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## *Review*

# Current understanding of orofacial tissue derived mesenchymal stem cells: an immunological perspective

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**Summary.** Mesenchymal stem cells (MSCs) are multipotent non-hematopoietic progenitor cells able to differentiate into multiple lineages, holding the potential for replacing damaged and diseased tissues by tissue regeneration and immunomodulatory functions. So far, MSCs have been successfully isolated and characterized from a variety of orofacial tissues, including dental pulp, periodontal ligament, root apical papilla, gingiva, etc. In addition to their self-renewal and multipotent differentiation properties, these orofacial tissue derived MSCs are also capable of profound immunomodulatory effects *in vitro* and *in vivo*, thus providing a foundation for their utilization in allogeneic application and in treating autoimmune diseases and inflammatory disorders. In this paper, we will review the current research progress of the immunomodulatory properties of orofacial tissue derived MSCs and the underlying mechanisms, emphasizing the effect of these MSCs on immune cells, which will facilitate the use of such cells in clinical treatment.

**Key words:** Mesenchymal stem cells, Tooth, Immunomodulation, Cell therapy, Regenerative medicine

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## Introduction

Mesenchymal stem cells (MSCs) are a heterogeneous population of fibroblast-like cells possessing self-renewal capacity and could differentiate into a variety of cell types, including, but not limited to osteoblasts, adipocytes, chondrocytes (Prockop, 1997; Pittenger et al., 1999; Bianco et al., 2001). Until now, MSCs have been isolated and cultured in a wide spectrum of post-natal organs and tissues, such as bone marrow, adipose tissue, placenta, umbilical cord, and orofacial tissues, etc (da Silva et al., 2006; Haniffa et al., 2007; Wang et al., 2012). In addition to their ability of multi-potent differentiation and regeneration and/or replacement of diseased tissues, MSCs are capable of regulating the functions of various immune cells (Di Nicola et al., 2002; Djouad et al., 2003; Krampera et al., 2003, 2006; Beyth et al., 2005; Jiang et al., 2005; Corcione et al., 2006; Sotiropoulou et al., 2006; Spaggiari et al., 2006), making them promising cell sources for the treatment of human autoimmune diseases and inflammatory disorders (Le Blanc et al., 2004; Ringdén et al., 2006; Sun et al., 2009; Akiyama et al., 2012; Xu et al., 2012). Recently, studies have demonstrated that orofacial tissues contain several distinct MSC populations, including dental pulp stem cells (DPSCs), stem cells from human exfoliated decidous teeth (SHED), periodontal ligament stem cells (PDLSCs), stem cells from root apical papilla (SCAP), gingiva mesenchymal stem cells (GMSCs), and orofacial bone/bone-marrow-derived MSCs (OMSCs), etc (Gronthos et al., 2000; Miura et al., 2003; Seo et al.,

2004; Sonoyama et al., 2006; Zhang et al., 2009; Yamaza et al., 2011), which are easily obtained and provide a new hope for regenerative therapy in dental clinics (Rodríguez-Lozano et al., 2011). These cells are in accordance with the minimal criteria for defining multi-potent MSCs set by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy, i.e. they are plastic-adherent, are positive for CD105, CD73 and CD90 and negative for expression of hematopoietic lineage surface molecules, and could differentiate to osteoblasts, adipocytes and chondroblasts *in vitro* (Dominici et al., 2006). Meanwhile, these orofacial tissue derived MSCs also possess immunomodulatory characteristics. In this review, we explored the current understanding of the immunomodulatory characteristics of orofacial tissue derived MSCs in depth, and compared the immune capabilities of orofacial tissue derived MSCs with those of bone marrow mesenchymal stem cells (BMSCs) and adipose mesenchymal stem cells (AMSCs), two intensively studied MSCs possessing profound immunomodulatory properties. The immune features of these MSCs, and application in pre-clinical models and patients, are listed in Table 1.

**Table 1.** Immunological properties and clinical potential of MSCs.

| cell type | cell source | immunological properties | pre-clinical models | clinical application | references |
|---|---|---|---|---|---|
| BMSCs | bone marrow | suppress proliferation of T cells; promote proliferation of regulatory T cells (Tregs); inhibit the proliferation, cytokine production, and cytotoxic activity of natural killer cells; suppress differentiation, maturation and function of dendritic cells; most studies demonstrated that BMSCs inhibit B cells proliferation, differentiation, and antibody secretion, whileas some studies showed that BMSCs promote B cells proliferation and antibody secretion | systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis, Crohn's disease, diabetes mellitus (DM), systemic sclerosis, graft rejection, encephalomyelitis, etc | graft-versus-host disease(GVHD), SLE, systemic sclerosis, Sjogren's syndrome, etc | Le Blanc et al., 2004; Sun et al., 2009; Akiyama et al., 2012; Wang et al., 2012; Xu et al., 2012 |
| AMSCs | adipose tissue | suppress T cell proliferation induced by mitogens and mixed lymphocyte reaction (MLR); improve the production of anti-inflammatory cytokines and decrease the production of inflammatory cytokines | multiple sclerosis, Crohn's disease, DM, Alzheimer's disease, autoimmune hearing loss, etc | GVHD, DM, RA, ulcerative colitis, multiple sclerosis, etc | Puissant et al., 2005; Fang et al., 2010; Zhou et al., 2011; Leto Barone et al., 2013 |
| DPSCs | dental pulp of permanent tooth | fail to stimulate allogeneic T cell proliferation and inhibit mitogen-triggered T cell proliferation and MLR; induce activated T cells apoptosis; up-regulate levels of Tregs | DM, Parkinson disease, myocardial infarction, colitis, regeneration of bio-root, SLE, etc | — | Pierdomenico et al.,2005; Demircan et al., 2011; Zhao et al., 2012 |
| SHED | dental pulp of deciduous tooth | suppress T cell proliferation; reduce levels of Th17 cells and IL-17 | periodontitis, SLE | — | Yamaza et al., 2010; Ma et al., 2012; Fu et al., 2014 |
| SCAP | apical papilla of immature teeth | inhibit one-way MLR and T cell proliferation stimulated by mitogen | — | — | Ding et al., 2010a,c |
| PDLSCs | periodontal ligament | fail to stimulate allogeneic T cell proliferation; inhibited mitogen-stimulated proliferation of allogenic and xenogenic T cells, or an allogeneic MLR; suppress proliferation, differentiation and immunoglobulin production of B cells | periodontitis | — | Wada et al., 2009; Ding et al., 2010b; Liu et al., 2013; Tang et al., 2014 |
| GMSCs | gingival tissue | suppress T cell proliferation and induce expression of immunosuppressive factors; up-regulate levels of Tregs; turn macrophages into an anti-inflammatory M2 phenotype; suppress the differentiation of dendritic cells and activation of mast cells | colitis; allergic contact dermatitis; skin allograft model; cutaneous wound | — | Zhang et al., 2009, 2010; Mitrano et al., 2010; Su et al., 2011; Xu et al., 2013 |
| DF-MSCs | dental follicle | suppress proliferation of T cells by secretion of TGF-β; TLR3/ TLR4 agonist enhance the suppressive effect of DF-MSCs and improved TGF-β and IL-6 secretions | — | — | Tomic et al., 2011 |
| OMSCs | orofacial bone/bone-marrow | suppress activated splenocytes; inhibit spontaneous apoptosis of naïve splenocytes | — | — | Yamaza et al., 2011 |
| PL-MSCs | periapical lesions | inhibit T cell proliferation; suppress the production of pro-inflammatory cytokines | — | — | Dokić et al., 2012 |
| OMLP-PCs | oral mucosal lamina propria | inhibit lymphocyte proliferation induced by mitogen and one-way MLR | — | — | Davies et al., 2012 |

### Bone marrow mesenchymal stem cells (BMSCs)

BMSCs are a population of non-hematopoietic stromal cells with self-renewal and multi-differentiation capabilities (Friedenstein et al., 1970; Wang et al., 2012). It is well known that BMSCs possess immunomodulatory properties as shown that BMSCs were capable of interacting with almost all subsets of immune cells, including T cells, B cells, dendritic cells, natural killer cells, macrophages, and neutrophils via secretion of soluble factors and/or cell-cell contact (Liu et al., 2012a,b), which providing a foundation for the clinical treatment of a variety of inflammatory and immune related diseases, such as graft-versus-host disease (GVHD), systemic lupus (SLE), systemic sclerosis, and Sjogren's syndrome, etc (Le Blanc et al., 2004; Sun et al., 2009; Akiyama et al., 2012; Xu et al., 2012).

### Adipose mesenchymal stem cells (AMSCs)

Abtained from adipose tissue, AMSCs expressed STRO-1, CD29, CD44, CD71, CD90, and CD105, which are considered as markers for MSCs, did not express the hematopoietic lineage markers CD31, CD34, and CD45, and could differentiate *in vitro* into osteogenic, adipogenic, chondrogenic, and myogenic cells under lineage-specific induction conditions (Zuk et al., 2001, 2002). Immunologically, AMSCs did not express human leukocyte antigen (HLA)-DR, which renders them less immunogenic, while they were capable of suppressing mixed lymphocyte reaction (MLR) and lymphocytes proliferation induced by mitogens (Puissant et al., 2005; Cui et al., 2007; Leto Barone et al., 2013). AMSCs were also shown to improve the production of anti-inflammatory cytokines and decrease the production of inflammatory cytokines (Gonzalez-Rey et al., 2010). In pre-clinical studies and clinical trials, AMSCs successfully treated a variety of diseases, including autoimmune inner ear disease, GVHD, rheumatoid arthritis, ulcerative colitis, etc (Fang et al., 2010; Garcia-Olmo et al., 2008; Gonzalez-Rey et al., 2009; Zhou et al., 2011).

### Dental pulp stem cells (DPSCs)

Gronthos et al. described a clonogenic, rapidly proliferative population of cells from adult human dental pulp and named them DPSCs (Gronthos et al., 2000). The investigtors characterized the self-renewal capability, multi-lineage differentiation capacity, including advancing into odontoblasts, adipocytes, and neural-like cells, of DPSCs. When DPSCs were transplanted into immunocompromised mice with hydroxyapatite/tricalcium phosphate (HA/TCP) as a carrier, they generated a dentin-pulp-like tissue, demonstrating their *in vivo* regeneration capability (Gronthos et al., 2000; Gronthos et al., 2002). Furthermore, DPSCs can also be used to treat systemic diseases, including diabetes, Parkinson disease, myocardial infarction, etc (Gandia et al., 2008; Govindasamy et al., 2011; Nesti et al., 2011).

Pierdomenico et al. first explored the immunomodulatory properties of DPSCs and compared the immunosuppressive function of DPSCs with that of BMSCs tested by co-culturing phytohemagglutinin (PHA)-stimulated allogeneic T cells with or without DPSCs/BMSCs for 3 days, and found that the addition of DPSCs or BMSCs resulted in 91% and 75% inhibition of T cells response, respectively, assessed by a 3H-thymidine assay (Pierdomenico et al., 2005). However, in another study, it was reported that BMSC-mediated suppression of PHA-induced proliferative response of peripheral blood mononuclear cells (PBMCs) was significantly more pronounced than that of DPSCs (Djouad et al., 2010).

A variety of studies showed that human or swine DPSCs failed to stimulate allogeneic T cell proliferation and significantly inhibited PHA- or Concanavalin A (Con A)-triggered T cell proliferation and MLR both in cell-cell contact culture and Transwell culture, thus implying that DPSCs have low immunogenicity and immunosuppressive actions on T cells, and soluble factor(s) may play important roles in the immunosuppressive process (Wada et al., 2009; Demircan et al., 2011; Tang et al., 2011; Tomic et al., 2011). Furthermore, conditioned media (CM) derived from DPSCs pre-treated with interferon (IFN)-γ partially suppressed PBMC proliferation when compared to CMs without IFN-γ stimulation (Wada et al., 2009). Elevated expression levels of HLA-G, hepatocyte growth factor-β1, intracellular adhesion molecule-1, interleukin (IL)-6, IL-10, transforming growth factor (TGF)-β1, vascular adhesion molecule -1 ,vascular endothelial growth factor and indolamine-2,3-dioxygenase-1 (IDO), and decreased expression levels of pro-inflammatory cytokines, including IFN-γ , IL-2, IL-6 receptor, IL-12, IL-17A, and tumor necrosis factor (TNF)-α, were detected in the co-culture of DPSCs with activated PBMCs. Meanwhile, expression patterns of CD4+CD25+Foxp3+ regulatory T cells (Tregs), were significantly induced (Wada et al., 2009; Demircan et al., 2011). Tomic et al. found that DPSC-mediated proliferation suppression of PBMCs could be neutralized with anti-TGF-β antibody, suggesting that DPSCs possess immunosuppressive properties mediated, at least in part, by TGF-β (Tomic et al., 2011).

Some reports showed that DPSCs were capable of inducing activated T cell apoptosis *in vitro* (Demircan et al., 2011; Zhao et al., 2012), and this effect was associated with the expression of Fas ligand (FasL), a transmembrane protein that plays an important role in inducing the Fas apoptotic pathway (Zhao et al., 2012). To examine *in vivo* immunomodulatory properties of DPSCs, Shi group generated a colitis model in mice by oral administration of 3% dextran sulfate sodium (DSS), and found that systemic infusion of DPSCs, but not FasL-knockdown DPSCs, protected mice from colitis-related tissue injuries and reduced overall disease

severity compared with the colitis group, along with a marked rescue of body weight and decrease in histological score. Also, the DPSCs treatment group significantly ameliorated colonic transmural inflammation, decreased wall thickness, suppressed epithelial ulceration, and restored normal intestinal architecture. In contrast, the FasL knockdown DPSCs group failed to ameliorate histological phenotypes of colitis. These data suggest that FasL is required for DPSC-mediated immune therapy in the colitis mouse model (Zhao et al., 2012).

As the first study to investigate the feasibility of allogeneic MSCs in bio-root engineering in a pre-clinical model, Wei et al. used allogeneic DPSCs to regenerate bio-root, and then installed a crown on the bio-root to restore tooth function 6 months later in miniature pig. No immunological response to the bio-roots was observed (Wei et al., 2013).

Human supernumerary tooth-derived stem cells (SNT-DPSCs) suppressed not only the viability of T cells, but also the differentiation of IL-17-secreting helper T (Th17)-cells *in vitro*. In addition, systemic SNT-DPSCs transplantation ameliorated the shortened lifespan and elevated serum autoantibodies and nephritis-like renal dysfunction in SLE model by suppressing *in vivo* increased levels of peripheral Th17 cells and IL-17. Adoptive transfer experiments demonstrated that SNT-DPSC-transplanted MRL/lpr mouse-derived T-cell-adopted immunocompromised mice showed a longer lifespan in comparison with non-transplanted MRL/lpr mouse-derived T-cell-adopted immunocompromised mice, indicating that SNT-DPSC transplantation suppressed the hyper-immune condition of MRL/lpr mice through suppressing T cells (Makino et al., 2013).

### Stem cells from human exfoliated decidous teeth (SHED)

SHED are isolated from human naturally deciduous tooth, an easily accessible resource, and are identified to be a population of highly proliferative, clonogenic cells capable of differentiating into a variety of cell types including neural cells, adipocytes, and odontoblasts (Miura et al., 2003). In different *in vivo* transplantation conditions, SHED were found to be able to induce bone formation and repair critical-size calvarial defects, generate dentin, produce dental pulp-like tissue, suggesting that SHED might be a suitable resource for orofacial bone regeneration and dental pulp tissue engineering (Cordeiro et al., 2008; Seo et al., 2008).

In addition, SHED was also shown to possess an immunomodulatory property (Yamaza et al., 2010). When SHED was co-cultured with anti-CD3/CD28 antibodies-activated naïve T cells, levels of Th17 cells and IL-17 were significantly reduced in SHED groups compared to the naïve T cells group. The authors also found that SHED transplantation is an effective approach for treating SLE disorders in SLE-like mice, which resulted in a significant reduction in serum levels of SLE associated antibodies. Histological analysis revealed that SHED transplantation led to recovery of SLE-associated renal disorders, such as nephritis with glomerular basal membrane disorder and messangial proliferation in MRL/lpr mice. Mechanically, SHED transplantation provided a remarkable reduction of Th17 cells and IL-17 level in MRL/lpr mice, thus up-regulating the ratio of Treg and Th17 cells (Yamaza et al., 2010). SHED from inflamed tissues is highly dysfunctional in terms of their immunomodulatory properties, which is less effective in suppressing PHA-induced T cell proliferation in comparision to SHED isolated from healthy dental pulp tissues. In addition, levels of several cytokines, such as TNF-$\alpha$, TNF-$\beta$, and IL-2, were drastically suppressed in SHED isolated from healthy dental pulp tissues compared to SHED from inflamed tissues (Yazid et al., 2014). SHED isolated from cryopreserved deciduous pulp tissues is also capable of improving SLE-like disorders, and the therapeutic efficacy of SHED from cryopreserved deciduous pulp on immune and skeletal disorders was similar to that of SHED from fresh deciduous pulp (Ma et al., 2012).

Previous studies showed that stem cells from miniature pig exfoliated deciduous teeth were able to successfully regenerate the critical-size mandible bone defects, produce the dentin-like structures, and nearly completely restored the pulp chamber roof defects in swine models (Zheng et al., 2009, 2012). In addition, allogeneic stem cells from miniature pig exfoliated deciduous teeth can effectively repair hard and soft tissue loss brought about by periodontitis in a swine model, in which clinical indices showed significant periodontal restoration, CT scans showed that 75% of the samples had successful hard-tissue regeneration, and histologic examination demonstrated remarkable regeneration of periodontal tissues (Fu et al., 2014).

### Periodontal ligament stem cells (PDLSCs)

Isolated from periodontal ligament tissue, a soft connective tissue embedded between the cementum and the alveolar bone, PDLSCs maintain MSCs characteristics, including putative stem-cell marker expression of STRO-1 and CD146, and single-colony-strain generation. Under defined culture conditions, PDLSCs could differentiate into cementoblast-like cells, adipocytes, and collagen-forming cells. When transplanted into immunocompromised rodents, PDLSCs showed the capacity to generate a cementum/periodontal ligament-like structure and contribute to periodontal tissue repair, whether PDLSCs are isolated from fresh or cryopreserved periodontal ligament tissues (Seo et al., 2004, 2005). Using miniature pig, a large animal model whose oral maxillofacial region is similar to that of humans in anatomy, development, physiology, and pathophysiology (Wang et al., 2007), Liu et al explored the potential of using autologous PDLSCs to treat periodontal defects,

and found that PDLSCs were capable of regenerating periodontal tissues post-transplantation, demonstrating the feasibility of PDLSC-mediated therapy for periodontal diseases (Liu et al., 2008). Autologous PDLSCs also displayed the efficacy of reconstructing periodontal intrabony defects in patients, and all treated patients showed no adverse effects during the follow up (Feng et al., 2010).

PDLSCs expressed HLA-I, but were negative for immune phenotype markers including HLA-II DR, CD80 and CD86, and pre-irradiated PDLSCs failed to stimulate allogeneic T cell proliferation (Ding et al., 2010b). Human, swine, and canine PDLSCs significantly inhibited Con A- or PHA-stimulated proliferation of allogenic and xenogenic PBMCs, or an allogeneic MLR in a cell dependent manner (Wada et al., 2009; Ding et al., 2010b; Kim et al., 2010). Further, osteogenic differentiated PDLSCs also could not stimulate allogeneic T cell proliferation and suppressed PHA-triggered T cell proliferation to the same extent as undifferentiated PDLSCs (Tang et al., 2014). Strikingly, PDLSCs are capable of suppressing PBMC proliferation 2 days post-PHA stimulation, thus displaying their profound immunosuppressive capabilities (Ding et al., 2010b). Cell cycle assessment showed that PDLSCs suppressed the cell division of PBMCs (Wada et al., 2009; Kim et al., 2010). Mitogen stimulated-PBMCs co-cultured with PDLSCs did not undergo apoptosis, as determined by using trypan blue uptake method and flow cytometric analysis, and PHA or IL-2 re-stimulation of T cells pre-suppressed by PDLSCs resulted in vigorous proliferation (Wada et al., 2009; Ding et al., 2010b; Kim et al., 2010). T cell proliferation was equally inhibited by cell-cell contact culture and the Transwell culture, suggesting that inhibition was independent of cell-cell contact and might involve soluble factor (Wada et al., 2009; Ding et al., 2010b). As for the soluble factors responsible for PDLSC-mediated immunosuppression, Wada et al found that IDO took part in the suppression of PBMC proliferation (Wada et al., 2009), whereas our group found that prostaglandin E2 (PGE2) is a key inhibitor of T cell proliferation mediated by PDLSCs (Ding et al., 2010b; Tang et al., 2014).

It was demonstrated that PDLSCs in inflamed periodontal ligaments (PDLSC-IPs) had markedly dysfunctional immunomodulatory properties. Compared to healthy cells, PDLSC-IPs showed significantly diminished inhibition of T cell proliferation. Tregs production and IL-10 secretion were less induced in the presence of PDLSC-IPs compared with healthy PDLSCs, and meanwhile, suppression of Th17 differentiation and IL-17 production by PDLSC-IPs was significantly less compared to healthy cells (Liu et al., 2012a).

When allogeneic PDLSC sheet was transplanted into the lesion areas of miniature pig periodontitis models, periodontal tissue regeneration mediated by allogeneic PDLSCs was close to nearly normal level 12 weeks post-transplantation, comparable to that in the autologous PDLSCs group, as evidenced by intraoral photographs, periodontal examination, CT scanning and histological evaluation. There were no significant differences between allogeneic and autologous PDLSCs groups as to the immunological reaction markers, such as T cell-related markers, routine blood and biochemical tests (Ding et al., 2010b). In a Merino sheep model of surgically created zero-wall dehiscence defects, allogeneic PDLSCs were well tolerated by recipient animals and at the same time, new alveolar bone, new cementum and new Sharpey's fibers were successfully regenerated in the allogeneic PDLSCs group (Mrozik et al., 2013).

In addition to inhibiting T cell proliferation, human PDLSCs also suppressed B cell proliferation in a cell-number-dependent manner, suppressed B cells' differentiation into plasma cells and immunoglobulin production, while intriguingly, PDLSCs increased B cell viability by secreting IL-6 (Liu et al., 2013). In contrast to their regulation of B cell proliferation and differentiation, PDLSCs failed to affect the expression of HLA-DR, and costimulatory molecules CD40, CD86, and CD80 on B cells. After allogeneic PDLSCs transplantation therapy for periodontitis in miniature pig model, there were no significant differences regarding the percentage of CD20+ or CD25+ B cells in peripheral blood between the allogeneic and autologous PDLSCs groups, suggesting there were no B cell related immunological rejections in the animals that received allogeneic PDLSCs transplantation (Liu et al., 2013).

### Stem cells from root apical papilla (SCAP)

Another type of MSCs isolated from the apical papilla of human immature permanent teeth was SCAP (Sonoyama et al., 2006). SCAP express many surface markers including STRO-1, alkaline phosphatase, CD24, CD29, CD73, CD90, CD105, CD106, CD146, and CD166, but are negative for CD34, CD45, CD18 and CD150. SCAP are capbable of forming adherent clonogenic cell clusters, differentiating into odontoblasts, osteoblasts, and adipocytes. Additionally, SCAP express a wide variety of neurogenic markers such as nestin and neurofilament M upon stimulation with a neurogenic medium (Sonoyama et al., 2008). SCAP showed a significantly higher rate of cell proliferation, increased number of population doublings, an elevated tissue regeneration capacity, higher telomerase activity, and an improved migration capacity, when compared to DPSCs from the same tooth (Sonoyama et al., 2006). A root/periodontal complex capable of supporting a porcelain crown was regenerated when human SCAP and PDLSCs were transplanted into miniature pig model, resulting in normal tooth function. Thus, SCAP hold potential utilization for pulp/dentin regeneration and bio-root engineering (Sonoyama et al., 2006, 2008; Huang et al., 2008).

As for the expression profiles of immune related molecules, swine SCAP were positive for a low level of

swine leukocyte antigen (SLA) class I molecules and were negative for SLA class II DR molecules. Moreover, swine SCAP could inhibit one-way MLR, and autologous or allogeneic T cell proliferation stimulated by PHA in a dose-dependent manner, with or without mitomycin C pre-treatment. Soluble factors may be involved in SCAP-mediated immune suppression because T cell proliferation was equally suppressed in both cell-cell contact culture and Transwell culture. After a 5-day co-culture of swine SCAP, allogeneic T cells, and PHA, few T cells were apoptotic. These data indicated that swine SCAP were weakly immunogenic and suppressed T cell proliferation *in vitro* through an apoptosis-independent mechanism (Ding et al., 2010a). Further, we found that cryopreservation did not affect the immunomodulatory functions of SCAP. Cryopreserved human or swine SCAP did not induce allogeneic T cell proliferation and could suppress two-way MLR in a dose dependent manner, to the same suppressive extent in comparison to freshly isolated SCAP (Ding et al., 2010c).

### Gingiva mesenchymal stem cells (GMSCs)

As a population of stem cells isolated from human gingival tissues, one kind of tissue source easily accessible from the oral cavity, GMSCs exhibited clonogenicity, self-renewal capacity, and could differentiate into adipocytes, osteoblasts, chondrocytes, endothelial cells, and neural cells (Zhang et al., 2009; Mitrano et al., 2010; Ge et al., 2012). It was reported that around 90% of GMSCs are derived from cranial neural crest cells (CNCC) and 10% from the mesoderm (Xu et al., 2013). GMSCs were negative for CD45, but consistently expressed SSEA-4, Oct-4, STRO-1, CD29, CD73, CD90, and CD105. When GMSCs were transplanted s.c. into the dorsal surface of immunocompromised mice with HA/TCP as a carrier, connective tissue-like transplants were regenerated 4 weeks later. Most importantly, GMSCs have also been shown to possess the immunomodulatory functions, which were capable of suppressing PBMC proliferation and inducing expression of a wide panel of immunosuppressive factors, including IL-10, IDO, inducible NO synthase, and cyclooxygenase 2 in response to IFN-γ. Intravenous administration of GMSCs in experimental animal models of DSS-induced colitis significantly alleviated both clinical and histopathological severity of the colonic inflammation, restored the injured gastrointestinal mucosal tissues, reversed diarrhea and weight loss, and suppressed the overall disease activity in mice. The positive outcomes following infusion of GMSCs may be attributed to the down-regulation of inflammatory infiltrates and the up-regulation of Tregs infiltration and anti-inflammatory cytokine IL-10 at the colonic sites (Zhang et al., 2009).

In comparison with mesoderm MSCs (M-GMSCs), CNCC-derived GMSCs (N-GMSCs) show an elevated capacity to induce activated T cell apoptosis *in vitro*, which is associated with up-regulated expression of FasL. When transplanted into mice with DSS-induced colitis, N-GMSCs showed superior effects in ameliorating inflammatory-related disease phenotype in comparison with the M-GMSCs treatment group, which is also associated with the up-regulation expression of FasL (Xu et al., 2013). GMSCs have also been demonstrated to be capable of turning macrophages into an anti-inflammatory M2 phenotype after co-culture, characterized by an increased expression of mannose receptor and secretory cytokines IL-10 and IL-6, a suppressed production of TNF-α, and decreased ability to induce Th17 cell expansion. *In vivo*, systemically infused GMSCs could home to the wound site in a tight spatial interaction with host macrophages and significantly enhanced wound repair (Zhang et al., 2010). In addition, GMSCs were capable of directly suppressing the differentiation of dendritic cells and phorbol 12-myristate 13-acetate-stimulated activation of mast cells under co-culture condition of direct cell-cell contact or via Transwell system (Mitrano et al., 2010). Systemic infusion of GMSCs could dramatically suppress murine contact hypersensitivity, an experimental model for human allergic contact dermatitis, manifested as a decreased infiltration of dendritic cells, CD8+T cells, Th17 cells and mast cells, suppression of a variety of inflammatory cytokines, and increased infiltration of Tregs and expression of IL-10 at the regional lymph nodes and the allergic contact areas (Su et al., 2011).

GMSCs isolated from cyclosporine A-induced human gingival hyperplasia tissues also could down-regulate the proliferation of PBMCs stimulated with allogenic PBMCs by increasing the ratio of Tregs. Using a highly immunogenic murine skin allograft model, the authors found that the allograft rejection was delayed in the group of systemic infusion of hyperplastic gingival tissues-derived GMSCs compared to the un-treated control group (Tang et al., 2011).

### MSCs from dental follicle (DF-MSCs)

DF-MSCs showed colony-forming ability and expressed typical MSC markers. DF-MSCs proliferated faster, contained cells larger in diameter, exhibited a higher potential to form adipocytes and a lower potential to form chondrocytes and osteoblasts, when compared with DPSCs from the same tooth. DF-MSCs could produce TGF-β and suppressed the proliferation of PBMCs, which could be neutralized with anti-TGF-β antibody. The treatment with TLR3 agonist or TLR4 agonist enhanced the suppressive effect of DF-MSCs and improved TGF-β and IL-6 secretions by DF-MSCs. These results suggest that DF-MSCs are also one kind of functionally immunosuppressive agents that may be utilized for the management of immune-mediated disorders (Tomic et al., 2011).

### Orofacial bone/bone-marrow-derived MSCs (OMSCs)

Yamaza et al. isolated and cultured mouse OMSCs from mandible and examined the biological and immunological properties of OMSCs. OMSCs failed to express the hematopoietic markers including CD14, CD34, and CD45, but were positive for MSC-associated markers, such as CD73, CD105, CD106, SSEA-4, and Oct-4. Colony forming efficency, population doublings, and cell proliferation rate of OMSCs were higher than those of BMSCs, which was used as a control. OMSCs showed osteogenic, adipogenic, and chondrogenic differentiation potential under inductive culture medium. Post transplantation subcutaneously into immuno-compromised mice with HA/TCP as carrier, OMSCs were capable of forming de novo bone and bone marrow structures (Yamaza et al., 2011). When compared the immunosuppressive capacity of OMSCs with that of BMSCs, OMSCs had a stronger immunosuppressive effect than BMSCs in the co-culture containing anti-CD3 antibody-activated splenocytes and different ratios of OMSCs and BMSCs, and the inhibition effect of OMSCs could be partially blocked by anti-IFN-γ antibody and inducible NO synthase inhibitor. At the same time, Pan T cells activated by anti-CD3 antibody were able to cause OMSCs death, and Fas/FasL pathway is thought to contribute to the apoptosis of OMSCs since treatment with anti-FasL antibody blocked OMSCs death (Yamaza et al., 2011).

### Tooth germ stem cells (TGSCs)

Human tooth germs contain TGSCs, which can be easily isolated, expanded, and are positive for CD73, CD90, CD105 and CD166, but negative for CD34, CD45 and CD133 (Yalvaç et al., 2010a,b). Under specific culture conditions, TGSCs differentiated into osteogenic, adipogenic, chondrogenic, neurogenic cells, endothelial- and epithelial-like cells, as well as formed tube-like structures in Matrigel assay (Yalvaç et al., 2010a,b, 2011; Doğan et al., 2014), suggesting that TGSCs is an attractive candidate for future cell-based therapy. In addition, TGSCs hold neuro-protective secretome, and could increase the activity of a number of anti-oxidant enzymes and reduced the apoptosis of brain cells exposed to various toxic materials (Yalvaç et al., 2013). At the same time, it remains to be dertermined whether TGSCs also possess the immunomodulatory properties, which is necessary for the allogeneic use of TGSCs.

### MSCs from periapical lesions (PL-MSCs)

Isolated by collagenase/DNAse digestion from surgically extracted periapical lesions, PL-MSCs had typical MSC phenotype, clonogenicity and self-renewal rate, and possessed the potential to differentiate into adipocytes, osteoblasts and chondrocytes *in vitro*. PL-MSCs were capable of inhibiting PHA-induced proliferation of PBMCs and production of IL-2, IFN-γ and IL-5 in the co-culture via TGF-β-dependent mechanisms. PL-MSCs also suppressed the production of IL-1β, IL-6, TNF-α, and IL-8 by periapical lesions-inflammatory cells (Dokić et al., 2012).

### Oral mucosal lamina propria-derived progenitor cells (OMLP-PCs)

A robust progenitor cell population was identified from the human oral mucosa lamina propria, named OMLP-PCs (Marynke-Kalmani et al., 2010). OMLP-PCs expressed the MSC markers Oct4, Sox2, Nanog, CD44, CD90, CD105, and CD166, but were negative expression of CD34 and CD45, and this novel stem cell population showed multipotency by differentiation into mesenchymal (chondrogenic, osteoblastic, and adipogenic), endodermal, and neuronal (neuron and Schwann-like cells) cell lineages (Davies et al., 2010, 2012).

OMLP-PCs did not constitutively express HLA-II and costimulatory molecules including CD40, CD80, CD86, CD154, and CD178. OMLP-PCs could inhibit lymphocyte proliferation by PHA and one-way MLR in a contact-independent and dose-independent manner, and annexin V staining confirmed that this immunosuppressive effect was not due to the induction of lymphocyte apoptosis (Davies et al., 2012).

### Conclusions

In comparision to BMSCs or MSCs derived from other sources, orofacial tissue derived MSCs are more easily accessible with minimal trauma and have higher proliferative abilities (Gronthos et al., 2000; Miura et al., 2003; Seo et al., 2004; Sonoyama et al., 2006; Zhang et al., 2009; Yamaza et al., 2011). Because of their excellent tissue regenerative capabilities *in vivo*, orofacial tissue derived MSCs are regarded as ideal candidates in cell-based therapy for oral diseases (Wang et al., 2012; Wada et al., 2013; Liu et al., 2014). Moreover, the low immunogenicity and immuno-suppressive functions possessed by orofacial tissue derived MSCs render them attractive for allogeneic application and for the treatment of immune-related diseases. However, several questions should be clearly addressed before clinical applications: (1) It was reported that immune cells may have an profound effect on BMSCs, and tissue regeneration capacity of BMSCs may be influenced by recipient immune cells such as T cells, which are shown to govern BMSC-based tissue regeneration via IFN-γ and TNF-α (Yamaza et al., 2008; Liu et al., 2011, 2012b). The effect of immune cells on orofacial tissue derived MSCs is largely unknown. (2) There is still some discrepancy among results of some studies, for example, the immunesuppressive ability of DPSCs versus BMSCs, etc. Therefore, it is necessary to further clarify these inconsistent issues. (3) The underlying mechanisms responsible for orofacial tissue

derived MSC-mediated immunomodulation should be fully understood, which is critical for the use of these MSCs in clinics and will be the focus of future studies. (4) It is required to conduct well-designed pre-clinical studies to explore the immunosuppressive properties of orofacial tissue derived MSCs in large animal models in the future. (5) The development of standardized protocols for production and quality control processes of MSCs, according to Good Manufacturing Practice (GMP), plays a crucial role in cell-based therapy (Sensebé et al., 2011). All the cell products should be prepared in compliance with current GMP guidelines for human application (Rustichelli et al., 2013). Clinical-grade DPSCs were also isolated and expanded according to GMP conditions, including contamination test, karyotype, and tumor formation in immunodeficient mouse, to ensure excellent quality control (Iohara et al., 2013; La Noce et al., 2014). However, to our knowledge, few studies on GMP application of other types of orofacial tissue derived stem cells are reported. After the above mentioned questions are well answered, orofacial tissue derived MSCs can bring benefit for orofacial tissue regeneration and immune-related disease efficaciously and safely.

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