Keywords: Dental pulp stem cells; Periodontal ligament stem cells; Progenitor cells

Introduction

To restore the damaged tissue or organs, it is critical to understand the developmental process behind the tissue engineering and regenerative medicine. Stem cells play essential roles in tissue engineering for organ development and tissue repair. Two main types of stem cells have been studied including embryonic stem cells (ES cells) and postnatal or adult stem cells (AS cells). ES cells derived from the blastocyst have the ability to differentiate into multipotent stem/progenitor cells, including epithelial, mesenchymal, and other tissue-specific stem cells [1,2]. The specialized functional tissue and organs of human body can be generated by the interactions among these stem cells [3,4]. Once the organ matures, the pluripotent ES cells evanesce. Some multipotent AS cells however remain in the developed tissue and have the ability to regenerate and/or repair injuries [5], for example mesenchymal stem cells (MSCs) derived from bone marrow [6], dental pulp [7], adipose tissue [8], dermis [9] and umbilical cord [10].

Oral stem cells mainly include dental pulp stem cells (DPSCs) [7], stem cells from exfoliated deciduous teeth (SHED) [11], stem cells from apical papilla (SCAP) [12], periodontal ligament stem cells (PDLSCs) [13], mesenchymal stem cells from gingiva (GMSCs) [14] and progenitor cells from oral mucosal lamina propria (OMLP-PCs) [15]. They are widely applied in not only regenerative dentistry but also tissue and organ repair and/or regeneration of other systems.

We showcase in this mini-review the past development and current status of two representative oral stem/progenitor cells: DPSCs and OMLP-PCs, and showcases their potential in not only regenerative dentistry but also regenerative treatments for other systems and immunotherapies.

Dental Pulp Stem Cells

DPSCs are mesenchymal-derived cells residing within the peri-invasive niche of dental pulp and are considered to originate from migrating cranial neural crest cells. These cells are multipotent stem cells which have advantages for clinical applications [16]. Compared with other MSCs derived from bone marrow, adipose tissue, peripheral blood, and umbilical cord blood, DPSCs has a distinct advantage of easy accessibility with entirely non-invasive procedures and little ethical issues. This is because healthy adult teeth, particularly impacted molars are often extracted during orthodontic treatment. Rather than discarding the teeth, pulp tissue can be excavated and cryogenically preserved. DPSCs can subsequently be isolated from such tissue, following thawing, with no detrimental effects in viability, proliferation or differentiation [17]. This has led to the establishment of pulp tissue banks for patients to store and use their own progenitor cell samples if and when required. A number of studies also demonstrated that the immunogenic reaction to DPSCs may be suppressed by the inhibited activation and induced apoptosis of T-cells [18-20]. This suggests that in addition to patient-matched autologous applications, DPSCs may be tolerated as an allogeneic cell transplant without the need for immunosuppression [21].

The presence of DPSCs was first proposed by Gronthos et al. [7]. DPSCs have been demonstrated to express a range of mesenchymal stem cells related markers such as CD44, CD73, CD90, CD105, stem cell antigen (SCA1) and stro-1 [22], therefore they are generally classified as mesenchymal derived stem cells. DPSCs can be readily induced to differentiate in vitro into the classic MSC lineage cell types of osteoblasts/odontoblasts, chondrocytes and adipocytes using established protocol [23,24]. However, the differentiation potential of DPSCs may not be strictly limited to mesenchymal cell types, since the expression of pluripotency markers such as nanog, oct4, sox2 and ssea4 can be found in cultured DPSCs [25]. Additionally, DPSCs have been demonstrated to express markers related to tissues derived from other germ layers such as muscle (α-smooth muscle actin) [22] and neurons (Nestin, βIIItubulin and Map2) [25]. A number of studies have demonstrated the potential for endothelial [26], myogenic [27], hematopoietic [28] and melanocytic differentiation [29]. Due to this potential versatility, a variety range of therapeutic applications have been proposed for DPSCs. In vivo, a number of DPSCs based studies...
have been conducted to repair or regenerate non-dental tissues. De Mendonca et al. and Riccio et al. investigated that new bone could be formed in critical-size calvarial bone defects [30,31], while Yamada et al. and Zheng et al. found that critical-size mandibular bone defect could be repaired using DPSCs [32,33]. Ito et al. compared the osteogenic potential of three types of cells-DPSCs, bone marrow stem cells (BMSC) and periosteal cells (PC), for the osseointegration of dental implants and tissue engineered bone by transplanting these cells into the alveolar bone defect of dogs. Interestingly, the osteogenic ability of DPSCs was shown to be more superior than that of bone marrow-derived stem cells, indicating that DPSC was a useful cell source for tissue-engineered bone in dental implants [34]. Additionally, some of studies demonstrated that DPSCs play a potential role in the nerve repair and/or regeneration. DPSCs formed blood vessels and myelinating tissue and contributed to the promotion of peripheral nerve regeneration when transplanted into a gap in the rat facial nerve [35]. They could also induce neuroplasticity within a recipient host nervous system by secreting factors that coordinate axon guidance [36]. Apart from above, DPSCs also have positive effects in the modulation and enhancement of adult neurogenesis. The co-culture with DPSCs significantly protected primary cultures of hippocampal and ventral mesencephalic neurons in-vitro models of Alzheimer’s and Parkinson’s disease [37,38]. Additionally, DPSCs exert protective effect on dopamine (DA) neurons by increasing number of spared tyrosine hydroxylase (TH)+ cells [38]. These all proved that DPSCs can be deemed as possible candidates for cell-based therapy in adult neurodegenerative disorders in future. DPSCs have also demonstrated its potential in the Spinal Cord Injury (SCI) repair, which promoted the recovery of hind limb locomotor function in rats by inhibiting the SCI-induced apoptosis of neurons/astrocytes/oligodendrocytes and multiple axon growth inhibitors, and by replacing lost cells through differentiating into mature oligodendrocytes [39]. Furthermore, DPSCs have been proved to be a new stem cell source for cell-based therapy to stimulate angiogenesis/vasculogenesis during tissue regeneration. The cells have been shown to secrete new vasculature and express several proangiogenic factor, such as VEGF-A, G-CSF, GM-CSF, and MMP3 when transplanted into the model of mouse hind limb ischemia [26], and exhibited therapeutic potential for the repair of Myocardial Infarctions (MI) in a MI mouse model [40]. The cells from human deciduous teeth were also reported to accelerate wound healing in combination with basic fibroblast growth factor (b-FGF) [41].

OMLP-PCs

Oral Mucosal Lamina Propria progenitor Cells (OMLP-PCs) are progenitor cells of neural crest origin isolated from oral mucosal lamina propria (OMLP) [42] (Figure 1). OMLP-PCs which were first proposed by Stephens et al. have been demonstrated to express a range of stem cell markers including CD44, CD90, CD105 and CD166 [15]. These cells are generally classified as neural crest derived stem cells due to the present of Jagged 1 and the expression of a number of neural crest markers, such as Snail, Slug, Sox10 and Twist [15]. These progenitor cells have demonstrated their multipotency in-vitro models of Alzheimer’s and Parkinson’s disease [37,38]. Additionally, DPSCs exert protective effect on dopaminergic neurons (DA) neurons by increasing number of spared tyrosine hydroxylase (TH)+ cells [38]. These all proved that DPSCs can be deemed as possible candidates for cell-based therapy in adult neurodegenerative disorders in future. DPSCs have also demonstrated its potential in the Spinal Cord Injury (SCI) repair, which promoted the recovery of hind limb locomotor function in rats by inhibiting the SCI-induced apoptosis of neurons/astrocytes/oligodendrocytes and multiple axon growth inhibitors, and by replacing lost cells through differentiating into mature oligodendrocytes [39]. Furthermore, DPSCs have been proved to be a new stem cell source for cell-based therapy to stimulate angiogenesis/vasculogenesis during tissue regeneration. The cells have been shown to secrete new vasculature and express several proangiogenic factor, such as VEGF-A, G-CSF, GM-CSF, and MMP3 when transplanted into the model of mouse hind limb ischemia [26], and exhibited therapeutic potential for the repair of Myocardial Infarctions (MI) in a MI mouse model [40]. The cells from human deciduous teeth were also reported to accelerate wound healing in combination with basic fibroblast growth factor (b-FGF) [41].

Figure 1: Multilineage differentiation capacity and potential clinical applications of dental pulp stem cells and oral mucosal lamina propria progenitor cells.
with induced differentiation into both mesenchymal (chondrogenic, osteoblastic, and adipogetic) and neuronal (neuron and Schwann-like cells) cell lineages [15]. Additionally, the expression of pluripotency markers such as nanog, oct4 and sox2 can be found in cultured cells, which may indicate that the differentiation potential of OMLP-PCs may not be strictly limited to mesenchymal and neuronal cell types but can also be induced into other cell types derived from other germ layers. Therefore the potential differentiation of these progenitor cells offers a new approach for allogeneic tissue engineering application. Compared with other current stem cells sources, OMLP-PCs offers distinct advantages including easy accessibility, and minimally invasive isolation from the buccal mucosa, which heals without scar formation. Most importantly, OMLP-PCs has shown no inherent immunogenicity with minimal expression of costimulatory molecules (CD40, CD80, CD86, CD154 and CD178) or human leukocyte antigen (HLA) class II [43]. They can efficiently suppress the proliferation of peripheral blood lymphocytes in a dose dependent manner [43]. Compared with other mesenchymal stem cells, these characteristics make OMLP-PCs more advanced for allogeneic tissue engineering as well as in the treatment of immunological disorders such as chronic graft-versus-host disease [43].

Conclusion and Perspectives

The rapid expansion of the current research on oral stem/progenitor cells has shown encouraging outcome in tissue engineering and preclinical investigation. Stem cells from oral epithilum and mesenchymal tissue with advantages of easy accessibility and non-invasive extraction procedures offered minimal ethical issues in clinical application. Oral stem/progenitor cells demonstrated high proliferation rate, excellent regeneration capacity, multipotential of differentiation as well as little inherent immunogenicity, which guarantees their future clinical application potential in not only dentistry but also various other fields of regenerative medicine. However, several major objectives must be addressed before clinical application to be conducted, as below:

1. Oral stem/progenitor cells have been demonstrated to be a promising cell source for the treatment of various pathological conditions, however the long-term fate of transplanted oral stem/progenitor cells needs to be systematically clarified in the in-vitro models. It has been reported that in the spinal cord injury repair model, that massive cell death occurred at the grafting site with only a few transplanted cells survived, and most of them could not integrate into the local tissue [39]. The successful transition from in-vitro/ex-vivo to in-vivo is one of the key factors determining the efficiency of cell based therapy, in particular the standardized verification of the data from in-vitro/ex-vivo models in the in-vivo situation where cells respond to the endogenous environment largely differently compared to the in-vitro/ex-vivo conditions. Under in-vitro/ex-vivo situation, the multipotency and differentiation capacity of stem/progenitor cells could be very well controlled with the aim of added growth factors and etc in the artificial culture system, which is very difficult to achieve once the cells are transplanted in-vivo. The multipotent differentiation capacity of oral stem/progenitor cells in-vitro does not necessarily reflect the response in-vivo, therefore the homing and transdifferentiation capacity and efficiency of oral stem/progenitor cells into a specific tissue type deserves more attention for investigation. Many pre-clinical/translational studies reported encouraging outcome of the treatments, however some of these claims were based on marginal improvement (even though statistically meaningful), which might not be sufficient enough in the real clinical combat. Therefore, more stricter quantitative analysis should be put in place to evaluate the real therapeutic effect of these transplanted cells for tissue regeneration and injury repair.

2. There is still an intense debate on the neuronal/glial differentiation of MSCs-like cells. An increasing number of studies have demonstrated that DPSCs have potential of neuronal/glial differentiation both in-vitro or in-vivo. In-vitro, multi-step pharmacological transdifferentiation protocols are proved to be efficient than simple differentiation procedures. These studies unequivocally showed neuronal morphological differentiation, and increased expression of neuronal/glial markers at both gene and protein levels [24,44-49]. Additionally, some of the studies even provide evidence for the presence of at least some functional elements that are necessary for neuronal behaviour, such as specific calcium, sodium, and potassium channels [44,46]. However, the functional activation of these channels showed great variability depending on the different protocols of differentiation. Most of protocols could only induce one or two functional channels [44], therefore, most of DPSCs just differentiate into the neuronal/glial like cells in-vitro. Few of them demonstrate functional differentiation. In-vivo, DPSCs have been used to transplant into a brain injury or a SCI in animal model [39,50]. Although DPSCs transplantation therapy could promote recovery of neuronal functions, little is known about the mechanism of how DPSCs work for the neuron regeneration. Therefore further in-depth investigations are desired to be conducted in order to fully figure out whether DPSCs are able to replace lost cells through their differentiating capacity into mature neurons/oligodendrocytes. Perhaps the alternative explanation could be that DPSCs could release growth factors which induce the local neural stem cells migration and differentiation to replace the lost neurons/oligodendrocytes.

3. Developing a safer and more reliable system to deposit/deliver oral stem/progenitor cells, which also closely relates to the discussion above. Grafted cells will be challenged by the adverse endogenous environment post transplantation which would trigger auto-differentiation of the cells into non-therapeutic cells; therefore it is vitally important to provide a smart delivery system ideally with the capacity of conjugate favourable biomedical factors essential to maintain the multipotency of the grafted cells. Besides, for target specific replacement therapy stem/progenitor cells cannot be delivered in single cell suspension, otherwise the grafted cells will be either lost quickly or diffused without control, therefore a 3D niche of porous structure which constrains the grafted cells would be desirable. From these point of views, nanomaterials based 3D scaffold has been proposed for many years to serve these targets, by rational design of nanomaterials based biocompatible/biodegradable 3D scaffolds with the binding capacity of multiple biological factors essential for multipotency and differentiation. Despite many successful studies showcased the huge potential of such functionalised nanoscaffold in treating multiple cell types based replacement therapy, such application with oral stem/progenitor cells is still yet to be explored further.

4. The microenvironment of transplantation sites should be analyzed more closely because the interaction between the transplanted stem/progenitor cells and endogenous cells may play a critical role in determining the fate of the grafted cells and consequently the outcome of transplantation. Ex-vivo models of stem/progenitor cells transplantation is very useful to disclose the potential interactions between grafted cells vs. endogenous ones, however these can never fully mimic the genuine in-vivo situation where multiple biological factors (both positive and negative) are secreted as part of the pathological response. Therefore such microenvironmental interactions would need to be dealt with using the in-vivo transplantation models, which is inevitably time consuming for the screening of multiple factors regulating the cellular interactions. System biology approach is another very useful tool in the exploration of such intercellular reactions.
involving multiple factors, which is largely neglected and requires more attention in our research community.

(5) The advantage of immunomodulatory properties of oral stem/progenitor cells deserves more attention in the stem cell replacement therapy. Studies have demonstrated that some of the oral stem/progenitor cells (OLMP-PCs) have no inherent immunogenicity [43], therefore making OMLP-PCs one of the desirable stem cell sources in cell transplantation therapy for reduced immune rejection response. However such suppressed immune responses of the recipient should be fully characterized before clinical applications, and further investigation should be conducted in order to systematically explore the critical roles of oral stem cells in immunomodulation, as well as the interactions between oral stem cells and the immune system.

(6) The mechanism for the regulation of oral stem cells differentiation, proliferation and generation of specific tissue should be fully investigated both in-vitro and in-vivo. More investigation should be performed on the expression of particular genes and sequential activation of associated signaling pathways in the control of such cellular response. The outcome of such studies can be combined with the approaches mentioned above to promote the oral stem cells based regeneration into the desired tissue/cell types with proper functions both in-vitro and in-vivo.

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