Mesenchymal Stem Cells and Tooth Engineering

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Abstract
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Tooth loss compromises human oral health. Although several prosthetic methods, such as artificial denture and dental implants, are clinical therapies to tooth loss problems, they are thought to have safety and usage time issues. Recently, tooth tissue engineering has attracted more and more attention. Stem cell based tissue engineering is thought to be a promising way to replace the missing tooth. Mesenchymal stem cells (MSCs) are multipotent stem cells which can differentiate into a variety of cell types. The potential MSCs for tooth regeneration mainly include stem cells from human exfoliated deciduous teeth (SHEDs), adult dental pulp stem cells (DPSCs), stem cells from the apical part of the papilla (SCAPs), stem cells from the dental follicle (DFSCs), periodontal ligament stem cells (PDLSCs) and bone marrow derived mesenchymal stem cells (BMSCs). This review outlines the recent progress in the mesenchymal stem cells used in tooth regeneration.

Keywords mesenchymal stem cell, tooth engineering, dental pulp stem cell

Introduction

A commonly applied definition of tissue engineering, as stated by Langer and Vacanti, is “an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ” (Langer and Vacanti, 1993). Tissue engineering has also been defined as “understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use” (MacArthur and Oreffo, 2005). Tissue engineering aims to stimulate the body either to regenerate tissue on its own or to grow tissue outside the body which can then be implanted as natural tissue.

Stem cells are characterized by the ability to renew themselves through mitotic cell division and differentiate into a diverse range of specialized cell types. According to developmental stages, stem cells can be divided into embryonic stem cells and adult stem cells. Differentiation and proliferation of embryonic stem cells constitute the basis of animal development. The further differentiation of adult stem cells is the prerequisite of tissues and organs’ repair and regeneration. Embryonic stem cells are the progenitors of undifferentiated cells, which are “totipotent” (totipotency is the ability of a single cell to divide and produce all the differentiated cells in an organism, including extraembryonic tissues) and can differentiate into a variety of cells to form various organs, also known as the “all-competent cells”. In the process of cell differentiation, they can gradually differentiate into a stable form of “pluripotent stem cells”. With the features of highly proliferative capacity and plasticity, stem cells are regarded as a new source of seed cells in tissue engineering in a wide range of applications.

There is no doubt that the description of tissue engineer offers a new hope to both patients who
suffer with tooth loss and the dentists as well. The exploration of tooth tissue engineering mainly focuses on three parts: seeding cells, scaffolds and growth factors. Here we summarized the recent studies on tooth engineering using mesenchymal stem cells. Numerous attempts have been made to “create” tooth and very promising results have been made. Single cell suspensions obtained from rat, pig or mice tooth germs or bone marrow were seeded onto the surface of biodegradable polymer scaffolds (e.g. collagen-coated polyglycolic acid, calcium phosphate material, collagen sponges, PGA/PLLA scaffolds) and the cell/polymer constructs were successfully re-implanted into a suitable immunocompromised host, so a sufficient blood supply could support the growth of higher ordered structures (Duailibi et al., 2004; Honda et al., 2006, 2007a, 2007b; Hu et al., 2006). All these reports described the formation of dentin or enamel or even both of them. Besides dissociated cells, the dissociated tooth tissues were also used to bioengineer complex tooth crowns resembling those of naturally developing teeth successfully (Young et al., 2002). This indicated that the seed cells or tissues could differentiate properly into odontoblast-like and ameloblast-like cells. However, these bioengineered teeth were produced in ectopic sites and lacked of some essential elements such as the complete root and periodontal tissues which allow their correct anchoring into the alveolar bone (Bluteau et al., 2008). Recently, a three-dimensional organ-germ culture method has been proposed for growing teeth in the mouse mandible (Nakao et al., 2007). In this study, epithelial and mesenchymal cells were sequentially seeded into a collagen gel drop and then implanted into the tooth cavity of adult mice. With this technique the bioengineered tooth germ generated a structurally correct tooth, showing all dental structures such as odontoblasts, ameloblasts, dental pulp, blood vessels, crown, periodontal ligament, root and alveolar bone (Nakao et al., 2007). Thus, the development of bioengineered organ replacement strategies and the appropriate seeding cells, plus biodegradable polymer scaffolds and proper microenvironment ensure a substantial advance in tooth engineering science.

In the field of tooth engineering, efforts have been made to explore mesenchymal stem cells (MSCs) such as stem cells from human exfoliated deciduous teeth (SHEDs), adult dental pulp stem cells (DPSCs), stem cells from the apical part of the papilla (SCAPs), stem cells from the dental follicle (DFSCs), periodontal ligament stem cells (PDLSCs), bone marrow derived mesenchymal stem cells (BMSCs) and epithelium-originated dental stem cells (Bluteau et al., 2008). The recent advances of MSCs in tooth engineering were reviewed as followings.

**Stem cells from human exfoliated deciduous teeth**

The discovery of stem cell in deciduous teeth (Miura et al., 2003) sheds a light on the intriguing possibility of using dental pulp stem cells for tissue engineering (Murray and Garcia-Godoy, 2004; Sloan and Smith, 2007). The obvious advantages of SHEDs (stem cells from human exfoliated deciduous teeth) are: higher proliferation rate compared with stem cells from permanent teeth (Miura et al., 2003), easy to be expanded in vitro, high plasticity since they can differentiate into neurons, adipocytes, osteoblasts and odontoblasts, readily accessible in young patient (Miura et al., 2003), especially suitable for young patients with mix dentition (Nör, 2006). Miura demonstrated that SHEDs could not differentiate directly into osteoblasts but did induce new bone formation by forming a template to recruit murine host osteogenic cells (Miura et al., 2003). SHEDs are distinctive with the osteoinductive ability and high plasticity. Cordeiro seeded SHEDs in PLLA (porous poly L-lactic acid) prepared within human tooth slice scaffolds and transplanted them into the subcutaneous tissue of immunodeficient mice. They observed that SHEDs differentiated into odontoblast-like cells and showed morphologic characteristics resembled those of odontoblast cells. Moreover, an increase in microvessel density was found in the co-implantation. They also verified that the transplanted SHEDs were capable of differentiating into blood vessels that anastomosed with the host vasculature (Cordeiro et al., 2008). These studies proved that SHEDs might be an ideal resource of stem cells to repair damaged tooth structures and induce bone regeneration.
Adult dental pulp stem cells

The regenerative capacity of the human dentin/pulp complex enlightens scientists that dental pulp may contain the progenitors that are responsible for dentin repair. Gronthos first identified adult dental pulp stem cells (DPSCs) in human dental pulp in 2000 and found DPSCs could regenerate a dentin-pulp-like complex, which is composed of mineralized matrix with tubules lined with odontoblasts, and fibrous tissue containing blood vessels in an arrangement similar to the dentin-pulp complex found in normal human teeth (Gronthos et al., 2000). The same group further verified DPSCs possessed striking features of self-renewal capability and multi-lineage differentiation by finding that DPSCs were capable of forming ectopic dentin and associated pulp tissue in vivo and differentiating into adipocytes and neural-like cells (Gronthos et al., 2002). An in vivo study showed that DPSCs produced bone when implanted into subcutaneous sites in immunocompromised mice with HA/TCP powder as carrier. In addition, the potential of DPSCs for long-term storage was analyzed. They found that even after storage for 2 years, DPSCs were still capable of differentiating into pre-osteoblasts and produced woven bone tissues. In addition, DPSCs still expressed certain surface antigens, confirming cellular integrity (Papaccio et al., 2006, Otaki et al., 2007). Scientists have been working to find an efficient scaffold that can be loaded with DPSCs and an appropriate microenvironment to promote the differentiation of DPSCs. In a recent study DPSCs were seeded onto different 3-dimensional (3-D) scaffold materials (a spongyous collagen, a porous ceramic, and a fibrous titanium mesh) and implanted in nude mice for 6 or 12 weeks, the formed tissue was not dentin-pulp-like complex but something resembled connective tissue (Zhang et al., 2006). These studies indicate the potential of DPSCs in tooth tissue engineering.

Stem cells from dental follicle

The dental follicle is a mesenchymal tissue that surrounds the developing tooth germ. During tooth root formation, periodontal components, such as cementum, periodontal ligament (PDL), and alveolar bone, are created by dental follicle progenitors (Yokoi et al., 2007). Stem cells from dental follicle (DFSCs) have been isolated from follicle of human third molars and express the stem cell markers: Notch1, STRO-1 and nestin (Morsczeck et al., 2005). DFSCs were found to be able to differentiate into osteoblasts/cementoblasts, adipocytes, and neurons (Kémoun et al., 2007, Yao et al., 2008, Coura et al., 2008). In addition, immortalized dental follicle cells were transplanted into immunodeficient mice and were able to recreate a new periodontal ligament (PDL)-like tissue after 4 weeks (Yokoi et al., 2007). These cells may be a useful research tool for studying PDL formation and for developing regeneration therapies. Wu showed that dNCPs (dentin non-collagenous proteins) extracted from dentin could stimulate DFSCs to differentiate into cementoblast lineage (Wu et al., 2008). Tsuchiya reported that Col-1 facilitated the differentiation of DFSCs along the mineralization process (Tsuchiya et al., 2008). Kémoun proved that enamel matrix derivatives (EMD) activated human dental follicle stem cells (hDFSCs) toward the cementoblastic phenotype. hDFSCs acquired cementoblast features under stimulation of BMP-2/-7 and EMD in vitro (Kémoun et al., 2007). These studies provide new insights into the mechanism of cementogenesis. In addition, Luan indicated that DFSCs lines were heterogeneous. The three main lineages were highly undifferentiated state of periodontal ligament-type and cementoblastic or alveolar bone osteoblastic lineage. The profound cellular heterogeneity of DFSCs suggests that heterogeneous cellular constituents might play a role in tissue regeneration as much as the individual lineages might do (Luan, 2007).

Bone marrow derived mesenchymal stem cells

Human bone marrow-derived stem cells (hBMSCs) originate from cell populations in the bone marrow and are capable of differentiating along multiple mesenchymal lineages. Bone regeneration has long been the critical point of BMSCs’ research. In the
aspect of tooth engineering, current research focused on the fields of tooth-like structures formation and periodontal regeneration. The bone marrow derived (BMD) cells are a mixed population which consist of fibroblasts, osteoblast, adipocyte progenitors and up to 0.01% stem cells (Pereira et al., 1998; Pittenger et al., 1999). Most tooth engineering researches were carried out with cell populations of purified stem cells, however, Ohazama challenged them by proving that bone and soft tissues could also be formed from a heterogeneous population such as BMD cells. They reported that tooth structures could also be formed when intact explants which formed from BMD cells were transferred into renal capsules. Meanwhile, they recombined embryonic oral epithelium with three non-dental mesenchymal cells such as embryonic stem cells, neural stem cells and adult BMD cells, and transferred the recombinations into adult renal capsules and adult jaw. The results showed tooth structures and associated bone were developed in the adult environment (Ohazama et al., 2004). Li came to the coincident conclusion with that of Ohazama group. They verified that the recombination of BMSCs with oral epithelial cells derived from rat embryos expressed odontogenic genes such as Pax9, DMP1, and DSPP and demonstrated the presence of tooth-like structures (Li et al., 2007). BMSCs have been tested for their ability to recreate periodontal tissue and restore periodontal defects. It was proved that auto-transplantation of BMSCs are able to form in vivo cementum, periodontal ligament, and alveolar bone after implantation into defective periodontal sites. Thus, bone marrow provides an alternative source of MSC for the treatment of periodontal diseases (Kawaguchi et al., 2004). Interestingly, when BMSCs were regarded as a source of mesenchymal seed cells, Hu and his colleagues investigated the possibility that BMSCs give rise to different types of epithelial cells and their potential to serve as a source for ameloblasts. Their results showed, for the first time, that BMSCs can be reprogrammed to give rise to ameloblast-like cells (Hu et al., 2006). They offered BMSCs a novel possibility for tooth-tissue engineering and could be induced into both mesenchymal and epithelium cells in tooth tissue engineering. Not all the scientists identify BMSCs with the ideal seeding cells for tooth engineering. Jing pointed out that the differentiation abilities of BMSCs decrease significantly with the increasing age of donors. They hold the opinion that adipose derived stem cells could be induced into odontogenic lineage and might be used as suitable seeding cells for tooth regeneration to replace the lost tooth of elderly patients (Jing et al., 2008).

**Periodontal ligament stem cells**

The periodontal ligament is a specialized connective tissue, derived from dental follicle and originated from neural crest cells. Recent studies have shown that mesenchymal stem cells obtained from periodontal ligament (PDLSCs) are multipotent cells with similar features of the BMSCs and DPSCs, capable of developing different types of tissues such as bone and tooth associated-tissues. It was reported that PDLSCs could differentiate into cells that can colonize and grow on biocompatible scaffold, suggesting an easy and efficient autologous source of stem cells for bone tissue engineering in regenerative dentistry (Trubiani et al., 2008). Orciani verified the osteogenic ability of PDLSCs and pointed out that differentiating cells were also characterized by an increase of Ca$^{2+}$ and nitric oxide production. The authors demonstrated that local reimplantation of expanded cells in conjugation with a nitric oxide donor could represent a promising method for treatment of periodontal defects (Orciani et al., 2008). Besides osteogenic ability, differentiation of PDLSCs to the cementoblastic lineage was also emphasized. The conditioned medium from developing apical tooth germ cells (APTG-CM) was shown to be able to provide a cementogenic microenvironment and induce differentiation of PDLSCs along the cementoblastic lineage. When transplanted into immunocompromised mice, the induced PDLSCs showed tissue-regenerative capacity to produce cementum/periodontal ligament-like structures, characterized by a layer of cementum-like mineralized tissues connected with periodontal ligament-like collagen fibers. This has important implications for periodontal engineering (Yang et al., 2008). Dentin noncollagenous proteins (DNCPs) were also proved to increase proliferation and adhesion ability of HPDLSCs. Induced HPDLSCs
presented several features of cementoblast differentiation (Ma et al., 2008).

Moreover, some studies paid more attention to the identification and character of cells produced from human periodontal ligament. There is evidence that human periodontal ligament, with its mesodermal derivatives, produced neural crest-like cells. Such features suggested a recapitulation of their embryonic state. The human periodontal ligament revealed itself as a viable alternative source for possible primitive precursors to be used in stem-cell therapies (Coura et al., 2008).

Adipose-derived stromal cells

Adipose-derived stromal cells (ADSCs) are considered to contain a group of pluripotent mesenchymal stem cells and manifest multilineage differentiation capacity, including osteogenesis, chondrogenesis and adipogenesis (Liu et al., 2008). ADSCs exhibit stable growth and proliferation kinetics in vitro. Adipose tissue can be obtained by less invasive methods and in larger quantities than bone marrow cells, making the use of hADSCs as a source of stem cells very appealing (Zuk et al., 2002). In 2005, a research team first proposed the hypothesis that adipose derived stem cells could be induced into odontogenic lineage and might be used as suitable seeding cells for tooth regeneration to replace the lost tooth of elderly patients (Jing et al., 2008). The team holds the opinion that the seeding cells for tooth regeneration such as odontoblasts from dental germ, stem cells from dental pulp and deciduous teeth, and ectomesenchymal cells from the first branchial arch are difficult, even impossible to harvest in clinic. Bone marrow mesenchymal stem cells have odontogenic capacity, but their differentiation abilities significantly decrease with the increasing age of the donors. Therefore, the cells mentioned above are not practical in the clinical application of tooth regeneration in the old. They tried to find ideal alternative seeding cells and an appropriate inducing method to overcome the problems mentioned above. The team reported that overexpression of DSPP enhanced expression of genes related to mineralization, such as Msx1, Msx2, Lhx7 and Pax9, which implied that these cells may differentiate into functional odontoblast-like cells (Wu et al., 2008). In addition, the osteogenesis potential of ADSCs has prompted wide attention. It was reported that ADSCs expressed bone marker proteins including alkaline phosphatase, type I collagen, osteopontin, and osteocalcin and produce mineralized matrix. In the current study, the ADSCs ability to form osteoid matrix in vivo was determined, proved them a novel therapeutic for bone repair and regeneration (Hicok et al., 2004). Kakudo investigated the possibility of using honeycomb collagen scaffold to culture ADSCs in bone tissue engineering. It showed that the scaffold was filled with the grown ADSCs and calcification materials. When the ADSCs-loaded honeycomb collagen scaffolds were subcutaneously transplanted into nude mice, bone formation in vivo was identified after 8 weeks (Kakudo et al., 2008).

From this review, we can conclude that tooth engineer seeding cells may come from both dental stem cells and non-dental stem cells, which share the similar features such as high proliferation rate, multi-differentiation ability, easy accessibility, high viability and easy to be induced. There has been great interest in mesenchymal stem cells and their roles in maintaining the physiological structure of tissues. The progress of tooth engineering can not be made without the support of other research of organ regeneration. There is a comment interpreting the relationship: “the accessibility of teeth, together with the fact that they are not essential organs, mean that teeth provide an attractive organ with which to test the practicalities and feasibility of tissue-engineered organ replacement (Ohazama et al., 2004).

Since these cells are considered as candidates for regenerative medicine, the knowledge of the cell differentiation mechanisms is imperative for the development of tooth engineering. Further studies will be carried out to elucidate the molecular mechanisms involved in their maintenance and differentiation in vitro and in vivo.

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