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Review

Mesenchymal stem cell-mediated treatment of oral diseases

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Summary. In the oral maxillofacial region, there are significant demands for repairing severe tissue defects caused by congenital malformations, oncologic resection, post-traumatic loss, and pathologic degenerative destruction such as periodontitis. Mesenchymal stem cells (MSCs) are adult stem cells whose multipotency has been investigated for therapeutic applications. This review highlights the main MSCs involved in the tissue regeneration of oral maxillofacial region and recent advances in dental MSC-based tissue regeneration and treatments in this region. MSCs isolated from oral maxillofacial sources have higher proliferation rates and are more capable of forming bone and dental tissues. Large animal models of oral diseases or defects were established and treated with MSCs. Miniature pigs or dogs more closely mimic disease in humans and provide a useful means for translating research into clinical applications. MSCs exert other beneficial effects, including immuno-modulation and paracrine processes. The immuno-regulatory properties of MSCs facilitate their application to oral diseases and tissue regeneration. Besides autologous MSCs being an excellent cell source for tissue engineering and regenerative medicine, allogeneic MSC-based treatment also provides a safe and effective therapeutic modality, the use of allogeneic MSCs in highly standardized clinical trials could lead to a better understanding of their real-life applications, which sheds light on potential clinical applications for treating oral diseases.

Key words: Mesenchymal stem cells, Cell transplantation, Allogeneic, Tissue engineering, Regeneration medicine, Oral diseases, Miniature pig

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Introduction

Tissue engineering is now considered an alternative to traditional medical treatments and may help alleviate the shortcomings of conventional therapeutic options (Langer and Vacanti, 1993). In the oral maxillofacial region, there are significant demands for repairing severe tissue defects caused by congenital malformations, oncologic resection, post-traumatic loss, and pathologic degenerative destruction such as periodontitis. Current therapeutic approaches have often resulted in unsatisfactory clinical outcomes. Therefore, the concept of MSC-based tissue engineering has been integrated into research and in applications aimed at managing damaged and lost oral tissues through reconstruction and regeneration of the periodontium (Bartold et al., 2000), dentin-pulp complex (Nör, 2006), and maxillofacial bone (Steinhardt et al., 2008). Large animal models, such as miniature pigs or dogs, of oral diseases or defects have been established, which more closely mimic the disease in humans. MSCs were used to treat these diseases or defects, which can be translated from research to clinical applications.

Biological and immunoregulatory functions of MSCs in the oral maxillofacial region

Biological functions of MSCs in the oral maxillofacial region

The tooth and the periodontium are embedded in the alveolar bone of the maxilla or the mandible (Nakashima and Reddi, 2003). Following tooth development, some of the periodontal and dental tissues, as well as the bone marrow, exhibit regenerative or reparative capacity (Duailibi et al., 2006), which is thought to be mediated by the presence of MSCs (Kim et al., 2012). Recent

studies in the dental field have identified many MSC sources in the oral and maxillofacial region (Fig. 1). These cells were confirmed to express the surface molecules CD105, CD73, CD90 and to lack the expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR surface molecules (Shi and Gronthos, 2003; Horwitz et al., 2005; Dominici et al., 2006). MSCs are also capable of differentiating into both mesenchymal and nonmesenchymal cell types, including adipocytes in dental and bone-associated tissues to replace damaged and diseased tissues in the affected areas (García-Gómez et al., 2010). In human oral tissue-derived MSCs, such as orofacial BMSCs, the proliferation and osteogenic differentiation capacity is high, whereas the adipogenic potential is lower (Akintoye et al., 2006). The biological characteristics of MSCs derived from maxillofacial tissues have advantages and disadvantages, as listed in Table 1.

Immunoregulation of MSCs from the oral maxillofacial region

In addition to tissue repair and regeneration, immunomodulatory properties have also been identified in MSCs. Although the precise molecular mechanism remains unclear, it is well known that MSCs have immunosuppressive and immunomodulatory properties *in vitro* and *in vivo* (Liu et al., 2012). MSCs can interact with T cells, B cells, natural killer cells, monocytes and macrophages, dendritic cells, and neutrophils (Krampera et al., 2003; Jiang et al., 2005; Corcione et al., 2006). Moreover, human oral tissue-derived MSCs, such as dental pulp stem cells (DPSCs) (Pierdomenico et al., 2005), stem cells from exfoliated deciduous teeth (SHED) (Yamaza et al., 2010), periodontal ligament stem cells (PDLSCs) (Liu et al., 2013), stem cells from the apical papilla (SCAP) (Ding et al., 2010), and gingiva-derived MSCs (GMSCs) (Zhang et al., 2009) have immunomodulatory properties similar to bone marrow mesenchymal stem cells (BMSCs). Notably, allogeneic PDLSCs exhibit immunosuppressive activities on activated T-cells *in vitro* (Ding et al., 2010). A further study demonstrated that PDLSCs can modulate B-cell functions *in vitro* (Liu et al., 2013). These findings may represent a novel therapeutic strategy for immune-related disorders.

MSC sources for oral tissue regeneration

Sources of stem cells

To date, the main MSCs involved in oral maxillofacial region tissue regeneration come from the following sources: bone marrow, adipose tissue, and as

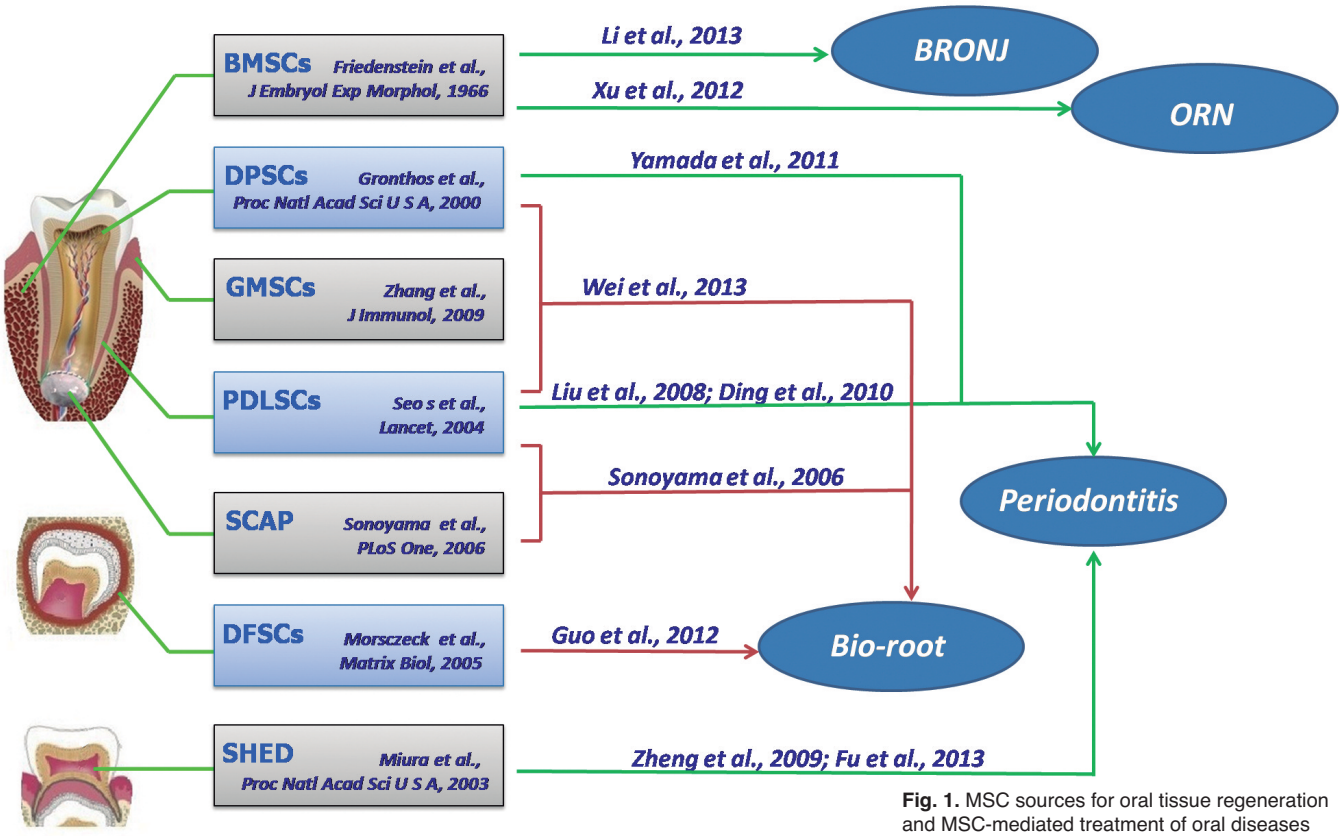

Fig. 1. MSC sources for oral tissue regeneration and MSC-mediated treatment of oral diseases

dental derived MSCs, which have been identified and extensively characterized (Table 1).

BMSCs

BMSCs (Friedenstein, et al., 1966) can be harvested from the sternum, iliac crest, and maxilla or mandible (Derubeis and Cancedda, 2004). BMSCs from the iliac crest have been extensively studied and have been shown to differentiate along the osteogenic, chondrogenic, adipogenic, myogenic, or non-mesenchymal neurogenic lineages (Bianco et al., 2001). In the area of periodontal regeneration, it is apparent that BMSCs have the capacity to enhance periodontal regeneration through generation of alveolar bone (Kawaguchi et al., 2004), new cementum (Yang et al., 2010), and the periodontal ligament (Hasegawa et al., 2006).

Notably, clinical observations (Han et al., 2009) and experimental animal studies (Donovan et al., 1993) have consistently indicated that there are functional differences between orofacial and iliac crest human

Table 1.

Cell type	*In vivo* tissue formation capacity	Protein markers expression (Positive)	Protein markers expression (Negative)	Advantages	Disadvantages	References
BMSCs	Bone; Alveolar bone; Cartilage; Muscle; Cementum; Periodontal ligament	CD10, CD13, CD29, CD44, CD49, CD54, CD55, CD59, CD73, CD90, CD105, CD271, STRO-1	CD11b, CD14, CD19, CD34, CD45, CD79a, HLA-DR	Rich resource; Easy isolation and expansion; Broadly multipotent and well characterized	Trauma during harvesting, age-related decline in the osteogenic potential of BMSCs isolated from the human iliac crest and femur	Derubeis and Cancedda, 2004
ASCs	Bone; Alveolar bone; Cartilage; Dentin; Periodontal ligament	CD9, CD10, CD13, CD29, CD44, CD49, CD54, CD55, CD59, CD73, CD90, CD105, CD106, CD146, CD166, HLA-I, Fibronectin, Endomucin, Asthma, Vimentin, Collagen-1	CD11b, CD14, CD19, CD31, CD34, CD45, CD79a, CD80, CD117, CD133, CD144, HLA-DR, C-kit, MyD88, STRO-1, LIN, HLA-II	Rich source; Easy isolation and expansion; Less invasive surgical harvesting procedure	Uncertainty about the true clinical potential of human ASC	Hung et al., 2011; Locke et al., 2011
DPSCs	Dentin; Pulp; Muscle; Alveolar bone	CD9, CD10, CD13, CD29, CD44, CD49d, CD59, CD73, CD90, CD105, CD106, CD146, CD166, STRO-1, Nestin	CD14, CD31, CD34, CD45, CD117, CD133	Easy isolation and expansion; Clinical abundance	Limited differentiation potentials	Gronthos et al., 2002; Zhang et al., 2008
SHED	Dentin; Bone; Alveolar bone; Vessel	CD13, CD44, CD73, CD90, CD105, CD146, STRO-1, Oct-4, Nanog, Nestin, SSEA-3, SSEA-4	CD14, CD19, CD34, CD43, CD45	Rich and suitable autologous stem cell source	Limited differentiation potentials *in vivo*	Miura et al., 2003; Cordeiro et al., 2008; Yamaza et al., 2010
PDLSCs	Cementum; Periodontal Ligament; Alveolar bone	CD9, CD10, CD13, CD29, CD44, CD49d, CD59, CD73, CD90, CD105 CD106, CD146, CD166, STRO-1, Scleraxis	CD31, CD34, CD45	Broadly multipotent; Ideal for periodontal tissue regeneration	Difficult to harvest a large quantity of PDLCs from the teeth; Requirement of a professional technician is higher for extraction, isolation, and culture	Seo et al., 2005; Gronthos et al., 2006; Liu et al., 2008
GMSCs	Bone; Cartilage; Muscle; Epithelia; Neural tissue	CD29, CD44, CD73, CD90, CD105, CD106, CD146, CD166, STRO-1, Oct-4, Nanog, Nestin, SSEA-4, HLA-ABC, Sox-2, Tra2-49, Tra2-54	CD34, CD45, CD117	Easy isolation and expansion; clinical abundance	Limited differentiation potentials	Zhang et al., 2009; Wang et al., 2011a
DFSCs	Cementum; Periodontal ligament; Alveolar bone	CD9, CD10, CD13, CD29, CD44, CD49d, CD59, CD73, CD90, STRO-1, HLA-I	CD31, CD34, CD45, CD133	Immature stem cells; Easy isolation and expansion; broadly multipotent	Limited accessibility	Morsczeck et al., 2005; Yokoi et al., 2007;Yao et al., 2008
SCAP	Dentin; Pulp	CD49d, CD51/61, CD56, CD73, CD90, CD105, CD106, CD146, CD166, STRO-1, Nestin, Survivin	CD14, CD18, CD34, CD45, CD117, CD150	Immature stem cells; Better regeneration of the dentin matrix	Limited accessibility; Limited differentiation potentials	Sonoyama et al., 2006; Abe et al., 2007; Huang et al., 2008

BMSCs (Akintoye et al., 2006; Chung et al., 2009). The ability to expand iliac crest BMSCs *in vitro* appears to be limited (Derubeis and Cancedda, 2004). In orofacial BMSCs, on the other hand, the cells' gene expression patterns seem to be little affected by the age of the donor (Han et al., 2009). These properties of orofacial BMSCs may prove advantageous for orofacial tissue engineering and regeneration (Egusa et al., 2012).

Adipose tissue-derived stem cells (ASCs)

ASCs can be easily harvested from adipose tissue. ASCs share similar differentiation characteristics with BMSCs (Yarak and Okamoto, 2010). Thus, ASCs are anticipated to be an alternative source of MSCs for oral maxillofacial bone regeneration (Pieri et al., 2010; Mizuno et al., 2012). In dentistry, periodontal (Tobita et al., 2008) and dental pulp tissue regeneration (Ishizaka et al., 2012) using ASCs has been demonstrated in animal models. ASC implants were also able to grow dentin, periodontal ligaments, and alveolar bone in adult rabbit extraction sockets (Hung et al., 2011).

However, there is considerable uncertainty about the true clinical potential of human ASCs, since the differentiation of ASCs into cell lineages apart from adipocytes has not been conclusively demonstrated in many studies. Hence, the clinical potential of ASCs will require more extensive investigations of their fundamental biology.

DPSCs

DPSCs exhibit features similar to those of BMSCs, with the capacity to regenerate dentine⁄ pulp complexes *in vivo* (Gronthos et al., 2000). DPSCs have the potential to differentiate into cells of odontogenic (Gronthos et al., 2002), adipogenic (Alipour et al., 2010), myogenic (Zhang et al., 2008a,b), and neurogenic lineages (Arthur et al., 2009). These features make DPSCs attractive for therapeutic uses in regenerative endodontic and other tissues (Nakashima et al., 2005). Autologous DPSCs were used to prevent postoperative alveolar bone loss (D'Aquino et al., 2009). DPSCs may enhance alveolar bone regeneration through the generation of well-formed mature bone with neovascularization (Yamada et al., 2011).

However, results obtained by different research groups (Ji et al., 2010; Park et al., 2011; Yamada et al., 2011) using DPSCs implanted into various periodontal defects in dogs are inconsistent. There was little difference in the extent of regeneration between defects receiving DPSCs and control defects, which did not receive any stem cells (Park et al., 2011).

SHED

SHED are one of the more readily available sources of oral maxillofacial MSCs (Arora et al., 2009). SHED can differentiate into several cell types *in vitro*, including adipocytes, chondontocytes, osteoblasts, and neurons (Miura et al., 2003). After *in vivo* implantation, implanted SHED formed markedly more new bone in the defect site (Zheng et al., 2009). Furthermore, allogeneic SHED transplantation into bone defects in the mandibles of dogs generated well-formed neovascularized mature bone in the defect sites (Yamada et al., 2011). In contrast to DPSC, however, SHED failed to produce a dentin pulp-like complex (Miura et al., 2003). Dentin regeneration using SHED was achieved in miniature pigs (Zheng et al., 2012). These studies provide evidence that SHED could serve as an alternative source of MSCs for stem cell-based approaches to alveolar bone and dentin regeneration. Importantly, frozen allogeneic SHED could be a more suitable source, with sufficient stem cell numbers for use in regenerative procedures.

PDLSCs and GMSCs

PDLSCs were first isolated from the periodontal ligaments of human third molars (Seo et al., 2004). These MSCs have multilineage differentiation potential and are able to differentiate into adipogenic, osteogenic, and chondrogenic phenotypes *in vivo* (Seo et al., 2005). It is well known that PDLSCs represent a novel stem cell population in terms of their *in vivo* capacity to differentiate into cells similar to cementoblasts and collagen-forming cells. The characteristics of PDLSCs may depend on the harvest location (Wang et al., 2011a,b). PDLSCs from the surface of alveolar bone may have a synergistic effect on PDLSCs from the root surface, and display superior alveolar bone regeneration compared with PDLSCs from the root surface. The ability to regenerate cementum, periodontal ligaments, and alveolar bone in experimental animal models (Gronthos et al., 2006) make PDLSCs highly amenable for use in periodontal regeneration. Moreover, allogeneic PDLSCs can indirectly regenerate periodontal tissues *in vivo* by regulating B lymphocyte function (Liu et al., 2013).

Human GMSCs were characterized and found to have clonogenicity, self-renewal, and multipotent differentiation capacities similar to those of BMSCs (Zhang et al., 2009). GMSCs can be easily obtained, proliferate faster than BMSCs, and display a stable morphology (Tomar et al., 2010). The multipotency of GMSCs and their clinical abundance, ease of isolation, and expansion provide great advantages as a stem cell source for potential clinical applications.

Dental follicle stem cells (DFSCs)

In 2005, DFSCs were first isolated from the dental follicle of human third molars (Morsczeck et al., 2005). The dental follicle, which contains the developing tooth and differentiates into the periodontal ligament, contains stem cells with the ability to regenerate periodontal tissues (Yao et al., 2008). *In vitro* studies demonstrated

that DFSCs have the capacity to differentiate into odontoblasts, cementoblasts, osteoblasts, and other cells implicated in the tooth. When implanted into immunodeficient mice, DFSCs also have the capacity to generate periodontal ligaments *in vivo* (Yokoi et al., 2007). DFSCs were used to assess the ability of such cells to contribute to the formation of the tooth root (Guo et al., 2012). DFSCs implanted into three different microenvironments in rats showed that DFSCs contributed to the formation of root-like tissues with a pulp-dentin complex and a periodontal ligament connecting a cementum-like layer to host alveolar bone. These results also demonstrate the potential of DFSCs in tooth regeneration.

SCAP

SCAP were first isolated from the apical papilla and are capable of forming odontoblast-like cells *in vivo* (Sonoyama et al., 2006). When transplanted into immunocompromised mice in an appropriate carrier matrix, a typical dentin pulp-like structure was formed by SCAP; however, SCAP demonstrate better proliferation *in vitro* and better regeneration of the dentin matrix compared with DPSCs. SCAP (Abe et al., 2007) were found in the papillar tissue in the apical part of the roots of developing teeth. SCAP, together with PDLSCs, are also able to form a root-like structure when seeded onto the hydroxyapatite-based scaffold and implanted in pig jaws (Huang et al., 2008), suggesting their potential utilization for pulp/dentin regeneration and bio-root engineering.

MSC-mediated treatment of oral diseases

Large animal models

Compared with small animal models like rodents, large animal models are superior in many aspects for the study of oral diseases and pre-clinical therapies. Miniature pigs are increasingly used in studies of the oral maxillofacial region because of the similarity to humans in their anatomic, developmental, physiological, pathophysiological, and disease occurrence (Wang et al., 2007).

Many different types of miniature pigs have been bred, and some spontaneously develop diseases seen in humans. Wang et al. (1998) used Chinese experimental miniature pigs for oral disease studies. This kind of miniature pig was derived from swine from Guizhou Province, China, in 1985. Its characteristics include inherent small size, early sexual maturity, rapid breeding, and ease of management (Yu et al., 2003). The application of MSC therapy based on miniature pigs has been widely discussed for periodontal diseases (Liu et al., 2008; Ding et al., 2010; Fu et al., 2014), orofacial bone defects (Zheng et al., 2009), tooth regeneration (Sonoyama et al., 2006; Wei et al., 2013), osteoradionecrosis (Xu et al., 2012), and bisphosphonate-related osteonecrosis of the jaw (BRONJ) (Li et al., 2013).

Autologous MSC-based treatment for oral diseases

Repair of oral tissue defects that arise as a result of disease is often accomplished via transfer of autologous MSCs. Recently, clinicians turned to the fields of MSC-based tissue engineering and regenerative medicine to develop cellular strategies for regenerating oral tissues such as periodontal tissue and maxillofacial bone (Fig. 1). As an easily accessible stem cell source, the proliferative potential and multilineage differentiation capacity make autologous MSCs an excellent source for tissue engineering and regenerative medicine. Autologous MSCs have also been associated with regenerative capacity owing to their unique immune modulatory properties. Their immunosuppressive capability defines their application in the treatment of oral diseases with a pathogenesis involving uncontrolled activity of the immune system.

Treatment of periodontitis and bone defects

Periodontal disease can result in irreversible destruction of periodontium, leading to loss of attachments between teeth and their supporting tissues. MSCs are a promising resource for regenerating periodontal structures such as the periodontal ligament, cementum, and alveolar bone. Isolated from the periodontal ligament, PDLSCs are a population of MSCs with the capacity to differentiate into cells similar to cementoblasts and collagen-forming cells. The ability to regenerate cementum, the periodontal ligament, including Sharpey's fibers, and alveolar bone in experimental animal models (Gronthos et al., 2006) make PDLSCs an ideal MSC source for periodontal regeneration. Liu et al. (2008) explored the potential of using autologous PDLSCs to treat periodontal defects in a miniature pig model of periodontitis. In their study, autologous PDLSCs were obtained from extracted teeth from miniature pigs and transplanted into surgically-created periodontal defect areas. The PDLSCs were then shown to be capable of regenerating periodontal tissues. Moreover, Zheng et al. (2009) isolated SHED from miniature pig deciduous teeth then engrafted them into critical-sized bone defects generated in miniature pig mandible models. They indicated that autologous SHED were able to engraft and regenerate bone to repair these critical-sized mandibular defects. Similarly, Lendeckel et al. (2004) used autologous ASCs to treat the calvarial defect along with an iliac crest bone graft, revealing a new method for difficult reconstructive procedures.

Treatment of osteoradionecrosis

Osteoradionecrosis (ORN) of the mandible is a common and severe complication of radiation therapy for head and neck cancers (O'Dell and Sinha, 2011). To

date, the clinical management of ORN has been considered complex and unsatisfactory. Recent studies demonstrated that BMSCs have therapeutic potential in irradiated tissues (Lange et al., 2011). BMSCs also have the potential to regulate immune responses (Matysiak et al., 2013). The therapeutic potential of BMSCs was achieved in irradiated tissues (Zhang et al., 2008a,b). Advanced ORN was cured and tissue reconstruction was established in miniature pig models (Xu et al., 2012), suggesting that advanced ORN could be ameliorated by treatment with autologous BMSCs. BMSC transplantation is assumed to treat ORN via two mechanisms: by active tissue regeneration, in which the BMSCs assist in the recovery of bone tissue; and by organization of recipient origin bone marrow, in which the BMSCs assist in tissue revitalization. Additionally, microvessel regeneration is suggested to play an important role in the treatment of ORN due to the observation of marked microvessel regeneration in BMSC-mediated tissue regeneration in ORN (Mendonca and Juiz-Lopez, 2010). These findings may yield important preclinical information about the application of stem cell-based therapy for treating human mandibular ORN.

Allogeneic MSC-mediated treatment of oral diseases

The limited number of completely characterized autologous MSCs available represents a major obstacle for their use in adult stem cell therapy. In certain situations like older-aged patients, sources of autologous MSCs are limited, which largely impedes the clinical application of this approach. Thus, it is critical to develop a feasible allogeneic MSC-based method for the treatment of oral diseases. The use of allogeneic MSCs from controlled donors under optimal conditions and their application in highly standardized clinical trials could lead to a better understanding of their real-life applications and reduce the time to clinical translation (Fig. 1).

Treatment for periodontitis

A large body of research has been conducted to assess the capacity of MSCs to enhance periodontal regeneration, and some promising results have been obtained (Feng et al., 2010). MSCs have been extensively studied with regard to their capacity to aid periodontal regeneration (Hynes et al., 2012). In the treatment of periodontitis-induced bone defects in miniature pigs, allogeneic PDLSC sheets provided appropriate therapy for periodontitis, with significant periodontal tissue regeneration and low immunogenicity (Ding et al., 2010a,b). Research has also been conducted with other easily accessible MSC populations, such as BMSCs, SHED (Fu et al., 2014), and DPSCs (Yamada et al., 2011). These MSC populations have the capacity to enhance periodontal regeneration through enhanced generation of well-formed mature alveolar bone and neovascularization. Although there is an overwhelming body of evidence to support the notion that MSCs can be used for periodontal regeneration, other issues such as appropriate delivery devices and immunogenicity are important considerations that should not be overlooked.

Treatment for BRONJ

Patients on high-dose bisphosphonate therapy have an increased risk of BRONJ (Kühl et al., 2012). Despite the severity of this disease, appropriate therapy has not been established. Necrotic bone is often found adjacent to areas of local inflammatory infiltrates, suggesting an association between inflammation and tissue necrosis in BRONJ (Ruggiero et al., 2004). To investigate the pathogenesis of BRONJ, disclose the immune response-based mechanism of BRONJ-like disease, and observe the treatment effect of allogeneic MSC transplantation, Kikuiri et al (2010) established a preclinical mouse model of BRONJ. They demonstrated that systemic infusion with MSCs prevents and cures BRONJ-like disease, possibly via induction of peripheral tolerance. These findings provide evidence of the immune-based mechanism of BRONJ-like disease, and support the rationale for *in vivo* immunomodulatory MSC-based therapy to treat BRONJ. Recently, a large-animal model of BRONJ was established in miniature pigs and the impaired biological and immunological properties of BMSCs in this animal model were observed (Li et al., 2013). Furthermore, after allogeneic BMSC transplantation via intravenous infusion, mucosal healing and bone reconstruction were observed; interleukin (IL)-17 levels were reduced and Tregs were elevated (Li et al., 2013). Thus, MSC-based immunotherapy could potentially offer a safe and effective novel therapeutic modality for preventing the development of BRONJ disease.

Treatment for tooth loss

A functional tooth root that can support a natural or artificial crown is very important. In 2006, SCAP and PDLSCs were used to form a bio-root, which was encircled with periodontal ligament tissue and appeared to have a natural relationship with the alveolar bone (Sonoyama et al., 2006). In a follow up study, Vc-induced allogeneic PDLSCs were used for periodontal-like tissue regeneration and allogeneic DPSCs for dentin-like tissue regeneration (Wei et al., 2013). The regenerated bio-root exhibited the characteristics of a normal tooth after 6 months of use, including dentinal tubule-like and functional periodontal ligament-like structures. Recent advances in MSC biotechnology and cell-based bioengineered tooth regeneration have encouraged researchers to explore their potential for regenerating living functional teeth (Ikeda et al., 2009). The outcomes of these studies suggest great potential for

biological and functional tooth regeneration in humans.

Conclusions and perspective

The past few years have witnessed a growing optimism and progress in stem cell biology and tissue engineering, suggesting that MSC-mediated tissue regeneration may have expanded clinical applicability in the future. Moreover, MSCs also exert other beneficial effects, including immunomodulation and paracrine processes. It is important to clarify the possible role of MSCs in promoting immunosuppression when they are locally/systemically implanted, and how these effects can be counterbalanced in order to maintain homeostasis in the recipient. Properly understanding the relationship between the host's immune system and donors' MSCs will provide a foundation for improving the therapeutic effect of MSC-based tissue regeneration (Liu et al., 2012).

More work is needed and evidence from long-term studies is absolutely required to validate the nature of MSC-mediated therapy. There are still several important issues linked to the clinical use of MSCs. It is important to establish effective and reliable protocols to characterize donor MSCs prior to clinical application. Banking teeth as an autologous cell source and the potential use of allogeneic stem cells all require further research to determine the ultimate benefits to our patients.

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