10.5005/jp-journals-10011-1258
REVIEW ARTICLE

Stem Cell Therapy: Dental Aspects

Venkatesh Elluru, DNSV Ramesh

ABSTRACT

Stem cells have the remarkable potential to develop into many different cell types in the body. Serving as a sort of repair system for the body, they can theoretically divide without limit to replenish other cells as long as the person or animal is still alive. Presently dental treatment for missing teeth largely utilize partial or complete dentures and titanium implants which are not equivalent, neither in function nor esthetics, to natural teeth. However, progress in stem cell biology and tissue engineering may present new options for replacing heavily damaged or lost teeth, or even individual tooth structures. This review summarizes the emerging concepts of whole-tooth replacement strategies and challenges in the field of dentistry.

Keywords: Stem cells, Teeth, Dental.

How to cite this article: Elluru V, Ramesh DNSV. Stem Cell Therapy: Dental Aspects. J Indian Aca Oral Med Radiol 2012;24(1):45-50.

Source of support: Nil

Conflict of interest: None declared

INTRODUCTION

Dental exfoliation in humans is a genetically regulated event during childhood. In humans, tooth loss can lead to physical and mental suffering that compromise an individual's self-esteem and quality of life.[1] Dental carries and periodontal diseases are major causes for tooth loss. If the permanent teeth are damaged or lost, they do not regenerate. Edentulism not only results in reduced oral and social functions, but also remains a major public health issue.[2] Traditional methods to treat edentulism include complete denture therapy, which is associated with complications, such as denture-induced stomatitis, soft tissue hyperplasia, traumatic ulcers, altered taste perception and burning mouth syndrome.[3] However, progress in stem cell biology and tissue engineering may present new options for replacing heavily damaged or lost teeth, or even individual tooth structures.

Fundamentally, two means of regenerating teeth are described. The first is conventional tissue engineering, in which the application of cells in a carrier material *in vitro* under the influence of a stimulus leads to tissue regeneration. The second is the much more innovative process of tooth regeneration using dental epithelium and mesenchymal cells *in vivo* after direct implantation, representing a kind of tissue engineering in the broader sense, based on knowledge of general embryogenesis and physiological tooth development during childhood.[4]

Regeneration of teeth can be broadly divided into:

- Regeneration or *de novo* formation of the entire, anatomically correct teeth.
- Regeneration of dental pulp.
- Regeneration of dentin based on biological approaches and potentially as biological fillers that may replace current restorative dentistry.
- Regeneration of cementum as a part of periodontium regeneration or for loss of cementum and/or dentin resulting from trauma or orthodontic tooth movement.
- Regeneration of the periodontium including cementum, periodontal ligament and alveolar bone.
- Regeneration or synthesis of enamel-like structures that may be used as biological substitute for lost enamel.

Tissue engineering is an emerging interdisciplinary science, which aims at developing strategies for regeneration of damaged organs and tissues, based on principles of engineering and life sciences.[5] This field of science is grounded in the interplay of three essential components: Scaffolds, responsive cells and morphogens.[5]

Responsive cells are generally stem cells. They are undifferentiated cells with varying degrees of potency and plasticity, capable of self-renewal and multilineage differentiation.[5] There are two basic categories of stem cells classified according to their potential of differentiation: Embryonic stem cells (ESC) and somatic stem cells (also called adult stem cells or mesenchymal stem cells-MSC). While the use of ESC is limited by ethical issues, somatic stem cells constitute a more favorable cellular source to be used in tissue engineering. Postnatal stem cells have been isolated from several tissues including, brain, skin, hair follicles, skeletal muscle, bone marrow and dental tissue, and five types of dental MSC are isolated and characterized: Dental pulp stem cells (DPSC) from pulp of permanent teeth; stem cells of human exfoliated teeth (SHED) and immature dental stem cells (IDPSC) from primary teeth; periodontal ligament stem cells (PDLSC); stem cells from apical papilla (SCAP), and dental follicle progenitor cells (DFPC).[6,7]

DENTAL EPITHELIAL STEM CELLS

The embryonic oral epithelium induces odontogenesis.[8] The dental enamel is formed from ameloblasts, which arise from epithelial stem cell. They are the only cells of ectodermal origin which play a role in odontogenesis. They are lost after tooth eruption, thus leaving no adult human ectodermal stem cells available for cell therapy.

DENTAL MESENCHYMAL STEM CELLS

With the exception of ameloblast progenitor cells, all stem cells involved in odontogenesis originate in mesenchyme.

Dental Pulp Stem Cells

Dental pulp stem cells (DPSCs) are isolated from the dental pulp, can either regenerate new stem cells or undergo a differentiation process. In the dental pulp, there are different progenitor cell subpopulations, which differ in terms of self-renewal ability, proliferation rate and differentiation potential. Dental pulp can be acquired from third molars or pulpectomized teeth left *in situ*. *In vitro*, DPSCs can differentiate to odontoblasts, osteoblasts, endothelocytes, smooth muscle cells, adipocytes, chondrocytes and neurons. When seeded in scaffolds casted within the pulp chamber of tooth slices, DPSCs were able to produce a pulp-like tissue.[9,10]

Stem Cells from Human Exfoliated Deciduous Teeth (SHEDs)

Human exfoliated deciduous teeth are a relatively easily accessible source of adult stem cells. SHEDs can be isolated from the coronal pulp of exfoliated deciduous teeth. It is assumed that in addition to their role in the eruption of permanent teeth, they also influence the osteogenesis associated with the same. *In vitro*, they can differentiate odontogenically, osteogenically, adipogenically, chondrogenically, or neurally, depending on different conditions. *In vivo*, these multipotent stem cells have the potential to differentiate into neurons, adipocytes, odontoblasts, and osteoinductive and endothelioid cells.[11]

Stem Cells from the Apical Papilla (SCAP)

SCAP were isolated from dental papilla,[12] and is located in tip of growing tooth roots.[12] SCAP, similarly to DPSC and SHED, comprise a heterogeneous population capable of osteoblastic and odontoblastic differentiation, and to a lesser extent adipogenic differentiation.[13] Furthermore, SCAP show consistent capacity for dentin regeneration. Since the dental papilla is the precursor tissue for radicular pulp, it is possible that SCAP convert into DPSC, and therefore SCAP may constitute a population of earlier stem cells.[14] Recent studies have shown that SCAP have the capacity to produce vascularized pulp-like tissue *in vivo* into 5 to 6 mm long root canals.

Periodontal Ligament Stem Cells (PDLSCs)

The periodontal ligament contains stem cells which have the potential to form periodontal structures, such as cementum and ligament. It can be harvested from the roots of extracted teeth. *In vitro*, PDLSCs differentiate into osteoblasts, cementoblasts and adipocytes. *In vivo*, after transplantation into mice, structures resembling bone, cementum, cartilage and PDL have been found. Combined with SCAPs from impacted third molars, PDLSCs on a hydroxyapatite-tricalcium scaffold were transplanted into the alveoli of juvenile miniature pigs. A root and a periodontal complex were formed that were able to support a ceramic crown, thus fulfilling the function of a natural tooth.[9]

Dental Follicle Stem Cells (DFSCs)

The dental follicle surrounds the developing tooth. It plays a major role in the genesis of cementum, periodontal ligament and alveolar bone. DFSCs can be isolated from the follicles of impacted third molars. DFSCs cultivated *in vitro* exhibit characteristics of cementoblasts and osteoblasts, and can differentiate neurally. *In vivo*, tissue similar to dental cementum and differentiation into PDL progenitor cells have been observed.[15]

Nondental Stem Cells

Dental tissue can also be regenerated from nondental adult multipotent stem cells.[16] Embryonic oral epithelium can stimulate an odontogenic response in mesenchyme which does not have a dental origin.[8] Bone marrow derived mesenchymal stem cells (BMSCs) can replicate themselves and be differentiated into osteoblasts, chondrocytes, myoblasts, adipocytes and neuron-like cells.[17] Embryonic oral epithelium induces BMSCs to express odontogenic genes. The *in vivo* development of tooth-like structures with bone has been observed. In humans, BMSCs are already being used therapeutically in bone augmentation by sinus lifts.[18] Stem cells isolated from the bone marrow of the mandible (MBMSCs) possess a high osteogenic potency.[19] From umbilical cord blood, cartilage, the cornea, mammary glands and adipose tissue stem cells can be obtained.[19,20] In dentistry, hair follicles have been studied as an easily accessible source of mesenchymal stem cells.

INDUCED PLURIPOTENT STEM CELLS

Despite the fact that SHED can be obtained from exfoliated primary teeth and DPSC can be retrieved from third molars indicated for extraction, there are limitations to the use of these cells. For example, primary teeth are only available in children and frequently the amount of pulp tissue available is minimal. The method for generation of human iPS cells was first described in 2008.[21] Using different transcription factors (Oct4, Sox2, Klf4, Myc), adult human cells were

reprogrammed to generate embryonic stem-like cells.[21] IPS constitutes a new approach for the generation of stem cells for regenerative therapies, apparently devoid of issues related to immunocompatibility.[22] However, there are significant risks involved with the use of IPS cells for tissue regeneration. Perhaps the most serious one is that some of the transcriptional factors used to reprogram the cells (e.g. c-Myc) are very well-known oncogenes. Another issue is related to the fact that many times viruses are used to transfect these genes into the cells. It is well known that viruses that integrate into the host cell genome (e.g. retroviruses) have also intrinsic risks in regards to cell transformation. Nevertheless, if these issues are solved, IPS cells may constitute an attractive cellular source for tissue engineering.[23]

SCAFFOLDS FOR DENTAL PULP TISSUE ENGINEERING

New strategies for dental pulp regeneration require the development of compatible biomaterials. As in any other tissue, the engineering of the dental pulp demands the association of appropriate cells with a conducive microenvironment.[24] Scaffolds are three-dimensional structures that provide an initial framework for cells and can be used to deliver morphogenic molecules.[25] After a scaffold has achieved these goals, they should degrade.[26] The use of appropriate 3-D scaffolds allows cells to attach, grow and differentiate. The role of the scaffold becomes even more critical, if one takes into account the challenges imposed by the quest for engineering a connective tissue within the confines of the human dental root. The necessity of recruiting blood vessels and neuronal structures solely through the apical foramina make the development of the scaffolds a critical step toward the goal of translating laboratory results into clinical use.[25,26] Scaffolds have been developed for research purposes (proof-of-principle), as well as for clinical use.[27] The tooth slice/scaffold model using poly-L-lactic acid PLLA has become a very useful model for mechanistic studies.[28] Another approach involves the use of a copolymer, i.e. poly-lactic-co-glycolic acid (PLGA), in the tooth slice/scaffold.[29]

MORPHOGENIC SIGNALING MOLECULES

Morphogenetic signaling molecules are proteins that bind to specific cell membrane receptors and induce a cascade of processes that results in the generation of a new tissue.[25] Growth factors control the activity of stem cells, e.g. by regulating the rate of proliferation, inducing differentiation into another cell type, or by stimulating cells to synthesize mineralizable matrices.[30] BMP are members of the transforming growth factor (TGF)-beta family. They were originally identified as regulators of cartilage and bone formation and they play an important role in embryogenesis and morphogenesis of various organs and tissues, including teeth.[31] It has been demonstrated that human recombinant BMP (rhBMP-2, rhBMP-7) induce dentinogenesis.[32] The response of dental pulp cells to BMPs suggests that the cells present receptors for these bioactive molecules. BMP receptors (BMPR) are serine/threonine kinases that include type I receptors (BMPR-IA, BMPR-IB) and the type II receptor (BMPR-II). It was demonstrated that dental pulp cells (SHED, DPSC, fibroblasts) express BMPR-IA, BMPRIB and BMP-II receptors.[33] The growth factors BMP-2, BMP-4, BMP-6, BMP-7 and Gdf11 play an important role in the biology of pulp cells. Studies have shown that the expression of BMP-2 is increased during terminal differentiation of odontoblasts,[32] and that BMP-7 promotes the formation of reparative dentin mineralization in animal models.[34]

VASCULAR NETWORKS

Successful tissue engineering relies on the establishment of an effective vascular network, able to supply the tissue with oxygen, nutrients and immune cells, while removing by-products and waste.[35] Considering the anatomical characteristics of the root canal system, the development of strategies that enhance neovascularization is a major challenge in the field of dental pulp tissue engineering. To overcome the problem of vascularization, strategies, such as embedding angiogenic factors into the scaffold to promote ingrowth of microvessels, fabrication technologies to create polymers containing vessel-like networks and prevascularization of matrices prior to cell seeding have been proposed.[36] Alternatively, one could exploit the inherent vasculogenic potential of endothelial cells (EC).[37] Another group reported that stromal stem cells from human dental pulp (SBP-DPSC) implanted subcutaneously into immunodeficient rats generated bone with its own blood supply, suggesting the stem cell differentiation into endotheliocytes.[38] Several growth factors and environmental conditions are able to affect vascular network formation. VEGF is the prototypic proangiogenic factor and studies have shown that VEGF induces stem cell differentiation into endothelial cells. When SHED were cultured in collagen matrices, they organized themselves into capillary structures that resembled microvessels. In addition, VEGF enhanced the differentiation of SHED into vascular endothelial cells. Notably, areas within tissue engineering constructs exhibit hypoxia, which is a very well-described physiological condition in which angiogenesis is stimulated through the activation of transcriptional factors (e.g. HIF-1 alpha) and induction of VEGF expression.[39]

Periodontal Tissue Regeneration

Periodontal tissue regeneration represents the ultimate goal of periodontal therapy and entails the formation of all components of the periodontium, including gingiva, PDL, cementum and alveolar bone. Commonly used growth factors for PDL regeneration therapies include bone morphogenetic proteins, platelet-derived growth factor, emdogain and recombinant amelogenin protein. The resultant improved regenerative capability could be related to increased recruitment of progenitor MSCs, which subsequently differentiate to form PDL tissue. Transplanted cell seeded polyglycolic acid sheets regenerated new bone, cementum and well-oriented collagen fibers when inserted into root surfaces, thus proving multipotency of PDLSC. The combined use of transplanted MSCs and added exogenous signaling molecules could accelerate the directed differentiation of MSCs *in vivo*, providing more effective promotion of periodontal tissue regeneration. Successful therapies for PDL tissue regeneration will not only facilitate the treatment of periodontal diseases, but may also be used to improve current dental implant therapies.[40]

Whole-Tooth Regeneration

Whole-tooth regeneration efforts largely consist of two approaches: One involves *in vivo* implantation of immature tooth structures grown *in vitro* from dental progenitor cells, while the other uses in vitro expanded, cultured dental progenitor cell populations seeded onto polymer scaffolds and implanted *in vivo*. Early studies showed that it was possible to regenerate tooth crowns from intact or partially dissected tooth germs, if suitable environments were provided, such as *in vitro* organ culture, *in vivo* grafting on chick chorioallantoic membrane, ocular or subrenal grafts or subcutaneous transplants.[41] Each of these implant sites provides nutrients and oxygen to nurture tooth germ differentiation into mature teeth. Thus, there are several choices for cultivating small tooth primordia prior to implantation into their anatomically relevant site in the jaw. By applying traditional tissue engineering methods, tooth-like structures can be produced from biodegradable polymer scaffolds seeded with dissociated tooth germ cells obtained from postnatal pigs or cultured rat tooth bud cells grown in the omentum of immunocompromised mice.[42] Recently, it was found that dental cell-seeded tooth scaffold constructs grown in a coculture system appeared to result in an improved ability to control tooth size and shape.

Dental Stem Cell Markers

Stem cell markers help identify, characterize and isolate stem cells. STRO-1, a trypsin-resistant cell-surface antigen, is a commonly used dental stem cell marker for all dental MSCs. It is expressed, for example, from bone marrow mesenchymal cells. Another stem cell marker, Stro-4, binds to heat shock protein–90 beta of multipotent MSCs and is also suited to identifying stem cells. Markers for differentiated cells can also be used to characterize stem cells. For instance, the osteoblast marker osteocalcin is also a stem cell marker of DPSCs. In addition to mesenchymal stem cell markers, immature dental pulp stem cells also express markers of embryonic stem cells, such as Oct-4, Nanog, SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81.[43,44]

Challenges in Dental Tissue Engineering

A sufficient number of cell sources currently exist including DPSCs, SHED, SCAP and PDLSCs. In contrast, human dental epithelial stem cell sources are limited for the following two reasons. First, dental epithelial cells undergo apoptosis after enamel formation is completed and therefore are no longer present in erupted teeth. Undifferentiated wisdom or impacted teeth are thus the only available sources of human dental epithelium. Second, *ex vivo* dental epithelial expansion can be difficult, due to the fact that it is inherently more difficult to expand epithelial cells in culture as compared to mesenchymal cells. Therefore, in order to routinely bioengineer human whole teeth containing functional enamel, alternative dental epithelial cell sources will have to be identified.

It is currently accepted that the dental follicle plays an important role in the tooth eruption process, as indicated by published reports demonstrating that teeth lacking dental follicles cannot erupt.[45] Therefore, the creation of bio-engineered tooth organs with an associated *de novo* dental follicle may likely be the optimal design for successful tooth eruption.

Successful whole-tooth regeneration requires the formation of both functional tooth crown and root structures. To date, bioengineered tooth root formation with accompanying functional PDL tissues has proved to be quite challenging, with only a few reports of success, indicating that increased effort will be needed to achieve this goal.

Finally, potential immune responses to bioengineered human dental implants have yet to be examined and remain virtually unknown at this time.[46] Ideal tooth replacement therapies would use autologous cells harvested from the patient, thereby avoiding potential immunological rejection responses.

CONCLUSION

Dental tissue regeneration provides an attractive alternative to traditional, synthetic tooth restoration therapies. The vision

of natural dental restorations generated from stem cells, or the stem cell-based autologous regeneration of tissues is what makes stem cell research interesting for dentistry. The development of such 'test tube teeth' requires precise regulation of the regenerative events in order to achieve proper tooth size and shape, as well as the development of new technologies to facilitate these processes. Several existing challenges in regenerative dentistry still need to be overcome, including the need to establish reliable ways to control tooth size, shape and color and to create suitable jaw implantation site that enable tooth development *in vitro*. Finally, methods to control proper functional tooth eruption in jaws must also be defined. It has not yet been possible to find a source of human adult ectodermal stem cells to regenerate enamel posteruptively. Based on the current efforts, interest and progress, it is tempting to speculate that clinically relevant bioengineered functional tooth therapies for humans may be available in the near future.

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ABOUT THE AUTHORS

Venkatesh Elluru

Reader, Department of Oral Medicine and Radiology, Navodaya Dental College, Raichur, Karnataka, India

DNSV Ramesh (Corresponding Author)

Professor and Head, Department of Oral Medicine and Radiology, Navodaya Dental College, Raichur, Karnataka, India
e-mail: dr_eva1@yahoo.com