

Trends in and Future of Regenerative Therapy

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Regenerative therapy is expected to be one of the novel clinical systems in the 21st century. In recent decades, stem cells have been identified in various tissues (Fig. E1 and E2 SC-A), contributing to the developing concept that our bodies are maintained throughout our lifetime by a system of stem cells. The approaches to creating the first generation of regenerative therapy have been influenced by our understanding of embryonic development, stem cell biology, and tissue engineering technology. In particular, the identification of hematopoietic stem cells and their successful clinical application in the treatment of leukemia have led to the concept of stem cell transplantation therapy. Indeed, in order to restore the partial loss of organ function, stem cell transplantation therapy has been developed as a cure for various diseases such as leukemia, Parkinson's disease, and cardiac infarction (Fig. E1 and E2 SC-A, A').

Identification of embryonic stem (ES) cells also provides a novel concept that these cells can be developed into various cell species and organs for regenerative therapy and investigation of the molecular functions of various genes *in vivo* using transgenic and knock-out mice (Fig. E2, SC-B). Furthermore, the development of induced pluripotent stem (iPS) cells has been a breakthrough not only for biological investigations, but also for medical application as a source of cells for future regenerative therapy (Fig. E2 SC-C). In the case of multipotent stem cells, such as ES cells (Fig. E2 SC-B) and iPS cells (Fig. E2 SC-C), the cells would ideally be manipulated by *in vitro* cell processing and then transplanted into the patient (Fig. E1). In particular, the utilization of iPS cells for the creation of bioengineered tissues and organ reconstitution will contribute to the future development of clinical systems for organ replacement regenerative therapy (Fig. E2, right-directed arrow and T-E, O-F).

The ultimate goal of regenerative therapy is to develop fully functioning bioengineered organs that can replace organs damaged by disease, injury, or aging (Fig. E1). This concept has essentially been demonstrated by various organ transplantations as treatments for injury and disease. It is expected that bioengineering technology will be developed that will allow the reconstruction of fully functional organs *in vitro*, constructed by the precise arrangement of several different cell species (Fig. E2 right). However, current bioengineering technologies have not yet achieved three dimensional reconstruction of fully functioning organs. To achieve the functional replacement of lost or damaged tissues and organs, three dimensional bioengineered tissues have been developed which are comprised of a single cell type and biodegradable materials (Fig. E2 T-B) and uniform

cell sheet (Fig. E2 T-A, C). These bioengineered tissues have been used clinically for the treatment of corneal dysfunction, myocardial infarction, and hepatic insufficiency using oral mucosal epithelial cells, myocardial cells and liver cells, respectively, with favorable clinical results. Continued use of these methods will systematically increase the relative technology level and its value for clinical applications (Fig. E2 T-D, E).

A novel concept has also been proposed to develop a bioengineered organ that properly reproduces the developmental process of organogenesis. Most organs arise from organ germ, which are influenced by reciprocal interactions between the epithelium and mesenchyme of developing embryos. Along these lines, the existence of organ-inductive stem cells in the adult body has been reported for hair follicles and the mammary gland (Fig. E2 O-A). However, most organs are induced by epithelial-mesenchymal interactions during embryo development and would not be induced by organ-inductive stem cells, which have yet to be identified yet in the adult. Furthermore, it was also reported that chimeric human organ was developed by the transplantation of human stem cells into an organ germ of rat developing embryo (Fig. E2 O-B). Therefore, it is expected that development of bioengineered technology to reconstitute organ germs between epithelial and mesenchymal cells *in vitro* will be required for adult organogenesis.

We have adopted tooth replacement regenerative therapy as a future organ replacement therapy. Tooth replacement regenerative therapy, which is also induced by the typical reciprocal epithelial and mesenchymal interactions, is thought to serve as a feasibility study for future clinical application of bioengineered organ replacement. The strategy for the development of a bioengineered third set of teeth after the loss of deciduous and permanent teeth utilizes the proper recapitulation of the processes that normally occur during embryonic development through the reconstitution of a bioengineered tooth germ *in vitro*. We have recently developed a novel three dimensional bioengineered organ germ method which can be applied to ectodermal organs such as the tooth and the whisker follicle (Fig. E2 O-C). However, it remains to be explored whether the bioengineered tooth can achieve fully functioning organ in applications such as masticatory performance, biomechanical cooperation with tissues in the oral and maxillofacial regions, and proper response to sensory receptor detection of noxious stimulations in the maxillofacial region. To date, there have been no reports of successful organ replacement with a bioengineered organ in the appropriate place.

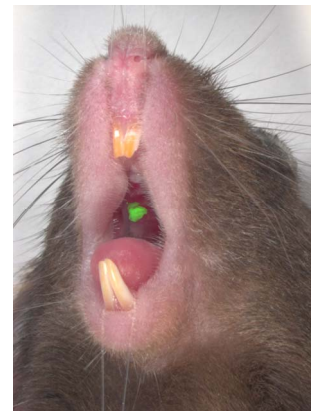


Figure 1 Erupted a bioengineered tooth in adult mouse transplantation model for a missing tooth.

In our recent study, we demonstrate the successful replacement of a fully functioning tooth through transplantation of bioengineered tooth germ into the alveolar bone of a lost tooth region in adult mice (Fig. 1). The bioengineered tooth, which erupted and reached occlusion in the oral environment (Fig. 2), had the correct tooth structure, hardness of mineralized tissues for mastication, and responses to experimental orthodontic treatment and noxious stimulations in cooperation with tissues in the oral and maxillofacial regions. These results significantly increase the current understanding of the potential to use bioengineered organ replacement in future regenerative therapies (Fig. E2 O-D). Further development of the technologies required to properly construct and culture fully functioning organs will contribute to the development of future systems for organ replacement therapy (Fig. E2 O-E). Furthermore, the identification of the proper seed cells for creation of a bioengineered tooth germ and the utilization of specific molecules with inductive potential will also provide valuable methods for application in future organ replacement therapies (Fig. E2 O-F).

Bioengineered tooth

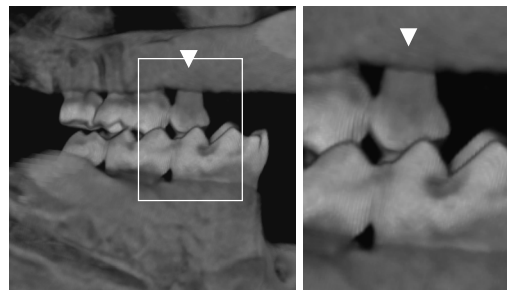
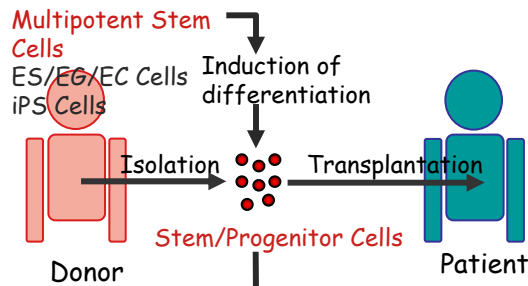


Figure 2 Occluded a bioengineered tooth analyzed by Micro-CT.

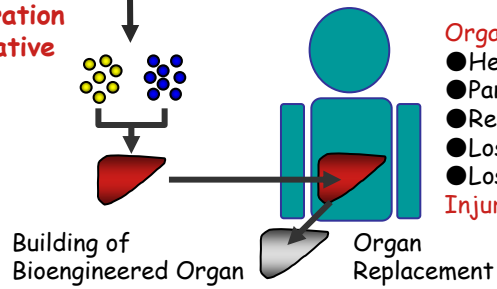
1st Generation of Regenerative Therapy



Stem/Progenitor Cells-Diseases

- Hematopoietic Stem Cells-Leukemia
- Neural Stem Cells-Parkinson disease
- Mesenchymal Stem cells-
Cardiac Infarction, Bone Diseases
- Hepatic Stem Cells
Hepatic Insufficiency, Hepatic cancer
- Pancreatic Cells-Diabetes
- Skin, Corneal Cells
(Cell Sheet transplantation)

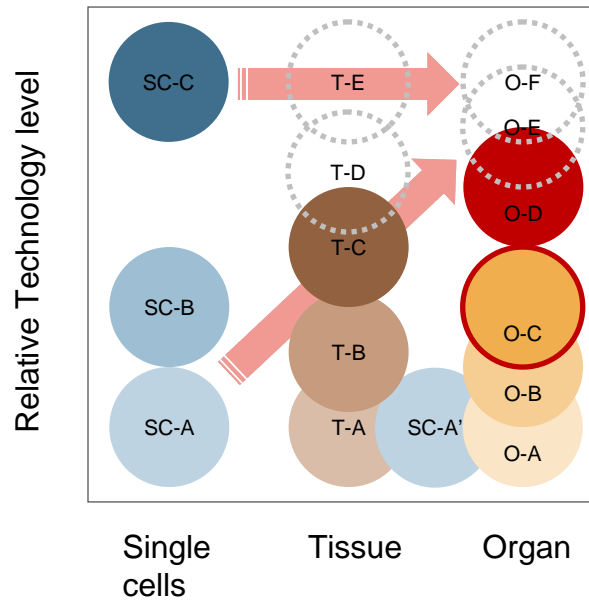
Next Generation of Regenerative Therapy



Organ Insufficiently, Diseases

- Hepatic Insufficiency
 - Pancreatic Insufficiency
 - Renal Insufficiency
 - Loss of Hair
 - Loss of Tooth
- Injury, Aging

Tsuji T. Fig. E1



SC-A: Isolation of tissue stem cells

SC-A': Stem cell transplantation therapy for the partial damaged organ

SC-B: Identification of embryonic stem cells

SC-C: Establishment of iPS cells from patient's own cells

Putative identification of pluripotent stem cells from patient's own cells

T-A: Two-dimensionally skin cell sheet by Green method

T-B: Three-dimensionally tissues using a biodegradable materials

T-C: Two/three dimensionally cell sheet engineering using stem cells

T-D: Three-dimensionally functional tissues (such as bone and cartilage)

T-E: **Putative three-dimensionally tissue engineering using an iPS cells**

O-A: Organ development using an organ-inductive stem cells

O-B: Chimeric organ using embryo or organ-deficient animals

O-C: Reconstitution of organ germ using epithelial and mesenchymal cells

O-D: Fully functioning organ replacement using a bioengineered organ germ

O-E: Putative fully functioning full-size organ replacement cultured *in vitro*

O-F: Putative fully functioning organ replacement using iPS and patient's own cells