At a glance on stem cell-based therapies in dentistry

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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 and tissue engineering therapies in dentistry and continue to attract increasing clinical interest. This article reviews the various types of intra- and extra-oral tissue-derived stem cells with regard to clinical availability and applications in dentistry. Appropriate sources of stem cells for regenerative dentistry and stem cell-based therapies in dentistry are also discussed.

Key words: stem cell, embryonic stem cells, adult stem cells, pluripotent stem cell, regenerative dentistry

1. Introduction

Stem cells are immature, unspecialized cells that have the potential to develop into many different cell lineages via differentiation. 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. According to their unique abilities, stem cells are particularly important for developing innovative technologies for tissue engineering strategies to regenerate or replace damaged, diseased or missing tissues and even organs by in vitro cell manipulation.

In dentistry, tissue engineering is also considered to be a new frontier in the regeneration of missing oral tissues/organs. For example, various degrees of alveolar bone resorption occur after tooth loss/extraction because of periodontal disease, severe caries, root fractures or accidental trauma. In addition, the bone resorption in the residual ridge, particularly in the mandible, continues throughout life in many edentulous patients. The severe bone resorption in edentulous areas makes it difficult to restore the missing teeth with dental implants or denture treatment. Therefore, stem cell and tissue engineering therapies are expected to provide a novel capability to regenerate large defects in periodontal tissues and alveolar bone, and to ultimately replace the lost tooth itself [2].

Many basic and translational studies with stem cells 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 trial in orofacial bone tissue regeneration [3]. This review focuses on the types and sources of stem cells in dentistry from the viewpoint of clinical availability. We also discuss the current state of stem cell research and clinical trials in dentistry.

2. Literature review

2.1. Characterstics of stem cells

1. Totipotency: Produce all types of cells as well as germ cells.
2. Pluripotency: Produce all types of cells apart from cells of the embryonic membrane.
3. Multipotency: Distinguish into more than one adult cell.
4. Unipotency: (dedicated progenitors): produce one particular cell type.

2.2. Types of stem cells

1. Embryonic stem cells
2. Adult stem cells [4]

2.3. Sources of stem cells in dentistry

2.3.1. Embryonic stem (ES) cells

There are two primary sources of stem cells: embryonic stem (ES) cells and adult stem cells which are naturally present in the human body. In addition to these stem cells, induced pluripotent stem (iPS) cells have been recently generated artificially via genetic manipulation of somatic cells [5]. 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.

Embryonic stem cells are imitative from embryos that are 2-11 days old known as blastocysts. They are best developed from supernumerary embryos obtained from in vitro fertilization centers. They are totipotent - cells practically capable of differentiating into any type of cell as well as the germ cell.

ES cells are considered eternal as they can be propagated and maintained in an undifferentiated state forever. These stem cells have the maximum potential to regenerate and repair unhealthy organs and tissues in the body. However, the therapeutic advantage of ES cells is increased ethical and moral concerns. Further, it is hard to control the expansion and differentiation of the embryonic stem cells posing risk of teratoma formation and tumorogenicity. ES cells are not so far used therapeutically and have only remained an excellent platform for research[4].

2.3.2. Induced pluripotent stem cells (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. Just a year after the mouse study was reported, the findings were replicated in human skin cells [6], 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. 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 Stem cells from the apical papilla (SCAP), dental pulp stem cells (DPSCs) and stem cell from exfoliated deciduous teeth (SHED), tooth germ progenitor cells (TG-

PCs), buccal mucosa fibroblasts, gingival fibroblasts and periodontal ligament fibroblasts [7]. 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.

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 [8]. However, the scientific understanding of iPS cells and how to control their differentiate fate is still limited.

2.3.3. Adult stem cells

Adult stem cells are also called somatic stem cells or postnatal stem cells and these are multipotent i.e., they can only differentiate into a limited number of cell types. 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. 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 (MSCs).

In cell culture, MSCs can be identified and isolated based on their adherence to tissue-culture-treated plastic. MSCs are among the most promising adult stem cells for clinical applications and they were originally found in the bone marrow but also been isolated from many other adult tissues, including skin, adipose tissue and various dental tissues [9]. The concept of using adherent fibroblastic cells isolated from the bone marrow was originally reported in 1970 by Friedenstein et al. [10]. In 1999, Pittenger et al. [11] 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. Finally, MSCs must be able to differentiate to osteoblasts, adipocytes and chondroblasts in vitro.

2.3.3.1. 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 [12].

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.

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.

Clinical observations and experimental animal studies [13, 14] have consistently indicated that grafted bone obtained from the craniofacial area (membranous bone) for autologous bone grafting at craniofacial sites provides bet-

ter results and significantly higher 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.

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) [15] 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.

2.3.3.2. 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 [16] 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. This niche may be specific to rodents 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 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 [17]. To date, several MSC sources have been identified in dental tissues, and the isolated stem cells have been extensively characterized.

In 2000, adult human dental stem cells were first identified in the dental pulp (dental pulp stem cells; DPSCs: (Fig. 1A) [18], and these cells had phenotypic characteristics similar to those of BMSCs. MSC-like cells were subsequently also isolated from the dental pulp of human deciduous teeth (stem cells from human exfoliated deciduous teeth; SHED). DPSCs and SHED possess definitive stem cell properties, such as multi-differentiation and self-renewal. Importantly, these cells have the specific ability to regenerate the dentin–pulp complex when transplanted into immunocompromised mice.

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 [19].

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. 1B), 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.

Stem cells from the apical papilla (SCAP) were found in the papilla tissue in the apical part of the roots of developing teeth (Fig. 1C). 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 availabili-

ty. However, these cells are heterogeneous with various differentiation states, as they include true ''stem'' cells, progenitor cells and possibly fibroblasts. Therefore, it is necessary to effectively classify and purify these cells to prevent unexpected clinical results.

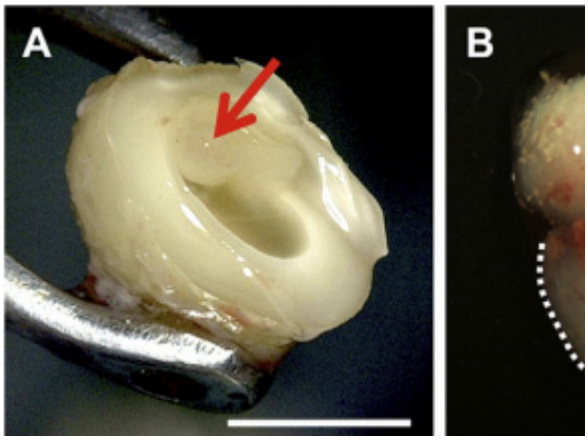

Fig. 1. Sources of adult stem cells in dental tissues. (A) After a tooth was cut horizontally, the pulp tissue (arrow) in the pulp chamber was exposed; this pulp provides dental pulp stem cells (DPSCs). (B) Extracted impacted third molar (10-year-old female) containing the dental follicle (dotted line) that provides dental follicle stem cells (DFSCs). Bar: 5 mm. (C) Extracted impacted third molar (18-year-old male) containing root apical papillae (asterisks) that are a source of stem cells from the apical papilla (SCAP). Bars: 5 mm.
Source - Egusa, H, et al. 2012 [20]

2.3.3.3. Oral mucosa-derived stem cells

The oral mucosa is composed of stratified squamous epithelium and underlying connective tissue. 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 mm). 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 which suggests that they may be useful for intra-oral grafting [21].

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 (Fig. 2). 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. [22] 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. 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.

2.3.3.4 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 [23], 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, the relationship between the vasculature and the perioste-

um and the periosteal osteogenic capacity. Histologically, the periosteum is composed of two distinct layers and up to five distinctly different functional regions when it is dissociated enzymatically and cultured [24].

The outer area contains mainly fibroblasts and elastic fibers, and the inner area contains MSCs osteogenic progenitor cells, 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. These cells are capable of differentiating into osteoblasts, adipocytes and chondrocytes and expressing the typical MSC markers. In addition, De Bari et al. [25] 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 [26].

Agata et al. [27] 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 [28], 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 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 and reduced postoperative waiting time after implant placement [29]. Therefore, the periosteum is a large source of stem/progenitor cells for bone regeneration, particularly for large defects.

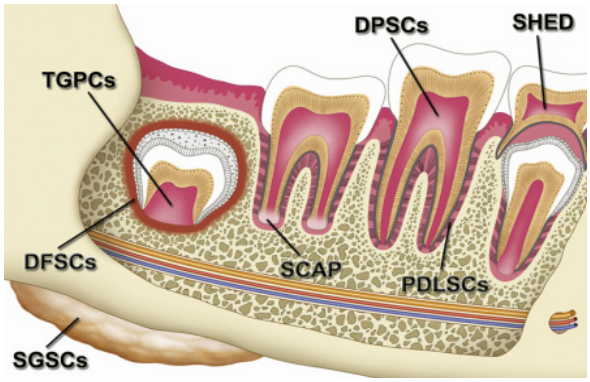

Fig. 2. Sources of adult stem cells in the oral and maxillofacial region. BMSCs: bone marrow-derived MSCs from orofacial bone; DPSCs: dental pulp stem cells; SHED: stem cells from human exfoliated deciduous teeth; PDLSCs: periodontal ligament stem cells; DFSCs: dental follicle stem cells; TGPCs: tooth germ progenitor cells; SCAP: stem cells from the apical papilla; OESCs: oral epithelial progenitor/stem cells; GMSCs: gingiva-derived MSCs, PSCs: periosteum-derived stem cells; SGSCs: salivary gland-derived stem cells.

Source- (Egusa et al., 2012) [20]

2.3.3.5. 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. Kishi et al. [30] 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. [31] 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. In addition, the intra-glandular transplantation of cells isolated from mouse submandibular glands successfully rescued the salivary function of irradiated salivary glands [32], and These reports suggest that the salivary gland is a promising stem cell source for future therapies targeting irradiated head and neck cancer patients.

2.3.3.6. 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, as liposuction is one of the most common cosmetic procedures. Although the intrinsic characteristics of ASCs appear to be different from those of BMSCs, ASCs exhibit robust osteogenesis and are thus expected to be an alternative source of MSCs for bone regeneration in dentistry.

Pieri et al. [33] 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 [34].

In addition, Ishizaka et al. [35] demonstrated that ASC transplantation induced pulp regeneration in the root canal after pulpectomy in dogs. Hung et al. [36] 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.

3. Conclusion

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.

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. Studies on ES/ iPS cells may reveal the complex developmental process of oral organs, such as the teeth and salivary glands. Therefore, Stem cells are also a promising tool for dental reconstruction and regeneration.

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