

Craniomaxillofacial Sources of Mesenchymal Stem Cells: A Brief Review

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ABSTRACT

Tissue specific mesenchymal stem cells are of great scientific interest across diverse fields in stem cell biology and medicine. Sources of mesenchymal stem cells (MSC) in the craniomaxillofacial skeleton are numerous and are of significant interest to craniofacial and oral surgeons, among other medical and dental specialties. Mesenchymal stem cells are defined by their characteristic cell surface marker expression, multipotentiality and ability for self-renewal. Bone marrow derived MSC (BMSC) are most commonly studied, but are of low abundance in the craniomaxillofacial (CMF) skeleton. Alternative CMF sources of MSC are diverse and include dental pulpal stem cells (DPSC), periodontal ligament stem cells (PDLSC), suture-derived mesenchymal stromal cells (SMC), gingival mesenchymal stem cells (GMSC) and adipose-derived MSC (ASC). This brief review will introduce various MSC sources derived from the head and neck, along with a discussion of their identity, characteristics and results of preclinical studies in tissue engineering.

Key words: Periodontal ligament stem cells; Suture-derived mesenchymal stromal cells; Dental pulpal stem cells; Gingival mesenchymal stem cells; Adipose-derived MSC

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INTRODUCTION

Mesenchymal stem cells (MSC) are best known for their isolation from bone marrow^[1]. Bone marrow derived MSC (BMSC) are defined by their adherence to culture, ability for self-renewal, multipotency and cell surface marker expression^[2]. MSC can utilize a number of pathways to elicit effects, including endocrine, paracrine, immunomodulation, direct differentiation, and induction of angiogenesis among others (Figure 1)^[3]. Tissue-specific MSC have gained much attention as the presumptive native precursor cells for their respective tissue types and cells responsible for tissue-specific regeneration. Of these, adipose tissue-derived MSC (otherwise termed ASC) have been most thoroughly studied as an alternative and abundant MSC cell population^[3].

Craniomaxillofacial sources of MSC are of interest to multiple disciplines, including plastic and reconstructive surgeons, dentists and dental subspecialties including orthodontists and oral pathologists, as well as stem cell biologists. MSC among craniomaxillofacial sources are diverse, unique and variably well characterized. These include dental pulp stem cells (DPSC)^[4], periodontal ligament stem cells (PDLSC)^[5], suture-derived mesenchymal stromal cells (SMC)^[6] and gingival mesenchymal stem cells (GMSC)^[7] (Figure 2). Also discussed are ASC when derived from fat tissues of the head and neck^[8]. This review will introduce each MSC type, its identity, unique attributes and studies to date in pre-clinical efforts in tissue engineering.

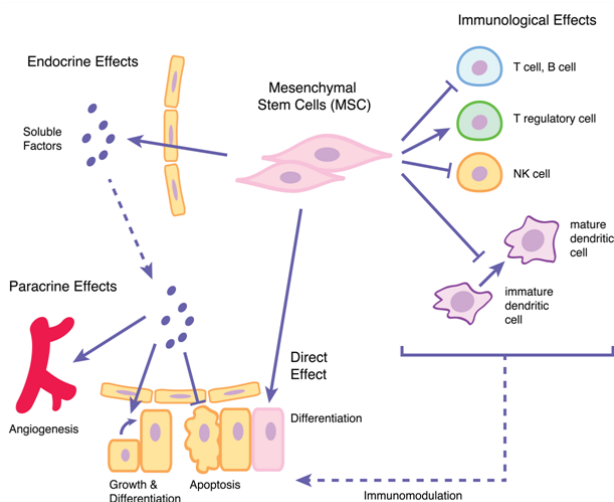


Figure 1 Mesenchymal Stem Cell (MSC) Mechanism of Action.

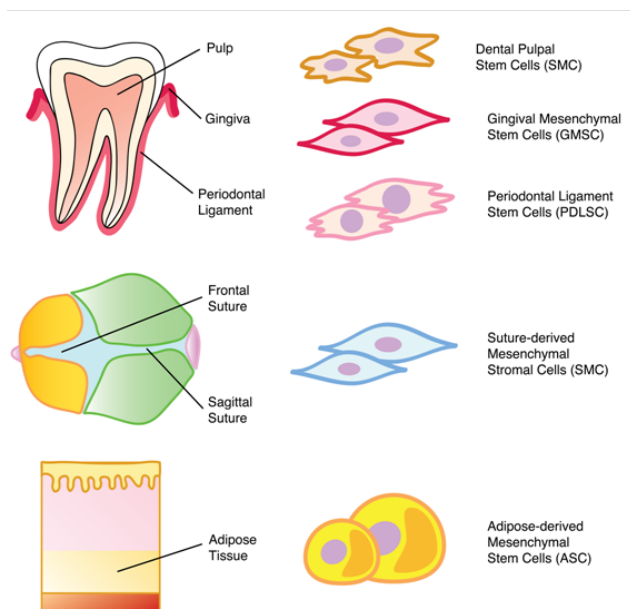


Figure 2 Different Craniomaxillofacial Sources of Mesenchymal Stem Cells (MSC).

DENTAL PULP STEM CELLS (DPSC)

Background and Applications

Dental pulp stem cells (DPSC) are multipotent cells of neural crest origin, found in the cellular rich region of the dental pulp^[4]. Under different stimuli, DPSC can differentiate into adipocytes, myocytes, neurons and osteoblasts among other cell types^[9]. With their capacity to differentiate into osteoblasts, DPSC have seen increasing use in the field of endodontics as they promise a more reliable, regenerative tissue approach for treatment of pulpal necrosis of the permanent dentition^[10]. Thus far, DPSC have been shown to contribute to dentin-like tissue formation *in vitro* and bone repair in mandibular defects^[11], showing promise in the field of hard-tissue engineering both for bone and dentin.

Profiling and Identity

In terms of practicality, DPSC are typically obtained from the dental pulp of deciduous dentition or from molars after extraction. Conventionally, DPSC have been isolated and characterized in subjects under 30 years of age^[12]. However, investigators have recently confirmed that DPSC persist in older subjects (tested at up to 45 years of age) and when enriched via FACS selection (for CD117, STRO-1, CD34, CD44 and RUNX2) showed a population with comparable osteogenic potential to that of younger individuals^[9,13,14]. Further studies have shown that DPSC express members of the insulin-like growth factor signaling family (including IGF-1, IGFBP-3 and IGF-1 receptors)^[15]. Notably, IGF signaling plays a large role in the regulation of the later stages of tooth development and pulpal differentiation^[15]. DPSC identity and fate is also regulated by dentin sialophosphoprotein (DSPP), a precursor protein that is cleaved into Dentin sialoprotein and Phosphophoryn protein. DSPP expression is required for normal differentiation towards the odontoblastic lineage, whereas absence of DSPP leads to dental pulp cell development into chondrocyte-like cells^[16]. Similarly, DPSC can veer away from an odontoblastic lineage under continuous passage towards a pericyte or myofibroblast-like phenotypes, although both early and late passage DPSC will typically maintain their capacity for odontogenic differentiation^[17]. Likewise, DPSC have been shown to undergo adipogenic differentiation or myogenic differentiation under appropriate culture conditions^[9,14].

Pre-Clinical Studies

DPSCs have been most thoroughly studied for their ability to form dentin and bone. A number of investigators have examined the ability of DPSC to undergo odontogenic differentiation^[18-21]. Ectopic (subcutaneous) implantation of DPSC in a mouse model resulted in dental pulp tissue formation, when exposed to dentin matrix protein 1^[22]. In terms of application, this model serves as a good candidate for hard tissue formation^[22]. Treatment of DPSC with FGF-2 (Fibroblast growth factor-2) likewise demonstrated a role for FGF-2 as a differentiation factor in repairing pulpal tissue, along with a positive regulator of inflammatory cytokine expression among DPSCs^[23]. Injury to odontoblasts, such as from a deep cavity preparation, was found to activate DPSC proliferation^[24]. Moreover, DPSC odontogenic differentiation was found to increase with dynamic hydrostatic pressure^[25] including a decrease in DPSC adhesion, increase in DPSC mineralization and increase in BMP-2 responsiveness^[25].

DPSC have likewise been examined for their osteogenic potential and *in vivo* bone formation^[9,26,27]. DPSC will typically form dense, calcified nodules *in vitro*, but when grafted *in vivo*, will mineralize to form bone or dentin-like tissue^[26]. Laino *et al* demonstrated that DPSC differentiated into osteoblasts and secreted extracellular mineralized matrix, which later formed three-dimensional woven bone^[13,14]. Other investigators have found that subcutaneous implantation of living autologous fibrous bone, derived from DPSC *in vitro*, in immunocompromised rats can lead to lamellar bone formation that also contains entrapped osteocytes, resembling human bone^[9]. Similarly, DPSC-mediated bone formation has been observed to have a similar pattern of vascularization as that of native human bone^[28]. In fact, investigators have shown that DPSC may play a role in paracrine-mediated angiogenesis, potentially via VEGF-R2 (Vascular endothelial growth factor receptor 2) expression^[27]. Thus, in addition to pulpal regeneration, DPSC have demonstrated the ability to form either bone or dentin-like tissue, depending on the model and context.

PERIODONTAL LIGAMENT STEM CELLS (PDLSC)

Background & Applications

The periodontal ligament contains groups of connective tissue fibers in the alveolar bone and cementum with progenitors for mineralized tissue-forming cell lineages, termed periodontal ligament stem cells (PDLSC). Perhaps the most defining characteristic of PDLSC is their multipotency, with the most well-studied being their ability to undergo osteogenesis and cementogenesis^[29,30]. Further, PDLSC have been shown to be a viable, alternative source to autologous Schwann cells, in one instance promoting the regeneration of the mental nerve^[31]. Lastly, like other MSC, PDLSC also show paracrine induction of angiogenesis *in vitro* and *in vivo*^[32]. Thus, in addition to their multilineage potential, the possibility of an immortal cell line has made PDLSC highly promising for tissue engineering applications^[5].

Profiling and Identity

As expected, PDLSC express numerous markers characteristic of other MSC sources, including CD146, CD105, CD90, CD29 and STRO-1^[33]. Under certain conditions, such as culture with BMP-2 or -7, PDLSC also express MSC markers such as CD44 and CD73^[30]. PDLSC also express cementum protein 1, which is associated with a cementoblastic phenotype and reduced osteoblastic differentiation^[30]. While PDLSC show mineralization under osteoinductive culture conditions, they also show multilineage differentiation potential and undergo adipogenesis or chondrogenesis under appropriate culture conditions^[33].

PDLSC differ in important ways from the previously discussed DPSC, although they are often interchanged. DPSC typically exhibit higher rates of proliferation and telomerase activity in comparison to PDLSC^[34]. In fact, DPSC show higher osteogenic, chondrogenic and angiogenic potential, as indicated by increased expression of BMP-2, BMP-6, sex-determining region Y-box (SOX9), integrin alpha 6 (ITGA6), melanoma cell adhesion molecule (MCAM) among others^[34]. By contrast, PDLSC play a more important role in inflammation, immunomodulation and tissue remodeling, expressing higher levels of matrix metalloproteinase 2 (MMP2), interleukin-6 and -10^[34].

Several molecular signaling pathways are of interest in PDLSC biology, including Notch, Wnt and BMP signaling. The Notch signaling pathway is known to play a crucial role in the osteogenic differentiation of PDLSC, as determined in rat studies^[35]. Notch signaling is associated with self-renewal and cell-fate determination, with both mRNA and protein expression of Notch1 and Jagged1 upregulated when PDLSC were cultured in osteogenic conditions^[35]. Inhibition of Notch signaling in PDLSC, via treatment with γ -secretase inhibitor (a protease that cleaves the Notch protein), showed decreased osteogenic differentiation^[35]. In addition, the canonical Wnt signaling pathway, which typically regulates bone homeostasis, has been shown to modulate PDLSC osteogenesis^[36,37]. Although TNF- α -induced inflammation inhibited osteogenic differentiation, PDLSC osteogenic potential was restored by blocking Wnt via use of recombinant Dkk-1^[37]. Likewise, under a chronic inflammatory microenvironment, PDLSC showed reduced Runx2 expression and enhanced NF- κ B activity in comparison to BMSC^[37,38]. PDLSC have also been implicated in the formation of cementum, one of the three mineralized substances of the tooth. In differentiation towards a cementogenic lineage, Torii *et al.* found that BMP-7 is required to induce cementum protein 1 (CEMP1) via GC-rich Smad-binding elements^[29]. This follows a mechanism distinct to

that of an osteogenic or odontogenic lineage^[29].

Pre-Clinical Studies

A common focus in the literature is the comparison between, or combination of, PDLSC and BMSC. For example, Yu *et al.* compared PDLSC and BMSC from the same canine donor in subcutaneous and critical-sized defects in the rat calvarium^[39]; PDLSC demonstrated increased bone forming efficacy in comparison to BMSC^[39]. However, in another group using a canine peri-implant defect model, BMSC showed superior alveolar bone regeneration^[40]. Furthermore, PDLSC microencapsulation in RGD-coupled alginate enhanced MSC viability, proliferation and osteogenic differentiation both *in vitro* and *in vivo*^[41]. The combination of PDLSC with MSC demonstrated greater reossification when placed in a mouse calvarial defect model^[41]. Overall, mature woven bone formation was observed with lamellate structure and osteocytes with lacunae as seen in healthy human bone^[41].

A variety of other animal studies have examined the potential importance of PDLSC in orthodontic tooth movement. For example, under cyclic tension, osteogenic differentiation is upregulated in PDLSC and serves to maintain the homeostasis of periodontal tissues, a property utilized in orthodontic tooth movement^[42]. PDLSC have been shown to modulate root resorption of human primary teeth primarily through RUNX2 upregulation of RANKL and downregulation of OPG. Collectively, this enhances root resorption and is one of the responsible mechanisms in exfoliation of primary teeth^[43]. Conversely, in a positive-feedback manner, root dentin normally expresses high levels of dentin sialoprotein (DSP), which has been found to stimulate both PDLSC proliferation and differentiation. In sum, the understanding of PDLSC biology not only is of interest in the field of tissue engineering, but also in orthodontics and the mechanisms of tooth movement.

SUTURE-DERIVED MESENCHYMAL STROMAL CELLS (SMC)

Background and Sources

Given the similarities in the craniofacial development process between mice and humans, the murine model has been a popular choice to study cranial suture fusion. Two primary sutures of interest include the posterofrontal and sagittal suture, located between the frontal bones and parietal bones, respectively, both from which suture-derived mesenchymal stromal cells (SMC) are obtained^[44]. The posterofrontal suture, which is analogous to the metopic suture in humans, typically undergoes endochondral ossification and fuses within 8 to 10 days postnatally^[6]. By contrast, the sagittal suture, along with all others remains patent throughout adulthood^[45]. Suture-derived mesenchymal stromal cells (SMC) have been isolated from each of these midline cranial sutures and have found to have distinctly different properties, as discussed below.

Profiling and Identity

Both posterofrontal and sagittal suture-derived mesenchymal stromal cells (SMC) possess osteogenic potential and will express much of the same osteoblastic markers. However, they differ in that posterofrontal SMCs have been reported to express significantly higher levels of Osteopontin (Opn)^[6], Runx2, Col 1 α and Alkaline phosphatase (ALP) activity^[46]. With generally higher levels of bone nodule formation^[47], posterofrontal SMC demonstrate greater osteogenic potential than sagittal SMC. Furthermore, compared to their sagittal-derived counterparts, posterofrontal SMC show elevated

expression of Collagen II (Col II), a chondrogenic component of the extracellular matrix^[6], thus indicating greater chondrogenic potential. These *in vitro* findings recapitulate the *in vivo* fate of the posterofrontal suture, which undergoes endochondral ossification and fusion in comparison to the sagittal suture, which remains patent. Importantly SMC from either suture have not been investigated as rigorously as previously described stem cell population. As cell surface markers in SMC are as yet unknown, these populations are still referred to as ‘stromal cells’ rather than ‘stem cells.’

Pre-Clinical Studies

A number of studies using animal models have underlined the effects of various major cytokines and hormones on SMCs, including TGF- β , FGF-2, BMP and estrogens. Transforming growth factor (TGF)- β is known to control cell growth, proliferation and differentiation, so it has an expected involvement in SMC. Interestingly, SMC derived from posterofrontal suture of postnatal mice show approximately five times the expression level of TGF- β 1 than sagittal suture^[6,46,47]. TGF- β 1 has been shown to not only upregulate chondrogenic marker expression in posterofrontal SMC^[46], but also significantly downregulates proliferation and osteogenic differentiation of both populations, whereas TGF-3 significantly increases posterofrontal SMC proliferation^[48]. Both TGF- β 1 and TGF- β 3 will also induce expression of fibroblastic growth factors (FGF-2, FGF-18) and cytokines associated with posterofrontal suture fusion^[48]. Consistent with the pro-chondrogenic anti-osteogenic effects of TGF- β 1, posterofrontal SMC express higher levels of FGF-2^[6], which severely reduces osteogenesis (downregulated of Col I)^[47] while stimulating a dose-dependent upregulation of Sox9 and OB-cadherin, indicative of a chondrogenic fate^[47].

Bone morphogenetic protein (BMP) also plays a role in determining suture fate since it is implicated in ectopic bone formation^[49]. BMP type 1b receptor (BMP-R1B) induces osteogenesis at different stages of differentiation and is expressed at higher levels in posterofrontal SMC^[6]. Conversely, sagittal SMC exhibit enhanced levels of BMP-3, the only osteogenic inhibitor in the BMP family and Noggin, a BMP antagonist^[6]. This suggests that BMP plays a role in maintaining patency in sagittal sutures. Furthermore, since estrogens are associated with growth plate fusion of endochondral bones, estrogen and estrogen receptor alpha (ER α) were found to be necessary for normal posterofrontal suture fusion^[50]. Increased ER α transcript abundance corresponded with posterofrontal suture fusion, whereas ER α knockout (α ERKO) mice exhibited delayed posterofrontal suture fusion. *In vitro*, addition of 17- β estradiol, an estrogen analog, enhanced osteogenic and chondrogenic differentiation in both SMC populations. Furthermore, *in vivo* administration of the estrogen antagonist Fulvestrant diminished calvarial osteogenesis and inhibited suture fusion in mice. Thus, estrogen, its respective receptor and BMP play a crucial role in osteogenic differentiation of SMC.

Clinical Applications

With the osteogenic and chondrogenic potential of SMC, they hold potential therapeutic implications in the field of cranial suture biology. The association between SMC and various signaling pathways can be used to develop novel osteoregenerative therapies, with one of the most well-studied being craniosynostosis, a fairly common disease characterized by the premature ossification of cranial suture leading to stunted calvarial growth and brain development^[51,52]. One known cause, not exclusive to just this craniofacial abnormality, is retinoid exposure while *in utero*^[53-55]. Looking at retinoid-induced

suture fusion, posterofrontal and sagittal SMC cultured with all-trans retinoic acid expressed retinaldehyde dehydrogenase (Raldh1, Raldh2, Raldh3) along with retinol-binding protein 4 (Rbp4), all of which are essential for retinoic acid synthesis, binding and signal transduction. Addition of retinoic acid was also found to enhance osteogenic differentiation, exemplified via upregulated expression of Runx2, Hedgehog and BMP signaling activity and enhanced ALP activity. The craniosynostosis model thus provides an excellent platform for SMC to not only further elucidate the mechanism of the pathologic pathway, but to also provide a potential therapeutic option.

GINGIVAL MESENCHYMAL STEM CELLS (GMSC)

Background and Application

Gingival mesenchymal stem cells (GMSC) are derived from gingival tissue, an oral mucosal barrier that exhibits scarless healing after injury^[33]. Due to their multilineage differentiation potential and implication in tumor suppression, immunoregulation and wound healing, GMSC are a promising new candidate for the field of regenerative therapy.

Profile & Identity

GMSC express high levels of the MSC surface markers CD13, CD44, CD73, CD90, CD105, STRO-1 and SSEA-4, but do not express hematopoietic markers CD14, CD19, CD34, CD38, CD45 and CD54^[56-58]. With their ease of accessibility from clinically resected gingival tissues during routine dental procedures, GMSC are an attractive alternative to other MSC^[7,33]. Furthermore, GMSC isolated from inflamed tissues demonstrate similar multilineage differentiation potential as compared to those retrieved from healthy tissue^[59]. As well, investigators have found that GMSC are non-tumorigenic and maintain normal karyotype and telomerase activity^[33,60]. Multiple studies have demonstrated that GMSC tend to proliferate at a higher rate than either PDLSC or BMSC^[7,33,61].

GMSC are multipotent and can differentiate towards the osteogenic, adipogenic and chondrogenic lineages. Culturing of GMSC in osteoinductive medium leads to mineralization of extracellular deposits^[56] and formation of calcium deposits^[57]. Compared to PDLSC and DPSC, GMSC express intermediate levels ALP activity and expression of Runx2, OCN and Col I^[7]. Wu *et al.* examined the use of enamel matrix derivative (EMD) treatment to promote GMSC proliferation and osteodifferentiation, finding a dose dependent increase in Runx2, Alp and Ocn expression with EMD treatment^[60]. Conversely, GMSC cultured in adipogenic medium express PPAR γ -2^[7] and form lipid globules characteristic of adipogenic differentiation^[57]. In comparison to other CMF sources of MSC, GMSC undergo a greater degree of adipogenesis as compared to PDLSC, but less so than DPSC^[56]. GMSC also exhibit the capability to differentiate towards the chondrogenic lineage, as evidenced by strong staining for the chondrogenic markers aggrecan and collagen type II α 1^[56]. Such successfully differentiated progenitor cells derived from gingival connective tissue exhibit increased proteoglycan production^[57].

Given their location, it is not surprising that GMSC can differentiate toward the odontogenic lineage under the proper stimulation of embryonic tooth germ cell-conditioned medium (ETGC-CM)^[7]. For example, ETGC-CM induction promoted not only proliferation of GMSC, but also higher expression of odontogenic genes, including Alp, Opn, Bsp and Dental matrix protein (Dmp1).

Pre-Clinical Studies

GMSC have been considered promising for tissue engineering-based therapies, as seen in various pre-clinical studies. For example, Wang *et al.* implanted GMSC seeded on type I collagen gel in a mandibular and critical-sized calvarial defect rat model, demonstrating that GMSC could repair either wound type within two months post-surgery through formation of new bone^[62]. Likewise, Fawzy El-Sayed *et al.* induced bilateral periodontal defects in the premolar and molar regions of a miniature pig model, showing that GMSC possessed the capability to regenerate bone, cementum and periodontal ligament^[63]. Furthermore, Xu *et al.* demonstrated that GMSC are able to migrate towards site of injury^[64]. In fact, GMSC transplanted via the tail vein in mice promoted bone regeneration in the mandibular defect region^[64]. Thus, these studies exhibit the promising regenerative potential of GMSC, which can be applied towards tissue engineering.

Animal studies have also examined the effects of GMSC in tumor inhibition and immunoregulation, among other areas. For example, Xia *et al.* investigated the efficacy of GMSC as a vehicle for tumor-targeted therapy, via the use of GMSC expressing tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)^[61]. GMSC showed a tendency to migrate toward tumor cell lines (including Tca8113 and Cal27) and TRAIL expressing GMSC induced tumor cell necrosis and apoptosis *in vitro*. Moreover, TRAIL expressing GMSC inhibited tongue squamous cell carcinoma growth in mice when delivered in either a local or systemic fashion^[61].

In another study, Mitrano *et al.* evaluated the immunoregulatory capacity of GMSC on proliferation of peripheral blood mononuclear cells (PBMC) in response to mitogen by culturing PBMC for five days in either the absence or presence of GMSC at different proportions^[57]. Their results showed that GMSC possessed immunosuppressive properties and inhibited PBMC proliferation in a dose-dependent manner. This is consistent with the findings of other studies, which have investigated the immunosuppressive role of MSC. In fact, MSC derived from adipose tissue, bone marrow, skeletal muscle and umbilical cord inhibit cell proliferation of T cells, B-cells, natural killer cells and dendritic cells, producing a state of division arrest anergy^[65]. MSC also inhibit the processes of antigen presentation and antibody secretion^[65]. Furthermore, when GMSC and PDLSC were cultured with inflammatory cytokines TNF- α and IL-1 β , the osteogenic potential of both cell types decreased, but the amount of mineralized matrix formation, ALP activity and expression of OCN, Runx2 and Col I declined to a lesser degree in GMSC^[66]. These data in aggregate suggest that GMSC may be of more utility in inflamed or infected tissues, such as in the context of periodontitis. In another study, Li *et al.* investigated whether GMSCs mediate inflammation-induced hyperplasia in gingiva by differentiating towards a pro-fibrotic phenotype^[58]. They successfully isolated GMSCs from both normal (N-GMSC) and inflamed (I-GMSC) tissues^[58]. They found that GMSC from inflamed tissues showed lower expression of matrix metalloproteinases (MMP1 and MMP2). These data suggested that GMSC may be in part responsible for the fibrotic phenotype of gingival inflammatory hyperplasia. In summary, these animal studies elucidate both the anti-tumor and immunosuppressive properties of GMSC.

ADIPOSE-DERIVED STEM CELLS (ASC)

Adipose-derived stem cells (ASC) are most commonly derived from areas where cosmetic removal of fat tissue is desired, including the abdomen and thighs. Although less frequently studied, fat tissue from the head and neck is a source of ASC like other fat depots^[67-69].

Numerous studies in rat^[70], pig^[71] and human models^[72] have demonstrated the multipotentiality of head and neck-derived ASC. The depot-specific differences in ASC has been studied in multiple models^[8,73]. For example, rat ASC from the neck show lower rates of proliferation than other sources^[8]. These same investigators found, however, that neck-derived ASC were the most responsive to FGF-2 and/or PDGF (Platelet derived growth factor) induced proliferation^[8]. In another study, Kim *et al.* showed the potential application of neck-derived ASC for the treatment of head trauma-related olfactory dysfunction in terms of regeneration epithelium^[70]. Olfactory epithelial degeneration was first induced in a rat model through unilateral transection of the olfactory nerve; regeneration of olfactory epithelium was then observed following systemic transplantation of neck-derived ASC^[70].

Derived from the head and neck, neural crest cells consist of heterogeneous progenitor mesenchymal cells which can give rise to craniofacial cartilage, bone, dermis, adipose tissue and vascular smooth muscle cells^[74]. Neural crest-derived adipose stem cells (NCDASC) are technically a subpopulation within ASC, showing similar MSC markers as their non-neural crest derived counterparts, but demonstrate a multipolar morphology for neural crest progenitors (i.e. Nestin and Sox2) as well as preadipocytes (CD24, CD34, S100)^[75,76]. However, while NCDASC maintain their predilection towards an adipogenic lineage, they show reduced osteogenic and chondrogenic potential^[75]. Another group also confirmed that a subset of adipocytes originate from the neural crest; as well, the neural-crest derived subpopulation under the proper stimulation will differentiate into adipocytes^[77]. Taken together, neck-derived ASC and NCDASC show multipotentiality, but reduced osteogenic and chondrogenic potential compared to ASC derived from other sites.

CONCLUSION

Tissue-specific mesenchymal stem cells (MSC) represent a promising and more abundant alternative to the current standard of autologous bone grafting, among other applications. Adipose-derived stem cells (ASC) are currently the most well-studied of those discussed here, offering great abundance in addition to multilineage potential in various tissue environments. ASC derived from neck fat have shown both osteogenic and adipogenic potential, although further studies are needed for this sub-population. Dental pulpal stem cells (DPSC) under the appropriate stimuli have also demonstrated exceptional multipotency, including adipocytes, myocytes, neurons and osteoblasts^[9]. In terms of application, DPSC have seen increasing use in the field of endodontics as regenerative treatment of pulpal necrosis of the permanent dentition, with studies indicating dentin-like tissue formation both *in vitro* and in mandibular defects^[10]. Periodontal ligament stem cells (PDLSC) also demonstrates multipotency, with the most notable being osteogenesis and cementogenesis^[29,30]. Interestingly, both its induction of nerve regeneration and angiogenesis contributes to its unique versatility in generating mature woven bone seen in healthy human tissue. Suture-derived mesenchymal stromal cells (SMC) are derived from cranial sutures, so its predilection towards a chondrogenic and osteogenic path are not surprising. Lastly, gingival mesenchymal stem cells (GMSC) hold multilineage differentiation potential and even a hypothesized role in tumor suppression, immunoregulation and wound healing. Taken together, these various tissue-specific MSC types all demonstrate variable degrees of multipotency, with effective therapeutic application requiring further study. To conclude, though the majority of literature has focused on isolation of MSC from bone

marrow, the craniomaxillofacial skeleton represents an alternative source of various stem cell populations with significant potential in the field of bone tissue engineering. Furthermore, craniomaxillofacial sources of MSC are an area of interest in a multitude of medical and dental disciplines, including stem cell biology, plastic and reconstructive surgery, orthodontics and oral pathology. Thus, craniomaxillofacial MSC populations are a promising area of investigation and the transition from pre-clinical to clinical studies will elucidate potential therapeutic applications.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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