

# Using Dental Stem Cells to Regenerate Tooth Tissue and Whole Tooth Replacement

Peretz Rapoport

Peretz Rapoport graduated January 2018 with a BS in Biology and is starting Touro School of Dental Medicine in fall 2018

## Abstract

*Irreversible dental problems such as dental carries and periodontal disease create a plethora of general and oral health issues. Although there are solutions to treat these different problems, an emphasis has been placed on finding a solution to these points with the help of bioengineering and stem cells. Using stem cells to treat these problems could result in a more permanent fix than the methods dentists employ now. Two novel approaches to fixing irreversible dental problems via stem cell therapy are tooth tissue regeneration and whole tooth replacement. This paper reviews the advantages and disadvantages of using dental pulp stem cells, stem cells from human exfoliated deciduous teeth, periodontal ligament stem cells and stem cells of the apical papilla for these novel techniques. Ultimately, these methods provide a promising future for dental patients, particularly with the use of stem cells from of the apical papilla.*

## Introduction

The tooth is a complex ectodermal organ consisting of various hard tissues as well as soft connective tissue. The hard tissues found in the tooth include: enamel, dentin, and cementum. Teeth also contain soft connective tissues like pulp and a periodontal ligament composed of fibrous connective tissue with both vascular and neural sections. Whole tooth loss and oral diseases such as dental carries, oral trauma, periodontal disease create many problems that impact proper oral function. Issues with enunciation, mastication, occlusion and general health issues are known to be induced by tooth loss or the onset of oral disease. (Oshima et. al, 2014)

Currently, there are many procedures using artificial materials to replace missing or damaged teeth. Dental implants, bridges, dentures and crowns all help to replace missing teeth, however, many believe that the optimal replacement is to re-grow tooth tissue or an even an entire tooth in place of the missing tooth.

Due to extensive, active research in embryonic development, stem cell biology, and tissue engineering, regenerative therapy is a promising method for the restoration of function in missing or damaged organs including teeth. There are numerous tooth-tissue derived stem cells that have been found to aid in the development of new teeth allowing for the evolution of teeth regeneration techniques. Tooth-tissue regenerative therapy involves transplanting a bioengineered tooth germ grown in vitro via stem cells. At the time of implantation, these bioengineered specimens must contain all the components of a naturally growing tooth in order to be able to function as one in the future. Whole tooth replacement transplants a complete, bioengineered tooth to replace the missing tooth. Many believe that tooth replacement via stem cell therapy will be the treatment of choice for missing or severely damaged teeth. Several other methods have been proposed to replace missing teeth including three-dimensionally bio engineered teeth and the cell-aggregation method. Additional reports have been released regarding fully functional bioengineered teeth with correct tissue orientation and the masticatory capabilities of regular teeth. This review will discuss the most recent findings on tooth tissue engineering and whole tooth regeneration, and will provide the most viable method for the near future.

## Methods

This review is based on the analysis of scientific articles and original works found on the internet. Several different internet databases were used including Touro college's online library and PubMed.

## Discussion: Tooth Development

To ensure the success of tooth regeneration techniques, it is important to first understand how a biological tooth is formed and attempt to mimic those natural steps in the lab. As a tooth develops, it undergoes four distinct morphological phases; the initiation, bud, cap, and bell stages (Yildirim, 2013). During initiation, support structures such as the alveolar bone and periodontal ligament begin to develop. The tooth germ is first observed as a thickening and propagation of the cells of the oral epithelium and these cells form a bud that extends to the dental mesenchyme. The dental epithelium continues to undergo extensive proliferative activity, eventually forming a cap-like structure and completing tooth development.

During tooth development, the cells differentiate into three different sections: the inner and upper layers and the central cell layer that forms the stratum intermedium and stellate reticulum. As they do so, the enamel knot signaling center can be identified. At this point, ectomesenchymal cells of the dental papilla condense, giving rise to both the dentine and pulp of the tooth. The dental follicle forms around the dental papilla and enamel, giving rise to the periodontal tissues. The final bell stage is heavily defined by the continued proliferation and tissue differentiation. In this process, the inner epithelial cells adopt a cube shape and produce a considerable amount of glycogen, while the cells of the stratum intermedium produce alkaline phosphatase and the stellate reticulum adopts a star shape, encircled by the outer epithelial layer.

As the tooth continues to grow and differentiate, odontoblasts from dental mesenchyme differentiate and build up the dentin matrix, while ameloblasts from epithelial cells produce the enamel matrix. Once the crown of the tooth has formed, tooth root structures start to develop from Hertwig's epithelial root sheath, creating periodontal ligament, dentin, cementum and alveolar bone.

Throughout the entire tooth development, the only portion that does not have regenerative properties is the dental enamel.

Enamel is the only mineralized tooth tissue sourced from the dental epithelium. All other mineralized tooth tissue, such as dentin, periodontal ligament, alveolar tissue and cementum have some form of regenerative properties. These tooth tissues originate from neural crest-derived dental ectomesenchyme. Dental stem cells are believed to play an important role in the regenerative properties of these tissues (Dannan 2009).

### Dental Stem Cells

Unlike other cells found in the body, stem cells have unique capabilities. They are unspecialized, yet can produce specialized cell types, even after prolonged periods of inactivity. Stem cells are able to proliferate and can be isolated based on unique cell surface markers. It is important to note that *in vitro* culture conditions may alter the cells, thus causing the cells to behave in a different manner than they would have *in vivo*. There are two categories of stem cells that can be used: embryonic or somatic stem cells. Being that the use of embryonic stem cell is ethically controversial, researchers work with strictly somatic, or adult human stem cells for clinical application. Somatic stem cells are easily accessible and without the controversy of their embryonic counterparts, making them the optimal choice for use in dentistry.

There are several types of stem cells that can be found in teeth. Dental stem cells are found in the periodontal ligament, apical papillae, and the pulp of both adult and children's teeth. Although these stem cells differ in their growth rate in culture, cell differentiation and gene expression, they most likely share a common lineage. Dental stem cells are derived from neural crest cells and all have generic mesenchymal stem cell-like properties. (Volponi et. al, 2010)

### Dental Pulp Stem Cells

Dental pulp stem cells (DPSCs) are a mesenchymal type of stem cell located in the center of the tooth. Dental pulp stem cells are considered a great candidate for stem cell therapy for many reasons. DPSCs are easily accessible for collection and have a low morbidity once extracted. They are also known to generate more dentin than other stem cells, being easily conserved via cryopreservation (Yan, et. al. 2010). There are various methods to isolate DPSCs from the dental pulp including size-sieved isolation, stem cell colony cultivation, magnetic activated cell sorting (MACS), and fluorescence activated cell sorting (FACS). In addition, DPSCs were found to generate a dentine structure *in vivo* and differentiate *in vitro* (Gronthos et.al, 2000). These features make DPSCs a prime source of stem cells for 3-dimensional tooth regeneration.

### Stem Cells from Human Exfoliated Deciduous Teeth

Stem cells from the pulp of human baby teeth, also known as stem cells from human exfoliated deciduous teeth (SHED),

were found to differentiate into non-dental mesenchymal cell derivatives *in vitro*, produce dentine, and induce bone formation. SHED differs from DPSCs with respect to their higher rate of proliferation, sphere-like cell clustering and, most importantly, their inability to produce a dentin-pulp-like complex. It is believed that the reason for these differences is due to SHED being a multipotent stem cell that is less mature than DPSCs. SHED were reported to not be able to differentiate into osteoblasts, yet managed to form an osteoinductive template, thus leading to new bone formation. Following transplantation, SHED were found to aid in bone formation and generate dentin. SHED were found to be a highly proliferative cell population, capable of differentiating into various cells such as neural and glial cells as well as odontoblasts. Although there are better types of stem cells for use in dentistry, due to its penchant for widespread differentiation there is still much potential for SHED stem cells in other areas of regenerative medicine, such as in the brain and nervous system. (Miura et. al, 2003).

### Periodontal Ligament Stem Cells

The periodontal ligament (PDL) has been viewed as a potential source of stem cells for a long time. The PDL is a connective tissue that acts as a shock absorber during chewing and is found between the cementum and the inner wall of the alveolar socket, anchoring both to each other. These stem cells have characteristics of mesenchymal stem cells and have potential to be used in periodontal regeneration. It is important to note that unlike other stem cells, PDLSCs do not have a unique cell marker that differentiates them from mesenchymal stem cells. Therefore, PDLSCs are not an optimal choice for stem cell research and dental regeneration (Zhu and Liang, 2015).

### Root Apical Papilla Stem Cells

Stem cells of the apical papilla (SCAP) are found by the tips of growing tooth roots. These cells are only present during root development, prior to the eruption of the tooth. Stem cells of the apical papilla were reported to differentiate into both adipocytes and odontoblasts. When compared to the proliferative potential of DPSCs *in vitro*, SCAP were noted to proliferate at a higher rate, making them a very promising source of stem cells for stem cell therapy. Current implant-based methods to replace whole teeth have failed to reproduce the necessary root structure, causing jaw-bone resorption around the implant due to the force of chewing (Volponi et. al, 2013). When combined with periodontal ligament stem cells and transplanted into mini pigs, researchers noted formation of periodontal ligament and dentine. These results suggest that the necessary technology to implant a biological root and place an artificial dental crown on it has been attained.

Another point of advocacy is that SCAP can be harvested without the ethical issues of stem cell harvesting in embryos.

# Using Dental Stem Cells to Regenerate Tooth Tissue

In addition, an important attribute of SCAP is that they are believed to be the source of root dentin, while DPSCs are the source of the replacement odontoblasts. SCAP could be distinguished from other mesenchymal stem cells in a number of ways. The most significant difference is that while all other mesenchymal stem cells test negatively for hTERT (human telomerase reverse transcription) activity, SCAP were found to test positive for it. This suggests that there is a difference in the genetic lineage of stem cells from the apical papilla compared to that of other dental stem cells. SCAP seem to come from an early population of progenitor cells and may be a better option for tissue regeneration (Huang et al, 2008).

## Using Dental Stem Cells for Tooth Tissue Regeneration

When stem cells isolated from the apical papilla of human teeth and human periodontal ligament were transplanted into mice and mini pigs, the successful regeneration of the root and periodontal complex capable of supporting a crown was achieved. Being that pigs have an orofacial tissue organization similar to humans, the successful regeneration of a bio root and transplantation into pigs shows the tremendous potential of this method for use in humans. Unlike other experiments to regenerate tooth tissue, this method used human stem cells and not stem cells from mice or pigs, proving that human stem cells were capable of producing the above mentioned successful results (Sonoyama et al, 2006).

## Whole Tooth Regeneration

Whole tooth regeneration is another approach to fix the issues associated with irreversible tooth damage. This method of tooth regeneration attempts to grow a whole tooth in vitro and transplant it into the mouth. To successfully regenerate a whole tooth, the same process that the tooth undergoes when it is first formed in the developing embryo must be replicated. Organogenesis of the tooth takes place by the interaction of mesenchymal and epithelial progenitor stem cells. Once the bioengineered tooth germ is produced in vitro, it may be transplanted it into the area of tooth loss, thus regenerating a new tooth. The biggest challenge is to develop a technique that will allow for the mesenchymal-epithelial stem cell interaction while in vitro. So far, there are several suggested methods that will allow scientists to use dissociated stem cells to bioengineer a tooth germ (Oshima et al, 2014).

## The Scaffold Method

The first method which allows for the necessary mesenchymal-epithelial stem cell reaction, is using a biodegradable scaffold. Scaffold technology has provided us with a method to regenerate specific tissue by sowing cells on degradable materials. The scaffold method has been used to generate tissues in cartilage

and bone regeneration therapies. Experiments using a collagen sponge as the scaffold material have reported successful formation of small teeth. These lab-grown teeth had all the characteristics found in biological teeth including dentin, enamel and dental pulp as well as forming the correct shape and size (Young et al, 2002). Despite the scaffold method's ability to regenerate teeth, there are some significant problems with this technique. It has a low frequency of tooth formation. This approach fails to provide the many precise intercellular reactions, causing irregularities in the created tooth tissues, such as in the enamel-dentine complex. For proper tooth structure, there needs to be many precise junctions between the enamel, cementum and dentine for normal tooth development. These complex junctions are the result of very specific mesenchymal-epithelial stem cell tissue interactions, resulting in a very precise combination of ameloblasts, cementoblasts and odontoblasts, a process the scaffold method has yet to mimic perfectly (Thesleff, 2003).

## The Cell Aggregation Method

A second approach is known as the cell aggregation method. This procedure is used to reconstruct bioengineered organ germ and has been able to produce the proper mesenchymal-epithelial cell interaction during the developmental process that the scaffold method has failed to produce. Recent studies have reported that transplanted bioengineered mammary gland stem cells successfully caused organ regeneration in vivo with the proper structure and cellular arrangement (Zheng et al, 2005). The cell aggregation method was also used to successfully regenerate hair follicles (Zheng et al, 2005). There has been much research done using the cell aggregation method to successfully regenerate teeth with correct structures. Currently, this technique has only been able to create teeth with the proper structure, but to date, only at a low frequency. Although the cell aggregation method replicated the organogenesis of biological teeth partially, a method that has a higher frequency of success is being sought (Nakao and Tsuji, 2008).

## The Organ Germ Method

The interaction between the epithelial and mesenchymal stem cells in the embryo gives rise to the organ germ. It can be concluded from this that to properly reproduce organs, one needs to reproduce the steps and conditions that created them in the first place. Both tooth and whisker follicle germs were used as a source of the disassociated epithelial and mesenchymal stem cells. Although there has been successful regeneration of organs in the past, they were all produced in vitro. The organ germ method allows for the regeneration of teeth in vivo as well as in vitro.

Self-reorganization of the mesenchymal and epithelial stem cells is the first step in multicellular aggregation. Self-reorganization is accomplished by these stem cells moving and adhering themselves to select cells until there is the necessary

equilibrium. After the proper cell configuration has been accomplished through self-reorganization, the next step is the initiation of organogenesis by the mesenchymal and epithelial stem cells. These stem cells regulate the morphogenesis and differentiation as well. It is important to note that the frequency of successful self-reorganization and tissue regeneration is dependent on the source of the stem cells used in this procedure.

In research performed by Nakao, the organ germ method successfully produced tooth germ with the correct structure, resulting in transplantation that yielded tooth growth. Similarly, when growing whole teeth *in vitro* and then transplanting them, the bioengineered teeth were able to attach themselves to the mouth and form both nerve fibers and blood vessels from the implanted stem cells. This method used completely dissociated mesenchymal and epithelial stem cells to generate the necessary tooth germ resulting in successful tooth transplantation both *in vivo* and *in vitro*.

When the experiment was first conducted, single cells from the incisor tooth germ at cap stage from the lower jaws of mice were used. These explants failed to produce a complete tooth, yet managed to generate bone or keratinized oral epithelium-like structures. The explants that failed to form cell compartmentalization at high cell density, failed to produce the correct tooth structure as well. To form a tooth with the correct cell compartmentation of epithelial and mesenchymal stem cells, the cells that could compartmentalize at high density were then collected and injected into the adjacent areas via a collagen gel drop. After just one day, there was already evidence of tooth germ formation with the correct compartmentalization of stem cells. This bioengineered tooth germ was then transplanted into sub renal capsules in mice. After transplantation, the formed tissue was observed histologically and was found to contain odontoblasts, dentin, enamel, dental pulp, Tomes' process, root analog, alveolar bone, periodontal ligaments and blood vessels in the same layout as natural teeth. This transplantation produced successful results 100% of the time in 50 different transplants.

Analysis of the regenerated teeth showed mRNA for specific markers for periodontal ligaments and ameloblasts. To find out if tooth germ taken from bioengineered teeth would also produce the same successful results, the origin of the mesenchyme and epithelium cells from the tooth germ of GFP-transgenic mice were analyzed. The mesenchymal cells derived from GFP-transgenic mice were found to contain dental pulp and odontoblasts as well as produce alveolar bone and periodontal follicles, which are normally derived from dental follicles. The epithelial cells from these mice produced ameloblasts. It was also determined that bioengineered tooth germ did not develop into teeth as frequently as natural tooth germ. This is a distinct indication that the developmental stage of the natural tooth germ is vital for successful regeneration of tooth germ.

To prove that bioengineered tooth germ successfully produces teeth *in vitro* with the correct cell types, time-course images were observed. These images proved that the tooth germ successfully produced the necessary cell types in proper fashion. After successfully growing a tooth in the renal capsule, the next step was to check if it can be successfully transplanted and develop into a fully functioning tooth. Bioengineered teeth were allowed to grow in the sub renal capsule for a duration of two weeks. After transplanting the tooth into the oral cavity of a mouse, there were successful results. There was formation of a correct tooth structure with all the components that natural teeth have including dental pulp, dental root, enamel, dentin, and periodontal ligaments. The size of these regenerated teeth was found to be within a 1.1-fold increase, thus ensuring that we have attained a method for successful whole tooth replication with the proper proportions as well as with the necessary components. (Nakao et. al, 20007)

Although these results provide us with a viable method to regenerate teeth successfully in mice, there are still many things that need to be investigated before this method can be considered for successful whole-tooth regeneration in humans. There are a few more things that this experiment did not determine, such as the hardness of the regenerated tooth and its response to mechanical stress (Ikeda et. al, 2008).

Although there have been no reported cases of transplanting a tooth grown with the organ germ method into a human, there have been studies that prove that teeth grown in this manner and implanted in mice exhibit the appropriate hardness and normal function. To determine whether the regenerated teeth were as hard as natural teeth, the Knoop hardness test was performed. It is used to determine the hardness of brittle materials. By testing multiple times throughout the growth of the bioengineered teeth, the result values showed the same significant increase in dentin and enamel hardness as is found in natural teeth between three and nine weeks, proving that they can successfully take the place of missing teeth.

In order to determine if the regenerated tooth successfully replaces the missing tooth, the regenerated tooth's response to mechanical stress must be examined. It is believed that in order to regrow a fully functioning tooth, the tooth must interact with the oral and maxofacial regions by means of the periodontal ligament. This is because the periodontal ligament is known to induce alveolar bone remodeling when under mechanical stress (Ikeda et. al, 2008). Histochemical analysis of the periodontal ligament revealed a connection between the tooth and alveolar bone, showing the successful interaction between the tooth and the oral and maxofacial regions via the PDL. When the regenerated tooth was subjected to mechanical stress, localization of osteoblasts and osteoclasts occurred. These cells have been found when the PDL of normal teeth was subjected to mechanical stress as well, proving that the

# Using Dental Stem Cells to Regenerate Tooth Tissue

regenerated tooth is able to properly respond to mechanical stress. (Ikeda et. al, 2008)

There are those that believe the successful regeneration of teeth in mice does not translate into successful results in regenerating human teeth. Unlike humans, mice are known to only grow molars and incisors separated by a toothless region known as the diastema. Mice also only possess one set of teeth that grow continuously throughout their life. Thus, mice may not be the best model for tooth regeneration in humans (Huysseune and Thesleff, 2004). Being that whole teeth were successfully regenerated in the diastema as well as in the lower jaw-bone of mice there is evidence to suggest that despite notable differences between human and mouse mouths, the regeneration of teeth in mice is not exclusive to mice and may be done in humans as well.

## Conclusion

Although there have been no reports of successful whole tooth regeneration in humans, scientists appear to be on the right track. The research presented above indicate that although the cell aggregation method is a promising technique to regenerate whole teeth, there is still much work to be done before this will be the treatment of choice to replace missing teeth. Being that a bio root composed of human stem cells has been reported to be successfully transplanted into mini pigs, there is strong reason to believe that this method may be applied to humans in the near future. This review highlights differences between mice and humans that may affect the success of regeneration in humans. Despite there being extensive research and roadblocks ahead in the development of tooth regeneration techniques, progress and success with model organisms has introduced an element of hope for science to provide cures and resolutions to damaging oral diseases and encourage improved overall dental health.

## References

Dannan A. Dental-derived Stem Cells and whole Tooth Regeneration: an Overview. *Journal of Clinical Medicine Research*. 2009;1(2):63-71. doi:10.4021/jocmr2009.03.1230.

Gronthos S, Mankani M, Brahmi J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proceedings of the National Academy of Sciences of the United States of America*. 2000;97(25):13625-13630.

Huang GT-J, Sonoyama W, Liu Y, Liu H, Wang S, Shi S. The Hidden Treasure in Apical Papilla: The Potential Role in Pulp/Dentin Regeneration and BioRoot Engineering. *Journal of Endodontics*. 2008;34(6):645-651. doi:10.1016/j.joen.2008.03.001.

Huysseune A, Thesleff I. Continuous tooth replacement: the possible involvement of epithelial stem cells. *BioEssays*. 2004;26(6):665-671. doi:10.1002/bies.20039.

Ikeda E, Morita R, Nakao K, et. al. Fully functional bioengineered tooth replacement as an organ replacement therapy. *Proceedings of the National Academy of Sciences*. <http://www.pnas.org/content/106/32/13475.full>. Published June 30, 2008. Accessed May 12, 2017.

Miura M, Gronthos S, Zhao M, et. al. SHED: Stem cells from human exfoliated deciduous teeth. *Proceedings of the National Academy of Sciences of the United States of America*. 2003;100(10):5807-5812. doi:10.1073/pnas.0937635100.

Nakao K, Morita R, Saji Y, et. al. The development of a bioengineered organ germ method. *Nature Methods*. 2007;4(3):227-230. doi:10.1038/nmeth1012.

Nakao K, Tsuji T. Dental regenerative therapy: Stem cell transplantation and bioengineered tooth replacement. *Japanese Dental Science Review*. 2008;44(1):70-75. doi:10.1016/j.jdsr.2007.11.001.

Oshima M, Tsuji T. Functional tooth regenerative therapy: Tooth tissue regeneration and whole-tooth replacement. *Odontology*. 2014;102(2):123-36. <https://search.proquest.com/docview/1553301568?accountid=14375>. doi: <http://dx.doi.org/10.1007/s10266-014-0168-z>.

Sonoyama W, Liu Y, Fang D, et. al. Mesenchymal Stem Cell-Mediated Functional Tooth Regeneration in Swine. *PLoS ONE*. 2006;1(1). doi:10.1371/journal.pone.0000079.

Thesleff I. Epithelial-mesenchymal signalling regulating tooth morphogenesis. *Journal of Cell Science*. 2003;116(9):1647-1648. doi:10.1242/jcs.00410.

Volponi AA, Kawasaki M, Sharpe PT. Adult Human Gingival Epithelial Cells as a Source for Whole-tooth Bioengineering. *Journal of Dental Research*. 2013;92(4):329-334. doi:10.1177/0022034513481041.

Volponi AA, Pang Y, Sharpe PT. Stem cell-based biological tooth repair and regeneration. *Trends in Cell Biology*. 2010;20-206(12-6):715-722. doi:10.1016/j.tcb.2010.09.012.

Yan M, Yu Y, Zhang G, Tang C, Yu J. A Journey from Dental Pulp Stem Cells to a Bio-tooth. *Stem Cell Reviews and Reports*. 2010;7(1):161-171. doi:10.1007/s12015-010-9155-0.

Yildirim S. *Dental pulp stem cells*. New York, NY: Springer Science Business Media, LLC; 2013.

Young C, Terada S, Vacanti J, Honda M, Bartlett J, Yelick P. Tissue Engineering of Complex Tooth Structures on Biodegradable Polymer Scaffolds. *Journal of Dental Research*. 2002;81(10):695-700. doi:10.1177/154405910208101008.

Zheng Y, Du X, Wang W, Boucher M, Parimoo S, Stenn KS. Organogenesis From Dissociated Cells: Generation of Mature Cycling Hair Follicles From Skin-Derived Cells. *Journal of Investigative Dermatology*. 2005;124(5):867-876. doi:10.1111/j.0022-202x.2005.23716.x.

Zhu W, Liang M, Periodontal Ligament Stem Cells: Current Status, Concerns, and Future Prospects. *Stem Cells International*. <https://www.hindawi.com/journals/sci/2015/972313/cta/>. Published March 16, 2015. Accessed May 11, 2017.