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REVIEW ARTICLE

Adult Stem Cells of Orofacial Origin: Current Knowledge and Limitation and Future Trend in Regenerative Medicine

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Abstract Stem cell research is one of the most rapidly expanding field of medicine which provides significant opportunities for therapeutic and regenerative applications. Different types of stem cells have been isolated investigating their accessibility, control of the differentiation pathway and additional immunomodulatory properties. Bulk of the literature focus has been on the study and potential applications of adult stem cells (ASC) because of their low immunogenicity and reduced ethical considerations. This review paper summarizes the basic available literature on different types of ASC with special focus on stem cells from dental and orofacial origin. ASC have been isolated from different sources, however, isolation of ASC from orofacial tissues has provided a novel promising alternative. These cells offer a great potential in the future of therapeutic and regenerative medicine because of their remarkable availability at low cost while allowing minimally invasive isolation procedures. Furthermore, their immunomodulatory and anti-inflammatory potential is of particular interest. However, there are conflicting reports in the literature regarding their particular biology and full clinical potentials. Sound knowledge and higher control over proliferation and differentiation mechanisms are prerequisites for clinical applications of these cells. Therefore, further standardized basic and translational studies are required to increase the reproducibility and reduce the controversies of studies, which in turn facilitate comparison of related literature and enhance further development in the field.

Keywords Orofacial stem cells · Adult stem cell · Regenerative medicine · Stem cell therapy

1 Introduction to stem cells, types and potential applications

The stem cell engineering is a rapidly growing field in the area of regenerative medicine. Stem cells are being used extensively for understanding development and progression of diseases. Currently, stem cell therapy is one of the bravest and promising moves for successful treatment of various medical conditions. This field is rapidly expanding as different clinical trials reveal their tremendous therapeutic potentials. Stem cells have been investigated as potential therapy for various medical conditions and diseases such as; cerebral ischemia, parkinson's disease, alzheimer's disease, retinal disease, diabetes type 1 and 2, myogenic disease [1]. It is also applied for neuronal, cardiovascular and bone regeneration [1–3].

Although various stem cells have been isolated and defined, they share common general features which make them distinctive among other mammalian cells. The main interesting key feature of stem cells is their undifferentiated nature with a potential to either retain their stemness through self-renewal (symmetric division) or give rise to differentiated daughter cells (asymmetric division) [4]. In general, stem cells stay in a quiescent state inside adult tissue, where upon stimulation they enter the cell cycle for division [5, 6]. Two types of cell division mechanism

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follow the local physiological request, as asymmetric or symmetric cell division [7]. Asymmetric division allows maintenance of a constant stem cell population, while symmetric division is in response to tissue injury or disease conditions [8]. This is controlled by multiple complex biological pathways that maintain the balance; however, the exact mechanism is unknown.

The main three types of stem cells investigated extensively for potential therapeutic and clinical applications in medicine are: embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult stem cells (ASCs) (Fig. 1). Stem cells are characterized by their ability of self renewal with maintenance of this proliferation potential for a long period, and their unspecialized state with the ability to differentiate (pluripotency) into multiple specialized cell lineages. However, multipotent adult stem cells (ASCs) have lower differentiation potential than pluripotent stem cells (ESCs and iPSCs) [9].

ESCs are originated from the inner cell mass of embryonic blastocyst in the early pre-implantation stage after *in vitro* fertilization. They can differentiate into most cell types from all three germ layers [9]. A regulatory system of transcription factors maintains ESCs in a pluripotent and unspecialized state as long as they are cultured under appropriate conditions [10]. ESCs offer a great potential for clinical applications but their exact differentiation mechanism is still unclear.

iPSCs are generated through genetic reprogramming of somatic cells by forced expression of genes and transcription factors (i.e. Sox2, c-Myc, and KFL-4) to maintain defined properties of ESCs [11]. However, they differ from ESCs in their cellular epigenetic memory that may divert their differentiation potential toward donor cell lineages

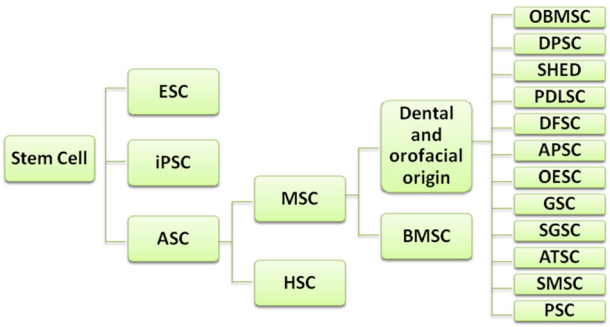

Fig. 1 Main categories of stem cells. *ESC* embryonic stem cell, *iPSC* induced pluripotent stem cell, *ASC* adult stem cell, *MSC* mesenchymal stem cell, *HSC* hematopoietic stem cells, *BMSC* bone marrow stem cell, *OBMSC* orofacial bone marrow mesenchymal stem cell, *DPSC* dental pulp stem cell, *SHED* exfoliated deciduous teeth stem cell, *PDLSC* periodontal ligament stem cell, *DFSC* dental follicle stem cell, *APSC* adult pulp stem cell, *OESC* oral epithelium stem cell, *GSC* gingival stem cell, *SGSC* salivary gland stem cell, *ATSC* adipose tissue stem cell, *SMSC* Schneiderian membrane stem cell, *PSC* periosteum stem cell

[12]. iPSCs are relatively easy to generate and they provide useful tools for drug investigation and *in vitro* modeling of specific diseases using patient derived cells [13]. However, the viral transfection is used to introduce the reprogramming factors into adult somatic cells which may alter iPSCs in a negative way and limit their applications. This necessitates careful controlling before any clinical applications. Recent studies investigate other non-viral mean of inducing iPSCs using miRNA or small molecules to enhance their stability and transduction efficacy [14, 15].

Stems cells are valuable natural source for therapeutic and regenerative medicine. The main goal is to control the cellular fate by diverting the differentiation pattern to the desired lineage and abolish undifferentiated cells population. However, the ability to control the cellular fate to the lineage of choice is a challenging issue for successful therapeutic applications. The critical drawbacks for clinical use of ESCs and iPSCs are their potential for immune rejection, teratoma formation and critical ethical regulations [11]. Therefore, the extensive body of literature is focused on study of adult stem cells (ASC) and their potential clinical applications. Hereby, we provide a detailed update on different types of adult stem cells, their features and clinical potentials with specific focus on new resources of ASC from dental and orofacial origin.

2 Adult stem cells

2.1 Definition, types, and basic characteristics

It is known that adult stem cells (somatic stem cells or postnatal stem cells) reside in specific location of each tissue in a specialized microenvironment known as the "stem cell niche". In cell-based regenerative medicine, adult stem cells can be expanded in an undifferentiated state *in vivo* for a limited number of passages before differentiation into specialized cells of mesodermal origin. These multipotent progenitor cells allow immortalization for desired periods and can express a range of genes after genetic engineering. However, their isolation (from adult tissue and organ of body) and expansion are more difficult than ESCs and they have differentiation potential which is limited to cell range of the original tissue [16].

The two main types of adult stem cells are hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) (Fig. 1). HSCs are blood-derived and they may provide signaling molecules and growth factors that enhance function of other cells through paracrine mechanisms. MSCs were first recognized in bone marrow by Friedenstein, and play a crucial role in tissue regeneration following stress and injury impacts [17]. They are responsible for the maintenance of connective tissues by

differentiation into several cell lineages such as; osteoblasts, chondrocytes, adipocytes, and myoblasts [18, 19].

The term MSC may be scientifically inaccurate to be applied to plastic adherent cells isolated from bone marrow or other sources since some of the recognized biological properties of cells may not match the generally accepted criteria for stem cell activity. Therefore, to clarify the terminology and avoid inconsistency between nomenclature and biologic properties, *International Society for Cellular Therapy* (ISCT) has proposed a new nomenclature "multipotent mesenchymal stromal cells" to be applied to the fibroblast-like plastic-adherent cells, regardless of the tissue of origin. However, the term "Mesenchymal stem cells" should be used only for cells that meet specified stem cell criteria while the commonly used acronym "MSC" can be still applied for both cell populations [20]. Furthermore, for more uniform identification, ISCT has proposed minimal four criteria to characterize human MSC. These criteria are as follow; (1) MSC must adhere to plastic tissue culture plate in standard culture conditions, (2) MSC must express surface definitive markers CD105, CD73 and CD90, (3) MSC should not express CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR, (4) MSC should be able to differentiate *in vitro* into different cell types of osteoblasts, adipocytes and chondroblasts [21].

2.2 Isolation and culture

MSCs are commonly isolated from bone marrow aspirate using adherent culture technique. MSCs represent only 1–5 cells in 1×10^4 nucleated cells of bone marrow but they can be expanded *in vitro* for research in tissue regeneration [22].

Bone marrow aspirate from the iliac crest is the most commonly used and documented procedure for MSC isolation in regenerative medicine. The isolated MSC from the iliac crest or femur proved to have a great potential in bone tissue engineering; however, this procedure is invasive for the patients [23]. Another important factor to consider is increasing donor age that can have a negative impact on biological behaviors of MSC by reducing their proliferation status, viability, multilineage differentiation potentials, expression kinetics, and immunoregulatory features [24, 25]. It has been reported that kinetics of cell growth is slower in MSC derived from old rats (>15 months old) compared with young ones (4 weeks old) [24]. There are many reports about the impact of donor age on clinical efficacy of MSC in bone regeneration where the osteogenic potential of these cells decline with the age increasing [26–28]. Furthermore, *in vitro* multipotent capability is also reduced by increasing culture period and repeated passaging [23]. In an effort to explain the underlying mechanism of age related impacts, MSC derived from old donors (18 months rat model) were found to have increased susceptibility to reactive oxygen species induced adhesion impairment and apoptosis compared to young donors (8–10 week old rat model). In addition, MSC from old rats donor showed more rapid reduction of survival rate after transplantation into the region of myocardial infarction of rat models [29]. Therefore, the fact that therapeutic capability of MSC is dependent on donor age, remains as a challenge and limitation in therapeutic and regenerative medicine that requires further investigation with special focus on different sources of MSC and their clinical differences. In particular, further studies are required to determine which types of MSC are less dependent on donor age.

Isolation of bone marrow MSC is not limited to iliac crest or femur as the orofacial bone marrow is also a valuable source of MSC (Fig. 1). Orofacial bone marrow stem cells (OBMSC) can be obtained from the bony maxilla or mandible during various intraoral or extraoral surgical procedures such as; dental implant, surgical exodontia of impacted tooth, enucleation of cyst, and orthognathic surgery. OBMSC can be isolated from all ages and it seems that its gene expression pattern is not affected by donor age [30].

It is known that craniofacial bones (membranous bone) provide a better quality bone for autologous grafting compared to other sources of endochondral bones (i.e. iliac crest, rib, femur) [31–33]. This is due to significantly higher reported bone volume, higher bone stability, and lower resorption rate after grafting from membranous bones, which implies that different donor tissues may express different regenerative potentials [34, 35]. The differences in embryonic origin can result in functional variations and behaviors of MSC originated from iliac crest and those of orofacial bones [36]. In fact, the functional difference between MSC of different sources has been well documented in several studies. MSC from orofacial bones demonstrated distinctive differentiation potential and expression pattern and were reported to have higher proliferation rate and osteogenic differentiation potential, are able to produce higher quantity (more volume) and quality (more mineralization) bone with less chondrogenic or adipogenic potential during osteogenesis when compared to those originated from other sources [37–40]. These properties, make OBMSC a better choice than MSC from other sources for craniofacial bone regeneration, however, the main disadvantages is limitation of available and collectable bone marrow volume from orofacial bones (about 0.1–3 ml) that necessitate a reliable *in vitro* cell expansion technique before their clinical applications [30, 40].

2.3 Potential clinical applications

MSCs have been used extensively for transplantation studies in animal model and human therapeutic trials. Their potential advantages include; ability to release bioactive molecules, represent specific receptors on their surface, allow genetic modification, and present low immunogenicity and minimum ethical concerns [41]. However, because MSC are maintained physiologically in a quiescent state within the tissue and organ, their study in the active growing state is more critical compared to pluripotent stem cells. Nevertheless, emerging evidence indicates the presence of both quiescent and active MSCs in several tissues (i.e. hair follicle, gut, bone marrow) in separate locations [6]. Generally, the main disadvantage of MSCs is the absence of definitive *in vivo* markers thus, they are still not clearly characterized *in vitro* [22]. Furthermore, when compared to embryonic stem cells, MSCs have limited proliferation and differentiation potential that decrease with passage and age [42].

In tissue engineering and regenerative medicine, MSCs are considered an attractive cell source as they can be rapidly expanded *in vitro* for several lineages while maintaining their differentiation potential [43]. Furthermore, they can be delivered using different natural or synthetic biomaterials for pre-clinical and clinical studies [44, 45]. It has been reported that MSCs can retain their stemness upon bioencapsulation that is linked to their hypoimmunogenic feature and limited alloantigen expression [46].

MSCs can also play an important role in angiogenesis by promoting the ability of adjacent endothelial cells in migration and tube-like formation [47, 48]. In addition, recent reports have shown their potential for differentiation into tissue-specific cells following systematic infusion [49, 50].

Various bioactive factors are released by MSCs such as cytokines and chemokines that produce paracrine effects. This signaling mechanism may possess an immunomodulatory role by allowing cell homing, migration and attachment of immune cells to injured cells. Currently, the mechanism for immunosuppressive potential of MSCs is not fully understood but it holds a great promise for treatment of auto-immune inflammatory diseases [51].

More recently, direct reprogramming of adult stem cells provided a new horizon as a unique therapeutic strategy in regenerative medicine. The main aim is to instruct adult cells to convert into other required cell types for tissue repair using defined transcription factors in adult organs. This technique allows generating a range of cell types similar to those derived from pluripotent stem cells without reversion [52, 53]. The main advantages of this method are overall simplicity and speed of differentiation process that also allow *in situ* conversion of cell fate. However, reprogrammed cells have to be characterized *in vitro* using extracellular matrix (ECM) for survival and growth ability before potential applications [54].

3 MSC from dental and orofacial origin

3.1 MSC from dental tissues

MSC have been isolated and characterized from multiple sources of dental tissues [55] (Fig. 1). MSC from adult dental pulp origin (DPSC) or exfoliated deciduous teeth (SHED) were among the first to be identified [56, 57]. They had similar phenotypic features to those of bone marrow origin (BMSC) such as multipotency and self-renewal capacity [56–58] in addition to their ability to regenerate dentinal pulp complex (Table 1). Furthermore, SHED has shown a distinctive potential in regeneration of bone in critical size defects *in vivo* by active contribution to osteogenesis and inducing the host cells to differentiate into osteogenic cells [57, 59]. Thus, exfoliated teeth could be a unique resource for stem cell therapy including autologous stem-cell transplantation and tissue engineering.

MSC isolated from periodontal ligament (PDLSC) is another type of MSC of dental origin, with the capacity to regenerate periodontium (i.e. cementum, PDL and alveolar bone) *in vivo* [60]. It has been reported that this potential is also site specific, as PDLSC harvested from alveolar bone surface of PDL when compared to those isolated from root surface, displayed higher proliferation capability, greater osteogenic differentiation potential, higher ALP activity (a marker enzyme of osteoblast differentiation) and mineralization-related markers, and higher bone regeneration ability (Table 1). In addition, they have shown the potential to repair critical size defects of calvaria bone *in vivo* [61]. Transplantation of these cells, which can also be obtained from extracted teeth, holds a great promise for regeneration of periodontium after periodontal diseases. Furthermore, human PDL can be cryopreserved and recovered subsequently for post-natal stem cells isolation, thus providing a valuable approach in tissue engineering [62].

MSC are also isolated from dental follicle or dental sac (DFSC) [63, 64] and apical papilla (APSC) [65, 66] of developing teeth. DFSC and APSC can be found in impacted teeth, which are commonly discarded as medical waste in dentistry. Interestingly, DFSC showed higher osteocalcin (OC) expression and calcium deposit once compared to bone marrow MSC (4 weeks implantation in mice), that indicates their potency to be as an alternative cell source for bone tissue engineering [67]. Developing dental tissue may provide better source for MSC. It has

Table 1 Different types of adult stem cells from dental and orofacial origin and their main characteristic features compared to bone marrow cells

Stem cells	Source	Bone regeneration	Periodontium regeneration	Reported phenotypic markers	Advantage	Disadvantage	References
BMSC	Bone marrow aspirates, i.e. iliac crest, femur	Yes	None	Oct-4, Nanog, CD73, CD90, CD105, CD106,CD166, SSEA-4, CD9, CD13, CD146, Nestin, Notch-1, STRO-1, CD44,CD24	Availability	Invasive for patient, Donor age dependant, prolonged culturing reduce *in vitro* multipotent capability	[23, 26–28]
OBMSC	Orofacial bone marrow aspirates, i.e. maxilla, mandible	Yes, produce higher quantity and quality bone than BMSC	None	CD73, CD90, CD105	Donor age non-dependant, higher proliferation rate, less chondrogenic/ adipogenic potential	Limitation of available and collectable bone marrow volume	[30–40]
DPSC	Adult dental pulp	Yes	Yes	Oct-4, Nanog, CD73, CD90, CD105, CD166, SSEA-4, CD9, CD13, CD146, Nestin, Notch-1, STRO-1, CD44, CD29	Similar phenotypic features to BMSC, ability to regenerate dentinal pulp complex	Available after pulp exposure or extraction, requires selective isolation and characterization	[56, 58]
SHED	Exfoliated deciduous teeth	Yes	Yes	Oct-4, Nanog, CD73, CD90, CD105, CD166, SSEA-4, CD146, Nestin, STRO-1, CD44	Similar phenotypic features to BMSC, ability to regenerate dentinal pulp complex	Available after tooth shedding, requires selective isolation and characterization	[57, 59]
PDLSC	Periodontal ligament	Yes, potential to repair critical size defects	Yes	Oct-4, CD73, CD90, CD105, CD166, CD9, CD13, CD146, Nestin, STRO-1, CD44, CD29	Availability, simple procedure	Available after extraction, requires selective isolation and characterization	[59, 61, 62]
DFSC	Dental follicle of developing teeth	Yes, higher osteocalcin expression and calcium deposit than BMSC	Yes	CD73, CD90, CD105, CD166, SSEA-4, CD9, CD13, CD146, Nestin, Notch-1, STRO-1, CD44,CD24, CD29	Availability, simple procedure	Available after extraction, requires selective isolation and characterization	[63, 64, 67]
APSC	Apical papilla of developing teeth	Yes	Yes	Oct-4, CD73, CD90, CD105, CD106, CD166, SSEA-4, CD9, CD13, CD146, Nestin,STRO-1, CD44, CD24, CD29, CD80, CD86	Higher proliferation and regeneration capacity than DPSC	Available in impacted teeth, requires selective isolation and characterization	[65, 66]
OESC	Oral epithelium	Yes, stem cells of neural crest origin possess high osteogenic potential in the presence of BMP-2	Yes	CD73, CD90, CD105, CD44H,	Unlimited availability, oral keratinocyte stem cells regenerate only into oral mucosa *ex vivo*	Requires selective isolation and characterization	[68–70, 72]

Table 1 continued

Stem cells	Source	Bone regeneration	Periodontium regeneration	Reported phenotypic markers	Advantage	Disadvantage	References
GSC	Gingival tissue	Yes	None	Oct-4, Nanog, CD73, CD90, CD105, CD106, SSEA-4, CD9, CD13, CD146, Notch-1, STRO-1, CD44,CD24, CD29	Unlimited availability, faster proliferation rate than BMSC, immunomodulatory and anti-inflammatory potential	Less effective differentiation potential than PDLSC	[71, 74–77, 106]
PSC	Periosteum of maxillofacial bones, i.e. mandible	Yes, show preferential osteogenic differentiation, superior to BMSC in enhancing bone regeneration	None	CD73, CD90, CD105, CD106, CD166, CD9, STRO-1, CD44	Faster proliferative ability, faster bone remodeling, more sensitive to signaling molecules than BMSC, produce cortical bone compared to BMSC that produce cancellous bone	Available only during surgical exposure	[79, 81–85]
ATSC	Adipose tissue, i.e. buccal fat pad	Yes, support accelerated wound healing and bone repair	Yes	CD73, CD90, CD105, CD29, CD34, CD44, CD45, CD14, CD19, CD146, SSEA, CD10, CD13, CD166	Similar to DPSC but a higher proliferation rate, low donor site morbidity, induce regeneration of dental pulp	Requires selective isolation and characterization	[86–92]
SGSC	Salivary glands	Yes	None	CD90, CD117, CD34, CD44, CD29, ALDH, CD166, CD49f	Potential for regeneration of salivary gland is under investigation, isolated progenitor cells from stromal tissue can differentiate into osteoblast, chondrocytes and adipocytes	Requires selective isolation and characterization	[93–97]
SMSC	Schneiderian Membrane of maxillary sinus	Yes	None	CD44, VCAM-1, CD146, STRO-1, CD29, CD44, CK19, CD90, CD105, CD73	Multilineage differentiation capacity into osteoblast, adipocytes and chondrocytes	Available only during surgical exposure, requires selective isolation and characterization	[98–100]

ALDH aldehyde dehydrogenase, *CK* cytokeratin, *SSEA* stage-specific embryonic antigen, *STRO-1* stromal precursor antigen-1, *VCAM-1* vascular cell adhesion molecule 1

been reported that compared to DPSC, APSC demonstrate higher proliferation and regeneration capacity upon transplantation *in vivo* [66]. This could be of interest when a high regeneration capacity is required in critical applications with healing challenges.

3.2 MSC from lining mucosa of oral cavity

Another interesting source of adult stem cells is oral mucosa. To date, two types of MSC have been identified and isolated; oral epithelial stem cells (OESC) [68–70] and gingival derived stem cell (GSC) [71]. Oral keratinocyte stem cells could regenerate only into well-organized oral mucosa *ex vivo* and hold a promise to be used for intraoral grafting procedures [72]. Furthermore, stem cells of neural crest origin may also be located in craniofacial adult tissue as scattered islands of cells in oral tissue. These cells have been isolated *in vivo* from various oral tissues, i.e. palate, tongue and buccal mucosa. They possess osteogenic potential by differentiation into osteoblast cells, and have expressed high level of ALP enzyme and mineralization profile in the presence of BMP-2 [73], thereby providing a useful source for bone regeneration strategies (Table 1). The gingival overlying alveolar ridge and extracted teeth are frequently discarded but Zhang et al. [74] first characterized GSC which exhibited a stable morphology and characteristics at higher passage and a faster proliferation rate than bone marrow MSC, in addition they are not tumorigenic [75]. The inherent stemness of gingival cells, their multipotency, high reprogramming efficacy into iPSC, ease of isolation, clinical availability, and rapid expansion provide great potential for cell therapy in regenerative medicine and tissue engineering [76]. Interestingly, it has been shown that GSC exhibited fewer inflammatory-related changes during osteogenic differentiation both *in vitro* and *in vivo* when compared to PDLSC [77]. Most importantly, GSC were reported to be capable of immunomodulatory functions in experimental colitis animal model by suppression of lymphocyte proliferation and inflammatory cytokines, inducing expression of immunosuppressive anti-inflammatory factors (IL-10 and COX-2) and increasing infiltration of regulatory T cells at the colonic sites [74]. Therefore, GSC may further function as a promising alternative for immunomodulatory, anti-inflammatory and cytotherapeutic applications.

3.3 MSC from orofacial bony tissues

Periosteum-derived stem cells (PSC) are other interesting resources of stem cells. This is not only because of their physiological role in fracture repair but is also related to their unique osteogenic potential. The osteogenic capacity of inner layer of periosteum is addressed in multiple other studies after the initial report in 1932 [78]. The isolated heterogeneous cells from the periosteum show preferential osteogenic differentiation, however, they also have adipogenic and chondrogenic potential [79]. Comparative qualitative analysis of tissue-engineered bone comparing bone marrow MSC, alveolar bone cells, and periosteal cells have shown *in vivo* superiority of periosteal cells in enhancing bone regeneration [80]. Furthermore, histological comparison of newly formed bone after bone marrow and periosteal graft in rat calvarial defects has shown that bone marrow graft induced spongy bone formation, whereas periosteal graft produced cortical bone structure in defect. This finding suggests that quality of bone formation may also be affected by type and source of transplanted cells [81]. It has been found that under normal condition bone marrow MSC are more osteogenic than periosteal cells. However, periosteal cells have faster proliferative ability [82, 83], they are more sensitive to pre-treatment with some signaling molecules (i.e. basic fibroblast growth factor; bFGF and bone morphogenetic protein; BMP-2) before transplant hence, they are more osteogenic [83]. Cultured autogenous periosteal derived stem cells have also been tried clinically for alveolar ridge augmentation or maxillary sinus lift. The results of bone biopsy analysis have indicated prominent recruitment of osteoblasts and osteoclasts along with angiogenesis that suggested faster bone remodeling than conventional autogenous bone grafting. This can reduce postoperative healing phase after bone grafting or dental implant insertion by enhancing osseointegration. Furthermore, expanded periosteum-derived cells can offer a valuable source for cell based bone tissue engineering by reducing the required volume of autogenous bone graft by 40%, allowing less traumatic grafting procedures [84, 85]. Moreover, the concept of bone regeneration can be guided in a desired instructive way based on the use of biomaterials similar to periosteum itself in combination of appropriate construct that mimic ECM of bone.

3.4 MSC from adipose tissues and salivary glands

MSC derived from adipose tissue (ATSC) is another valuable source of progenitor cells in the field of regenerative medicine. ATSC can be easily harvested in large numbers from various sources and related to low donor site morbidity. Although ATSC originate from mesodermal lineages, but their applications can also be extended to ectodermal and endodermal tissues and organs [86]. Although subcutaneous adipose tissue is very abundant, the buccal fat pad could provide an accessible and rich source for stem cells. An animal study by Niada et al. [87] revealed that buccal fat pad contains progenitor cells with the ability to differentiate towards osteogenic lineage with

deposition of calcified ECM. Autologous ATSC are applied successfully for orofacial bone reconstruction after jaw bone resections in human. They supported accelerated wound healing and new bone formation following transplantation and further rehabilitation with dental implants [88, 89]. Furthermore, transplanted ATSC induced dental pulp regeneration [90] and regeneration of periodontal tissues including PDL and alveolar bone in extraction sockets of animal models [91]. An animal study comparing ATSC and dental pulp stem cells revealed that although ATSC had a higher proliferation rate and better senescence resistance in culture, but ATSC are very similar and useful as DPSC in regenerative dentistry [92]. Therefore, the use of discarded fat tissue as one of the richest source of adult stem cells in mammals, for isolation and clinical application of stem cells may offer a paradigm shift in providing alternative therapeutic approach in regenerative medicine and dentistry.

Stem cells have also been isolated from salivary glands in human (SGSC) [93]. The primary culture of salivary gland usually contains various cells of different sources including stromal, blood vessel and parenchymal cells. Therefore, selective isolation and characterization is required to obtain the primary cell of interest. Although, the capacity of salivary gland stem cells for regeneration of salivary gland function is under investigation [94–96], but isolated progenitor cells from stromal tissue can be guided to differentiate into osteoblast, chondrocytes and adipocytes [97].

3.5 MSC from lining of maxillary sinus

More recently, it was found that the Schneiderian membrane of the maxillary sinus (Schneiderian Membrane stem cell-SMSC) is also a source of MSC. *In vitro* studies have demonstrated the ability of these cells for high expression of MSC markers (STRO-1, CD29, CD44, CD73, CD90, CD105 and CD146) and multilineage differentiation capacity into osteoblast, adipocytes and chondrocytes [98–100]. Furthermore, SMSC have the capacity to form mineralized bone-like deposits and maintain their MSC features after *in vivo* transplantation, thus maxillary sinus can also be a candidate of MSC origin for functional bone regeneration [98]. SMSC can be a strong candidate as alternative treatment option to conventional maxillary sinus lifting and bone grafting prior to dental implant insertion. Implementation of these stem cells as native and already present cells for bone regeneration at maxillary sinus floor is a promise for close future. This can significantly reduce the need for bone grafting procedures at maxillary sinus floor and related surgical trauma and cost. Further researches are required to disclose the full characteristics and potentials of SMSC in order to facilitate their application in clinical practice.

4 Orofacial stem cells; regenerative and immunomodulatoy potentials for clinical applications

The ideal stem cells for regenerative medicine or dentistry should be reliable and safe by allowing complete control of cell fate in the body upon transplantation. Currently, adult MSC are applied clinically for bone or periodontal regeneration. However, the main concerns are the accessibility and feasibility, ease of isolation and characterization, possibility of directing the differentiation pathway into desired cells/tissue of target, and added immunomodulatory properties.

Bone marrow MSC or periosteal derived MSC are suitable sources of progenitor cells for orofacial bony reconstruction because of potential functional match between cell source and target tissue. However, the differentiation capacity of adult MSC is limited to mesenchymal lineages which exclude their application for complex organ regeneration in tissue engineering. Therefore, alternative stem cells such as iPSC may be the choice for complex application; however, the facts of immune rejection, unreliable fate control and ethical issue hinder their clinical applications. The patient-derived autologous iPSC cells may be an alternative approach to overcome these issues. However, in depth knowledge of developmental physiology is required for successful induction of these cells to form desired specific progenitor cells for targeted tissue/organ regeneration.

Introduction of alternative sources of stem cells within the orofacial region holds a significant promise for future clinical applications (Fig. 1). This is because the orofacial sources of stem cells are very rich and accessible and do not require clinician to undergo special training. These cells are not limited to single lineage and are able to regenerate complex organ structures. Among all available reported sources of stem cells in orofacial region, the GSC seems to be the most convenient and accessible source. The gingival tissue can be easily obtained from patient with minimum morbidity and ASCs [75] and iPSCs [76] can be isolated and expanded *in vitro* to the required cell passage. However, isolation of stem cells from other sources such as bone marrow, periosteum, adipose tissue, salivary glands and dental tissue, are not convenient for clinicians and require more traumatic surgical procedures that cause further donor site morbidity.

In addition to the regenerative potential of stem cells, their immunomodulatory potential has been also an issue of concern. Some other therapeutic effects have been attributed to immunomodulatory properties of stem cells such as angiogenesis, anti-inflammation and anti-apoptosis [101]. The low inherent immunogenicity of MSC, in addition to

their immunomodulatory properties is an attractive feature in cell transplant application. A multicentre, phase II experimental study in patients with steroid-resistant, severe, acute graft-versus-host disease (a life-threatening complication after allergenic transplantation with haemopoietic stem cells) confirmed the immunomodulatory capability of MSC. They revealed that infusion of MSC expanded *in vitro*, irrespective of the donor, might be an effective therapeutic strategy for these patients [102]. Therefore, MSC represent a promising alternative therapy for treatment and prevention of immune-mediated diseases [103].

With this regards, the human orofacial derived MSC have been also reported to possess immunomodulatory properties similar to those of BMSCs [104]. For example, oral mucosal lamina propria progenitor cells are capable of immunomodulation via a dose-independent pathway (unlike other MSC) by release of immunosuppressive molecules that indicates their potential application for wide range of immune-related diseases [105].

In particular, it has been shown that GSCs can function as an immunomodulatory and anti-inflammatory component of the immune system *in vivo* [74]. Zhang et al. [74] demonstrated that systemic infusion of GSC could home the cutaneous wound site by interaction with host macrophages and inducing their polarization to M2 macrophage (an anti-inflammatory phenotype of macrophages). Furthermore, they reduced the secretion of TNF-α by macrophages, therefore, contributing to a significantly promoted wound repair [106]. Other animal study models revealed that the systemic administration of allogeneic BMSC could prolong the survival of skin and cardiac allograft [107, 108]. This may be related to MSC inhibiting cytokine release and impairing function of natural killer cells and T and B lymphocytes.

Currently, bone marrow is the primary source of MSC, however, orofacial sources of stem cells and in particular, the human GSC have been reported to be superior to BMSC for cell based regenerative therapy [75]. They are reported to lack teratogenic potential and possess several advantages over other sources of MSC, i.e. ease of availability and isolation, homogenous and faster proliferation, stable morphology and characteristic at higher passages, maintenance of normal karyotype and telomerase activity in long-term cultures, high regenerative capacity, and high potential for immune modulation. These properties make them a promising source of stem cells for future clinical cell based therapeutic and regenerative applications [75].

5 Current challenges and future trend

Different studies investigated the potential clinical applications of stem cells from orofacial origin for regeneration of dental and non-dental tissues as well as cell-based immunotherapy. These include but not limited to- osseous [109–114], neural [115–119], and cardiac muscle regeneration [120], angiogenesis [121, 122], hepatocytes differentiation [123–125], corneal repair [126, 127], treatment of skeletal muscle dystrophy [128, 129] and diabetes mellitus [130, 131]. The interested readers can refer to other reviews on clinical applications of orofacial stem cells [127, 130, 132–134]. The clinical applications of these stem cells will expand in future; however, the current knowledge on their full features and potentials is limited.

Comparing and contrasting of the studies related to stem cells are very challenging because of several conflicting findings on stem cell behavior and phenotypic characteristics in the literature. For example, there are controversial reports with regards to expression of different antigens during stem cell culture. This may be attributed to differences in isolation techniques, culture protocol (culture medium and cell density), passage of stem cells, etc. [135]. It is important to note that younger and older passage of stem cells may also behave differently in their expression of antigen. For example, it is reported that percentages of expression of phenotypic marker are subjected to change in different passages of stem cells, where subsequent passaging may result in increase or decrease of relevant expression markers [136, 137]. With regards to stem cell isolation, the age of donor, body mass index, and exact anatomical location of isolated cell may also influence behavior of stem cells [37, 138, 139]. Furthermore, minor differences in isolation and culture condition may not influences phenotypic expression pattern of primary cells, but it may result in significant impact on differentiation profile of stem cells [140].

All of these variables contribute to controversies in current literature that necessitate development and adherence to standardized protocols for isolation, culture and characterization of stem cells (Table 2). For this purpose, several procedures have been suggested that results in more homogeneity in composition of initial cell culture, such as early washing procedure before cell culture, use of flow cytometric sorting, and use of immunomagnetic separation [141].

Although, the main focus of recent studies has been directed toward exploration and characterization of new alternative sources of stem cells, there is a clear need to investigate their particular biology at genetic and cellular levels as well as their potential clinical applications. More specifically, with regards to dental and orofacial stem cells, further studies are necessary to explore the differences in their immunophenotypic characteristics and their relationship with stem cell behaviors. Moreover, identification of a specific phenotypic marker for each type of stem cell is required to help in better isolation and utilization of these cells.

Table 2 The list of important parameters that need to be standardized and fully reported in studies involving stem cells

Parameter	Detail
Donor	
Source of tissue	Human, animal, species, genetically modified, purchased, *etc*.
Gender/age	Gender and age of donor at isolation time
Anatomical site	Description of exact anatomical location of tissue extracted for stem cell isolation
Donor status	General health, nutritional status, BMI, *etc*.
Stem cell	
Isolation method	Detailed description of surgical procedure, aspiration strategy, materials, *etc*.
Culture medium	Complete list of ingredients; type/source, batch number, *etc*.
Cell density	Initial cell density, cell density at passage time, *etc*.
Cell passage	Passage number selected for study
Characterization method	Detailed description of materials and equipments used to characterize stem cells and expression of their markers
Translation	Detailed method of seeding/injection into scaffold before grafting into recipient site
Biological signal	List of applied growth factors or proteins, i.e., BMP
Cell banking	The impact of banking procedure (i.e., technique and duration) on stem cell behavior

Furthermore, in order to utilize the maximum potential of newly introduced stem cells, it is required to be able to understand and control their differentiation fate. It is specially true for the stem cells of orofacial region because of their potential to regenerate complex tissues. Therefore, a new wheel of research is required to identify the proper method of controlling their fate and guiding them into the desired differentiation track. It has been shown that the orofacial sources of stem cells can differentiate into different cell types and tissues, for example they can differentiate into bone forming cells. However, the characteristic biological features of differentiated cells; osteoblast in this case, should be investigated in more detail compared to the same cells originated from other sources. In addition, the quality and quantity of produced tissues; bone in this case, need to be compared more precisely with the same tissues produced from other cell lines.

Furthermore, the genotype pattern of these new stem cells and the way of their expression in relation to exposure to different external stimuli is not fully investigated in the literature. The impact of extracellular matrix, the properties of cell local microenvironment, topographical features and other external stimuli on the stem cell behavior are other areas to be disclosed in detail. In general, since the appropriate studies at cellular and molecular levels are lacking, it is difficult to conclude about the real impact of these newly identified stem cells in clinical cases. Furthermore, there are other unknown issues such as risk of transmission of infection, rejection rate, quantity of useful stem cells and impact of hereditary disorders and long term storage [142] that need further investigations and comparison to other sources of stem cells [143].

Another interesting area that requires exploration could be the possibility of stem cell banking or tissue banking for future potential applications. In general, all stem cells can undergo standard storage procedure by cryopreservation. However, dental stem cells require special treatment to ensure safety and prevent damage. The usual procedures followed by stem cell banking parties include onsite tooth collection, shipment using tooth transport kit, stem cell processing and cryopreservation.

In summary, clinical applications of stem cells require greater options for regulation of stem cells growth and differentiation profile. For this purpose, several attempts have to be made to understand particular physiology of conventional and newly identified stem cells. The exact self-renewal mechanism of dental and orofacial stem cells needs to be explored in more detail. Furthermore, the mechanism of upregulation and downregulation of different phenotypic markers during proliferation and differentiation of these stem cells require further studies. The interaction between ASC and immune system requires further attention to utilize the benefit of their immunomodulatory potential. Moreover, the interaction between ASC and different microenvironment needs to be understood and further optimized for regeneration of desired tissue. Understanding the particular response of ASC to different signaling molecules and growth factors during deposition of extracellular matrix is another area of further research. Further studies are required to bridge the gap between basic and clinical science related to applications of these stem cells. In this way, the application of stem cells can be optimized based on targeted clinical requirement considering their differentiation potential.

6 Conclusion

Adult stem cells have interesting capabilities to generate differentiated cells as well as offer immunomodulatory potential. These unique features have motivated extensive studies to define their exact behavior as an initial step toward utilizing their great potentials for clinical applications. Emergence of adult stem cells originated from dental and orofacial tissues provided a novel promising alternative to the traditional procedures and resources of stem cells. These cells could offer full clinical potentials of adult stem cells. Moreover, their ease of access, simplicity, greater availability, and lower cost could provide additional advantages over others sources for cell banking or potential applications. GSC, among the orofacial sources of stem cells, may hold a strong promise because of its remarkable availability and accessibility while simulating other feature of traditional BMSC. However, the full potentials of these new sources and the real clinical differences between them and traditional sources are not yet fully understood. Therefore, further basic and translational studies are required following standardized protocols in isolation, culture and handling of stem cells in particular those of orofacial origin. This helps better understanding of their particular biology and full clinical potentials in therapeutic and regenerative medicine.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement There are no animal experiments carried out for this article.

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