Dental Application Potential of Mesenchymal Stromal Cells and Embryonic Stem Cells

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In the past decade, research on the potential applications of stem cells in dentistry has made great progress. There are at least five different types of multipotent mesenchymal stromal cells (MSCs) originating from exfoliated primary teeth, including dental pulp, apica papilla, periodontal ligament, and dental follicle. It has been reported that dental tissue-derived MSCs are able to generate dentine–pulp-like complexes as well as differentiate into periodontal and craniofacial progenitor cells. Similar to these dental tissue-derived MSCs, bone marrow-derived MSCs are also capable of developing into ameloblasts, odontoblasts and periodontal ligament progenitor cells, as well as regenerating cementum, alveolar bone, craniofacial bone and articular condyles. Besides adult stem cells, embryonic stem cells are an alternative cell source for dental tissue regeneration, but the current data are preliminary and are based predominantly on in vitro *data. In addition to these commonly reported stem cells, other progenitor cells with MSC properties are also found in salivary glands, tongue muscle, taste buds and oral mucosa, and these may play a role in recovering the function of the residing tissues. Other than these regenerative applications, many reports have demonstrated the utility of these stem cells in cytotoxicity testing, biocompatibility testing and developmental research. The present article summarises the above findings regarding the regenerative and other potential applications of both MSCs and embryonic stem cells.*

Key words: *dental application, embryonic stem cells, mesenchymal stromal cells, regeneration*

Stem cells represent a particular cell population that is at an unspecialised stage capable of self-renewal and differentiation into more committed lineages[1]. According to their origins and differentiation abilities, stem cells are broadly classified into two categories: embryonic stem cells (ESCs) and adult stem cells. The former is theoretically immortal and pluripotent, capable of producing all cell types of three germ layers and body[2]. However, the latter has a limited life span[3,4] and is capable of generating committed cell lineages of the resident tissues/organs[5,6]. Among various adult stem cells, mesenchymal stem cells have the widest distribution in the human body and have been isolated from diverse tissues/organs[7]. Unlike ESCs, there is no scientific consensus about the immunophenotype of the bona fide mesenchymal stem cells. The currently isolated 'mesenchymal stem cells' always present high levels of heterogeneity, which is suggestive of a mixture of stromal progenitor cells at various developmental stages[8-10] that can be maintained by a minor population of bona fide stem cells[9,11]. Therefore, the International Society for Cellular Therapy would like to name these cells *multipotent mesenchymal stromal cells* (MSCs) rather than *mesenchymal stem cells*[12]. Based on the above knowledge, a more practical definition for MSCs is the stromal-derived cells that can be propagated long-term *in vitro* and have multiple differentiation capacities.

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So far, it has been proposed that both MSCs and ESCs have great potential in the therapy of many diseases[7,9,13,14]. In the past decade, progress has been made in exploring the clinical potential of MSCs and ESCs in dentistry. However, most attempts focused on the regeneration of tooth and periodontal tissues by oral tissue-derived MSCs[15-17], which have been intensively reviewed. Additionally, other clinical and non-clinical application potentials of MSCs and ESCs will be examined in the present review.

Tissue/organ regeneration

Oral tissue defects and loss are major dental problems, greatly reducing patients' quality of life. The causes are various, including congenital malformation, aging, periodontitis, post-cancer ablative surgery, trauma, osteoporotic fractures and progressive skeletal disease. Currently in clinical dentistry, dental sealants, osseointegrated implants, dentures, bone grafting and osteoconductive biomaterials are common remedies to recover the anatomical structure of the damaged oral tissues. However, the ideal therapy for those lost or damaged tissues/organs is not only the recovery of their morphology but also the recovery of their physiological functions. Stem cell-based replacement therapy provides a promise to achieve this goal.

Tooth reconstruction

Among various MSCs originating from different tissues, bone marrow-derived MSCs are most extensively investigated for regenerating most tissue types all over the body. Evidence to support tooth reconstruction by these MSCs can be seen in a report by Ohazama et al[18]. Upon transplantation of a recombination of MSCs and embryonic oral epithelium, tooth crowns associated with bone and soft tissues formed in the renal capsule. A similar tooth structure was formed when this recombination was transferred into the jaw. These results were supported by Hu and coworkers, who mixed MSCs with embryonic dental epithelial cells followed by re-association with dental mesenchyme *in vitro*[19]. Results showed that c-kit positive MSCs could be differentiated into ameloblast- and odontoblast-like cells in contact with the epithelial–mesenchymal junction. In the same assay system, c-kit negative MSCs could also be induced to odontoblast-like cells. Both groups showed the independence of the amelo-differentiation of MSCs without cell fusion.

Despite feasible evidence that bone marrow-derived MSCs can reconstruct teeth, abundant data suggest dental tissue-derived MSCs to be an alternative cell source suitable for whole tooth regeneration[20]. These MSCs are separated from exfoliated primary teeth (SHED, stem cells from human exfoliated deciduous teeth)[21], dental pulp (DPSC)[22], apical papilla (SCAP)[23], periodontal ligament (PDLSC)[24], and dental follicle (DFSC)[25]. It is reported that both bone marrow-derived MSCs and dental tissue-derived MSCs reside in the perivascular area in their respective tissues, and express some common surface molecules, such as stro-1 and CD146[26]. By proteomic characterisation, the regulation of selected proteins, involving Hsp27, Annexin A4 and CRMP4, was conserved between different MSCs of different origins from different species[27]. Both bone marrow- and dental tissue-derived MSCs can differentiate into at least three committed lineages[16,28,29]: osteo/odontogenic, neurogenic and adipogenic lineages. Interestingly, similar to bone marrow MSCs, SHED, DPSC, SCAP and PDLSC display low immunogenicity and immunosuppressive activity[30-34]. Also, the mechanisms under this immunosuppressive activity are independent of apoptosis and are mediated by soluble molecules. These properties imply that these cells are suitable for allogeneic transplantation. However, dental tissue-derived MSCs are more committed to odontogenic cells[16,35,36], while bone marrow-derived MSCs are more osteogenesis-oriented under non-inductive conditions[16]. In comparison with bone marrow-derived MSCs, DPSC, SHED, SCAP and PDLSC have higher proliferation rates and more population doublings[16,26], except for DFSC[25]. With the colony-forming unit-fibroblast (CFU-F) assay, DPSC, SHED and PDLSC displayed higher incidence of the number of colonies after 10 to 12 days in the same culture conditions at the same plating cell numbers[26].

To date, SHED, DPSC and SCAP have been reported to be able to differentiate into odontoblast-like cells[21,33,37]. *In vivo*, both DPSC and SCAP can regenerate dentine–pulp-like complexes[26,32,33,38,39], while SHED can only form a dentine-like structure[21,26]. For dentine formation, DPSC predominantly produce reparative dentine, while SCAP is involved in the production of root dentine[32,33,39]. Besides these three dental MSCs, there are no data on the reconstruction of enamel, dentine and pulp by PDLSC and DFSC. Interestingly, outside bone marrow and dental tissues, dermal-derived MSCs are also able to be induced into odontogenic lineage cells by exposure to a conditioned medium of embryonic and neonatal tooth germ cells in culture[40].

Because of the finite *in vitro* expansion ability of MSCs[16,26], there is a requirement to identify other cell sources as possible replacements for large-scale clinical usage. ESCs are such immortal cells, which theoreti-

cally can be infinitely propagated *ex vivo* and are inducible to develop into all adult cell types of three germ layers. Unlike the gene-transformed immortal cell lines, they have a normal karyotype and strong expression of telomerase to maintain the gene stability[41]. This telomerase activity is normally absent in MSCs[42,43]. Recently, trials to induce ESCs to oral cell types were performed. It was found that ESCs could attach to the extracted tooth root slice surface and proliferate after 2 days of direct seeding. Upon co-culture with periodontal ligament cells in osteogenic inductive medium, ESCs could synthesise osteogenic markers, such as osteopontin and osteocalcin[44]. However, after the embryoid body formation, ESCs are easier to induce to odontogenic epithelial cells, which produce ameloblast-specific proteins, such as cytokeratin 14, ameloblastin and amelogenin[45].

Periodontal reconstruction

Periodontal tissues are composed of gingiva, periodontal ligament, cementum and alveolar bone. They serve as a proprioceptor to detect the force on teeth, and a glue to keep the teeth attached to alveolar bone. Periodontal diseases are highly prevalent worldwide and result in bone tissue destruction and subsequent tooth loss. It has been found that a mixture of autologous bone marrow-derived MSCs and atelocollagen could repair experimental class III periodontal defects by the regeneration of periodontal ligament and alveolar bone[46-48]. It has been hypothesised that cytokines and signalling molecules could accelerate the periodontal differentiation of MSCs[49,50]. In the presence of platelet-rich plasma, bone marrow-derived MSCs displayed prominent periodontal tissue regeneration[51]. When these MSCs were transfected with basic fibroblast growth factor (bFGF), the regeneration of periodontal bone tissue was accelerated[52]. In addition, bone marrow-derived MSCs were also able to produce cementum when they were cultured on root slices pre-treated with enamel matrix proteins[53] or implanted with dl-lactide-co-glycolide scaffold[54]. The regeneration of cementum, periodontal ligament, and alveolar bone was enhanced by the transfection of the sonic hedgehog gene[55].

Among dental tissue-derived MSCs, stro-1-positive PDLSC and DFSC displayed the regenerative abilities of cementum[24,56-58] and periodontal ligament[58-60]. Moreover, *in vivo*, PDLSC could form cementum–periodontal ligament-like structure and alveolar bone with/without being loaded onto scaffolds[24,26,61,62]. The differentiation to periodontal cells could be programmed by enamel matrix derivatives, bone morphogenetic protein 2 (BMP-2) and BMP-7[56,63]. So far, there is no evidence about the periodontal tissue regenerative ability of DPSC, SHED and SCAP. However, there have been two trials to induce ESCs to periodontal lineages by co-culturing with periodontal ligament fibroblasts and directly contacting with tooth root slices[44,64]. Only a minority of cells in colonies showed the characteristics of periodontal ligament progenitor cells.

Craniofacial skeleton and temporomandibular joint/condyle reconstruction

Bone regeneration is one of the prominent characteristics of MSCs suitable for clinical application[65,66]. Abundant studies have reported that bone marrow-derived MSCs are able to heal critical-sized segmental bone defects[67], cranial defects[11,68,69], alveolar bone destruction[11,68], and mandibular defects[70], as well as reconstruct mandibular condyle[71-73]. The formation of lamellar bone in maxillary sinus augmentation by bone marrow-derived MSCs provides a reliable base for dental implants[74]. Most of this progress was made in combination with the utility of MSCs, biomaterials and/or growth factors[26]. In the repair of the critical-sized mandibular bone defects, bone marrow-derived MSCs were loaded onto poly-dl-lactic-co-glycolic acid scaffolds[70]. In the reconstruction of articular condyles, bone marrow-derived MSCs were firstly differentiated into osteo- and chondro-genic cells followed by the encapsulation in poly(ethylene glycol)-based hydrogel layers[71]; the transfection of telomerase reverse transcriptase could enhance the osteogenesis of MSCs[42,43].

Among dental tissue-derived MSCs, SHED and PDLSC are well tested for osteogenesis. Currently, studies have shown that they could be directly inducible into osteogenic lineages[21,75-79]. PDLSC are sensitive to the induction of retinoic acid[75], pharmacological stimulation[77] and pulsating fluid shear stress[78]. In comparison with PDLSC, SHED are less sensitive to retinoic acid stimulation[75]. Besides the direct osteogenic differentiation, SHED can recruit host osteogenic cells to repair bone defects[76], but the bone formed by SHED lacks hematopoietic marrow elements, which are always observed in the bone formed by bone marrow-derived MSCs[76]. Besides SHED and PDLSC, there are a few trials to induce DPSC and DFSC to osteogenic progenitor cells[77,80,81,82]. Although the osteogenic markers could be detected on these cells after the osteoinduction, it needs *in vivo* data to consolidate these findings.

Compared with DPSC and DFSC, more solid evidence about the osteogenic differentiation potential of ESCs is available. ESCs can be inducible to osteogenic cells by the co-culture with periodontal ligament fibro-

blasts *in vitro*[83,84], as well as the inducement of a chemical complex with[85,86] or without[87] embryonic body formation. They are also able to form bone structure by transplanting with hydroxyapatite/tricalcium phosphate particles into tooth sockets, without the occurrence of teratoma[88]. Besides the osteogenesis, ESCs are also inducible to chondrogenic cells[89,90], which may facilitate the reconstruction of temporomandibular joints. These *in vitro* expandable ESC-derived chondrogenic cells repair critical-sized osteochondral defects without evidence of tumorigenicity[91].

Gland restoration

Gland damage will cause a deficiency of saliva and various oral problems, such as mastication and swallowing problems, dental caries, burning sensations, periodontitis, denture problems, and dysgeusia. However, there is a lack of effective treatment to recover gland function. In recent years, stem cells have been proposed as a good remedy[92,93]. Putative salivary epithelial stem cells[94-99] and intercalated duct stem cells[100,101] were identified from oral glands. These salivary stem cells share the same surface molecule profile as well as proliferation and tri-lineage differentiation abilities as pancreatic acini-derived stem cells and bone marrow-derived MSCs[97]. Also, c-kit is their common molecule expressed on the cell surface[95-98,101]. Upon isoprenaline stimulation, the intercalated duct cells in parotid glands may develop to acinar cells[100]. *In vitro*, these salivary stem cells were inducible to amylase-producing acinar cells, while after transplantation, they could restore the morphology and function of salivary glands[98,102]. To further explore the properties and future applications of the salivary stem cells, an immortalised cell line was established by the transfection of integrin α6β1[103]. These cells were able to achieve both acinar- and duct-like structures.

Other dental tissue regeneration

To date, there is one study suggesting that bone marrow-derived MSCs can be recruited into the cheek and differentiate into buccal epithelial cells without host-recipient cell fusion[104]. However, the identity of the particular cells for differentiation is unclear. There are stem cells detected in other oral tissues, such as tongue muscle[105], taste buds[106] and oral mucosa[107]. However, their effects in differentiating into dental cells *in vitro* and regenerating oral tissues *in vivo* are unknown. Notably, there are mucosa lamina propria-derived stem cells exhibiting multiple differentiation properties to mesodermal, endodermal and neural lineages[107]. They have the same immunophenotype as bone marrow-derived MSCs and neural stem cells, and have a low level of expression of ESC markers. After transplantation, they can form a tumour consisting of mesodermal and ectodermal tissues.

Non-regenerative application

Cytotoxicity testing for non-biological materials

With reference to the International Standard (ISO 10993 part 5, 1999), immortal cell lines, such as L-929, Balb/3T3 and WI-38 etc., are recommended for cytotoxicity testing. These cell lines have homogeneous morphologies and infinite proliferation, which provide good reproducibility for *in vitro* cytotoxicity screening. However, these cells are normally derived from tumours or are gene-modified, as well as being of animal origin. The lack of genetic integrity and the xeno-origin make their representative identities for normal human cells questionable. As human stem cells are able to self-renew, expand *in vitro*, have normal karyotype, and are usable for cell replacement therapy, they may represent alternative suitable cell sources for cytotoxicity testing[108-111].

By using bone marrow-derived MSCs, it was found that 2-vinyl-8-hydroxyquinoline derivatives could efficiently decrease oxidative stress-induced cell death[111]. By testing various resin-based sealers on DPSC, CMF bond adhesive showed a better supportive effect on cell viability and proliferation than Prime&Bond NT, Clearfil S(3) and XP Bond[110]. Being evaluated on the same stem cells, laser phototherapy displayed a positive effect on improving DPSC growth in a nutritionally deficient condition[112]. Ameloblast stem cells are sensitive to the anti-microtubule agent vinblastine. The cytotoxicity of vinblastine on ameloblast stem cells displayed a dose-dependent effect[113]. Recently, human ESC-derived fibroblasts were established and were reported to be more sensitive to the cytotoxicity of mitomycin C than L929[109], suggesting another stable cell source available for *in vitro* cytotoxicity testing. Theoretically, ESCs are immortal, and hence can potentially be an infinite source of fibroblasts.

Biocompatibility testing

Biocompatibility is a broad concept about the behaviour of biomaterials in various contexts. In regenerative medicine, the biomaterials are fabricated as a scaffold or matrix to repair a structural defect or to support physi-

ological activities of *in vitro* expanded cells in the host, without eliciting any undesirable effects on both cells and host. Good biocompatibility is a prerequisite for the clinical application of new biomaterials and their tissue-engineering products, including implants and scaffolds. The current compatibility testing follows the guidelines of ISO 10993 prior to clinical study. As suggested, various stem cells will be promising cell sources for future dental and medical application. Recently, some preliminary data about the biocompatibility of dental biomaterials were obtained based on cytotoxicity, genotoxicity and embryotoxicity tests on bone marrow-derived MSCs[114-120] and ESCs[121,122].

Developmental research

The initiation signals for tooth development are from the contact of oral epithelium with neural crest-derived mesenchyme[123]. These signals mainly involve the members of hedgehog, Wnt, FGF and the transforming growth factor-beta superfamilies. However, the particular molecules triggering and regulating this process are still unclear, and most of the evidence is based on studies of mouse embryos. Therefore, to address these questions, human tooth developmental models will be of great importance. In 2004, Ohazama and coworkers found that a complete tooth primordium could be produced from bone marrow-derived MSCs upon interaction with embryonic oral epithelium[18], suggesting that the neural crest-derived mesenchyme is an optional cell source during the development of teeth. Furthermore, an *in vitro* three-dimensional human DPSC-scaffold–dental epithelial cell model was created to investigate the epithelial–mesenchymal interactions during tooth morphogenesis[124]. In this model, DPSC and dental epithelial cells showed a different pace of differentiation and a different spatial expression of dentine sialoprotein in the absence of the differentiation medium. These results confirm the possibility and feasibility of establishing *in vitro* tooth developmental models.

Summary

In summary, the present review has reported the clinical and non-clinical application potential of bone marrow-derived MSCs, dental tissue-derived MSCs and ESCs in dentistry. Although the research progress is promising for regenerative medicine, it is not readily translatable to the clinic. Firstly, differentiation to desired committed lineages by these stem cells is not controllable. This differentiation uncertainty will subsequently lead to low efficiency and efficacy upon transplantation or transfusion. Secondly, the biosafety of these stem cells is a concern. Currently, all cells are expanded *ex vivo* under culture conditions with xeno-components. The possible contamination of unknown xeno-biohazard will put patients in danger. Additionally, in spite of limited life span and restricted differentiation ability, adult stem cells still have a high risk of being tumorigenic[125]. It was reported that long-term *in vitro* culture of stem cells could cross-present exogenous antigens[126]. These exogenous antigens can possibly trigger an immune response after being transplanted, thereby decreasing the viability of stem cell grafts. Thirdly, the topographic structure of tooth and other oral tissues/organs is not reproducible by using pure stem cells. It is necessary to apply scaffolds or other carriers. The biocompatibility of stem cells and scaffolds requires further testing. Fourthly, limitation in the availability of autologous stem cells will hinder its application in tissue/organ regeneration. The application of allogeneic adult stem cells as alternative cell sources is still debatable. Recently, induced pluripotent stem cells were successfully established from oral mucosa fibroblasts via retroviral gene transfer of *oct4*, *sox2*, *c-myc* and *klf4e*[127]. This is another solution towards achieving personalised regenerative cells. However, safety and gene integrity are questionable. Last but not least, the cost effectiveness of stem cell-based therapy should be another consideration.

Practically, stem cells are good tools for dental research and cytoxicity testing *in vitro*. Nevertheless, some time is required to establish a standard operation protocol for evaluating the cytotoxicity of dental/medical chemicals and medicine.

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