Postnatal Stem Cell Sources for Tooth Regeneration

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Abstract: *Tooth formation is a complex and dynamic process in which epithelial–mesenchymal interactions play a pivotal role in regulating tooth morphogenesis and subsequent development. Regeneration of a biocompatible tooth has been studied for many years with various results, but has never been totally satisfactory. Recent achievements from stem-cell biology, tissue engineering, bionics, developmental and molecular/cellular biology have made stem-cell-based tooth regeneration a novel approach that will hopefully replace missing teeth and metal implants in the foreseeable future. To avoid the legal and ethical dilemmas regarding the use of embryonic cells for therapeutic and clinical applications, the optimal approach towards clinical tooth regeneration should be mediated by multipotent postnatal stem cells. Here, this review outlines the potential candidate cells and their performances in postnatal stem-cell-based tooth regeneration.*

Key words: *tooth regeneration, postnatal stem cell, epithelial–mesenchymal interaction, odontogenesis*

Tooth loss due to periodontal disease, dental caries, trauma, cancer, or various genetic disorders adversely affects not only masticatory function, but also the aesthetics of one's face. To reconstruct these defects, current treatments rely largely on artificial dentures, autologous tissue grafts and metal implants. There are some limitations in these treatment methods, such as foreign-body sensation, insufficient biocompatibility, bone resorption, limited graft quantity, and donor-site morbidity. Thus, the exploration of new approaches to tooth replacement is clinically demanded.

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To date, the regenerative capacity of postnatal stem cells has been widely recognised, and stem-cell-based tissue regeneration has made steady progresses towards regenerating a biological tooth. Many studies have demonstrated that postnatal stem cells exist in various tissues, including bone marrow, neural tissue, skin, retina, and dental epithelium[1–4]. These stem cells display astonishing capacities for self-renewal and multi-lineage differentiation, which makes them very promising in tissue regeneration[5–7], such as skin, bone, articular cartilage, muscle, nerve, tendon and adipose tissues. Advances in postnatal stem cell biology have provided a great deal of impetus for the biomedical community to change these findings into clinical applications.

The making of a biocompatible tooth has been attempted for decades with various achievements. To avoid the legal and moral disputes concerning the clinical use of embryonic cells, the putative strategy for clinical tooth regeneration should be mediated by autogenous postnatal cells, particularly stem cells. Recently, tooth crown-like structures have been reconstructed using postnatal tooth bud cells recombined with artificial scaffolds[8–11]. However, it is impossible to get these early-stage tooth bud cells from old patients, the major popu-

Table 1 Comparison of different postnatal stem cells used in tooth regeneration

Cell type	Tissue origin	Specific markers	Multi-differentiation capacity	Potential contributions to tooth regeneration
DPSCs	dental pulp	unknown	identified	dental mesenchyme
SCAPs	apical papilla	unknown	identified	dental mesenchyme
SHEPs	primary tooth pulp	unknown	identified	dental mesenchyme
PDLSCs	periodontal ligament	unknown	identified	periodontal tissues
PAFSCs	dental follicle	unknown	identified	periodontal tissues
ABSCs	apical bud of rodent incisor	unknown	unidentified	dental epithelium
HERSCs	epithelial root sheath	unknown	unidentified	dental epithelium
BMSSCs	bone marrow	unknown	identified	dental epithelium/mesenchyme
ADMSCs	adipose tissue	unknown	identified	dental mesenchyme
NDESCs	integumentary epithelium	unknown	identified	dental epithelium

DPSCs: dental pulp stem cells; SCAPs: stem cells from apical papilla; SHEPs: stem cells from human exfoliated primary teeth; PDLSCs: periodontal ligament stem cells; PAFSCs: periapical follicle stem cells; ABSCs: apical bud stem cells; HERSCs: Hertwig's epithelial root sheath cells; BMSSCs: bone marrow stromal stem cells; ADMSCs: adipo-derived mesenchymal stem cells; NDESCs: non-dental epithelial stem cells.

lation suffering from the diseases of tooth loss. Many studies have demonstrated that postnatal dental stem cells exist in both fresh and cryopreserved dental tissues, and can be isolated according to their anatomical locations, colony-forming ability, expression of stem cell markers, and regeneration of dental structures in vivo[12–20]. These stem cells play a crucial role inpostnatal tissue development and provide an attractive cell source for tooth regeneration/engineering[21].

To date, knowledge gained from postnatal stem cell biology, especially associated with dentine, cartilage, and bone tissue reconstruction, has enabled us to contemplate further promising strategies for clinical bioodontogenesis. In this paper we will review some putative postnatal stem cell populations and their performances in tooth regeneration (Table 1).

Dental Stem Cells

Dental pulp stem cells (DPSCs)

DPSCs have been isolated from adult dental pulps as multipotent stem cells which can differentiate into adipocytes, neural-like cells, and odontoblast-like cells[15,16,22–24]. The expression profile of genes and proteins of human DPSCs is similar to that of bone marrow stromal cells (BMSCs)[18]. Both can express a variety of markers associated with endothelium (vascular cell adhesion molecule 1 and CD146), smooth muscle (α-smooth muscle actin), bone (alkaline phosphatase, type I collagen, osteonectin, osteopontin, and osteocalcin), and fibroblasts (type III collagen and fibroblast growth factor 2). Primary DPSCs do not express the haematopoietic markers CD14 (monocyte/macrophage), CD45 (common leukocyte antigen), CD34 (haematopoietic stem/progenitor cells, endothelium), and other markers such as MyoD (smooth muscle), neurofilament (nerve), collagen type II (cartilage), peroxisomal proliferator activated receptor gamma 2 (fat), dentine sialophosphoprotein, and bone sialoprotein (bone). The most striking feature of DPSCs is their capacity to differentiate into odontoblast lineages and to form irregular dentine-pulp-like tissues with tubular dentine when recombined with biodegradable scaffolds[15,16,25]. The quantity of newly formed dentine tissues appears to far exceed the dentine matrix secreted in situ during the lifetime of a particular tooth[16]. After co-culture with tooth germ cell conditioned medium, transplanted DPSCs in renal capsules can generate the dentine-pulp complex with regular shape[25]. However, untreated DPSCs in vivo bring about the formation of osteodentin-like structures rather than dentin–pulp complexes[25], indicating that the optimal strategy towards the normal dentinogenesis should not be mediated by naive DPSCs.

Generally, DPSCs are deemed as an ideal candidate for tooth regeneration, because of the following advantages: (i) a large number of autogenic DPSCs can be easily isolated from a single impacted molar or exfoliated primary tooth[16,17]; (ii) DPSCs keep the distinct morphogenetic information necessary for the crown formation; and (iii) DPSCs can produce a large amount of dentine tissues within a relatively short period, which make them more competent during stem-cell-based tooth regeneration than non-dental stem cells, such as BMSCs and bone marrow stromal stem cells (BMSSCs)[26].

Stem cells from apical papilla (SCAPs)

The apical papilla at the late stage of root development contains a special population of stem cells. These stem cells can express many surface markers, including STRO-1, ALP, CD24, CD29, CD73, CD90, CD105, CD106, CD146, CD166 and ALP, but are negative for CD34, CD45, CD18 and CD150. The CD24 may be a specific surface marker of these stem cells. Even though SCAPs and DPSCs can express osteo-/odonto-genic markers and generate mineralised tissue when transplanted into immunocompromised mice, they are two distinct mesenchymal stem cell populations. Many genes are differentially expressed between these two populations according to a cDNA microarray profile comparison. Moreover, compared with DPSCs, SCAPs show a significantly higher rate of bromodeoxyuridine uptake, increased number of population doublings, elevated tissue regeneration capacity, higher telomerase activity and an improved migration capacity in a scratch assay.

SCAPs *in vitro* can differentiate into several cell lineages under defined media, including odontoblasts, osteoblasts and adipocytes. When SCAPs and periodontal ligament stem cells (PDLSCs) are recombined with hydroxyapatite–tricalcium phosphate scaffold, these stem cells can bring about the formation of root–periodontal complex capable of supporting a porcelain crown and performing the masticatory function.

Together, SCAPs represent a unique population of postnatal stem cells that have striking advantages in regenerating biological tooth root[19].

Stem cells from human exfoliated primary teeth

The transition from primary teeth to adult permanent teeth is a dynamic process in which the development of permanent teeth coordinates with the resorption of primary tooth roots. Primary dental pulp is thought to be an approachable 'niche' of stromal stem cells, and an ideal source of odontoblasts as well as osteoblasts for tooth/bone regeneration. Human primary tooth contains multipotent stem cells that have been identified to be a population of highly proliferative, clonogenic cells capable of differentiating into different cell types, including neural cells, adipocytes, odontoblasts, chondrocytes, osteoblasts, and smooth and skeletal myocytes in the inductive media. These stem cells can express both embryonic stem cell markers (Oct-4, Nanog, SSEA-4, TRA-1-60 and TRA-1-81) and mesenchymal stem cell markers (STRO-1, CD146) *in vitro*. After *in vivo* transplantation, these stem cells have the capability to bring about bone formation, generate dentine, and survive in mouse brain along with the expression of neural markers. Primary teeth, therefore, may offer a unique stem cell source for tooth/bone regeneration, and even for the treatment of neural tissue injury or degenerative diseases[17,27,28].

PDLSCs

The periodontal ligament (PDL) is a kind of soft connective tissue embedded between the cementum and the inner wall of alveolar bone socket. PDL not only has an important role in supporting tooth structures, but also contributes to the tooth nutrition, homoeostasis, and alveolar bone repair. Nagatoma et al[29] have reported that approximately 30% of 400 periodontal ligament cells possess replicative potential and form single-cell colonies, and 30% of these colonies display positive staining against STRO-1, 20% differentiate into adipocytes and 30% drive into osteoblasts. Moreover, PDL contains multipotent postnatal stem cells that can express an array of cementoblast/osteoblast markers (alkaline phosphatase, bone sialoprotein, osteocalcin, transforming growth factor-β receptor type I) and mesenchymal stem cell markers (STRO-1, CD105, CD166, CD146)[20,29]. When transplanted into immunocompromised rodents, PDLSCs show the capacity to generate a thin layer of cementum/PDL-like tissues on the surface of hydroxyapatite/tricalcium phosphate ceramic particles, along with condensed collagen fibres resembling Sharpey's fibres[13,20]. In the periodontal defects of rat mandibular molars, human PDLSCs can integrate into the PDL compartment and sometimes attach to the surfaces of alveolar bone or teeth, indicating a potential ability of PDLSCs for periodontal tissue reconstruction[20]. These stem cells can also be recovered from cryopreserved human periodontal ligament, thereby providing a practical clinical approach to the preservation of PDLSCs[13]. In short, PDL represents a unique reservoir of stem cells and PDLSCs show a promising prospect for periodontal tissue regeneration.

Periapical follicle stem cells (PAFSCs)

PAFSCs represent a kind of mesenchymal stem cell located at the apical dental follicle of human developing roots. Cementogenesis is one of the most important issues in periodontal regeneration, in which cementum matrix is generated by highly differentiated cells termed cementoblasts. However, the origin of cementoblasts has not been fully elucidated. Handa et al[30] have reported that dental follicle cells isolated from the root surface of

bovine tooth germ contain cementoblast progenitors capable of differentiating into cementoblasts and secreting cementum-like matrix *in vivo*. These cells are distinct from the osteoblasts and PDL cells. Based on the above findings, PAFSCs have been isolated from the apical end of impacted third molars at the postnatal root-forming stage. PAFSCs present self-renewal capabilities, colony-forming efficiency, multi-lineage differentiation into adipocytes, osteoblasts, and other kinds of mineral nodule-forming cells. These stem cells have excellent proliferation rates and can express the mesenchymal stem cell markers CD29 and CD44. PAFSCs are more multipotent than other stem cells, including DPSCs, PDLSCs and stem cells from the surrounding mandibular bone marrow (MBMSCs). PAFSCs, therefore, may be another competent candidate cell in regenerating cementum and PDL[4].

Apical bud stem cells (ABSCs)

ABSCs represent a kind of special dental epithelial stem cell situated at the apical end of rodent incisors. Rodent incisors are continuously growing teeth that are maintained by cell proliferation at the apical end and attrition of the incisal edge. This type of tooth has a special bulbous epithelial protrusion termed an 'apical bud'[26,31]. An apical bud is a kind of stem cell niche located at the apical part of rat incisors[26,31,32] and acts as an eternal tooth bud necessary for the continuous growth of rat incisors throughout their life[31]. Molecular signals, such as Notch signals, bone morphogenetic proteins and fibroblast growth factors, which regulate cell differentiation and epithelial morphogenesis, are expressed permanently in the apical bud[31–35]. Our studies have demonstrated that these ABSCs can promote the odontoblastic differentiation of DPSCs and BMSSCs *in vitro*[26]. When recombined with postnatal ABSCs, both DPSCs and BMSSCs in vivo can bring about the formation of tooth- and dentine-pulp-like structures respectively, indicating that epithelial signals from ABSCs can induce odontogenic morphogenesis of dental and non-dental mesenchymal stem cells. Therefore, ABSCs provide an attractive dental epithelial stem cell source at the postnatal stages for tooth regeneration.

However, developmental dental epithelium at the postnatal stages is quite scarce in humans and almost disappears after tooth eruption[16,17,19]. Therefore, seeking alternative epithelial cells for tooth regeneration is a very exhausting task during the regeneration of entire human teeth. In theory, dental epithelial cells from other species can also be used as a candidate to reconstruct human teeth. For example, we can recombine patient's dental mesenchymal stem cells with dental epithelial cells of non-human origin to make a chimeric tooth. When the bio-teeth are formed in vitro, all dental epithelial components will disappear during the bio-odontogenesis. Then we can transplant these bio-teeth into patients' jaws and no graft rejection will be evoked because all dental tissues except enamel structure are generated by autologous self-cells. The non-self enamel is exposed in the patient's mouth and will cause no harmful immune responses. Recently, non-human dental epithelial cells have been under increasing investigation as potential candidate cells for human tooth regeneration.

Hertwig's epithelial root sheath cells (HERSCs)

Hertwig's epithelial root sheath (HERS) derives from, but is different from, enamel organ, which brings about the different functions in tooth development. After crown formation, the inner and outer enamel epithelial cells form a bilayered epithelial sheath, termed HERS. Recently, postnatal HERSCs have been isolated from HERS and cultured *in vitro* as a heterogenous cell population, in which a variety of signalling pathways contribute to their functional differentiation. These HERSCs may provide factors for the specific regulation of cemento-/osteo-genic differentiation of PDLSCs and play an important role in the formation of root/periodontal tissues, as well as in the maintenance of periodontal ligament space[36,37].

Moreover, HERSCs may undergo epithelial-mesenchymal transition to generate functional cementoblasts[37–39]. Following the fenestration and breakdown of HERS, these cells move away from the root surface and become the cellular aggregates of Malassez's epithelial cell rests, whose function may be associated with cementum maintenance and HERSC apoptosis[37,40].

The regeneration of root–periodontal complex has rearoused interest in the study of HERS. When intact postnatal HERS tissue is recombined with dissociated dental mesenchymal cells and transplanted into the renal capsules, typical root–periodontal complex can be observed. However, dissociated HERSCs reaggregated with dental mesenchymal cells can only form dentine-pulp complex and no periodontal tissue can be detected. These results indicate that the integrity of HERS is crucial for tooth root development and regeneration. However, whether postnatal HERSCs or their derivatives maintain the potent capability for root regeneration is still unclear.

Non-dental Stem Cells

BMSSCs

Cultured stromal cells derived from the bone marrow have been termed BMSCs, in which there exists a subset of multipotent stem cell populations called mesenchymal stem cells or stromal stem cells. Bone-marrow-derived stem cells are putative candidate cells for bone tissue engineering and can give rise to various types of epithelial and mesenchymal cells, including myocytes, adrenocortical cells, adipocytes, chondrocytes, odontoblasts, osteoblasts, myotubes and tenocytes, and different epithelial cells in the lung, liver, gastrointestinal tract, skin, buccal mucosa, and kidney[3,26,41–48]. Moreover, these bone-marrow-derived cells hold great potential in tooth regeneration. When these cells are recombined with embryonic dental epithelial cells and dental mesenchyme *in vitro*, they can be simultaneously reprogrammed into both ameloblast and odontoblast lineages with polarised appearances[49]. Recombinations between bone-marrow-derived cells and embryonic oral epithelium *in vivo* can bring about the development of typical tooth structures and associated bones[50]. It seems that dentinogenesis and osteogenesis mediated by BMSCs are performed through distinct mechanisms and ultimately bring about the different organisations of dentine and bone. Under the induction of apical bud epithelial cells *in vivo*, BMSCs cannot perform dentinogenesis, whereas BMSSCs can, indicating that the purification of heterogeneous bone marrow cells may be helpful for their specific differentiation into odontoblast lineages[26].

Adipo-derived mesenchymal stem cells (ADMSCs)

ADMSCs are readily obtained via lipo-aspirate and can be expanded rapidly *in vitro*. ADMSCs are similar to BMSSCs and mesenchymal stem cells from umbilical cords regarding the morphology, immune phenotype, colony frequency, and differentiation capacity[51,52]. These stem cells are thought to be competent candidates for bone tissue regeneration that can induce new bone formation similar in amount to that induced by either BMSSCs or osteoblasts[53]. ADMSCs present many clinical advantages over BMSSCs or BMSCs, such as the easy and repeating access to subcutaneous adipose tissues and the uncomplicated enzyme-based isolation procedures. More strikingly, their differentiation potential can be maintained with aging, while the differentiation ability of BMMSCs significantly decreases as the donor's age increases. It has been suggested that ADMSCs are multipotent and capable of differentiating into cells of mesodermal or nonmesodermal origin, including adipocytes, neural cells, endocrine pancreatic cells, hepatocytes, endothelial cells, skeletal muscle cells, cardiomyocytes, osteoblasts and chondrocytes[54–58].

These stem cells can also be used as a potential source for tooth regeneration. Our recent experiments have demonstrated that these ADMSCs can perform odontoblastic differentiation under the inductive medium of tooth germ cells. When recombined with apical bud cells in vivo, these stem cells can perform the typical tooth morphogenesis in the renal capsules. Therefore, ADMSCs do represent an alternative source of autologous adult stem cells for tooth regeneration that can be isolated repeatedly in large quantities from subcutaneous tissues under local anaesthesia with a little discomfort.

Non-dental epithelial stem cells (NDESCs)

At the postnatal stages, the cell source for human dental epithelial cells is very limited, mainly because of the insufficient donor tissues[35,41,47]. Since NDESCs from human integumentary tissues (such as skin, gut, and hair) have the capacity to differentiate into many kinds of cell lineages, we can use these non-dental stem cells as an appropriate substitute for dental epithelial cells and drive them into ameloblast lineages.

To test this idea, we have investigated the transition potential from postnatal skin epithelial stem cells to dental epithelial cells under the induction of dental papilla mesenchymal cells (DPMCs). After 6 days co-culture with DPMCs *in vitro*, skin epithelial stem cells express several specific markers of dental epithelial cells, including ameloblastin and amelogenin. Enamel–dentine-like structures can be observed in the recombinants between skin epithelial stem cells and DPMCs *in vivo*, in which the enamel-forming cells are of a skin origin, as indicated by cell tracing analysis. These results indicate that non-dental epithelium can be used as an alternative cell source for future tooth regeneration.

Furthermore, several studies have proved that embryonic stem cells and neural stem cells can respond to the odontogenic signals derived from oral epithelium of E10.0 mice by the expression of *Lhx7*, *Msx1*, *Pax9*, and *Dspp* genes[50,59], indicating that these non-dental stem cells can also be used as potential candidates for tooth reconstruction.

However, several large obstacles stand in the way of application of these non-dental stem cells in making a bio-tooth. The first is a technical handicap, i.e. the difficulty in stimulating non-dental stem cells to differentiate reproducibly and predictably into the desired cell lineages. Second, their intrinsic characteristics are non-

dental and usually short of dental morphogenetic information that can determine the tooth types. Moreover, these non-dental stem cells may convey unexpected characteristics that dental tissues do not have[26]. Finally, the insurmountable ethical dilemmas and debates regarding the application of embryonic stem cells are equally challenging puzzles that must be settled. Therefore, more work should be performed and many basic hurdles residing in the biology of stem cells must be removed prior to the clinical use of non-dental stem cells in coming tooth regeneration.

Prospects

Clearly, the recent recognition of stem cells and their role in tissue regeneration provide a strong basis upon which we can begin to practically impact on the clinical management of tooth loss. Basic strategies have been established whereby bio-teeth can be produced from stem cells of either dental or non-dental origin in the appropriate conditions. However, engineering a bio-tooth with normal functions and periodontal supportive tissues by stem-cell-based therapy may be more complicated than expected. This highlights the need for more research to understand potential perplexing factors, such as shape control, size regulation, *in vivo* eruption, and graft rejection of a bio-tooth.

Opportunities and challenges coexist in tooth reconstruction. Generally, there are two major challenges accompanying stem-cell-based tooth regeneration. The first is that key techniques have to be developed to reproduce the highly specialised arrangements of differentiated cells that constitute a bio-tooth. This is a huge challenge in itself, even if it is possible today to reconstruct such kinds of bio-teeth. The second challenge is perhaps even more daunting, namely testing these bio-teeth on patients. The very reason is that most research resources are concentrated on the major internal organs that are essential for life; thus, testing engineered teeth on patients may be a long and torturous process.

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