Stem cells in dentistry – Part I: Stem cell sources

Hiroshi Egusa DDS, PhD*a, Wataru Sonoyama DDS, PhDb, Masahiro Nishimura DDS, Phdc, Ikiru Atsuta DDS, Phdd, Kentaro Akiyama DDS, Phdb

a Department of Fixed Prosthodontics, Osaka University Graduate School of Dentistry, 1-8 Yamadaoka, Suita, Osaka 565-0871, Japan
b Department of Oral Rehabilitation and Regenerative Medicine, Okayama University Graduate School Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama 700-8525, Japan
c Department of Prosthetic Dentistry, Graduate School of Biomedical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan
d Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

Received 1 June 2012; accepted 14 June 2012

Abstract

Stem cells can self-renew and produce different cell types, thus providing new strategies to regenerate missing tissues and treat diseases. In the field of dentistry, adult mesenchymal stem/stromal cells (MSCs) have been identified in several oral and maxillofacial tissues, which suggests that the oral tissues are a rich source of stem cells, and oral stem and mucosal cells are expected to provide an ideal source for genetically reprogrammed cells such as induced pluripotent stem (iPS) cells. Furthermore, oral tissues are expected to be not only a source but also a therapeutic target for stem cells, as stem cell and tissue engineering therapies in dentistry continue to attract increasing clinical interest. Part I of this review outlines various types of intra- and extra-oral tissue-derived stem cells with regard to clinical availability and applications in dentistry. Additionally, appropriate sources of stem cells for regenerative dentistry are discussed with regard to differentiation capacity, accessibility and possible immunomodulatory properties.

Keywords: Dental stem cells; Induced pluripotent stem cells; Mesenchymal stem cells; Regenerative dentistry; Stem cell sources

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* Corresponding author at: Department of Fixed Prosthodontics, Division of Oromaxillofacial Regeneration, Osaka University Graduate School of Dentistry, 1-8 Yamadaoka, Suita-city, Osaka 565-0871, Japan. Tel.: +81 6 6879 2946; fax: +81 6 6879 2947.
E-mail address: egu@dent.osaka-u.ac.jp (H. Egusa).
1. Introduction

Stem cells are immature, unspecialized cells that have the potential to develop into many different cell lineages via differentiation. By the conventional definition, these cells can renew themselves indefinitely through “self-renewal” [1], and they vary in terms of their location in the body and the type of cells that they can produce. Recent studies have revealed that the oral tissues, which are easily accessible for dentists, are a rich source of stem cells. Given their unique abilities, stem cells are particularly important for developing innovative technologies for tissue engineering strategies [2] to regenerate or replace damaged, diseased or missing tissues and even organs by in vitro cell manipulation and design of the extracellular environment.

In dentistry, tissue engineering is also considered to be a new frontier in the regeneration of missing oral tissues/organs [3,4]. For example, various degrees of alveolar bone resorption occur after tooth loss/extraction because of periodontal disease, severe caries, root fractures or accidental trauma [5]. In addition, the bone resorption in the residual ridge, particularly in the mandible, continues throughout life in many edentulous patients [6]. The severe bone resorption in edentulous areas makes it difficult to restore the missing teeth with dental implants or denture treatment [7–9] (Fig. 1). Therefore, stem cell and tissue engineering therapies are expected to provide a novel capability to regenerate large defects in periodontal tissues [10] and alveolar bone [11–13], and to ultimately replace the lost tooth itself [14,15]. The tissues and organs targeted for such regenerative medicine strategies in dentistry include the salivary gland [16], tongue [17] and craniofacial skeletal muscles [18], as well as the condylar cartilage of the temporomandibular joint [19,20].

Many basic and translational studies with stem cells and the other key elements of tissue engineering, i.e., bioactive factors and extracellular matrix scaffolds [21,22], have been conducted in animal models to develop the concept of oral tissue and organ regeneration for clinical application in dentistry. In addition, stem cell-based tissue engineering has already been applied to clinical trials with demonstrated efficacy in orofacial bone tissue regeneration [11–13] (details are provided in Part II of this review). Despite these promising successes, recent findings that various types of stem cells can be obtained from the oral and maxillofacial region may lead to confusion regarding the role of stem cells and regenerative biology in dentistry, particularly with regard to the optimal type of stem cells for oral tissue and organ regeneration.

Part I of this review focuses on the types and derivation of stem cells in dentistry from the viewpoint of clinical availability. We also discuss appropriate stem cell sources in dentistry with regard to their differentiation capacity, accessibility and possible immunomodulatory properties. Part II subsequently describes the current state of stem cell research and clinical trials in dentistry.

2. Sources of stem cells in dentistry

There are two primary sources of stem cells: adult stem cells and embryonic stem (ES) cells. In addition to these stem cells, which are naturally present in the human body, induced pluripotent stem (iPS) cells have been recently generated artificially via genetic manipulation of somatic cells [23,24]. ES cells and iPS cells are collectively referred to as pluripotent stem cells because they can develop into all types of cells from all three germinal layers. In contrast, most adult stem cells are multipotent, i.e., they can only differentiate into a limited number of cell types. We herein outline the different types of stem cells under consideration for applications in dentistry in terms of their clinical availability.

![Fig. 1. Alveolar bone resorption. (A) Resorption of the labial bone plate in the maxillary anterior occurs after tooth extraction. Alveolar bone augmentation is required to place the dental implant. (B) Some alveolar bone resorption inevitably occurs after tooth loss (black arrow). The posterior alveolar ridge areas in the mandible (white arrows) are flattened by bone resorption after tooth loss. The figure was reproduced with permission from [9].](image-url)
2.1. Adult stem cells

Adult stem cells are also called somatic stem cells or postnatal stem cells, and they are found in many tissues and organs. Although very few of these cells are present in adult tissues, they undergo self-renewal and differentiation to maintain healthy tissues and repair injured tissues. Recent stem cell studies in the dental field have identified many adult stem cell sources in the oral and maxillofacial region (Fig. 2). These cells are believed to reside in a specific area of each tissue, i.e., a “stem cell niche”. Many types of adult stem cells reside in several mesenchymal tissues, and these cells are collectively referred to as mesenchymal stem cells or multipotent mesenchymal stromal cells (MSCs).

2.1.1. Introduction to MSCs

In cell culture, MSCs can be identified and isolated based on their adherence to tissue-culture-treated plastic [25]. MSCs are among the most promising adult stem cells for clinical applications; they were originally found in the bone marrow, but similar subsets of MSCs have also been isolated from many other adult tissues, including skin, adipose tissue and various dental tissues [26–28]. The concept of using adherent fibroblastic cells isolated from the bone marrow was originally reported in 1970 by Friedenstein et al. [29]. Those cells were referred to as colony forming units-fibroblasts, and their capability to differentiate to various mesenchymal tissues gave rise to the concept of MSCs [30]. In 1999, Pittenger et al. [31] characterized human MSCs from the bone marrow of the iliac crest as multipotent stem cells by demonstrating their isolation, expansion in culture and directed differentiation to osteogenic, adipogenic and chondrogenic lineages. Since then, extensive studies on MSCs have demonstrated their robust multipotency and even “stem cell plasticity”, as exemplified by the capacity of MSCs to differentiate into lineages that are not typical mesenchymal derivatives [32].

However, the definition of MSCs has been controversial because the populations of adherent cells isolated from the bone marrow are not homogeneous, and definitive markers for distinguishing MSCs have not yet been identified [33]. In 2006, the International Society for Cellular Therapy (ISCT) proposed minimal criteria to define human multipotent MSCs; notably, the ISCT termed MSCs as mesenchymal stromal cells, regardless of the tissue from which they are isolated [25]. According to the ISCT criteria, MSCs must be adherent to tissue-culture-treated plastic when maintained in standard culture conditions. Additionally, MSCs must express CD105, CD73 and CD90 and lack the expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules. Finally, MSCs must be able to differentiate to osteoblasts, adipocytes and chondroblasts in vitro [34]. Recently, other unique cell surface markers for human MSCs, such as CD271 [35] and MSC antigen-1 [36], have been reported. In addition to the use of surface marker analysis, the selection of MSCs using stable mRNA markers specifically expressed in the MSCs has been proposed [37,38].

2.1.2. Bone marrow-derived MSCs (BMSCs)

Adult bone marrow contains rare multipotent progenitor cells that are generally termed BMSCs. Despite their heterogeneity, BMSCs possess a high replicative capacity and have the capacity to differentiate into various connective tissue cell types. In addition, BMSCs robustly form bone in vivo, which makes them an appropriate stem cell source for bone regeneration therapy [39].

2.1.2.1. BMSCs from the iliac crest. BMSCs from the iliac crest have been extensively studied and demonstrated to
differentiate along osteogenic, chondrogenic, adipogenic, myogenic or non-mesenchymal neurogenic lineages \[31,40–42\]. BMSCs can be easily isolated from the bone marrow of the iliac crest by physicians, but the bone marrow aspiration procedure is invasive for the donors. Nonetheless, the stem cells most commonly used to date for bone regeneration in dental patients are BMSCs from the iliac crest (details are provided in Part II of this review). The bone marrow of the iliac crest is the most documented cell source for MSCs in regenerative medicine, possibly because it has long been routinely collected for bone marrow transplantation for leukemia treatment.

Because of their great potential for bone regeneration \[39\], BMSCs from the human iliac crest may be applicable to bone tissue engineering irrespective of the age of the patient \[43,44\]. However, several reports have demonstrated an age-related decline in the osteogenic potential of BMSCs isolated from the human iliac crest and femur \[44–46\], which suggests that donor age is an important factor for the clinical efficacy of bone formation. In addition, the in vitro expansion capability appears to be limited, as the cells tend to undergo senescence and lose their multi-differentiation potential with repeated passaging and culture time \[39\]. These limitations should be overcome to successfully utilize BMSCs for bone tissue engineering and regeneration.

2.1.2.2. BMSCs from orofacial bones. Although the iliac crest has served as the primary source of BMSCs to date, human BMSCs can also be isolated from orofacial (maxilla and mandible) bone marrow aspirates obtained during dental surgical procedures such as dental implant treatment, wisdom tooth extraction, extirpation of cysts and orthodontic osteotomy (Fig. 3). Orofacial bone-derived BMSCs can be obtained not only from younger patients (6–53 years of age \[47\]) but also from relatively aged individuals (57–62 years of age \[48\]), and the age of the donor seems to have little effect on the BMSC gene expression pattern \[48\].

Clinical observations \[49–51\] and experimental animal studies \[52–54\] have consistently indicated that grafted bone obtained from the craniofacial area (membranous bone) for autologous bone grafting at craniofacial sites provides better results and significantly higher resultant bone volume than bone harvested from the iliac crest or rib (endochondral bone). These observations imply that different skeletal donor tissues have site-specific regenerative properties that may depend upon the BMSC type and BMSC niche present in the graft. Embryologically, the maxilla and mandible bones exclusively originate from cranial neural crest cells \[55\], whereas the iliac crest bone is formed by mesoderm. These differences in embryological origin may result in functional differences between orofacial and iliac crest human BMSCs \[56,57\].

Indeed, it is well documented that the orofacial BMSCs are phenotypically and functionally different from iliac crest BMSCs. Igarashi et al. \[37\] reported that orofacial BMSCs have a discrete differentiation potential with distinct expression patterns for several MSC marker genes compared with tibia-, femur- and ilium-derived BMSCs. Akintoye et al. \[56\] demonstrated site-specific properties of orofacial and iliac BMSCs from the same individuals where higher proliferation and osteogenic differentiation capacity were observed for orofacial BMSCs compared with the iliac crest BMSCs. In addition, the orofacial BMSCs formed more bone in an in vivo mouse model, whereas the iliac crest BMSCs formed more compacted bone that included hematopoietic tissue \[56\]. An animal study also indicated that upon transplantation, BMSCs from the rat mandible formed larger bone nodules and more mineralized bone than BMSCs from long bones \[58\]. Furthermore, the adipogenic potential of orofacial BMSCs is less than that of iliac BMSCs \[47,56\], which may decrease unfavorable fat formation during bone tissue regeneration.

These properties of orofacial BMSCs may provide an advantage for orofacial bone regeneration. However, the collectable volume of orofacial bone marrow is less (0.03–0.5 ml \[47,48\]) than that of iliac crest bone marrow. Therefore, a reliable and safe cell expansion protocol should be established when orofacial BMSCs are used for clinical trials.

2.1.3. Dental tissue-derived stem cells

To date, two types of adult stem cells have been characterized in dental tissues, i.e., epithelial stem cells and MSC-like cells. An adult epithelial stem cell niche in teeth was first demonstrated in 1999 \[59\] via organ culture of the apical end of the mouse incisor. The niche is located in the cervical loop of the tooth apex and possibly contains dental epithelial stem cells, which can notably differentiate into enamel-producing ameloblasts. Although the epithelial stem cell niche is useful for analyses of the fate decision of stem cells in tooth development, no information is available for dental epithelial stem cells in humans. This niche may be specific to rodents.

**Fig. 3.** Alveolar bone marrow aspiration and BMSC isolation. (A) Alveolar bone marrow was aspirated from the right maxillary edentulous jaw before the drilling of the implant sites using a biopsy needle (GC Corporation, Tokyo, Japan). (B) Plastic-adherent BMSCs derived from the bone marrow after 5 days of culture (white arrows). (C) Expanded alveolar bone-derived BMSCs after 15 days of culture. Bars: 200 μm.
Table 1
Characteristics of human dental tissue and gingiva-derived MSCs.

<table>
<thead>
<tr>
<th>Stem cells (Ref.)</th>
<th>CD antigen expression</th>
<th>Other representative markers</th>
<th>PD</th>
<th>In vitro differentiation capacity</th>
<th>In vivo tissue formation capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DPSCs</strong> [71,73,74,85,184–186]</td>
<td>CD9, CD10, CD13, CD29, CD44, CD49d, CD59, CD73, CD90, CD105, CD106, CD146, CD166</td>
<td>STRO-1, Nestin</td>
<td>&gt;120</td>
<td>dent (od), mes (os, ad, cho, myo), ect (neu)</td>
<td>dent (dentin, pulp), mes (adipose, muscle)</td>
</tr>
<tr>
<td><strong>SHED</strong> [73,187–190]</td>
<td>CD13, CD44, CD73, CD90, CD105, CD146</td>
<td>STRO-1, Oct-4, Nestin, SSEA-3, SSEA-4</td>
<td>&gt;140</td>
<td>dent (od), mes (os, ad, cho, myo, endo), ect (neu)</td>
<td>dent (dentin), mes (bone, microvessel)</td>
</tr>
<tr>
<td><strong>PDLSCs</strong> [76,184,191,192]</td>
<td>CD9, CD10, CD13, CD29, CD44, CD49d, CD59, CD73, CD90, CD105, CD106, CD146, CD166</td>
<td>STRO-1, Scleraxis</td>
<td>ND</td>
<td></td>
<td>dent (cementum, PDL), mes (alveolar bone)</td>
</tr>
<tr>
<td><strong>DFSCs</strong> [184,193–195]</td>
<td>CD9, CD10, CD13, CD29, CD44, CD49d, CD59, CD73, CD90, CD105, CD106, CD166</td>
<td>STRO-1, HLA class I</td>
<td>ND</td>
<td>dent (cem), mes (os, ad, cho), ect (neu)</td>
<td>dent (cementum, PDL), mes (alveolar bone)</td>
</tr>
<tr>
<td><strong>TGPC</strong> [83,196,197]</td>
<td>CD29, CD44, CD73, CD90, CD105, CD106, CD166</td>
<td>STRO-1, Oct-4, Nestin, HLA class I</td>
<td>ND</td>
<td>mes (os, ad, endo), ect (neu), end (hep)</td>
<td>mes (bone)</td>
</tr>
<tr>
<td><strong>SCAP</strong> [85,86,198]</td>
<td>CD49d, CD51/61, CD56, CD73, CD90, CD105, CD106, CD146, CD166</td>
<td>STRO-1, Nestin, Survivin</td>
<td>&gt;70</td>
<td>mes (ad), ect (neu)</td>
<td>dent (dentin, pulp)</td>
</tr>
<tr>
<td><strong>GMSCs (CMSCs)</strong> [98,100,199,200]</td>
<td>CD29, CD44, CD73, CD90, CD105, CD106, CD146, CD166</td>
<td>STRO-1, Oct-4, Nestin, SSEA-4, HLA-ABC, Sox2, Tra2-49, Tra2-54</td>
<td>&gt;20</td>
<td>mes (os, ad, cho), ect (neu, glia cell), end (definitive endoderm cell)</td>
<td>mes (bone, cartilage, fat, muscle), ect (epithelia, neural tissue)</td>
</tr>
</tbody>
</table>

PD: population doubling; ND: not determined; PDL: periodontal ligament; differentiation lineages: dent (dentinogenic lineage), mes (mesodermal lineage), ect (ectodermal lineage), end (endodermal lineage), od (odontoblast), os (osteoblast), ad (adipocyte), cho (chondrocyte), myo (myoblast), neu (neuronal cell), cem (cementoblast), hep (hepatocyte).

because their incisors differ from all human teeth in that they erupt continuously throughout the life of the animal.

Mesenchymal progenitor or stem cells have also long been assumed to exist in dental tissues [60–62] because some dental tissues, such as periodontal tissues and dental pulp, can regenerate or form reparative dentin by a natural process if the environmental conditions are suitable after dental treatments [61–69]. To date, several MSC sources have been identified in dental tissues, and the isolated stem cells [70] have been extensively characterized (Table 1).

In 2000, adult human dental stem cells were first identified in the dental pulp (dental pulp stem cells; DPSCs: Fig. 4A) [71], and these cells had phenotypic characteristics similar to those of BMSCs [72]. MSC-like cells were subsequently also isolated from the dental pulp of human deciduous teeth (stem cells from human exfoliated deciduous teeth; SHED) [73]. DPSCs and SHED possess definitive stem cell properties, such as multi-differentiation and self-renewal [71,73,74]. Importantly, these cells have the specific ability to regenerate the dentin–pulp complex when transplanted into immunocompromised mice. In addition, SHED can specifically induce the formation of a bone-like matrix with a lamellar structure by recruiting host cells [73,75]. This distinct property of SHED for bone formation may be explained by the nature of deciduous teeth, whose root resorption is accompanied by new bone formation surrounding the root.

The periodontal ligament is another adult MSC source in dental tissues, and periodontal ligament stem cells (PDLSCs) can even be isolated from extracted teeth. PDLSCs have demonstrated the ability to regenerate periodontal tissues (cementum, periodontal ligament and alveolar bone) in experimental animal models [76,77]. A recent report suggested that the characteristics of the PDLSCs may depend on the harvest location because PDLSCs from the alveolar bone surface displayed superior alveolar bone regeneration compared with PDLSCs from the root surface [78].

MSC-like cells have also been identified in the “developing” dental tissues, such as the dental follicle, dental mesenchyme and apical papilla. The dental follicle (Fig. 4B), which is a dental sac that contains the developing tooth and differentiates into the periodontal ligament, contains dental follicle stem cells (DFSCs) with the ability to regenerate periodontal tissues [79–82]. Ikeda et al. [83] identified distinctive stem cells in the dental mesenchyme of the third molar tooth germ at the late bell stage (tooth germ progenitor cells; TGPCs) with high proliferation activity and the capability to differentiate in vitro into lineages of the three germ layers including osteoblasts, neural cells and hepatocytes. Stem cells from the apical papilla (SCAP) [84–86] were found in the papilla tissue in the apical part of the roots of developing teeth (Fig. 4C). Compared with DPSCs, SCAP demonstrate better proliferation in vitro and better regeneration of the dentin matrix when transplanted in immunocompromised mice. These findings suggest that “developing” dental tissues may provide a better source for immature stem cells than “developed” dental tissues.

It should be noted that these tissues are often discarded in the clinic as medical waste and therefore present a particularly attractive source for stem cells because of their availability. Many research groups have therefore used dental stem cells to
elucidate various biological phenomena and to establish potential clinical applications [85,87–91]. However, these cells are heterogeneous with various differentiation states, as they include true “stem” cells, progenitor cells and possibly fibroblasts [71,73,74,76,85,86]. Therefore, it is necessary to effectively classify and purify these cells to prevent unexpected clinical results.

2.1.4. Oral mucosa-derived stem cells

The oral mucosa is composed of stratified squamous epithelium and underlying connective tissue consisting of the lamina propria, which is a zone of well-vascularized tissue, and the submucosa, which may contain minor salivary glands, adipose tissue, neurovascular bundles and lymphatic tissues depending on the site [92]. To date, two different types of human adult stem cells have been identified in the oral mucosa. One is the oral epithelial progenitor/stem cells, which are a subpopulation of small oral keratinocytes (smaller than 40 μm) [93]. Although these cells seem to be unipotential stem cells, i.e., they can only develop into epithelial cells, they possess clonogenicity and the ability to regenerate a highly stratified and well-organized oral mucosal graft ex vivo [94,95], which suggests that they may be useful for intra-oral grafting [96].

Other stem cells in the oral mucosa have been identified in the lamina propria of the gingiva, which attaches directly to the periosteum of the underlying bone with no intervening submucosa (see the inset of Fig. 2) [97]. The gingiva overlying the alveolar ridges and retromolar region is frequently resected during general dental treatments and can often be obtained as a discarded biological sample. In 2009, Zhang et al. [98] first characterized human gingiva-derived MSCs (GMSCs), which exhibited clonogenicity, self-renewal and a multipotent differentiation capacity similar to that of BMSCs. GMSCs proliferate faster than BMSCs, display a stable morphology and do not lose their MSC characteristics with extended passaging [99]. Recently, Marynka-Kalmani et al. [100] reported that a multipotent neural crest stem cell-like population, termed oral mucosa stem cells (OMSCs), can be reproducibly generated from the lamina propria of the adult human gingiva and can differentiate in vitro into lineages of the three germ layers. The inherent stemness of gingival cells may therefore partly explain the high reprogramming efficiency of gingiva-derived fibroblastic cell populations during iPS cell generation [101]. The multipotency of GMSCs/OMSCs and their ease of isolation, clinical abundance and rapid ex vivo expansion provide a great advantage as a stem cell source for potential clinical applications.

2.1.5. Periosteum-derived stem/progenitor cells

The periosteum is a specialized connective tissue that covers the outer surface of bone tissue. The osteogenic capacity of the periosteum of long bones was reported in 1932 [102], and the periosteum membrane was found to form a mineralized extracellular matrix under the appropriate in vitro conditions. Several subsequent studies have addressed other aspects of periosteal osteogenesis, including long bone development and the periosteum [103], the relationship between the vasculature and the periosteum [104] and the periosteal osteogenic capacity [105].

Histologically, the periosteum is composed of two distinct layers and up to five distinctly different functional regions when it is dissociated enzymatically and cultured [106]. The outer area contains mainly fibroblasts and elastic fibers, and the inner area contains MSCs [107–109], osteogenic progenitor cells [110,111], osteoblasts and fibroblasts, as well as microvessels and sympathetic nerves. Although the heterogeneous cell population isolated from the periosteum seems to preferentially undergo osteogenic differentiation [110,111], these cells are capable of differentiating into osteoblasts, adipocytes and chondrocytes and expressing the typical MSC markers [107,109]. In addition, De Bari et al. [108] demonstrated that single-cell-derived clonal populations of adult human periosteal cells possess mesenchymal multipotency, as they differentiate to osteoblast, chondrocyte, adipocyte and skeletal myocyte lineages in vitro and in vivo. Therefore expanded periosteum-derived cells could be useful for functional tissue engineering, especially for bone regeneration.

A comparative analysis of canine MSCs/progenitor cells showed that the in vivo potential of periosteum cells to form bone was higher than that of ilium-derived BMSCs and alveolar
bone cells [112]. The phenotypic profiles of human maxillary/mandibular periosteum cells were comparable to those of maxillary tuberosity-derived BMSCs, and both cell populations formed ectopic bone after subcutaneous implantation in mice [113]. Agata et al. [114] reported that human periosteal cells proliferated faster than marrow stromal cells, and subcutaneous transplants of periosteal cells treated with a combination of recombinant growth factors formed more new bone than BMSCs in mice. Periosteal grafts have been shown to induce cortical bone formation, whereas bone marrow grafting induced cancellous bone formation with a bone marrow-like structure in a rat calvarial defect model [115], which implies that the source of the transplanted cells can influence the structural properties of the regenerated bone.

The robust osteogenic potential of periosteum-derived cells has inspired dentists to use the periosteum for orofacial bone regeneration. Indeed, the inverted periosteal flap technique [116] has been recommended for alveolar bone augmentation in conjunction with implant placement or in combination with bone graft surgery. Additionally, cultured periosteum-derived cells have been used for alveolar ridge or maxillary sinus floor augmentation in clinical research that successfully demonstrated enhanced bone remodeling and lamellar bone formation with subsequent reliable implant insertion [117] and reduced postoperative waiting time after implant placement [118]. Therefore, the periosteum is a source of stem/progenitor cells for bone regeneration, particularly for large defects.

2.1.6. Salivary gland-derived stem cells

Patients afflicted with head and neck cancer who receive radiotherapy suffer from an irreversible impairment of salivary gland function that results in xerostomia and a compromised quality of life. Therefore, stem cells in the adult salivary gland are expected to be useful for autologous transplantation therapy in the context of tissue engineered-salivary glands or direct cell therapy. The salivary glands originate from the endoderm and consist of acinar and ductal epithelial cells with exocrine function. After ligation of the salivary gland duct, the acinar cells undergo apoptosis, and the duct epithelium subsequently proliferates. Although the existence of salivary gland stem cells has been suggested by in vivo studies [119,120], a single stem cell that gives rise to all epithelial cell types within the gland has not yet been identified. Thus far, the isolation of stem cells in the salivary glands has been attempted through the cell culture of dissociated tissue. Kishi et al. [121] isolated salivary gland stem/progenitor cells from rat submandibular glands and found that the cells are highly proliferative and express acinar, ductal and myoepithelial cell lineage markers. Lombaert et al. [122] reported that an in vitro floating sphere culture method could be used to isolate a specific population of cells expressing stem cell markers from dissociated mouse submandibular glands. These cell populations could differentiate into salivary gland duct cells as well as mucin- and amylase-producing acinar cells in vitro. Progenitor/stem cells were also isolated from swine [123] and human [122,124] salivary glands. In addition, the intra-glandular transplantation of cells isolated from mouse submandibular glands successfully rescued the salivary function of irradiated salivary glands [122,125], and Neumann et al. [126] reported the long-term cryopreservation of integrin-α6β1 expressing cells as a sub-population of rat salivary gland progenitor cells. These reports suggest that the salivary gland is a promising stem cell source for future therapies targeting irradiated head and neck cancer patients. However, primary cultures of dispersed cells will always contain a number of cells with different origins, such as parenchymal cells, stromal cells and blood vessel cells, which makes it difficult to select salivary gland stem cells. Indeed, Gorjup et al. [127] isolated primitive MSC-like cells from the human salivary gland, but possibly from stromal tissue, which expressed embryonic and adult stem cell markers and could be guided to differentiate into adipogenic, osteogenic and chondrogenic cells. To obtain a genuine stem cell population that can be considered to be a true stem cell for the salivary gland, it is necessary to select cells carrying a specific marker or labeled with induced reporter proteins [128].

2.1.7. Adipose tissue-derived stem cells (ASCs)

Adipose tissue is an abundant source of MSCs and has been extensively studied in the field of regenerative medicine as a stem cell source. Adipose-derived MSCs can be readily harvested via lipectomy or from lipoaspirate from areas such as the chin, upper arms, abdomen, hips, buttocks and thighs in large numbers with low donor-site morbidity [129], as liposuction is one of the most common cosmetic procedures. Although the intrinsic characteristics of ASCs appear to be different from those of BMSCs [26,130–132], ASCs exhibit robust osteogenesis and are thus expected to be an alternative source of MSCs for bone regeneration in dentistry. Indeed, the feasibility of using autologous ASCs for orofacial bone regeneration and implant placement has been demonstrated [133,134]. Pieri et al. [135] demonstrated that the transplantation of autologous ASCs with an inorganic bovine bone scaffold (Bio-Oss®) enhanced new bone formation and implant osseointegration following vertical bone augmentation of the calvarial bone of rabbits, which suggests that ASCs may be useful for vertical alveolar bone augmentation for implant treatment.

Periodontal tissue regeneration using ASCs has also been successfully demonstrated in a rat experimental animal model [136], and an in vitro study showed that rat ASCs acquired cement blast features when cultured in dental follicle cell conditioned medium containing dentin non-collagenous proteins [137]. In addition, Ishizaka et al. [138] demonstrated that ASC transplantation induced pulp regeneration in the root canal after pulpectomy in dogs, and Hung et al. [139] demonstrated that ASCs implants were able to grow self-assembled new teeth containing dentin, periodontal ligament and alveolar bone in adult rabbit extraction sockets with a high success rate. Further studies on the isolation, characterization and application of ASCs to enhance their efficacy for bone and periodontal regeneration will provide a definitive protocol for the use of waste fat tissues in future clinical applications.
2.2. Pluripotent stem cells

Pluripotency is defined as the capacity of individual cells to generate all lineages of the mature organism in response to signals from the embryo or cell culture environment [140]. Because of their intrinsic pluripotency and unlimited self-renewal, dental applications of pluripotent stem cells are expected to primarily involve basic research on developmental biology, drug testing and regenerative therapies. Therefore, the differentiation of pluripotent cells towards clinically useful oral lineages is primary focus in dental research.

2.2.1. ES cells

ES cells are produced by culturing cells collected from the undifferentiated inner cell mass of the blastocyst, which represents an early stage of embryonic development after fertilization [141,142]. This embryonic origin is the major reason that ethical and moral questions are associated with the use of human ES cells [143]. Nonetheless, ES cells are of great interest to scientists and clinicians because of their developmental capacity to differentiate in vitro into cells of all somatic cell lineages as well as into male and female germ cells [143]. In the field of dentistry, ES cells are expected to provide an in vitro model system and transplantation substrate for animal models to study the controlled differentiation of pluripotent stem cells into specific lineages of oral tissues and organs, such as mucosa [144], alveolar bone [145], periodontal tissues [146] and teeth [147]. These approaches can be useful to obtain a better understanding of oral developmental biology and may lead to future strategies in regenerative dentistry that meet clinical needs. However, in addition to the ethical issues, the tissue engineering applications of ES cells are limited because the cells are allogenic and thus may be immunologically incompatible between donors and recipients. To overcome this issue, the creation of human ES cell banks with human leukocyte antigen (HLA) matching and the generation of customized, patient-specific ES cells via nuclear transplantation from the patient’s own cells have been proposed to enable combined gene and cell therapy [143]. However, these strategies rely on inefficient and expensive techniques and are tedious and ethically cumbersome, especially for dentists, unless a cooperative team can be organized with experts who routinely deal with patients’ embryos.

2.2.2. iPS cells

In 2006, Dr. Shinya Yamanaka discovered that normal mouse adult skin fibroblasts can be reprogrammed to an embryonic state by introducing four genetic factors (Oct3/4, Sox2, Klf4 and c-Myc), and the resulting cells were termed iPS cells [23]. Just a year after the mouse study was reported, the findings were replicated in human skin cells [24,148], which opened the door to generate a patient-specific ES cell equivalent from autologous somatic cells. This technology is expected to revolutionize medicine because of the capacity of iPS cells to develop into all tissues/organs and thereby support the emerging field of “personalized medicine”, which uses a patient’s own cells to provide biologically compatible therapies and individually tailored treatments.

For dental applications, iPS cells that can be efficiently generated from tissues that are easily accessed by dentists have great potential, and iPS cells have been generated from various oral mesenchymal cells, such as SCAP [149], DPSCs and SHED [149,150], TGPcs [151], buccal mucosa fibroblasts [152], gingival fibroblasts (Fig. 5) [101,153] and periodontal ligament fibroblasts [153]. Most of these cells have a higher reprogramming efficiency than the conventionally used skin fibroblasts (Table 2), possibly because of their high expression of endogenous reprogramming factors and/or ES cell-associated genes [150] as well as their high proliferation rate [101]. Therefore, cells of oral origin are expected to provide an ideal iPS cell source, especially for dentists and dental researchers.

These iPS cells may be of particular importance for developing innovative technologies to regenerate missing jaw bones, periodontal tissues, salivary glands and lost teeth [9]. In a mouse model, iPS cells combined with enamel matrix derivatives provided greatly enhanced periodontal regeneration by promoting the formation of cementum, alveolar bone and periodontal ligament [154]. Recent in vitro studies demonstrated the differentiation of mouse iPS cells into ameloblasts [155] and odontogenic mesenchymal cells [156], which may be useful approach for tooth bioengineering strategies.

However, the scientific understanding of iPS cells and how to control their differentiate fate is still limited. Despite the similarities between iPS cells and ES cells, it remains unclear whether these pluripotent stem cells are exactly equal. Recent studies have indicated that not all iPS cells are equal and that iPS cells retain an epigenetic memory of their former
Table 2
The reprogramming efficiency of human somatic cells from different tissues.

<table>
<thead>
<tr>
<th>Authors (Ref.)</th>
<th>iPS cell source</th>
<th>Reprogramming method</th>
<th>Efficiency</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takahashi et al. [24]</td>
<td>Skin fibroblasts</td>
<td>O/S/K/M (RV)</td>
<td>0.02%</td>
<td>25–30 days</td>
</tr>
<tr>
<td>Aasen et al. [164]</td>
<td>Foreskin keratinocytes, Foreskin fibroblasts</td>
<td>O/S/K/M (RV)</td>
<td>1%</td>
<td>14–21 days</td>
</tr>
<tr>
<td>Sun et al. [201]</td>
<td>ASCs</td>
<td>O/S/K/M (LV)</td>
<td>&lt;0.2%</td>
<td>16 days</td>
</tr>
<tr>
<td>Yan et al. [149]</td>
<td>SCAP, DPSCs</td>
<td>O/S/N/L (LV)</td>
<td>&lt;0.08%</td>
<td>21 days</td>
</tr>
<tr>
<td>Tamaoki et al. [150]</td>
<td>DPSCs</td>
<td>O/S/K (RV)</td>
<td>&lt;0.06%</td>
<td>30 days</td>
</tr>
<tr>
<td>Oda et al. [151]</td>
<td>Clonally expanded TGPCs</td>
<td>O/S/K (RV)</td>
<td>&lt;0.03%</td>
<td>30 days</td>
</tr>
<tr>
<td>Miyoshi et al. [152]</td>
<td>Buccal mucosa fibroblasts</td>
<td>O/S/K/M (RV)</td>
<td>0.02%</td>
<td>13–25 days</td>
</tr>
<tr>
<td>Wada et al. [153]</td>
<td>Gingival fibroblasts, Periodontal ligament fibroblasts</td>
<td>O/S/K/M (RV)</td>
<td>–</td>
<td>28–30 days</td>
</tr>
</tbody>
</table>


...phenotype that can limit their differentiation potential [157,158]. Therefore, strategies that bypass the epigenetic memory to create more ES-like iPS cells or that can identify iPS cell sources that are amenable to efficient guided differentiation to the target lineage may be necessary. To achieve this goal, the generation of more stringent markers of pluripotency and assays to determine the abilities of a given iPS cell line is critical [159].

In addition, the prevention of tumor formation upon in vivo implantation of iPS cells is critical for their clinical application. The original protocol to generate iPS cells [160] uses the c-Myc oncogene as one of the reprogramming factors and a retroviral vector for gene transfer, which raises concern about possible carcinogenic properties. Recent rapid progress in iPS cell research has virtually resolved these problems, e.g., by using L-Myc as a replacement for c-Myc [161] or via the application of small molecules rather than viral gene delivery [162,163], the generation of reprogramming protocols to enhance the reprogramming efficiency without requiring c-Myc [164] and the use of non-viral components such as protein [165], microRNA [166], synthetic mRNA [167] or episomal plasmids [168] for reprogramming. However, serious clinical problems can still arise when residual undifferentiated iPS cells remaining among the differentiated target cells uncontrollably proliferate to form teratomas in the transplanted site. To address this critical issue, several approaches are being investigated, such as a selective ablation method to remove teratomas via suicide genes and chemotherapy [169,170], as well as a cell sorting method to remove teratoma-forming cells using specific antibodies [171].

3. Suitable stem cells for regenerative dentistry

Stem cells suitable for regenerative medicine/dentistry must be subject to the complete control of cell fate in the body to ensure the safety of the patient. In this regard, only adult MSCs currently have realistic clinical potential. Indeed, the regeneration of bone and periodontal tissues by MSCs has been extensively evaluated, with some studies already reaching the clinic (see Part II of this review). Suitable stem cells for dental tissue engineering should also be able to differentiate into the target tissue/organ and should be easily collected and prepared, and possible immunomodulatory properties can be used to provide a further benefit.

3.1. Differentiation capacity

As described in the previous chapters, BMSCs, particularly those from the orofacial bone marrow, and periosteum-derived stem/progenitor cells may be suitable for alveolar bone regeneration because of the compatibility of the cell source with the target tissue. Similarly, dental tissue-derived MSCs may be appropriate for the regeneration of dental mesenchyme-derived tissues, such as dentin, pulp and periodontal tissues (see Section 2.1.3). However, the differentiation capacity of adult MSCs is principally limited to mesenchymal lineages, which hinders their application to the regeneration of complex oral organs, such as teeth and salivary glands, which are formed during development by the interaction of epithelial and mesenchymal tissues. One strategy for achieving organ regeneration has focused on identifying organ-specific stem cells based on the capacity of a single tissue-specific stem cell to form the epithelial components of mammary glands [172] or gastric units [173]. However, a single postnatal stem cell with organogenic capacity has not yet been identified in the teeth or salivary glands.

From this point of view, pluripotent stem cells are promising for the regeneration of complex organs. For example, the three-dimensional culture of mouse ES cell aggregates resulted in the efficient self-formation of the optic cup, which is a structural...
feature of morphologically diverse eye tissue [174], or adenohypophysis tissues [175], which suggests that ES cells have the capacity for balanced organogenesis. However, ES cells are unlikely to be appropriate for cell therapies because of the associated issues of immune rejection and medical ethics (see Section 2.2.1). Alternatively, autologous patient-derived iPS cells may be able to overcome these issues, although formidable technical challenges must be surmounted to make iPS cell-based therapies a reality in dentistry (see Section 2.2.2). In addition, the successful implementation of iPS cell therapy will require an understanding of how to induce these cells to form specific progenitor cells for the tissues and organs targeted for regeneration. In this scenario, studies on the developmental mechanisms of oral tissues and organs using tissue/organ-specific stem cells will also be necessary for the further development of iPS cell technology.

3.2. Accessibility

With regard to accessibility, bone marrow aspiration from the iliac crest and liposuction from extra-oral tissue is not an easy operation for dentists because of the limitations of the dental license and the dental specialization. In contrast, orofacial bone marrow, periosteum, salivary glands and dental tissues are accessible stem cell sources for dentists; however, the isolation of stem cells from these locations may still not be convenient because it requires surgical procedures or tooth or pulp extraction. Additionally, even if impacted wisdom teeth could be a cell source, not all adults require the extraction of the wisdom teeth. Furthermore, these adult stem cells are present in small quantities and can therefore be difficult to isolate, purify and expand homogeneously. In contrast, the gingiva, which is a tissue that is easily obtainable by dentists and whose cells can be easily expanded from patients with minimal discomfort, seems to be a promising source of adult stem cells [98–100] and iPS cells [101] in dentistry (see Sections 2.1.4 and 2.2.2). More studies are necessary to determine the regenerative abilities of gingiva-derived stem cells in oral tissues.

Research on all available stem cells in dentistry should be continued to permit their manipulation for the regeneration of oral tissues. Based on the accumulated knowledge, the type of stem cell to be used for a given application will be decided by considering a balance of the differentiation capacity with accessibility/availability, which may vary on a case-by-case basis.

3.3. Immunomodulation

In addition to tissue repair and regeneration, immunomodulatory properties have also recently been identified for MSCs in animals and humans that may be related to therapeutic effects such as angiogenesis, anti-inflammation and antiapoptosis [176]. Furthermore, recent reports suggest that MSCs have low inherent immunogenicity [177]. Therefore, the immunomodulatory properties of MSCs may make them more attractive than other types of stem cells for some applications in cell transplantation.

Previous reports demonstrated that human oral tissue-derived MSCs, such as DPSCs [178], SHED [179], PDLSCs [180], SCAP [181] and GMSCs [98], have immunomodulatory properties similar to those of BMSCs. In addition, systemically injected GMSCs have been shown to home to the wound site and promote wound repair [182], and oral mucosal progenitor cells appear to have a more fetal phenotype for immune recognition, with immunomodulation that occurs under a mechanism different from that of BMSCs [183]. Therefore, the gingiva is currently a promising stem cell source that may have wide-ranging potential for future immune-related therapies in addition to regenerative medicine.

4. Conclusions

Growing evidence has demonstrated that the oral and maxillofacial region is a rich source of adult stem cells. Many intra-oral tissues, such as deciduous teeth, wisdom teeth and the gingiva, are not only easily accessible from the oral cavity but can also often be obtained as a discarded biological sample. Therefore, dental professionals should recognize the promise of the emerging field of regenerative dentistry and the possibility of obtaining stem cells during conventional dental treatments that can be banked for autologous therapeutic use in the future. The discarded oral tissues can also be used to generate iPS cells that can be used not only for the autologous cell-based regeneration of complex oral tissues but also for the patient-specific modeling of oral diseases and the development of tailor-made diagnostic and drug screening tools for alveolar bone augmentation and oral cancer treatment.

Further studies are necessary to establish evidence-based practices to educate dentists and patients regarding the use of stem cells in autologous regenerative therapies. Studies on the relatively well-characterized stem cells, such as BMSCs and other adult MSCs, should be continued to identify factors responsible for the successful outcome of stem cell-based bone/periodontal tissue regeneration. The immunomodulatory properties of stem cells under consideration for applications in dentistry should be investigated to facilitate the grafting of the transplanted cells at inflamed sites. Studies on ES/iPS cells may reveal the complex developmental process of oral organs, such as the teeth and salivary glands. Research efforts on adult stem cells and pluripotent stem cells should be concomitantly performed with cross-communication to permit the development of new and effective strategies for regenerative dentistry.

Conflict of interest statement

All authors state that they have no conflicts of interest.

Acknowledgements

We are grateful to Prof. Songtao Shi (Center for Craniofacial Molecular Biology, University of Southern California) for critical reading of the manuscript. This review article was written as a project proposed by the Journal of Prosthodontic Research (JPR) Editorial Committee under the support of the
Japan Prosthetic Society (JPS). The authors thank Prof. Takuo Kuboki, the Editor-in-Chief of the JPR, and Prof. Kiyoshi Koyano, the President of the JPS, for their valuable support to accomplish this work. Support was also received from a Grant-in-Aid for Young Scientists (A22689049: H.E. and A22689050: W.S.) and for Scientific Research (B22390367: M.N.) from the Japan Society for the Promotion of Science.

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