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Review Article

Dentistry Section

# Test Tube Tooth: The Next Big Thing

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## ABSTRACT

Unlike some vertebrates and fishes, humans do not have the capacity for tooth regeneration after the loss of permanent teeth. Although artificial replacement with removable dentures, fixed prosthesis and implants is possible through advances in the field of prosthetic dentistry, it would be ideal to recreate a third set of natural teeth to replace lost dentition. For many years now, researchers in the field of tissue engineering have been trying to bioengineer dental tissues as well as whole teeth. In order to attain a whole tooth through dental engineering, that has the same or nearly same biological, mechanical and physical properties of a natural tooth, it's necessary to deal with all the cells and tissues which are concerned with the formation, maintenance and repair of the tooth. In this article we review the steps involved in odontogenesis or organogenesis of a tooth and progress in the bioengineering of a whole tooth.

**Keywords:** Bioengineering, Odontogenesis, Regeneration, Stem cells

## INTRODUCTION

The goal of regenerative medicine is to regenerate fully functional tissues or organs that can replace lost or damaged ones that resulted due to diseases, injury and ageing. It has been theorized that a functional bioengineered organ could be produced by reconstituting organ germs between epithelial and mesenchymal cells invitro, although the existence of organ-inductive stem cells in the adult body has not been fully achieved yet with the exception of hair follicles and the mammary gland [1,2]. As almost every organ originates from an organ germ, which is instigated by reciprocative interaction between the epithelium and mesenchyme in the developing embryo, it is only advisable to fully reconstitute these events to develop a bioengineered tooth. For more than three decades now, several approaches for successful replacement of missing teeth have been studied; these include three-dimensional bioengineered teeth and tooth germ generation using biodegradable materials and cell aggregation techniques [3]. Some studies in the last few years have reported tooth replacement by transplantation of fully functioning bioengineered teeth having the right tooth structure, masticatory performance, correct responsiveness to mechanical stress and neural function after transplantation into the edentulous area [4-6]. These findings indicate that bioengineered tooth generation techniques can contribute to the rebuilding of a fully functional tooth. In this article we review the current knowledge about the mechanisms involved in tooth morphogenesis and replacement.

**Organogenesis of tooth/Odontogenesis:** Tooth development relies on reciprocal tissue interactions between ectoderm-derived dental epithelium and cranial neural crest-derived mesenchyme [7]. It has been proven now that without epithelial cells, the mesenchymal cells differentiate into bones, and if mesenchymal cells are not present, the epithelial cells develop cornified structures when implanted under the kidney capsule [8]. At the initiation of tooth development, the epithelium provides the first instructional signals to the mesenchyme [9-11]. The mesenchyme then sends the signals back to the epithelum. To bioengineer a tooth primordium from non-embryonic tooth cells, either the epithelial or mesenchymal cells, should have the capability to provide these inductive signals to one another. For this reason two populations of stem cells need to be considered in the development of the tooth: Epithelial Stem Cells (EpSC) and Mesenchymal Stem Cells (MSC), EpSCs differentiate into ameloblasts whereas MSCs give rise to odontoblasts, cementoblasts, osteoblasts and fibroblasts of the periodontal ligament. At least five types of human postnatal mesenchymal stem cells of dental origin have been isolated so far [12], including Dental Pulp Stem Cells (DPSCs), Stem Cells from Exfoliated Deciduous Teeth (SHED), periodontal ligament stem cells (PDLSCs), Dental Follicle Progenitor Cells (DFPCs), and stem cells from the apical papilla (SCAP). Because these dental stem cells are derived from the neural crest, there origin is different from bone marrow-derived MSCs, which are derived from the mesoderm. On the contrary, epithelial stem cells have not been found in postnatal dental tissues. Till now, the existence of epithelial stem cells of dental origin was not considered a possibility, because ameloblasts are lost via apoptosis upon tooth eruption [13]. However Shinmura et al., recently demonstrated that at the stage of crown formation, subcultured epithelial cell rest of malassez (ERM) can differentiate into ameloblast-like cells and generate enamel-like tissues together with dental pulp cells. Once subcultured ERM were combined with subcultured dental pulp cells, ERM expressed cytokeratin and amelogenin proteins invitro. Additionally, eight weeks after the transplantation, subcultured ERM in conjunction with primary dental pulp cells seeded onto scaffolds showed enamel-like tissues. Simultaneously enamel-like tissues showed positive staining for amelogenin, indicating the presence of well-developed ameloblasts in the implants [14].

The odontogenic potential means the capability of a tissue to induce gene expression in an adjoining tissue and to commence tooth genesis. On the opposite hand, the odontogenic competence means the capability of a tissue to reciprocate to odontogenic signals and to support tooth genesis [7]. In the previous studies involving the dental epithelium and mesenchymal tissues of a mouse molar tooth, it has been established that the odontogenic potential is present initially in the dental epithelium and in the later stages it shifts to the mesenchyme [15,16]. Before and at the embryonic day 11.5, the potential to induce tooth formation resides in the presumptive dental epithelium, if it is combined with non-dental mesenchyme. However, only the mesenchyme derived from the cranial neural crest is capable of odontogenic competence. Experiments that involved recombining dental epithelium with non-neural crest derived mesenchyme were unable to create teeth [16].

It has been indicated by some studies that the epithelium derived from the first branchial arch when recombined with neural crest derived cells from second branchial arch mesenchyme or with premigratory trunk neural crest can induce formation of tooth [15,16]. This capability to instruct tooth formation in the epithelium is, however, lost by embryonic day 12, and the genetic information subsequently shifts to the mesenchymal component. Thus the dental mesenchyme from mouse embryonic cap and bell stage teeth can instruct tooth development when combined with non-dental epithelium [17,18].

Growth factors that mediate inductive interactions during Odontogenesis: Formation of a tooth is a step-wise procedure where mutual and subsequent interactions between compartments coordinate advancing morphogenesis and cell differentiation. The reciprocal action between the epithelium and the mesenchyme is controlled by an elaborate series of signal pathways and mediators. The conserved signal pathways mediating these interactions include the Transforming Growth Factor Beta (TGFß), Bone Morphogenetic Protein (BMP), Wingless-type MMTV integration site family member (Wnt), Fibroblast Growth Factor (FGF), Hedgehog and Eda (Ectodysplasin, a TNF signal) pathways and they are used repeatedly during subsequent tooth formation stages [7]. The expunction of gene function of necessitous components of these pathways often leads to errors in tooth formation including complete arrest of tooth formation [19]. Tooth development is also influenced by the presence of multiple modulators of the signal pathways. For example, bone morphogenetic protein inhibitors such as Follistatin and Ectodin/Sostdc1 [20,21] and of Fibroblast Growth Factors such as Sprouty [22] are required for development of the precise tooth number, accurate shape and optimal hard tissue production thus underlining the key role of fine-tuning in regulatory control. In addition to multiple signal molecules, their receptors and inhibitors, several transcription factors are known which are specifically expressed in the early tooth mesenchyme. These include Muscle specific Homeobox 1, 2 (Msx1,2), Distal-less gene 1, 2, 5 (Dlx1, 2, 5), Runt related transcription factor 2 (Runx2), Paired Box Gene 9 (Pax9), Lymphoid Enhancing Binding Factor 1 (Lef1), Glioma-associated oncogene homologue 1, 2, 3 (Gli1, 2, 3), LIM Homeobox Gene 6, 7, 8 (Lhx6, 7, 8), Peroxiredoxin gene 1,2 (Prx1, 2). The deletion of the function of any of these transcription factors leads to in most of the cases, a complete arrest of tooth development either prior to placode formation or before morphogenesis from bud to cap stage [19,23].

## DISCUSSION

Vital issues that need to be considered while engineering a tooth are whether the bioengineered tooth is capable of functions such as mastication [24] and it's capability to withstand mechanical stress [25,26] and noxious stimulations [27]. Eruption of the tooth in the oral cavity and occlusion are important first steps toward tooth genesis and successful incorporation into the oral and maxillofacial region [28,29].

In order to successfully create a bioengineered tooth that is in harmony with oral and maxillofacial region it is of paramount importance that it has an interaction between the alveolar bone which further requires successful formation of periodontal ligament [25,26]. Ohazama et al., were the first one to report the formation of a biotooth using adult non-dental cells from recombination between embryonic tooth epithelium and adult bone marrow stromal cells [30]. Their study showed that recombination between mesenchyme created invitro (by aggregation of non-dental cultured cells from different stem cell sources) and embryonic oral epithelium stimulated an odontogenic response in the mesenchyme, and that when such explants were transferred intact into adult renal capsules, they developed into teeth (crowns) with associated bone and soft tissues.

Angelova Volponi A et al., successfully demonstrated tooth formation on recombination between mouse embryonic tooth-inducing mesenchyme cells with isolated and cultured human adult epithelial cells from the oral mucosa [3]. Another study reported the establishment of clonal cell lines when dental mesenchyme at embryonic day 16.5 were recombined with oral epithelium of p53 deficient fetal mice at embryonic day 18. Further these clonal cell lines formed calcified tooth structures as seen in natural teeth [31]. In a similar study by Komine A et al., clonal cell lines established from dental epithelium of a molar tooth germ were able to generate well calcified teeth [32].

One major limitation in tissue engineering is the inability to develop a complete organ at its normal location in the adult body following transplantation of an embryonic primordium. Ikeda E et al., transplanted a bioengineered tooth germ into the alveolar bone of a lost tooth in a murine model. The transplanted tooth developed into a completely functional tooth that possessed adequate hardness for mastication and responded well to mechanical stress. The neural fibers that re-entered the pulp and periodontal ligament tissues of the bioengineered tooth showed positive perceptive potential in response to noxious stimulations such as orthodontic treatment and pulp stimulation [4]. Nakao K et al., in their experiment dissociated epithelium and mesenchyme from murine cap stage tooth germs to single cells and reaggregated them. These cells were then grown invitro to allow early morphogenesis, and were implanted to the jaw of the mouse, where they developed into a nearly normal tooth [5]. With this method, a correct tooth structure comprising enamel, dentin, root, dental pulp and bone could be observed, showing penetration of blood vessels and nerve fibers. This is a major advancement over artificial dental implants as it enables the tooth to recover neuronal ability related to the perception of mechanical forces [33]. However, the reconstituted pellets of dental mesenchymal and epithelial cells gave rise to multiple tooth germs invitro indicating that the control of the number of teeth developing from dissociated dental cells may present a problem. Ohazama A et al., in their study showed that transfer of embryonic tooth primordia into the adult mouse jaw resulted in complete tooth development, showing that an embryonic primordium can develop in its adult environment [30]. Duailibi MT et al., have demonstrated the formation of bioengineered tooth crown with normal soft and hard tissues by culturing rat tooth bud cells which were seeded onto biodegradable scaffolds and then implanted into the jaws of adult mouse [34]. Basic requirements that are relevant to construct tissues; include a rich source of progenitor cells and a scaffold which is conducive to cell attachment and maintenance of cell function. The function of the scaffold is to assist in the biosynthesis, proliferation and differentiation of cells and also to prevent any disturbing cells from invading into the site of implantation. Calcium orthophosphate scaffolds are porous ceramics which have a reasonable surface roughness to facilitate cell seeding, sufficient mechanical strength, high porosity and possess properties like biodegradability/ bioresorbability so that it breaks down leaving the newly formed tissue to take over it's place [35].

## CONCLUSION

Current methods to repair and/or replace dental tissues include dental implants, FPD and removable partial dentures. However successfully fabricated, these methods utilize synthetic materials whose biological, physical and mechanical properties are quite different from that of natural tooth. However, with the current research and advancement going on in the field of bioengineering, artificial tooth genesis seems to be an achievable and realistic option for replacement of lost tooth. The recent demonstration of bioengineered whole tooth crowns from pig and rat tooth bud cells provide promising evidence that regeneration of a whole tooth is achievable in the near future. The advent of porous ceramics has opened up new horizons in artificial tooth development. Although,

there are many hurdles that need to be crossed before successful regeneration and implantation of a whole human tooth, fast progress in molecular biology and advances in bioengineering will soon facilitate realization of bioengineered implantable tooth.

## REFERENCES


[1] Claudinot S, Nicolas M, Oshima H, Rochat A, Barrandon Y. Long-term renewal of hair follicles from clonogenic multipotent stem cells. *Proc Natl Acad Sci USA*. 2005;102 (41):14677–82.
[2] Shackleton M, Vaillant F, Simpson KJ, Stingl J, Smyth GK, Asselin-Labat ML, et al. Generation of a functional mammary gland from a single stem cell. *Nature*. 2006;439(7072):84–88.
[3] Angelova Volponi A, Kawasaki M, Sharpe PT. Adult Human Gingival Epithelial Cells as a Source for Whole-tooth Bioengineering. *J Dent Res*. 2013;92(4): 329-34.
[4] Ikeda E, Morita R, Nakao K, Ishida K, Nakamura T, Takano-Yamamoto T, et al. Fully functional bioengineered tooth replacement as an organ replacement therapy. *Proc Natl Acad Sci USA*. 2009;106(32):13475-80.
[5] Nakao K, Morita R, Saji Y, Ishida K, Tomita Y, Ogawa M, et al. The development of a bioengineered organ germ method. N*at Methods*. 2007;4(3):227–30.
[6] Oshima M, Mizuno M, Imamura A, Ogawa M, Nakao K, Yamazaki H, et al. Functional tooth regeneration using a bioengineered tooth unit as a mature organ replacement therapy. *PLoS One.* 2011;6(7):e21531.
[7] Zhang Y, Chen Z, Song YQ, Liu C, Chen Y. Making a tooth: growth factors, transcription factors and stem cells. *Cell Res*. 2005;15:301–16.
[8] Kollar EJ, Baird GR. The influence of the dental papilla on the development of tooth shape in embryonic mouse tooth germs. *J Embryol Exp Morph*. 1969;21(1):131–48.
[9] Thesleff I, Kera¨nen S, Jernvall J. Enamel knots as signaling centers linking tooth morphogenesis and odontoblast differentiation. *Adv Dent Res*. 2001;15:14–18.
[10] Hu B, Nadiri A, Bopp-Kuchler S, Perrin-Schmitt F, Lesot H. Dental epithelial histomorphogenesis invitro. *J Dent Res*. 2005;84:521–25.
[11] Lesot H, Brook AH. Epithelial histogenesis during tooth development. *Arch Oral Biol.* 2009;54 (Suppl1):S25-33.
[12] Huang GTJ, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources. Their biology and role in regenerative medicine. *J Dent Res*. 2009;88(9):792–806.
[13] Abiko Y, Nishimura M, Arai J, Kuraguchi J, Saitoh M, Kaku T. Apoptosis in the reduced enamel epithelium just after tooth emergence in rats: In situ labeling of Nuclear DNA Fragmentation and electron Microscopic Studies. *Med Electron Microsc*. 1996;29(2):84–89.
[14] Shinmura Y, Tsuchiya S, Hata K, Honda MJ. Quiescent epithelial cell rests of Malassez can differentiate into ameloblast-like cells. *J Cell Physiol*. 2008;217(3): 728-38.
[15] Mina M, Kollar EJ. The induction of odontogenesis in nondental mesenchyme combined with early murine mandibular arch epithelium. *Arch Oral Biol*. 1987;32(2):123-27.
[16] Lumsden AG. Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. *Development*. 1988;103 suppl:155-69.
[17] Kollar EJ, Baird GR. Tissue interactions in embryonic mouse tooth germs. 1. Reorganization of dental epithelium during tooth-germ reconstruction. *J Embryol Exp Morphol.* 1970;24:159–71.
[18] Kollar EJ, Baird GR. Tissue interactions in embryonic mouse tooth germs. 2. Inductive role of dental papilla. *J Embryol Exp Morphol*. 1970;24:173–86.
[19] Thesleff I. Epithelial-mesenchymal signaling regulating tooth morphogenesis. *J Cell Sci.* 2003;116:1647–48.
[20] Wang XP, Suomalainen M, Jorgez CJ, Matzuk MM, Werner S, Thesleff I. Follistatin regulates enamel patterning in mouse incisors by asymmetrically inhibiting BMP signaling and ameloblast differentiation. *Developmental Cell*. 2004;7(5):719–30.
[21] Kassai Y, Munne P, Hotta Y, Penttila E, Kavanagh K, Ohbayashi N, et al. Regulation of mammalian tooth cusp patterning by ectodin. *Science*. 2005;309(5743): 2067–70.
[22] Klein OD, Minowada G, Peterkova R, Kangas A, Yu BD, Lesot H, et al. Sprouty genes control diastema tooth development via bidirectional antagonism of epithelial-mesenchymal FGF signaling. *Dev Cell*. 2006;11(2):181–90.
[23] Duverger O, Morasso MI. Role of homeobox genes in the patterning, specification, and differentiation of ectodermal appendages in mammals. *J Cell Physiol*. 2008;216(2):337–46.
[24] Manly RS, Braley LC. Masticatory performance and efficiency. *J Dent Res*. 1950;29(4):448–62.
[25] Wise GE, King GJ. Mechanisms of tooth eruption and orthodontic tooth movement. *J Dent Res*. 2008;87(5):414–34.
[26] Shimono M, Ishikawa T, Ishikawa H, Matsuzaki H, Hashimoto S, Muramatsu T, et al. Regulatory mechanisms of periodontal regeneration. *Microsc Res Tech*. 2003;60(5):491–502.
[27] Byers MR, Narhi MV. Dental injury models: Experimental tools for understanding neuro-inflammatory interactions and polymodal nociceptor functions. *Crit Rev Oral Biol Med.* 1999;10(1):4–39.
[28] Sharpe PT, Young CS. Test-tube teeth. *Sci Am.* 2005;293(2):34–41.
[29] Yen AH, Sharpe PT. Regeneration of teeth using stem cell-based tissue engineering. *Expert Opin Biol Ther.* 2006;6(1):9 –16.
[30] Ohazama A, Modino SA, Miletich I, Sharpe PT. Stem-cell-based tissue engineering of murine teeth. *J Dent Res*. 2004;83(7):518-22.
[31] Takahashi C, Yoshida H, Komine A, Nakao K, Tsuji T, Tomooka Y. Newly established cell lines from mouse oral epithelium regenerate teeth when combined with dental mesenchyme. *Invitro Cell Dev Biol Anim*. 2010;46(5):457-68.
[32] Komine A, Suenaga M, Nakao K, Tsuji T, Tomooka Y. Tooth regeneration from newly established cell lines from a molar tooth germ epithelium. *Biochem Biophys Res Commun*. 2007;758–63.
[33] Masamitsu Oshima and Takashi Tsuji (2014). Whole Tooth Regeneration Using a Bioengineered Tooth, New Trends in Tissue Engineering and Regenerative Medicine – Official Book of the Japanese Society for Regenerative Medicine, Prof. HideharuHibi (Ed.), ISBN: 978-953-51-1724-7,2014; InTech, DOI: 0.5772/58908
[34] Duailibi MT, Duailibi SE, Young CS, Bartlett JD, Vacanti JP, Yelick PC Bioengineered teeth from cultured rat tooth bud cells. *J Dent Res*. 2004;83(7):523-28.
[35] Dorozhkin SV. Bioceramics of calcium orthophosphates. *Biomaterials.* 2010; 31:1465–85.



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