Chapter 9

Whole Tooth Regeneration Using a Bioengineered Tooth

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1. Introduction

The tooth, which is an ectodermal organ whose development is regulated by reciprocal epithelial-mesenchymal interactions (Jussila et al., 2013), contributes to oral functions associated with mastication and enunciation, which are important aspects of general health and quality of life (Proffit et al., 2004). Teeth have a three-dimensional multicellular structure composed of characteristic hard tissues, *e.g.*, enamel, dentin, cementum and alveolar bone. Teeth also have soft connective tissues, such as pulp and periodontal ligaments, which contain nerve fibres and blood vessels that are important for maintaining tooth homeostasis (Avery, 2002). Dental caries, periodontal disease and trauma, which have high prevalence rates in dental disorders, cause fundamental problems for oral function and are associated with oral and general health issues (Proffit et al., 2004). To restore occlusal function after tooth loss, conventional dental treatments based on replacing teeth with artificial materials, such as fixed or removable dentures, have been established. Dental implants, which are able to stand alone in the jawbone without invading the adjacent teeth, have been used for the rehabilitation of tooth loss. Although these artificial therapies are widely applied to treat dental disorders, recent advances in tissue regeneration have been made that enhance the functions of the biological tooth, allow for underlying tooth movement through bone remodelling and aid the ability to perceive noxious stimuli (Huang et al., 2009). Substantial advances in the development of regenerative therapies have been driven by our understanding of embryonic development, stem cell biology and tissue engineering technologies (Yelick & Vacanti, 2006). Currently, an important concept in regenerative therapy is the transplantation of tissue-derived stem cells or *in vitro*-manipulated induced pluripotent stem (iPS) cells (Volponi et al., 2010). These therapies are attractive therapeutic concepts that have the potential to repair damaged tissues and restore the partial loss of organ function (Korbling & Estrov, 2003). In

dental medicine, tooth tissue-derived stem cells and the cytokine network that regulates tooth development have been well characterised at the molecular level (Jussila et al., 2013). These advances can be applied to the repair of dental pulp and periodontal tissues, including the alveolar bone (Egusa et al., 2012, 2013). Organ replacement regenerative therapy, which involves constructing a fully functional bioengineered organ using three-dimensional cell manipulation *in vitro*, holds great promise for the replacement of dysfunctional organs following disease, injury or aging. Tooth regenerative therapy would also involve the replacement of a lost or damaged tooth with a bioengineered tooth, constructed with stem cells, that has the capacity to become a functional unit comprising the whole tooth and periodontal tissue (Yen & Sharpe, 2008). It is anticipated that tooth replacement therapy will be established in the near future as a novel biological treatment for the functional recovery of lost teeth to satisfy both aesthetic and physiological requirements (Fig. 1). Over the past three decades, many approaches for replacing missing teeth have been studied, including three-dimensional bioengineered teeth and tooth germ generation using biodegradable materials and cell aggregation methods (Volponi et al., 2010). Recently, studies have reported tooth replacement by transplantation of fully functioning bioengineered teeth having the correct tooth structure, masticatory performance, proper responsiveness to mechanical stress and neural function after transplantation into the region of tooth loss (Ikeda et al., 2009; Nakao et al., 2007; Oshima et al., 2011). In this chapter, we describe novel technologies for whole tooth replacement that have the potential to provide functional recovery and could someday replace current dental treatments based on artificial materials.

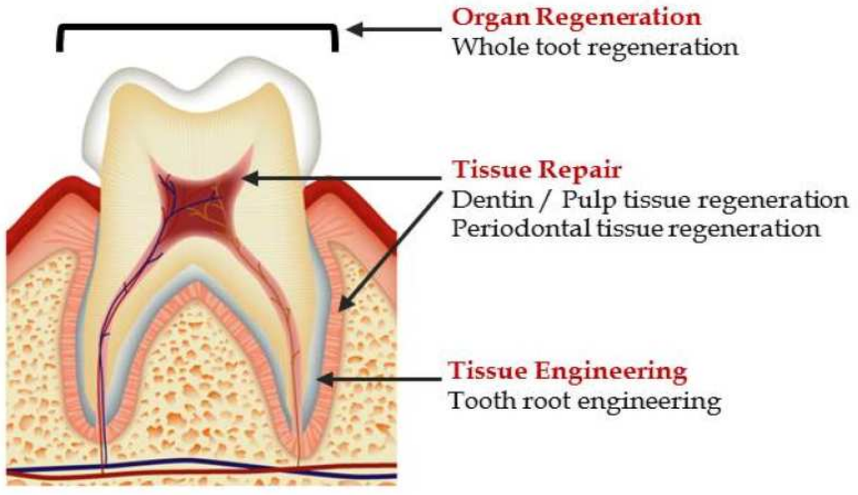

Figure 1. Concepts of tooth regenerative therapy

Recent approaches for tooth regenerative therapy have included tissue repair and whole tooth 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. The transplantation of dental stem cells has been examined for the treatment of dental caries, pulp injury and periodontal disease.

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2. The mechanisms of tooth development

Ectodermal organs, such as the teeth, hair and salivary glands, arise from their respective organ germs through reciprocal epithelial-mesenchymal interactions in the developing embryo. These interactions, which involve various signalling molecules and transcription factors, are the principal mechanism regulating organogenesis (Jussila et al., 2013). In tooth germ development, the dental lamina first thickens (lamina stage). This stage is followed by epithelial thickening (placode stage) at the future location of the tooth and subsequent epithelial budding to the underlying neural crest-derived ecto-mesenchyme. Tooth germ formation is initiated on embryonic days (EDs) 10-11 in mice by epithelial signals that include fibroblast growth factor (FGF) 8, bone morphogenetic protein (BMP) 4, sonic hedgehog (Shh), tumour necrosis factor (TNF) and Wnt10b. These signals induce the expression of several transcription factors in the dental mesenchyme that condense around the developing epithelial bud (bud stage) (Jussila et al., 2013; O'connell et al., 2013). At ED13.5-14.5, the first enamel knot, which acts as a signalling centre to orchestrate tooth development by controlling the gene expression of various signalling molecules and transcription factors, is formed in the dental epithelium (cap stage). At ED16-18, the epithelial and mesenchymal cells in the tooth germ terminally differentiate into the tooth-tissue progenitor cells, such as ameloblasts, odontoblasts, and dental follicle cells (bell stage). Ameloblasts and odontoblasts accumulate the enamel and dentin matrix, respectively, at the boundary surface between the epithelium and mesenchyme, while dental follicle cells differentiate into the periodontal tissues, which include the cementum, periodontal ligaments and alveolar bone (Avery, 2002).

3. A novel three-dimensional cell manipulation method for whole tooth regeneration

One current biological approach for the regeneration of three-dimensional organs is based on recapitulating organogenesis by mimicking the reciprocal epithelial-mesenchymal interactions that occur in the developing embryo, thereby developing fully functional bioengineered organs from a bioengineered organ germ generated from immature stem cells via three-dimensional cell manipulation *in vitro*. For tooth regeneration, one proposed concept has been to transplant a bioengineered tooth germ into the recipient jaw and allowing it to develop into a functional mature tooth *in situ* (Fig. 2, upper). It is also expected that it will be possible to transplant a bioengineered tooth unit that includes mature tooth, periodontal ligament and alveolar bone, which will achieve biological engraftment through bone integration with the recipient's jaw (Fig. 2, lower).

To realise whole tooth replacement, the first critical issue is to develop a three-dimensional cell manipulation method using completely dissociated epithelial and mesenchymal cells *in vitro*. Previously, it has been reported that using a polyglycolic acid and poly-L-lactate-co-glycolide copolymer (PLA/PLGA) or a collagen sponge as a tooth-shaped scaffold and seeding them with epithelial and mesenchymal cells isolated from tooth buds could generate small tooth structures (Honda et al., 2007; Yelick & Vacanti, 2006).

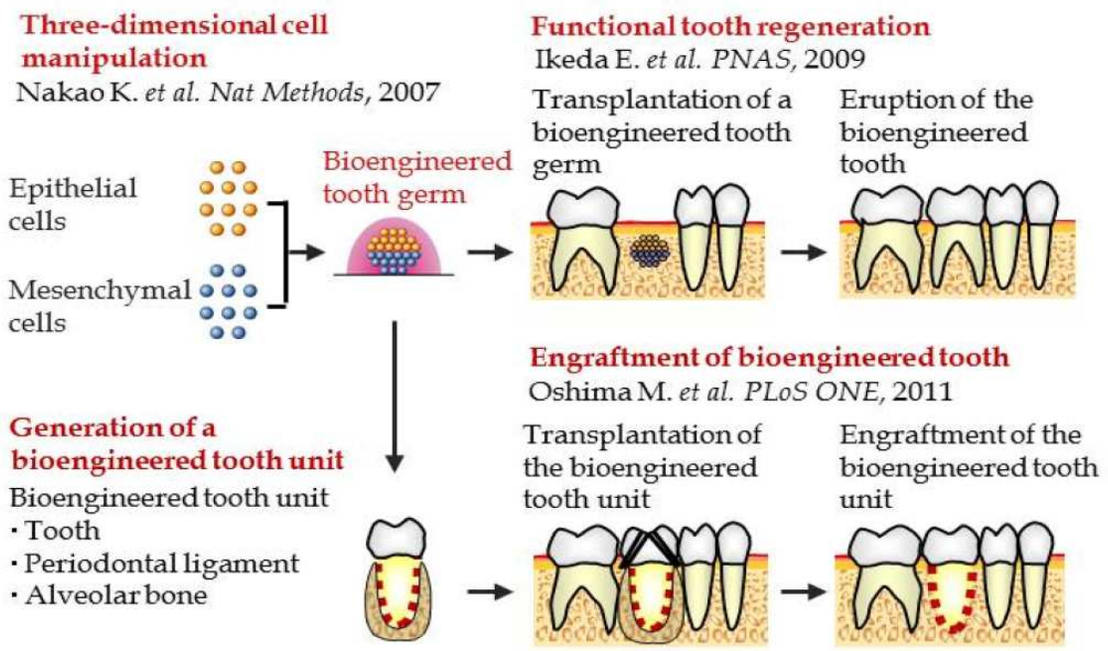

Figure 2. Strategies for whole tooth replacement via regenerative therapies. Functioning teeth can now be regenerated *in vivo* by transplanting bioengineered tooth germ generated from epithelial and mesenchymal cells via the organ germ method, or bioengineered tooth units with periodontal ligament and alveolar bone developed from bioengineered tooth germ.

In addition, the cell aggregation method, which aims to reconstitute a bioengineered organ germ, has been applied for the transplantation of cell aggregates constructed from dental epithelial and mesenchymal cells, and it has been reported that this approach can generate appropriate tooth formation (Hu et al., 2006). It has also been reported that mixed cell aggregates of tooth germ-derived epithelial and mesenchymal cells can develop into a tooth with the correct structure, following epithelial cell sorting and subsequent self-organisation of the epithelial and mesenchymal cells (Song et al., 2006). However, these approaches suffer from critical limitations, including a low frequency of tooth formation and irregularity of the resulting tooth tissue structures, for example with enamel-dentin complex formation and the arrangements of the ameloblast/odontoblast cell lineages.

To achieve precise replication of the processes in organogenesis, an *in vitro* three-dimensional novel cell manipulation method designated as the bioengineered organ germ method has been developed (Nakao et al., 2007). This innovative method is based on compartmentalisation of the epithelial and mesenchymal cells at a high-cell density in a type I collagen gel (Fig. 3A). Bioengineered tooth germ created by this technique, which could allow for large-scale organ development, mimics the multicellular assembly underlying epithelial-mesenchymal interactions during natural tooth development. This bioengineered tooth germ generates a correct tooth structure after transplantation in an organ culture *in vitro* as well as following placement into a subrenal capsule *in vivo*. The bioengineered tooth germ generated by this method was also found to develop in the oral cavity to form the proper tooth structure (Nakao et al., 2007). Furthermore, this unique technology can successfully generate a size-controlled

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bioengineered tooth unit comprising a mature tooth, periodontal ligament and alveolar bone after transplantation into the subrenal capsule (Fig. 3B). 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 therapy.

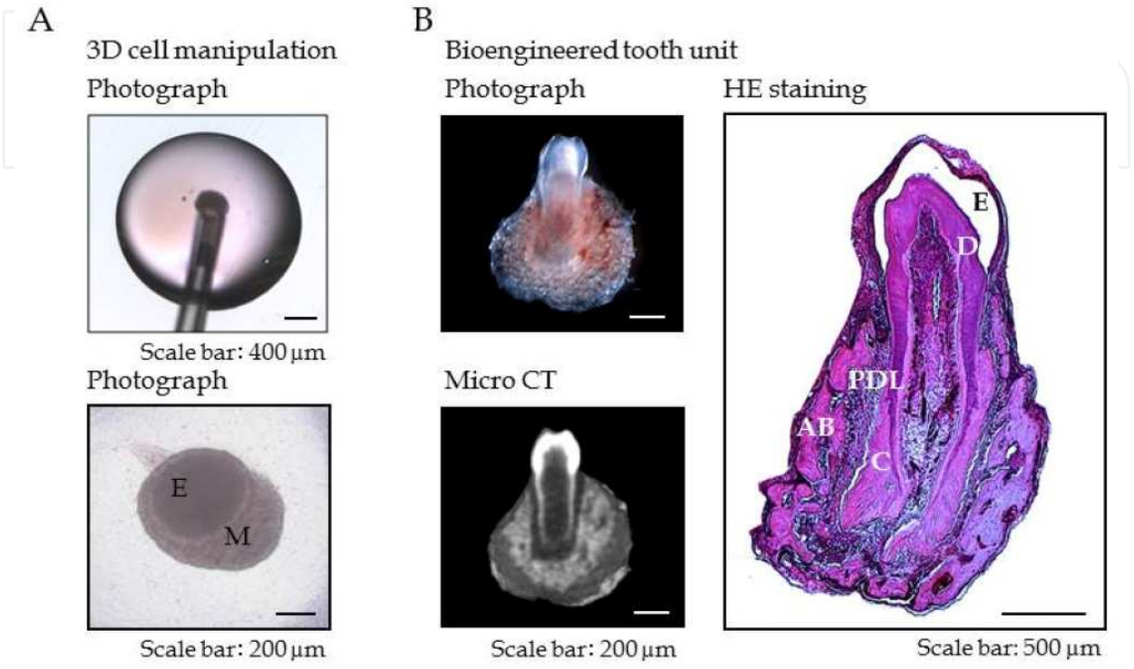

Figure 3. The organ germ method: three-dimensional cell processing A) Dissociated mesenchymal cells at a high density are injected into the centre of a collagen drop. Dissociated tooth germ-derived epithelial cells are subsequently injected into the drop adjacent to the mesenchymal cell aggregate (upper). Within 1 day of organ culture, bioengineered tooth germ formation with appropriate compartmentalisation between epithelial and mesenchymal cells and cell-to-cell compaction was observed (lower). B) By transplanting a bioengineered tooth germ into a subrenal capsule for 30 days (left panel), a bioengineered tooth unit comprising a mature tooth with the correct structural components such as enamel (E), dentin (D), periodontal ligament (PDL) and alveolar bone (AB) can be produced (right panel).

4. Functional tooth replacement therapy

Oral functions such as mastication, pronunciation, and facial aesthetics have an important influence on quality of life because they facilitate both oral communication and nutritional intake. These functions are achieved with the teeth, masticatory muscles and the temporomandibular joint, under control of the central nervous system. For the realisation of tooth replacement regenerative therapy, a regenerated tooth developing from bioengineered germ tissue or a transplanted bioengineered mature tooth unit must be capable of properly engrafting into the lost tooth region in an adult oral environment and acquiring full functionality, including sufficient masticatory performance, biochemical cooperation with periodontal tissues and afferent responsiveness to noxious stimulations in the maxillofacial region (Proffit et al., 2004).

4.1. Transplantation of bioengineered tooth germ or a bioengineered mature tooth unit as a tooth replacement therapy

The critical issue dictating the success of tooth regenerative therapy via the transplantation of bioengineered tooth germ tissue into the lost tooth region is whether the germ can erupt and occlude properly with the opposing tooth in an adult oral environment. It has previously been demonstrated that transplanted natural tooth germ erupts in a murine toothless diastema region (Ohazama et al., 2004). We have also reported that a bioengineered tooth germ can develop the correct tooth structure in an oral cavity and successfully erupt 37 days after transplantation (Ikeda et al., 2009). The bioengineered tooth subsequently reached the occlusal plane and achieved occlusion with the opposing tooth from 49 days onwards (Fig. 4A, B). In the case of a transplanted bioengineered mature tooth unit comprising mature tooth, periodontal ligament and alveolar bone, the most critical consideration is whether that unit can be engrafted into the tooth loss region through bone integration, which involves natural bone remodelling in the recipient. A bioengineered tooth unit transplanted at a position reaching the occlusal plane with the opposing upper first molar was successfully engrafted after 40 days and thereafter maintained the periodontal ligament originating from the bioengineered tooth unit through successful bone integration (Fig. 4C) (Oshima et al., 2011). The enamel and dentin hardness of the bioengineered tooth components were in the normal range when analysed by the Knoop hardness test (Ikeda et al., 2009; Oshima et al., 2011). These approaches demonstrate the potential to successfully recover masticatory performance and natural tooth tissue through state-of-the-art bioengineering technology.

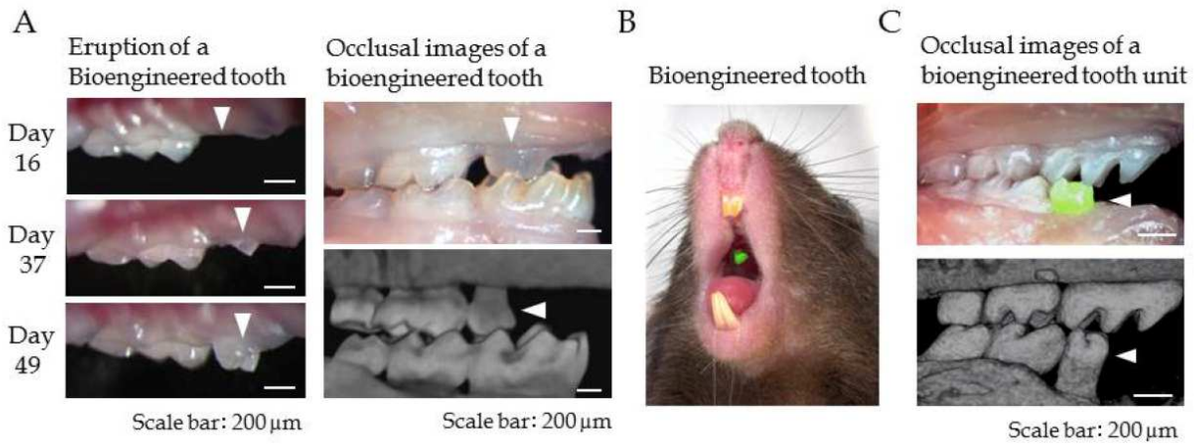

Figure 4. Regeneration of a bioengineered tooth in an adult oral environment A) A transplanted bioengineered tooth germ erupted and reached the occlusal plane with the opposing lower first molar 49 days after transplantation. B) GFP-labelled bioengineered tooth (right panel) erupted in the oral environment of adult mice. C) A bioengineered tooth unit was engrafted by bone integration and reached the occlusal plane with the opposing upper first molar at 40 days post transplantation.

4.2. Biological response of bioengineered teeth to mechanical stress

Biological oral functions require cooperation between teeth and the maxillofacial region through the connection of periodontal ligaments (Dawson, 2006). Tooth loss and periodontal disease cause fundamental problems for oral function, including mastication, as well as associated health issues. The periodontal ligament plays an essential role in the pathogenic and physiological tooth response to extreme mechanical forces from bone remodelling accompanied by orthodontic tooth movement (Proffit et al., 2004). Studies on autologous tooth transplantation have indicated that healthy periodontal tissue remaining on the tooth root can successfully restore physiological tooth function, including bone remodelling, and effectively prevent ankylosis. In contrast, the absence of a periodontal ligament in osseointegrated dental implants is associated with deficiencies in essential tooth functions and in the natural structural relationship between the tooth root and alveolar bone (Dawson, 2006). The periodontal ligament of bioengineered teeth that erupted following the transplantation of bioengineered tooth germ and mature tooth units achieved functional tooth movement comparable with that of natural teeth. Bioengineered teeth also successfully underwent bone remodelling in response to mechanical stress via the proper localisation of osteoclasts and osteoblasts, indicating that a bioengineered tooth can reproduce critical tooth functions by restoring and re-establishing cooperation with the surrounding jawbone (Ikeda et al., 2009; Oshima et al., 2011).

4.3. Perceptive neuronal potential of bioengineered teeth

The peripheral nervous system is established by the growth of axons that navigate and establish connections with developing target organs during embryogenesis (Guyton & Hall, 2000). The perceptive potential for noxious stimulation, including mechanical stress and pain, is important for proper organ function (Guyton & Hall, 2000). Additionally, it is believed that the recovery of the nervous system, which requires the re-entry of nerve fibres following organ transplantation, is critical for reconstituting organ function. Teeth are a peripheral organ for sensory and sympathetic nerves, both of which play important roles in tooth function and protection (Dawson, 2006). It is anticipated that tooth regenerative therapies will be able to recover the neuronal ability related to the perception of mechanical forces that are lacking in implant patients. Importantly, sensory and sympathetic nerve fibres innervate both the pulp and periodontal ligament of a bioengineered tooth following its eruption (Ikeda et al., 2009). Thus, these bioengineered teeth possess appropriate perceptive potential for nociceptive pain stimulations, such as pulp injury and orthodontic treatment, and can properly transduce these events to the central nervous system through c-Fos immunoreactive neurons (Ikeda et al., 2009; Oshima et al., 2011). In this way, bioengineered teeth can indeed restore the perceptive potential for noxious stimuli in cooperation with the maxillofacial region.

5. Future directions for tooth regeneration

To realise the use of tooth regenerative therapy in future clinical applications, one of the major research hurdles remaining is the identification of appropriate cell sources. The cell source may be optimised by using the patient's own cells for regenerative therapy to avoid immunological rejection. Tooth tissue-derived stem cells found in pulp and periodontal ligaments can differentiate into dental cell lineages and contribute to the supply of various progenitor cells (Egusa et al., 2012, 2013). While these tissues are good candidate cell sources for stem cell transplantation therapy for tooth tissue repair, epithelial-mesenchymal interaction driven tooth inductive potential has not been reported for these stem cells. Other candidate cell sources for whole tooth regeneration include embryonic stem (ES) cells and iPS cells, which are capable of differentiating into endoderm, ectoderm and mesoderm (Takahashi et al., 2006). Recently, iPS cells have been established from various oral tissues, and reprogramming procedures for dental epithelial and mesenchymal fates have been established (Arakaki et al., 2013; Otsu et al., 2013). Another important direction for future research on tooth regenerative therapies is the identification of key factors for reprogramming non-dental cells into dental epithelium and mesenchyme. Notably, the self-organisation of various tissues such as the optic cup and adenohypophysis using uniform pluripotent stem cells in three-dimensional culture has been reported (Eiraku et al., 2011; Suga et al., 2011). A three-dimensional *in vitro* organogenesis system using appropriately induced stem cells will be essential for the regenerative replacement of whole teeth and other organs (Sasai et al., 2013). These approaches will contribute to the realisation of future tooth replacement regenerative therapies.

6. Conclusion

The technology of regenerative medicine has progressed remarkably, and many patients and clinicians are anticipating the realisation of whole tooth regenerative therapy. Tooth regenerative therapy is now regarded as a crucial model for future organ replacement regenerative therapies for severe diseases and will contribute substantially to the understanding of tissue regeneration for more complex organs.

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