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STEM CELL-BASED APPROACHES IN DENTISTRY

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Abstract

Repair of dental pulp and periodontal lesions remains a major clinical challenge. Classical dental treatments require the use of specialised tissue-adapted materials with still questionable efficacy and durability. Stem cell-based therapeutic approaches could offer an attractive alternative in dentistry since they can promise physiologically improved structural and functional outcomes. These therapies necessitate a sufficient number of specific stem cell populations for implantation. Dental mesenchymal stem cells can be easily isolated and are amenable to *in vitro* expansion while retaining their stemness. *In vivo* studies realised in small and large animals have evidenced the potential of dental mesenchymal stem cells to promote pulp and periodontal regeneration, but have also underlined new important challenges. The homogeneity of stem cell populations and their quality control, the delivery method, the quality of the regenerated dental tissues and their integration to the host tissue are some of the key challenges. The use of bioactive scaffolds that can elicit effective tissue repair response, through activation and mobilisation of endogenous stem cell populations, constitutes another emerging therapeutic strategy. Finally, the use of stem cells and induced pluripotent cells for the regeneration of entire teeth represents a novel promising alternative to dental implant treatment after tooth loss. In this mini-review, we present the currently applied techniques in restorative dentistry and the various attempts that are made to bridge gaps in knowledge regarding treatment strategies by translating basic stem cell research into the dental practice.

Keywords: tooth, dental stem cells, mesenchymal stem cells, induced pluripotent cells, tooth regeneration, regenerative dentistry, clinical trials.

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Introduction

The tooth is composed of the highly mineralised tissues of enamel, dentin and cementum, as well as by the soft connective tissues of dental pulp and periodontium (Mitsiadis and Graf, 2009; Nanci, 2012). Enamel is formed by the epithelium-derived ameloblasts, while ectomesenchymal cells give rise to all other tooth components. Dental pulp cells differentiating into odontoblasts produce the dentin matrix, while periodontal cells are involved in cementum and alveolar bone formation. The periodontal space contains specific fibres (*i.e.*, periodontal ligament fibres) that stabilise teeth, since they connect root cementum to the alveolar bone, as well as a variety of cell types such as fibroblasts, epithelial rests of Malassez, neuronal and endothelial cells (Mariotti, 1993; Sonoyama *et al.*, 2007).

Traumatic injuries, periodontal disease and caries are mainly responsible for pathologies affecting teeth and their surrounding tissues (Caton *et al.*, 2011). These pathologies remain a major clinical challenge, due mainly to the limited self-healing capability of dental tissues. The reparative mechanisms following dental or periodontal lesion involve highly conserved genetic programs that are active during embryonic tooth development (Aberg *et al.*, 1997; About and Mitsiadis, 2001; Giannobile and Somerman, 2003; Jin *et al.*, 2004; Magloire *et al.*, 2001; Mitsiadis and Rahiotis, 2004; Ripamonti, 2007). For example, in severe dental pulp injury or inflammation (*i.e.*, pulpitis), stem cells or/and progenitors give rise to a new generation of odontoblasts that replace the disintegrated odontoblasts. Signalling molecules released at the pathologic sites may attract these stem cells and progenitors, thus initiating the healing process that includes the reparative dentin formation (Nakashima and Iohara, 2014). However, the reparative capability of the dental pulp and periodontium is often insufficient to restore the totality of the damaged tissues. If untreated, these lesions compromise tooth integrity that can lead to more severe pathologies and tooth loss.

The increased knowledge on the reparative events within dental tissues has contributed to the proposal of alternative methods for the treatment of dental pathologies. However, traditional treatments continue to be applied in dental clinics since most of the proposed therapeutic approaches are still at the experimental level. For example, partial dental tissue repair techniques involve specialised dental materials with uncertain effectiveness and durability, while high-tech dental implants are used for tooth replacement (Esposito *et al.*, 2013; Fron Chabouis *et al.*, 2013). These materials are often used in conjunction with growth factors and molecules to enhance the regenerative capacity of dental and periodontal tissues (Pilipchuk *et al.*, 2015). Recent advances in tissue engineering and regenerative

medicine offer the potential for a long-term solution by means of biological repair or replacement of damaged teeth. The aim of this mini-review is to present the currently applied tissue repair techniques and their limitations in restorative dentistry and to introduce stem cell-based approaches as promising tools for the regeneration of injured and pathological teeth. Therefore, the different sources of dental stem cells, their differentiation potential and the current state of stem cell-based strategies for dental tissue regeneration are discussed. From a translational point of view, we summarise the various preclinical models used for the evaluation of stem cell-based therapies in dentistry and report on the recent developments and challenges related to clinical applications of human stem cells in situations that necessitate pulp and periodontal tissue regeneration.

Current therapeutic interventions in dentistry

Contemporary techniques to replace damaged dental hard tissues consist of direct tooth restorations using resin-based composites, or indirect restorations using composite or ceramic inlays and onlays (Ferracane, 2011; Fron Chabouis *et al*., 2013). While adhesion of these materials to enamel is stable over time, adhesion to dentin is weaker and unstable because of the higher levels of organic matrix of dentin when compared to enamel (Lehmann *et al.*, 2009).

Endodontic therapy is a procedure implying the removal of contaminated or necrotic dental tissues within the pulp. In case of pulp exposure or infection, the damaged part of the pulp has to be removed, leaving intact the healthy part of the pulp at the tooth root level, a process called pulpotomy (DeRosa, 2006). In selected cases, this method preserves the vitality of pulp located at the root canal, thus allowing the accomplishment of the root growth (Fuks, 2008). Traditionally, the damaged pulp is entirely replaced with inorganic materials such as gutta-percha, after root canal treatment (Ricketts, 2001). Since dental pulp provides nutrition, sensation, and defence against the various pathogens, devitalised teeth are subject to various complications causing tooth fragility and fracture (Ricketts, 2001). Therefore, maintaining of dental pulp vitality is of prime importance and this is highlighted by the emergence of new stem cell-based techniques focusing on pulp regeneration (Potdar and Jethmalani, 2015).

Currently, missing teeth are replaced with dental implants (Esposito *et al*., 2013). Their retention requires close contact of implants with the alveolar bone, a process called osseointegration (Branemark *et al.*, 1977). Most of dental implants are made of biocompatible titanium alloy and they are inserted into the bone after surgical intervention. The clinical success of implants depends on alveolar bone quality and dimensions, primary implant stability, time of masticatory loading, infections, and implant surface characteristics (Esposito *et al*., 2013; Esposito *et al.*, 2007). Recent regenerative technologies using scaffolds, stem cells, and growth factor delivery have enhanced host tissue response and implant osseointegration (Naddeo *et al.*, 2015; Pilipchuk *et al*., 2015). Recent clinical trials have demonstrated that stem cells seeded in specific scaffolds are able to generate adequate amounts of bone in order to achieve primary implant stability (Kitamura *et al.*, 2011; Windisch *et al.*, 2012). These new approaches are contributing to the progress of dental treatments, but should be further studied using controlled randomised clinical trials.

Stem cells within teeth

The theoretical basis for dental tissue repair is the activation of stem and progenitor cells that will enhance the regenerative process (Bluteau *et al.*, 2008; Caton *et al.*, 2011). Mesenchymal stem cells (MSCs) were originally isolated from bone marrow (Friedenstein *et al.*, 1970). MSCs are fibroblast-like cells capable of adhering to plastic dishes, to form colonies derived from single cells (colony forming unit fibroblasts), and to differentiate into mature cells of mesenchymal lineages such as osteoblasts and chondrocytes (Caplan and Bruder, 2001; Friedenstein *et al.*, 1970; Pittenger *et al.*, 1999; Prockop, 1997; Sudo *et al.*, 2007; Weissman *et al.*, 2001). The discovery that human adult teeth contain cells with similar functions to MSC indicated that these organs are important reservoirs of adult stem cell populations (Gronthos *et al.*, 2000). Therefore, dental mesenchymal stem cells (DMSCs) can be used for regeneration of teeth, or other organs that have limited intrinsic repair potential (Di Scipio *et al.*, 2014; Gandia *et al.*, 2008; Graziano *et al.*, 2008; Kerkis *et al.*, 2008; Nosrat *et al.*, 2001). Besides their capacity to give rise to various cell types such as chondrocytes, osteocytes and adipocytes (Bluteau *et al*., 2008; Gronthos *et al*., 2000), DMSCs may act as cellular modulators to support endogenous reparative mechanisms tissue by secretion of bioactive molecules (Choi and Reddy, 2014; van den Akker *et al.*, 2013).

Cultures of DMSCs and MSCs are indistinguishable, and at present no markers permit selective identification of either cell type from culture-expanded DMSC populations (Pagella *et al.*, 2015). Likewise, it is not yet known whether DMSC properties reside in distinct cell subpopulations. Similarly to MSCs, DMSCs are heterogeneous in their phenotype, and this could possibly reflect a coexistence of functionally distinct cell subsets (Jiang *et al.*, 2002; Muraglia *et al.*, 2000). Markers alone would not be sufficient to rule out the presence of other than DMSCs within dental tissues. Studies using single cell-derived clonal populations will be needed to determine whether DMSCs differentiation potency is inherent in individual cells from dental tissues.

Despite similar phenotypic characteristics, DMSCs from different locations have significant functional heterogeneity both *in vitro* and *in vivo*, thus indicating distinct physiological roles within teeth (Caton *et al*., 2011; Pagella *et al*., 2015). In the dental pulp, DMSCs are located mainly in two niches: the apical niche and the perivascular niche (Mitsiadis *et al.*, 2011; Zhao *et al.*, 2014). In these two niches, DMSCs could have distinct functions and still be geographically interchangeable, but a temporo-spatial hierarchy between the two DMSC niches remains to be investigated. Furthermore, the identification

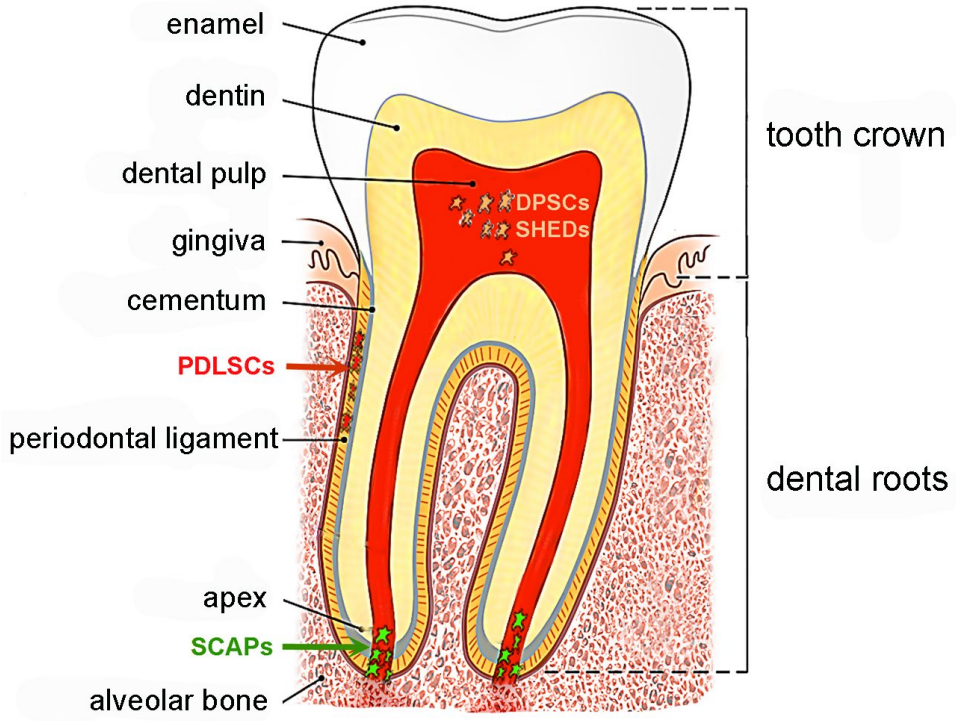

Fig. 1. Schematic representation of the main populations of mesenchymal stem cells found in human teeth. Abbreviations: DPSCs, dental pulp stem cells; PDLSCs, periodontal ligament stem cells; SCAPs, stem cells from the apical papilla; SHEDs, stem cells from human exfoliated deciduous teeth.

and characterisation of other dental stem cell niches, and the examination of how niche-derived signals are orchestrated towards tooth homoeostasis and repair is of prime importance.

The developmental origins of DMSCs in adult teeth are not known yet. They could be directly derived from dental tissues (*e.g.*, pulp, periodontium), but a contribution from blood-derived circulating MSCs has not to be excluded. Indeed, MSCs are found in the circulation and are likely to engraft in all tissues of the body (Kuroda *et al.*, 2014; Lemoli *et al.*, 2006). The embryonic origins of circulating MSCs are different from those of DMSCs (La Noce *et al.*, 2014; Pagella *et al.*, 2015), thus suggesting distinct properties and functions for these two stem cell populations. The various dental stem cell populations and their potencies are described in Fig. 1.

Dental pulp stem cells (DPSCs) were first isolated from human teeth in 2000 and are the most common source of DMSCs (Gronthos *et al.*, 2000). Due to the lack of specific DMSCs markers, generic MSC markers such as STRO-1, CD146 and CD44 are commonly used for the isolation and identification of DMSCs (Pittenger *et al.*, 1999). DPSCs are capable of differentiating into odontogenic (Gronthos *et al.*, 2000; Hayashi *et al.*, 2015; Miura *et al.*, 2003), osteogenic (d'Aquino *et al.*, 2009; de Mendonca Costa *et al.*, 2008), chondrogenic (Waddington *et al.*, 2009), adipogenic (Gronthos *et al.*, 2002; Waddington *et al.*, 2009), myogenic (Kerkis *et al.*, 2008; Pisciotta *et al.*, 2015), and neurogenic (Martens *et al.*, 2014; Nosrat *et al.*, 2001) cells *in vitro* and *in vivo*.

Stem cells from human exfoliated deciduous teeth (SHEDs) are isolated using the same procedure as for DPSCs. SHEDs express the surface molecules STRO-1 and CD146, and several neural and glial markers such as nestin and β-III tubulin (Miura *et al.*, 2003). SHEDs proliferate very fast, are capable of differentiating into odontogenic, osteogenic, chondrogenic, adipogenic, myogenic, and neurogenic cells *in vitro*, and induce bone and dentin formation *in vivo* (Kerkis *et al.*, 2008; Miura *et al.*, 2003).

Stem cells from the apical part of the dental papilla (SCAPs) are located at the root apex of the developing teeth, are highly proliferative, and exhibit increased migratory and regenerative potentials (Sonoyama *et al.*, 2006; Sonoyama *et al.*, 2008). SCAPs express the same DMSCs surface markers, as well as CD24 for which DPSCs are negative, and are able to form dentin *in vivo* (Huang *et al.*, 2009; Sonoyama *et al.*, 2006).

Periodontal ligament stem cells (PDLSCs) express the cell-surface markers STRO-1, CD146 and CD44, and are able to differentiate into adipogenic and osteogenic cells, under defined culture conditions *in vitro* (Seo *et al.*, 2004). PDLSCs can contribute to the regeneration of the periodontium by giving rise to cementum and PDL tissues *in vivo* (Seo *et al.*, 2004). Alveolar periodontal ligament stem cells (aPDLSCs) form another PDLSC population that locates close to the alveolar bone and shows great osteogenic and adipogenic capabilities (Wang *et al.*, 2011).

Stem cells from the dental follicle (DFSCs) are progenitor cells for the PDL, alveolar bone, and cementum, and express the STRO-1 and CD44 markers (Morsczeck

et al., 2005). DFSCs are able to form cementum and bone tissues *in vitro* and *in vivo* (Kemoun *et al.*, 2007; Yokoi *et al.*, 2006).

Human dental epithelial stem cells (hDESCs) can be isolated from the third molar that develops late after birth (Honda *et al*., 2007; Honda *et al.*, 2005). Another source of hDESCs is the epithelial root sheath that disintegrates into strands of epithelial cells, also known as epithelial rests of Malassez (ERM). ERM cells express epithelial stem cell markers such as Bmi-1, E-CAM, and p75, as well as embryonic stem cell markers such as Oct-4 and Nanog (Nam *et al*., 2011).

Induced pluripotent stem cells (iPSCs) may represent another source of hDESCs. Indeed, iPSCs have the capacity to differentiate into various cell lineages (Takahashi and Yamanaka, 2006) and can be technically produced from patient's cells. iPSCs technology can be progressively applied for the regeneration of dental tissues. iPSCs are able to differentiate into ameloblast-like cells in the presence of ameloblastin expressing cells (Arakaki *et al.*, 2012). Also, iPSCs are capable of differentiating into mesenchymal odontogenic cells (Otsu *et al.*, 2014).

Stem cell-based regenerative treatments in dentistry

Although basic research into dental stem cells is well documented, only very recently efforts are emerging to bridge the gap with translational research. Regenerative dentistry aims to regenerate the damaged dental tissues and to fully restore tooth anatomy and function. The functions of exogenously administered dental stem cells go beyond their differentiation potential and the replacement of cells lost due to injury or disease. Dental stem cells may create a repair-conducive microenvironment, stimulating the recruitment of endogenous stem cells or progenitors at the injury site. This insinuates that accurately designed bioactive scaffolds could generate effective dental tissue repair responses through activation and mobilisation of endogenous stem and progenitor cells, thus avoiding exogenous stem cell administration (Hayashi *et al*., 2015; Lee *et al.*, 2010; Mitsiadis *et al.*, 2012). Such innovative strategies would be easier to apply clinically and likely to encounter fewer regulatory obstacles. This raises the possibility of repairing entire dental tissues through stimulation of endogenous dental stem and progenitor cells. However, several studies in other organs – exploring the possibility of repairing tissues with the exclusive usage of scaffolds impregnated with chemotactic or growth factors – gave uncertain results, judging by the irregular and fibrotic appearance of the regenerated tissue (Lee *et al*., 2010; Zhang *et al.*, 2013).

The main approaches using stem cells for the repair of specific dental tissue, such as the pulp and the periodontium as well as for entire tooth regeneration, are described in Fig. 2.

Regeneration of pulp-dentin complex

Regenerative endodontics represents a new treatment modality that relies on the intracanal delivery of stem cells and focuses on re-establishment of pulp vitality and continued root development (Chrepa *et al*., 2015; Peters, 2014). Numerous attempts, using human DMSCs (hDPSCs), have been made in a variety of animal models in order to achieve complete pulp regeneration. Proper regeneration requires re-vascularisation and re-innervation of the pulp and allows new dentin formation (Peters, 2014). The very first experimental study using hDMSCs showed that these cells can differentiate into odontoblasts, which form dentin-like structures when transplanted together with HA/TCP ceramic powder in immunocompromised mice *ex vivo* (Gronthos *et al.*, 2000) (Fig. 2). Other, more recent studies, using hDPSCs and SCAPs seeded on poly-D,L-lactide/glycol scaffolds have confirmed the ability of human DMSCs to regenerate vascularised pulp tissues when transplanted into the empty mouse tooth root canal (Hayashi *et al*., 2015; Huang *et al.*, 2010; Volponi *et al.*, 2010) (Fig. 2). However, these experimental attempts using DMSCs transplantation were performed in ectopic locations and therefore, stem cell-based therapeutic approaches for entire pulp regeneration cannot be directly translated into the clinics. For this reason, new experimental strategies have been elaborated, where DMSCs or other stem cell population, combined with scaffolds and/or bioactive molecules, fully fill the empty pulp chamber after pulpotomy (partial pulp removal) or pulpectomy (total pulp removal) (Iohara *et al.*, 2011; Iohara *et al.*, 2013; Zheng *et al.*, 2012 ; Chrepa *et al.*, 2015; Lovelace *et al.*, 2011) (Fig. 2). Indeed, DPSCs transplanted together with granulocyte-colony stimulating factor (G-CSF), in pulpectomised teeth of dogs, were able to regenerate the entire pulp and to form new dentin (Iohara *et al.*, 2013). Similarly, bone morphogenetic proteins (BMPs) were used, a long time ago, in order to stimulate the regenerative response of the pulp. While these procedures appear to improve tissue regeneration, their true effectiveness for achieving durable repair is still unclear. For example, the fibrotic tissue that has been obtained in experiments focusing on dental pulp regeneration may not sustain a long-lasting therapeutic effect. Indeed, this fibrous pulp tissue can undergo degeneration over time or be replaced with bone.

Recent regenerative endodontic procedures that have been successfully applied in clinics are based on the bleeding technique, where the blood clot acts as a scaffold that delivers MSCs into the root canal of both immature teeth with pulp necrosis and mature teeth with apical lesions (Chrepa *et al.*, 2015; Deepak and Nandini, 2012; Lovelace *et al.*, 2011; Sonmez *et al.*, 2013). However, the current status of stem cell-based endodontic therapy is still characterised by an empirical approach (Peters, 2014).

Regeneration of periodontal tissues

Human PDLSCs have been shown to improve periodontal tissue regeneration when transplanted into immunocompromised mice, indicating their big potential for future cell-based therapies in dentistry (Seo *et al.*, 2004) (Fig. 2). The regenerative potential of autologous and allogeneic PDLSCs, as well as of DPSCs, SHEDs and bone marrow stem cells for the treatment of periodontitis has been also demonstrated in other animal models, such as the miniature swine and dog models (Ding *et al.*, 2010; Du *et al.*, 2014; Fu *et al.*, 2014; Khorsand *et al.*, 2013). In

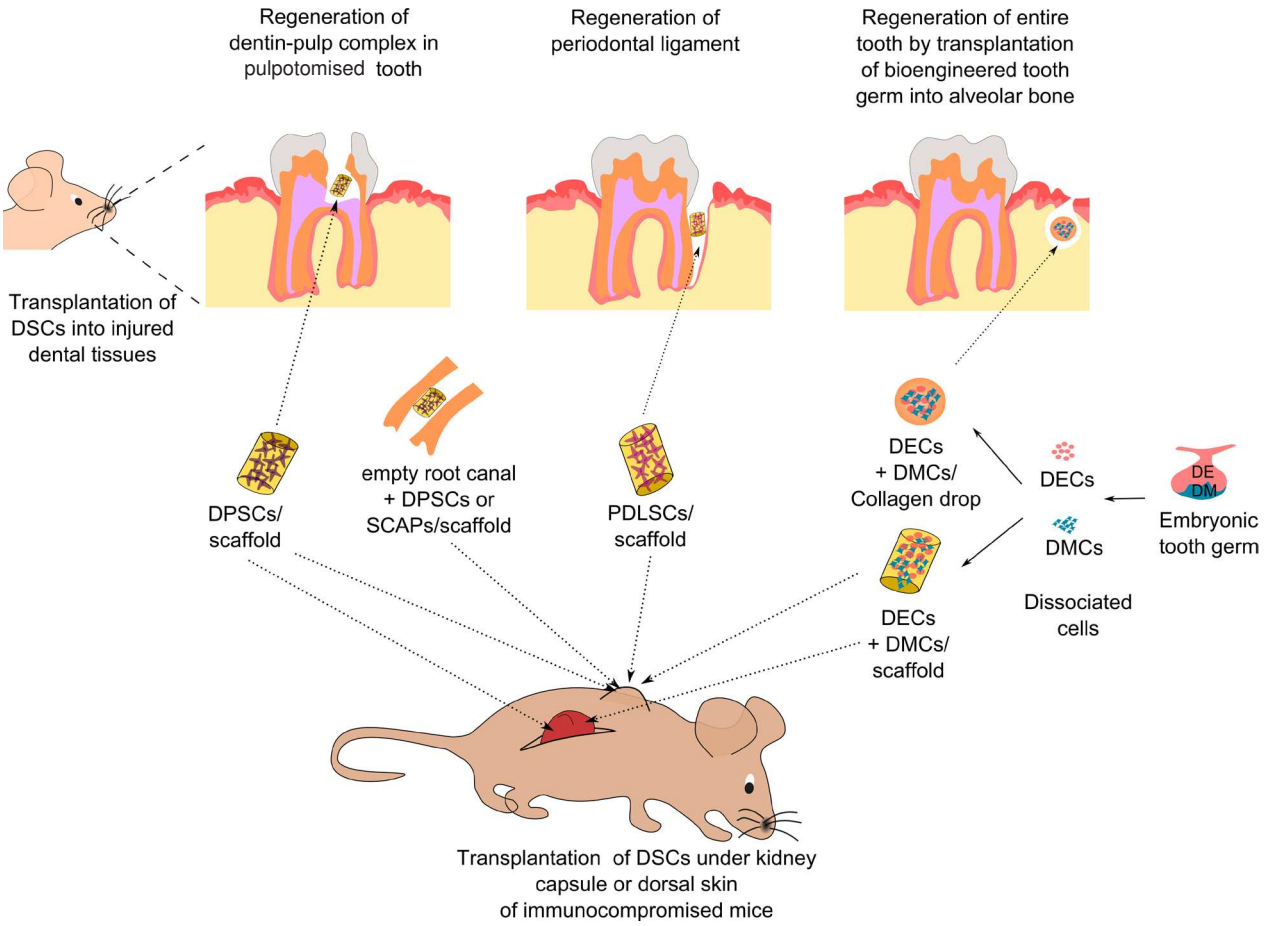

Fig. 2. Schematic representation of various stem cell-based strategies used for dental pulp, periodontium, and entire tooth regeneration. Transplantation of stem cells combined with scaffolds in the kidney or dorsal skin of immunocompromised mice is commonly used for regenerative purposes. Abbreviations: DE, dental epithelium; DM, dental mesenchyme; DECs, dental epithelial cells; DMCs, dental mesenchymal cells; DMSCs, dental mesenchymal stem cells; DPSCs, dental pulp stem cells; DSCs, dental stem cells; PDLSCs, periodontal ligament stem cells; SCAPs, stem cells from the apical papilla.

an attempt of improving stem cell-based therapies, growth factors such as platelet-derived growth factors (PDGFs) and BMPs have been used. PDGFs have been proven to stimulate periodontal regeneration (Howell *et al.*, 1997; Lynch *et al.*, 1989), while BMPs enhance alveolar bone and cementum formation (Selvig *et al.*, 2002). However, BMPs may have undesirable effects on periodontal tissues and provoke tooth ankylosis. Commercialised amelogenin extracts have been also used in dental clinics with success for periodontal tissue regeneration, but their mode of action is still unclear (Veis *et al.*, 2000).

Regeneration of alveolar bone defects, caused by periodontal diseases, is one of the major challenges for clinicians. The first clinical trial using autologous human DPSCs, combined with collagen scaffolds, for alveolar bone reconstruction was performed successfully several years ago (d'Aquino *et al.*, 2009). However, a three years follow-up study using in-line holotomography and conventional evaluation procedures has shown that the regenerated bone at the grafted sites was entirely compact and thus completely different from the normal spongy alveolar bone found in the mandibles (Giuliani *et al.*, 2013). Another study, with a significant and stable clinical outcome, was performed in a patient suffering from advanced periodontitis. Autologous bone marrow mononuclear cells (BMMNCs) embedded in a thermo-reversible gelation polymer scaffold were used successfully for alveolar bone regeneration, which was validated by clinical and radiographic evaluation in this three year follow-up trial (Sankaranarayanan *et al.*, 2013).

Regeneration of the entire tooth

Regeneration of the entire tooth would be the ideal therapeutic approach after tooth loss. The association of DESCs and DMSCs *in vitro* allows the formation of tooth germs that can be transplanted into the alveolar bone, where the germs will develop, erupt, and finally become functional teeth (Ikeda and Tsuji, 2008) (Fig. 2). In a similar assay, dental bud cells were seeded into platelet-rich fibrin scaffolds for tooth regeneration in the miniature swine model (Yang *et al.*, 2012). Another approach to obtain new brand teeth is the implantation into the jaw of tooth-shaped polymeric biodegradable scaffolds filled with DESCs and DMSCs (Oshima and Tsuji, 2014) (Fig.

2). The three-dimensional structure of the scaffolds should drive the differentiation of the transplanted stem cells into odontoblasts and ameloblasts. Indeed, bioengineered teeth using human stem cells have been formed, but only in ectopic sites, to date. Furthermore, these teeth are missing some essential tooth elements such as correct crown morphology and accomplished root formation. However, recent experiments in mice, using bioengineered approaches, have showed that it is possible to obtain functional teeth with entire roots (Oshima and Tsuji, 2014). In fact, tooth germs formed by dental epithelial and mesenchymal cells seeded into collagen drops gave rise to new functional teeth after their implantation into the mandible of adult mice. Formation of all dental tissues allows the eruption and full integration of these teeth into the recipient alveolar bone.

Recent studies have shown that re-aggregation of iPSCs-derived neural crest cells and mouse odontogenic epithelial tissues results to the generation of entire teeth *ex vivo* (Otsu *et al*., 2014). Although further technical improvements may be needed, the iPSCs technology is expected to open new horizons in regenerative dentistry.

However, such results have not yet been obtained with human cells. Various populations of human DMSCs are still under investigation, while human DESCs have not been fully studied. Moreover, time represents a great challenge for tooth regeneration: the whole process of odontogenesis in humans takes more than 7 years. This long-term physiological procedure may be discouraging for individuals missing teeth and look forward to immediate treatment outcomes.

Conclusions and perspectives

During recent decades, several stem cell lines with significant variability in potency have been isolated from human adult teeth. Considerable heterogeneity exists between individual cells isolated from the same dental stem cell pool that may affect the clinical outcomes. Therefore, the identification and purification of stem cell subpopulations with improved potency is a necessary step before application of cell-based treatment in dental clinics. In addition to the choice of dental stem cell populations, a variety of factors such as the lesion size and depth, health status of the surrounding tissues, as well as the delivery methods are also likely to impact on the success of therapy. There is still a need for understanding the mechanisms that control the fates and functions of stem cells after their transplantation into the pathological or injured dental pulp and/or periodontal tissues. Although applications using dental stem cells for pulp and periodontal regeneration have been reported in animal models, the number of clinical trials with long-term follow-up is very limited, if not inexistent. The translation of basic and preclinical stem cell research to the dental clinics is very slow, since technical, safety, regulatory and ethical concerns exist. It is obvious that patients will not benefit from these regenerative treatments until most of the above mentioned issues and concerns will be resolved, and the possible clinical restrictions will be well examined and taken seriously into consideration. Several clinical trials using autologous stem cells for pulp and periodontal tissue regeneration have already been approved and initiated, but the outcome of these studies has not yet been communicated. For example, a clinical trial sponsored by the Fourth Military Medical University in China will evaluate the effects of PDLSCs in periodontal tissue regeneration (https://clinicaltrials.gov/ct2/show/NCT01357785). Similarly, another clinical trial in Japan deals with dental pulp regeneration by transplantation of autologous pulp stem cells (http://www.stemcellsaustralia.edu.au/About-Stem-Cells/Stem-Cell-Clinical-Trials/Dental-treatments/Periodontitis.aspx).

It is obvious that stem cell-based regenerative approaches in dentistry are just at the beginning, but have the potential to benefit millions of patients worldwide. Other emerging technologies, such as nanotechnology, imaging systems and mathematical modelling should be incorporated in the stem cell research field in order to obtain faster, reliable and qualitative advancements and outcomes in dental clinics.

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Discussion with Reviewer

Jean Christophe Farges: What might be the most important hurdles and limits of regenerative endodontics in the future?

Authors: As in every dental speciality, since no generally accepted standardised protocol concerning therapies using stem cells in dentistry is available, the approaches may be very variable and as a result, the outcomes can be variable as well. For this reason, the most significant hurdles and limitations include:

1. Standardisation of clinical operation protocols for endodontic treatments using stem cells
2. Sources and standardisation of stem cells for use in clinics
3. Adaptation of endondontic techniques according to the specific anatomical shape of the roots, volume of the pulp chamber, age of the patient, status of the general health of the patient
4. Necessity of post-operative follow up
5. Cost of the treatment
6. Specialised manufactures (industrialisation) for covering large-scale treatment for all individuals

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