomia are mainly palliative approaches using artificial substitutes, a novel curable treatment based on a biological approach is required to recover the salivary gland functions [17,18•].

Attempts for salivary gland regeneration in previous studies

In the research field of salivary gland regeneration, many studies have traditionally been conducted using salivary gland-derived stem cells and biocompatible/biodegradable scaffolds, including collagen, fibrin, alginate, hyaluronic acid and PLGA, which could result in self-organization of 3D miniature tissues, termed salivary gland organoids [45–47]. Salivary gland organoids, which are generated by *in vitro* organoid culture following Wnt pathway activation through the addition of Wnt3A and R-Spondin, have demonstrated therapeutic potential for radiation-damaged salivary gland function with the saliva secretion and increase in functional acini *in vivo* [48,49]. Recently, it was reported that salivary gland function could be restored through *in vitro* self-renewal and organoid formation from human salivary gland stem cells [50]. Because irreversible xerostomia is caused by acinar cell damage and dysfunction related to post-radiation and aging, the development of organoid technology-based cell therapy is expected to realize salivary gland regeneration [51•].

Orthotopically functional salivary gland regeneration

For full regeneration of salivary gland impairment, bioengineered salivary glands must be able to restore the physiological secretory functions through functional replacement by orthotopic transplantation [18•]. Bioengineered salivary gland germ, which is generated by our Organ Germ Method, successfully underwent branching morphogenesis, followed by stalk elongation and cleft formation similar to the conventional 3D organoid method. To demonstrate successful salivary gland replacement therapy, a functional duct connection between the salivary gland ducts in the host and bioengineered salivary gland germ is critical for the differentiation of acinar formation and saliva secretion function [17]. Bioengineered salivary glands could be engrafted into the salivary gland defect model through successful duct connection and proper development of the acinar structure with myoepithelial encirclement and peripheral nervous innervation. Regenerated salivary glands could be demonstrated using the physiological secretory functions under afferent and efferent neuron network control in cooperation with the central nervous system [17,52] (Figure 2b). Bioengineered salivary glands could also be used to treat disorders resulting from salivary gland hypofunction, including dryness, bacterial infection and swallowing dysfunction. Our bioengineering technology for functional salivary gland regeneration represents a proof of concept for bioengineered secretory organ replacement therapy in the future [17,18•].

Future prospects for whole-organ regeneration of oral organs

Current progress in 3D organ regenerative technologies, including organoid models and the bioengineered organ

germ method, is remarkable regarding the potential in future organ replacement therapy [9•]. To address the clinical applications of complex oral organ replacement, organ-inducible stem cells, which can replicate epithelial–mesenchymal interactions in their respective organogenesis, must be identified from patients [7,18•]. Pluripotent iPS cells are considered candidate cell sources for oral organ regeneration and represent the potential to differentiate into dental epithelial or mesenchymal cells for the reconstitution of bioengineered organ germ [53,54]. In addition, notable recent research has demonstrated the bioengineered 3D integumentary organ system from iPS cells and includes appendage organs such as skin, hair follicles and sebaceous glands [55••]. This bioengineered 3D integumentary organ system also demonstrates the feasibility of available bioengineering technology in oral organs and the realization of whole-organ replacement therapy using iPS cells (Figure 1). Identically, morphological regulation of regenerated oral organs such as by size and shape is essential for full restoration of natural organ functions. Further studies are required to develop an *in vitro* bioengineering technology that can regulate organ morphology, including a 3D organoid model, tissue engineering using scaffolds and the utilization of morphogenesis-related molecules to achieve the appropriate organ morphogenesis [51•]. Complex oral organ replacement is now regarded as a viable model for studying future organ replacement therapies that can be applied to other complex organs, and it will contribute to developing 3D bioengineering technology in whole-organ regeneration [7,18•].

Conflict of interest

This work was partially funded by Organ Technologies, Inc. T. Tsuji is a director at Organ Technologies, Inc. This work was performed under an Invention Agreement between the Tokyo University of Science, RIKEN and Organ Technologies, Inc.

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This study successfully developed a novel *in vivo* transplantation model designated as a clustering-dependent embryoid body (CDB) transplantation method and generated a bioengineered 3D integumentary organ system, including appendage organs such as hair follicles and sebaceous glands, from induced pluripotent stem cells. This bioengineering technology has great potentials for application of 3D integumentary organ system that involves *in vitro* assay system, an animal model alternative, and a bioengineered organ replacement therapy.