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REVIEW

# Stem cells and tooth tissue engineering

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**Abstract** The notion that teeth contain stem cells is based on the well-known repairing ability of dentin after injury. Dental stem cells have been isolated according to their anatomical locations, colony-forming ability, expression of stem cell markers, and regeneration of pulp/dentin structures in vivo. These dental-derived stem cells are currently under increasing investigation as sources for tooth regeneration and repair. Further attempts with bone marrow mesenchymal stem cells and embryonic stem cells have demonstrated the possibility of creating teeth from non-dental stem cells by imitating embryonic development mechanisms. Although, as in tissue engineering of other organs, many challenges remain, stem-cell-based tissue engineering of teeth could be a choice for the replacement of missing teeth in the future.

**Keywords** Teeth · Stem cells · Tissue engineering · Odontogenesis · Epithelial-mesenchymal interaction

## Introduction

Teeth are structures that develop on the maxilla and mandible of mammals and serve eating, defence and phonetic purposes. Although the morphology of teeth varies depending on species and location, they are similar in structure, being composed of enamel, dentin, pulp and periodontium. Tooth development is characterized by a series of reciprocal epithelial-mesenchymal interactions that result in differentiation and the spatial organization of cells to form organs (Fleischmajer 1967; Thesleff 2003). Since gene expression comparisons during teeth development have shown only slight differences between human and mouse teeth, mice have been used as the major animal model for studying tooth development. Human genetic diseases that encompass loss of teeth also contribute to our understanding of tooth formation (Lin et al. 2007; Tucker and Sharpe 2004).

Modern dentistry for replacing missing teeth utilizes metal implants capped with a ceramic crown (Crubezy et al. 1998). Although these prostheses serve the purpose, factors that interfere with ossointegration may cause surgery failure (Esposito et al. 1998). With advances in stem cell biology and emerging concepts of tissue engineering (Langer and Vacanti 1993), biological teeth (Sharpe and Young 2005) may become an alternative for replacing missing teeth. The idea is to cultivate stem cells with odontogenic induction signals through epithelial-mesenchymal interactions, thereby programming the stem cells to adopt dental lineages and, with the help of scaffold/extracellular matrix, to become part of a tooth.

## Inspiration from tissue regeneration

The current concept of tooth tissue engineering is inherent in the belief that naturally occurring tissue regeneration can be reproduced in vitro. Experiments over 100 years ago by H. V. Wilson (Wilson 1907), showed that isolated sponge cells could reaggreagate to reform a functional sponge. Even in vertebrates, embryonic cells dissociated from animal tissues can autonomously aggregate and reassemble to reconstitute organs (Weiss and Taylor 1960). When tissues that normally form a particular structure are combined, they can rearrange to form a topographically correct resemblance

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of that structure. Moreover, some tissues appear preferentially to associate with others, such as mesoderm with either ecto- or endoderm. However, ectoderm and endoderm seem to avoid each other (Steinberg and Gilbert 2004; Townes and Holtfreter 1955). Active amoeboid movement has been shown to be responsible for the aggregation of dissociated sponges. In embryonic amphibian cells, cells aggregate by chance but the cohesion to form clusters occurs by tissue affinity (Moscona and Moscona 1952). This reaggregation character of cells has been demonstrated in regeneration experiments aimed at achieving de novo tissue-like organization. Examples include the use of hanging-drop culture to grow mouse embryos in vitro and the formation of "mini-eyes" following the reaggregation of avian embryonic retinal cells (Layer et al. 2002; Potter and Morris 1985). Another example is the dissociated tooth primordium implanted onto the chorio-allantoic membrane of chick embryos in ovo, where components of the epithelium and mesenchymal cells sort themselves out and arrange themselves in culture, forming a tooth when transplanted into recipient animals (Main 1966). The culturing of dissociated tooth germs to form teeth with improved tissue engineering procedures (Honda et al. 2007; Nakao et al. 2007) further demonstrates that reaggregated cells proliferate and differentiate into tooth structures.

Regeneration is a unique tissue reconstitution event that can occur in a more complicated manner than cell aggregation in certain adult tissues. Some amphibians exhibit the ability to regenerate tails or limbs following amputation. Hair, epidermis and the haematopoietic system are continually replenished in mammals. This phenomenon suggests indigenous populations of cells (stem cells) exist that are equipped with regenerative capability in adults.

The reiteration of these powerful natural reaggregation and regeneration processes in the laboratory is a key challenge. One answer could be to recreate early embryonic epithelial-mesenchymal interactions that constitute the origin of most organs.

## Epithelial-mesenchymal interactions are a prerequisite for tooth formation

Epithelial-mesenchymal interactions (Arias 2001; Fleischmajer 1967; Sanders 1988) are coordinate tissue interactions observed during the formation of most embryonic organs, such as lung, palate (Kaartinen et al. 1995), hair (Chuong et al. 1996; Kollar 1970), salivary gland (Cutler and Gremski 1991), feather (Eames and Schneider 2005), kidney (Aufderheide et al. 1987; Yu et al. 2004) and tooth (D'Souza et al. 1999; Dassule and McMahon 1998; Klein et al. 2006; Maas and Bei 1997; Slavkin et al. 1968). Tooth formation is controlled by reciprocal inductive signals between ectoderm-derived dental epithelium and neural-crest-derived ectomesenchyme. The odontogenic potential, which originally resides in the dental epithelium, is shifted to the underlying mesenchyme and the acquired potential in the mesenchyme is responsible for further epithelial morphogenesis and cytodifferentiation (Thesleff and Sharpe 1997).

These epithelial-mesenchymal interactions take place not only during normal development, but also in heterospecific experiments involving the interchange of tissues from different species. For example, teeth can be produced by chick embryonic epithelium combined with mouse embryonic molar mesenchyme as intraocular grafts (Kollar and Fisher 1980). This shows that signals can cross species boundaries and that gene loss in Aves does not prevent them from forming teeth if their tissue is arranged to allow appropriate interactions for odontogenesis (Kollar and Fisher 1980). Similar experiments have been carried out with adult lizard dental papilla recombined with quail skin; these recombinants show organized tooth structures after culture on chorioallantoic membranes of chick hosts (Lemus et al. 1986). This further demonstrates that adult tissue can be endowed with the plasticity of embryonic tissue via epithelial-mesenchymal interactions. The shifting reciprocal interactions between epithelium and mesenchyme are however dependent on the developmental stage, a key factor in devising tooth tissue engineering strategies.

The underlying molecular mechanisms of epithelial-mesenchymal interactions involve diffusible proteins such as bone morphogenetic proteins (BMPs; Chen et al. 2000; Vainio et al. 1993), fibroblast growth factors (FGFs; Kettunen and Thesleff 1998; Niswander and Martin 1992), sonic hedgehog (Shh; Cobourne and Sharpe 2005; Dassule et al. 2000), the tumor necrosis factor (TNF) family (Jernvall and Thesleff 2000; Tucker et al. 2000) and Wnts (Dassule and McMahon 1998; Sarkar and Sharpe 1999). Studies have demonstrated that transgenic intervention can change cell fate through epithelial-mesenchymal interactions, e.g. the induction of hairs in the oral epithelium in transgenic mice over-expressing the transcription factor Lef1 (Zhou et al. 1995). Exogenous FGF and BMP can mimic the effects of chick skin mesenchyme to induce epithelial appendages (Chen et al. 2000). FGF working via Dlx genes can lead to a heterotopic shift of tissue interactions in the evolution of the jaws (Shigetani et al. 2002). The iterative sequential exchanges of molecules are reused in various organs and developmental stages and their precise spatiotemporal expression govern specific organ formation.

## What can we learn from embryonic tooth development?

One scheme for stem-cell-based whole tooth tissue engineering is to use tooth development as a template (Figs. 1, 2).

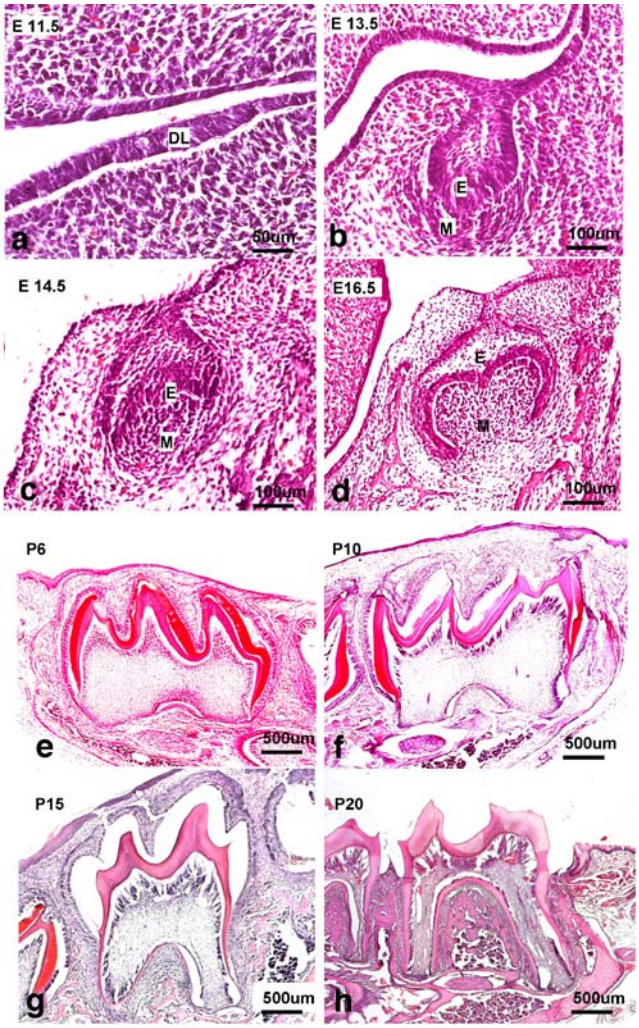


**Fig. 1** Early tooth development stages in mouse. Histological sections of molar tooth development. **a** Embryonic day 11.5 (E11.5); dental lamina (*DL*) formation. **b** E13.5; bud stage showing dental epithelium (*E*) and dental mesenchymal (*M*) condensation. **c** E14.5; cap stage. **d** E16.5; bell stage. **e** Postnatal day 6 (P6); enamel and dentine formation. **f** P10; the root starts to form. **g** P15; tooth about to erupt. **h** P20; erupted tooth with crown and root fully formed

The first sign of tooth development is a localized thickening of dental epithelium, the dental lamina, which subsequently invaginates into underlying neural-crest-derived ectomesenchyme forming a bud, following which proliferative mesenchyme condenses around the developing epithelial bud (Lumsden 1988; Thesleff and Sharpe 1997). The odontogenic signals pass from epithelium to mesenchyme at the tooth initiation stage. Mice tooth buds form at embryonic day (E) 10.5 to E11.5, one incisor and one molar primordia being initiated in each quadrant. Incisors and molars are separated by a toothless region called the diastema in which rudimentary tooth buds exist but fail to develop into teeth (Keranen et al. 1999). Mouse second and third molars form by successive thickening and budding of the epithelium from the first molars and second molars, respectively. Unlike human dentition, mice only form one set of teeth. Twenty primary tooth germs start to develop at 6 weeks after gestation, with 32 permanent tooth germs replacing the primary teeth thereafter.

The signals from the epithelium include FGF8, BMP4, Shh, and Wnt10b, which regulate the expression of several transcription factors including Barx1, Dlx1/2, Lhx6, Lhx7 Msx1, Pax9, Ptc, Gli, and Lef1 in the ectomesenchyme (Bei et al. 2000; Chen et al. 1996, 2000; Cobourne and Sharpe 2003; Dassule et al. 2000; Dassule and McMahon 1998; Grigoriou et al. 1998; Kettunen and Thesleff 1998; Maas and Bei 1997; Peters et al. 1998; Sarkar and Sharpe 1999; Vainio et al. 1993).

Tooth shape specification occurs early in tooth development, at the dental lamina stage, by homeobox genes,

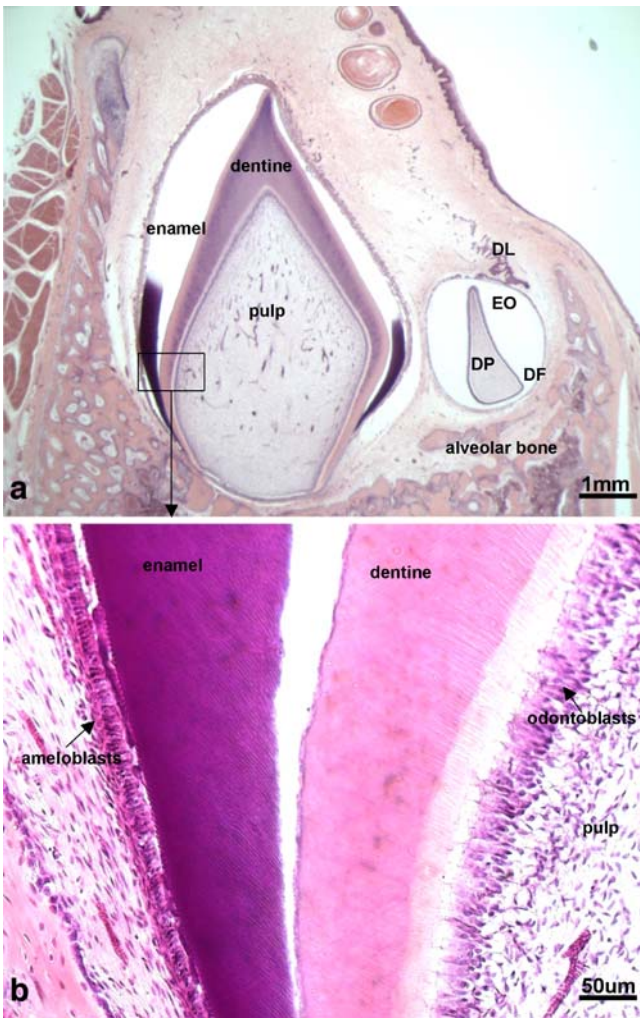


**Fig. 2** Tooth structures. **a** Early and late stage tooth germ formation showing dental follicle (*DF*), enamel organ (*EO*) and dental papilla (*DP*) with residual dental lamina (*DL*) connected to the oral epithelium. Note the enamel, dentin, pulp formation and adjacent alveolar bone. **b** Higher magnification of *boxed area* in **a**. Polarizing ameloblasts produce enamel, whereas odontoblasts in the pulp secrete dentin

which are specifically expressed in the pre-dental mesenchyme (Tucker et al. 1998). The physical morphological processes begin at the cap stage and are coordinated by enamel knots, via transient signalling centres that lie in the epithelium and that, in some way, are directed by the earlier expression of homebox genes (Jernvall et al. 1994; Thesleff et al. 2001; Vaahtokari et al. 1996). Additional enamel knots appear and pattern the crown. By the late cape stage, enamel knots disappear by apoptosis (Vaahtokari et al. 1996). After the mesenchyme receives the early odontogenic signals from the epithelium, the ectomesenchyme becomes the source of signals (Kollar and Baird 1970). The epithelium further differentiates into enamel-secreting ameloblasts, whereas the adjacent mesenchyme differentiates into dentine-secreting odontoblasts.

At the bell stage, a recognizable tooth germ is formed that consists of an enamel organ, dental papilla and dental follicle. The enamel organ is considered as a precursor of ameloblasts, being composed of the inner enamel epithelium, outer enamel epithelium, stellate reticulum and stratum intermedium. Ameloblasts can produce enamel, which is composed of more than 90% hydroxyapatite and is known to be the hardest tissue in the body (Eastoe 1960; Robinson et al. 1998). The cervical region of the inner enamel epithelium and outer enamel epithelium give rise to Hertwig's epithelial root sheath (HERS), which will initiate radicular dentin formation and determines root shape. The dental papilla will give rise to pulp tissue, which is a living connective tissue composed of fibroblasts, blood vessels, nerves, lymphatic ducts and odontoblasts. Odontoblasts are the cells derived from the mesenchymal cells in the dental papilla adjacent to the inner enamel epithelium (Ten Cate 2003). Differentiated odontoblasts are postmitotic cells that have withdrawn from the cell-cycle and cannot proliferate to replace irreversibly injured odontoblasts (Ruch 1998). Functional odontoblasts show polarized columnar morphology that shift into a resting state and become small and flat after primary dentin formation. However, odontoblasts remain functional throughout their life and can produce secondary dentin if trauma is mild (Smith and Lesot 2001; Sveen and Hawes 1968).

The dental follicle appears as a transient structure when teeth undergo morphogenesis. It is the origin of three major types of cells: cementoblasts, osteoblasts and fibroblasts. Cementoblasts secrete cementum, which is attached to the root surface. Osteoblasts produce bone around the roots of teeth. Fibroblasts produce collagen giving rise to periodontal ligaments (PDLs), which connect roots to the alveolar bone via the cementum. The PDL functions as a cushion when force is applied, as a source of sensation, and it is regarded as the main impetus for the tooth eruption process. The complex structure that includes the PDL, adjacent cementum and alveolar bone is called the periodontium.

After tooth eruption into the oral cavity, a tooth is clinically divided into two parts: crown and root. Crowns are the visible structures in the oral cavity, whereas roots are the regions that connect the surrounding alveolar bone with the cementum. Anatomically, crowns are the part covered by enamel and lie above the level of the cementoenamel junction (CEJ); roots are covered with cementum and lie below the CEJ. We have much more knowledge of the way that crowns are formed than the manner in which roots develop, little being known about the signal mechanisms of root development. Among the factors involved, FGF10 signaling stands out as a candidate for root initiation (Yokohama-Tamaki et al. 2006). A transcription factor NFI-C (nuclear factor I) is essential for root formation but currently little is known about its function (Steele-Perkins et al. 2003).

## Stem-cell-based tissue engineering

Prior to the concept of tissue engineering, partial teeth structures had been created. Dentin production was known to be induced by calcium hydroxide in the pulp-capping procedure, although the underlying mechanism was still elusive (Zander 1939). Guided tissue regeneration has been successfully used to regenerate periodontal tissues and has become a successful remedy in clinics (Aukhil et al. 1986). The emergence of tissue engineering has resulted from the needs for tissue/organ replacements and has been made possible by the marriage of biological and material sciences (Langer and Folkman 1976; Langer and Tirrell 2004; Langer and Vacanti 1993). The concept involves the use of ex-vivo expanded cells grown on a support of biocompatible material under appropriate environmental conditions to create tissue replacement and living prostheses.

Whole tooth regeneration has developed into two main streams: (1) in vitro cell culture and polymers for in vivo implantation; (2) in vivo implantation of engineered cells obtained by reiterating tooth embryonic developmental processes in vitro. The progress in stem cell biology and the identification of dental stem cells have made these cells good candidates for regenerating tooth germs by mimicking embryonic developmental processes.

## Stem cells

Stem cells are clonogenic cells that have the capacity for self-renewal and multilineage differentiation (Weissman 2000). The microenvironment in which stem cells reside is called a stem cell niche and is composed of heterologous cell types, extracellular matrix and soluble factors to support the maintenance and self-renewal of the stem cells

(Scadden 2006; Spradling et al. 2001; Watt and Hogan 2000). Stem cells can be divided into two main types; embryonic stem (ES) cells and adult stem cells. Pluripotent ES cells are derived from the inner cell mass of mammalian blastocysts and can be maintained indefinitely in culture (Evans and Kaufman 1981; Thomson et al. 1998). When injected back into blastocysts, these cells contribute to all tissues, including germ cells. The demonstration of the conversion of ES cells into differentiated neurons in an animal model of Parkinson's disease and into islet cells in diabetes, together with the successful isolation of human ES cells, has stimulated vigorous interest in their potential clinical applications (Kim et al. 2002; Lumelsky et al. 2001; Thomson et al. 1998). Human ES cells thus promise a renewable source of cells that can be stimulated to differentiate into precursors of any cell type. However, apprehension remains concerning possible tumorgenesis caused by undifferentiated ES cells when they are implanted into ectopic sites, together with ethical issues regarding the use of human ES cells.

Until recently, stem cells in adult tissues were generally assumed to be limited to specific cell fates. However, isolated adult stem cells such as bone-marrow-derived cells (Krause et al. 2001; Mezey et al. 2000), haematopoietic stem cells (Lagasse et al. 2000; Spangrude et al. 1988), neural stem cells (Bjornson et al. 1999; Gage 2000; McKay 1997) and mesenchymal stem cells (Pittenger et al. 1999; Prockop 1997) can differentiate into cell types that are usually derived from different germ layers, a feature defined as plasticity. Among these adult stem cells, bone-marrow-derived cells seem to have the largest capability to differentiate into diverse cell types, including endothelium and myoblasts (Asahara et al. 1997; Ferrari et al. 1998), and to become part of neural tissue, liver and heart (Lagasse et al. 2000; Mezey et al. 2000; Orlic et al. 2001; Petersen et al. 1999). The plasticity of adult stem cells however remains a controversial issue because of problems with adequate marker identification, tissue damage and irreproducible results (Krause et al. 2001; Morshead et al. 2002; Verfaillie 2002; Wagers et al. 2002).

## Dental pulp stem cells

Natural "reparative" dentin formation following deep caries and trauma suggests cells in the fully developed tooth can still function as odontoblasts to produce dentin-like hard tissue (Sveen and Hawes 1968). The use of tritiated thymidine to study cell division in the pulp by autoradiography following damage has revealed differences in labelling depending on the location related to trauma site (Fitzgerald et al. 1990). No labelling is observed in the existing odontoblast layer or in any specific pulp location, whereas perivascular labelling suggests that progenitor cells are located around the vessels (Tecles et al. 2005). The number of labeled cells in the pulp increases over time suggesting the existence of a continuous source of cells for replacement; the shift of labelled cells is consistent with the influx from deep tissue in the pulp to the periphery. These results support the theory that undifferentiated mesenchymal cells exist in the pulp and have the ability to differentiate into odontoblast-like cells, which are responsible for new dentin formation following dental injury (About et al. 2000; Tziafas 1995; Yamamura 1985).

Several populations of dental stem cells have been identified. Human dental pulp cells derived from developing third molars had been cultured under mineralization-enhancing conditions and form odontoblast-like cells that produce dentin-like structure in vitro and express nestin (About et al. 2000). Other studies have demonstrated that human dental pulp from adult teeth and exfoliated deciduous teeth contains dental pulp stem cells (DPSCs; (Batouli et al. 2003; Gronthos et al. 2000, 2002; Miura et al. 2003) characterized by multipotent differentiation, the expression of mesenchymal stem cell markers Stro-1 and CD146, dentin regeneration in vivo, and colony-forming ability in culture (Friedenstein et al. 1974). In addition to mineralization, DPSCs and stem cells from human exfoliated deciduous teeth (SHED) can express neural markers and have the potential to differentiate into adipocytes (Gronthos et al. 2002; Miura et al. 2003). Compared with DPSCs, SHED show higher proliferation rates and increased population doublings and can form spherical aggregations. SHED are thus considered distinct from DPSCs. When DPSCs are implanted subcutaneously into immunocompromised mice, dentin-pulp-like complex but not lamellar bone is formed. Dentin and bone can both be produced, but not dentin-pulp complexes by the transplantation of SHED in vivo. Although DPSCs and SHED seem to contain stem cells, they might also contain heterogeneous populations of cells from the pulp (Gronthos et al. 2000, 2002; Liu et al. 2006). Mice pulp stem cells have been investigated in transgenic mice that carry a green fluorescent protein (GFP) reporter expressed in dental mesenchymal cells; these studies indicate that mesenchymal cells from postnatal mice incisors can give rise to odontoblasts, osteoblasts and chondrocytes and functionally produce matrix when transplanted into kidney capsules (Braut et al. 2002, 2003). This osteogenic potential of mouse dental pulp is consistent with early studies from adult rodent pulp (Zussman 1966) and human DPSCs and SHED.

Odontoblast-like cells have been postulated to develop from perivascular cells (Senzaki 1980), and DPSCs are considered to express endothelial and smooth muscle markers (Gronthos et al. 2000). Since endothelium, pericytes and surrounding smooth muscle could be candidate

contributors to a perivascular niche for stem cells (Doherty et al. 1998; Nehls and Drenckhahn 1993; Shen et al. 2004), immunoselection by using STRO-1 and markers of smooth muscle cells, endothelial cells and pericytes has been performed to identify dental mesenchymal cells further. The evidence has shown that DPSCs represent a pericyte phenotype by demonstrating the co-expression of STRO-1 and pericyte-associated antigen 3G5 (Shi and Gronthos 2003). From this study, one can assume that the perivascular region in the pulp is the niche for DPSCs or that pericytes and DPSCs are the same entity.

A cell population from DPSCs/SHED has been sorted by mesenchymal markers but not haematopoietic markers (c-kit+/STRO-1+/CD34+[/CD45-]) and has been distinguished as a subpopulation that can efficiently produce woven bone without osteoinduction in vitro. These cells are able to grow into remodelling lamellar bone when implanted into immunocompromised rats (Laino et al. 2005, 2006a, b) and can also differentiate into adipocytes and myotubes. d'Aquino et al. (2007) and Laino et al. (2006b) have subsequently found that not only osteoblast differentiation markers are expressed, but also endothelial markers, in transplants derived from sorted DPSC. This subpopulation of cells might thus have potential as a source for bone regeneration.

Although Pittenger et al. (1999) have claimed that true mesenchymal stem cells can be characterized by a list of undefined markers, the resulting population still shows various degrees of multipotentiality. Moreover, clonal strains exhibiting the ability to regenerate bone in vivo can still demonstrate non-identical marker expression (Satomura et al. 2000). The isolation of dental mesenchymal stem cells faces the same difficulties as the isolation of other postnatal stem cells, i.e. the lack of specific stem cell markers. Teeth are however one of the most accessible organs for the derivation of stem cells, as DPSCs can be cryopreserved and retain their multipotential differentiation ability (Papaccio et al. 2006; Zhang et al. 2006). In addition, under appropriate conditions, DPSCs have a better immunoregulatory capacity that abolishes T-cell alloreactivity than bone marrow stem cells (BMSCs). This finding plus their high proliferative ability makes DPSCs a good candidate for organ transplantation (Pierdomenico et al. 2005). Dental mesenchymal stem cells can thus probably provide a population of autologous stem cells for therapeutic purposes (Reynolds and Jahoda 2004; Shi et al. 2005).

Another unique population of stem cells isolated from human developing root apex called SCAP (stem cells from root apical papilla) has been demonstrated to differentiate into odontoblasts and adipocytes. Its higher proliferative potential compared with DPSCs makes this population of cells suitable for cell-based regeneration and preferentially form roots (Sonoyama et al. 2006).

## PDL stem cells

In common with dental pulp, the PDL has a certain degree of regenerative ability after mild trauma (Amar and Chung 1994). Earlier studies have identified dividing cells emerging from wounded PDL as fibroblast-like heterogeneous cells, derived from the vicinity of blood vessels, but not of haematopoietic origin (Gould et al. 1977, 1980; Ivanovski et al. 2001; Limeback et al. 1983; McCulloch 1985). Undifferentiated paravascular progenitors are further suggested as the source of these fibroblasts (Gould 1983; McCulloch et al. 1987). Recently, multipotent progenitors from human PDL have been validated by using the same methods as those characterizing DPSCs/SHED by single-colony selection and magnetic activated cell sorting with STRO-1 (Seo et al. 2004). PDL stem cells (PDLSCs) are characteristically STRO-1 and CD146/MUC18 positive (Filshie et al. 1998). Under defined culture conditions, PDLSCs are multipotent and show a differentiation ability into cementoblast-like cells, adipocytes and fibroblasts. When PDLSCs are transplanted into immunocompromised mice, cementum/PDL-like structures are formed. These cells have been verified as maintaining their stem cell properties and tissue regeneration capacity, even after recovery from solid-frozen human primary tissue (Seo et al. 2005). This finding suggests that the preservation of PDL from extracted teeth could be used for therapeutic purposes in the future. The possibility of constructing the root/periodontal complex has further been successfully demonstrated by the use of a hydroxyapatite/tricalcium phosphate block containing SCAP coated with Gelofoam containing PDLSCs into tooth sockets in mini-pigs (Sonoyama et al. 2006).

## Dental follicle stem cells

The dental follicle has long been considered as a multipotent tissue because of its ability to generate cementum, bone and PDL from the homogeneous-like ectomesenchymal derived fibrous tissue (Handa et al. 2002a, b; Luan et al. 2006). Bovine dental follicle cells show a differentiation ability to form cementoblasts when transplanted into immunodeficiency mice (Handa et al. 2002a, b). Human dental follicle progenitor cells can be obtained from human third molars and are characterized by their plastic attachment in culture and expression of makers such as Nestin and Notch-1. These cells are considered to be able to differentiate into PDL-like structures, bone and cementum (Morsczeck et al. 2005). Further analysis has shown the presence of heterogeneous cell populations in developing dental follicles after analysis of their mineralization characteristics in vitro and the growth factor and matrix

protein gene expression patterns from several cloned cell lines under the same culture conditions (Luan et al. 2006). Committed progenitors contributing to various tissue formation might well have been isolated in this study, although the existence of a common stem cell that differentiates by induction cannot be excluded (Jo et al. 2007; Kemoun et al. 2007).

## Dental epithelial stem cells

Ectoderm-derived ameloblasts are unable to proliferate or regenerate after they reach the mature stage of development. Continuously growing mouse incisors and continuously growing molars in some mammalian species show replenishing populations of enamel organs composed of a core of stellate reticulum, stratum intermedium and surrounding enamel epithelial cells and provide models for the identification of dental epithelial stem cells and for the study of dental epithelium regeneration (Smith 1980).

Mice have an epithelial stem cell niche located at their incisor labial apical ends, known as the cervical loop; this is the junction of the inner enamel epithelium (IEE) and the outer enamel epithelium (OEE) at the apex of the enamel organ (Fig. 3). The cervical loop has been considered to be a determinative region in odontogenesis due its ability to produce enamel and dentin (Slavkin et al. 1968, 1989). From early morphological observations, most of the mitoses were found in IEE and stratum intermediate, whereas stellate reticulum and OEE had lower proliferation activity (Chiba 1965). It was noticed that there is one specialized structure found at apical region of the labial cervical loop in mouse incisors, the "apical bud" (Ohshima et al. 2003). Apical buds were suggested as stem cell compartments, they could differentiate into ameloblasts through interaction with mesenchymal cells, including DPSCs. (Harada et al. 1999; Morotomi et al. 2005; Yu et al. 2007). FGF10, Notch and Sprouty have been suggested to play roles in the continuous growth of the mouse incisors and maintenance of the stem cell niche (Harada et al. 1999, 2002; Klein et al. 2006; Tummers and Thesleff 2003; Yokohama-Tamaki et al. 2006).

## Current progression in tooth regeneration

From early studies, we know it is possible to regenerate tooth crowns if suitable environments are provided, such as in vitro organ culture, grafts on chick chorio-allantoic membrane, ocular grafts, subcutaneous transplants or kidney capsules (Hay 1961; Morio 1985; Slavkin and Bavetta 1968; Yamada et al. 1980; Yoshikawa and Kollar 1981). These culture sites provide nutrients and oxygen to nurture tooth germs. Thus, several choices exist for cultivating small-sized primordia, such as those of teeth, before they can be implanted into their anatomical sites. Optimally, the setting should reproduce cells in a three-dimensional (3D) organization, support the differentiating function, and avoid xenograft rejection. Organotypic

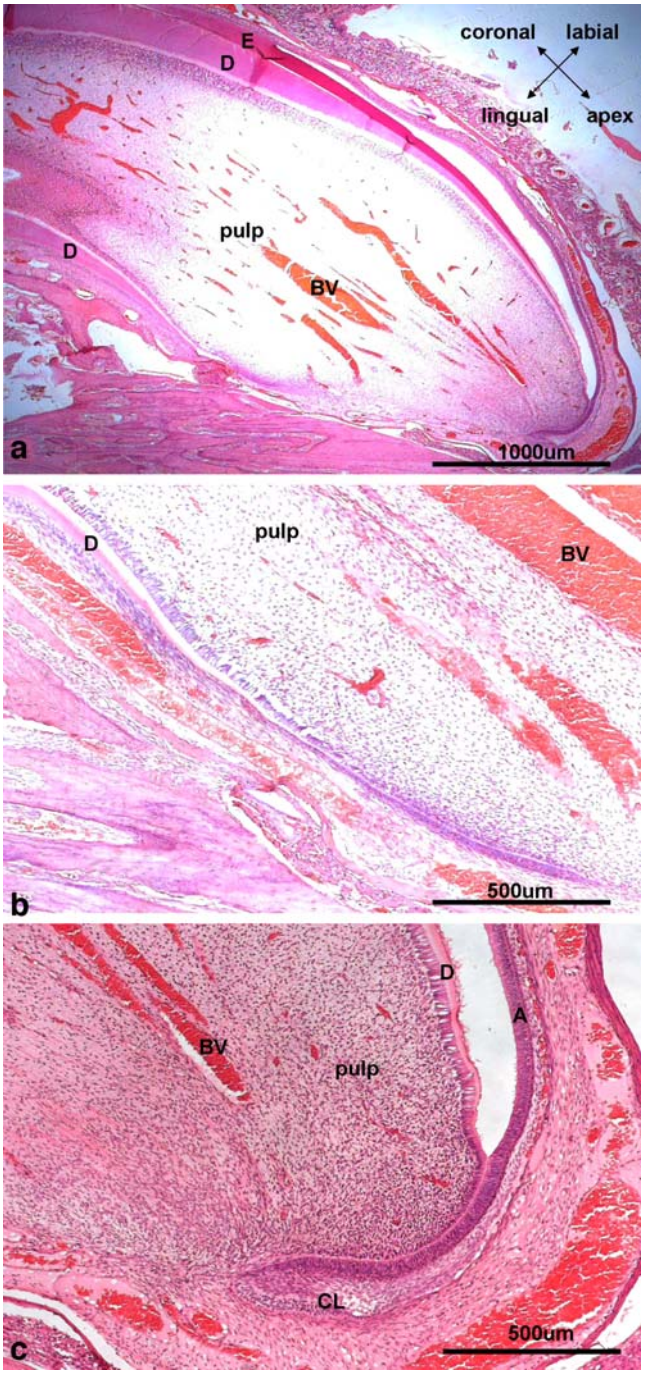


**Fig. 3** Apical end of tooth. **a** Vertical section of mouse incisor showing the labial side with both enamel (*E*) and dentine (*D*) and the lingual side with dentin only (*BV* blood vessels). **b** Higher magnification of the lingual side of the incisor. **c** Both odontoblasts and ameloblasts (*A*) are present in the labial side of the incisor. Note the cervical loop (*CL*) composed of ameloblasts and precursor cells

culture is therefore arguably the most relevant model system to grow teeth in vitro (Fig. 4).

Applying traditional tissue engineering methods, tooth-like structures can be produced from biodegradable polymer scaffolds seeded with dissociated tooth germs from postnatal pigs or cultured rat tooth bud cells and grown in the omentum of immunocompromized mice. The structures contain enamel, dentin and pulp but, in majority of the cases, form in a disoriented way and do not reach the expected size or shape of the scaffold (Duailibi et al. 2004; Honda et al. 2005; Young et al. 2002). These results have however confirmed the cell reaggregation ability of dissociated tooth germs.

Examination of the autonomous reaggregation capacity of cap-stage tooth germs has further disclosed that cap-stage mesenchyme can induce dental epithelial cell histogenesis, even when positional memories have been lost (Hu et al. 2005a, b). The tooth morphogenesis obtained here shows characteristic developmental features including functional odontoblasts, ameloblasts, pulp and cusp formation and, following longer culture, roots and PDL can also form (Hu et al. 2006a). These experiments have demonstrated that the cap-stage mesenchyme might control cusp number and have further reinforced the powerful mesenchymal and dental epithelium reaggregation ability.

Recently, cell-scaffold constructs in a co-culture system have appeared to improve the tooth shape control. Polyglycolic acid mesh and latter collagen sponge, together with the sequential seeding of dental mesenchyme in direct contact with dental epithelium, have demonstrated orga-

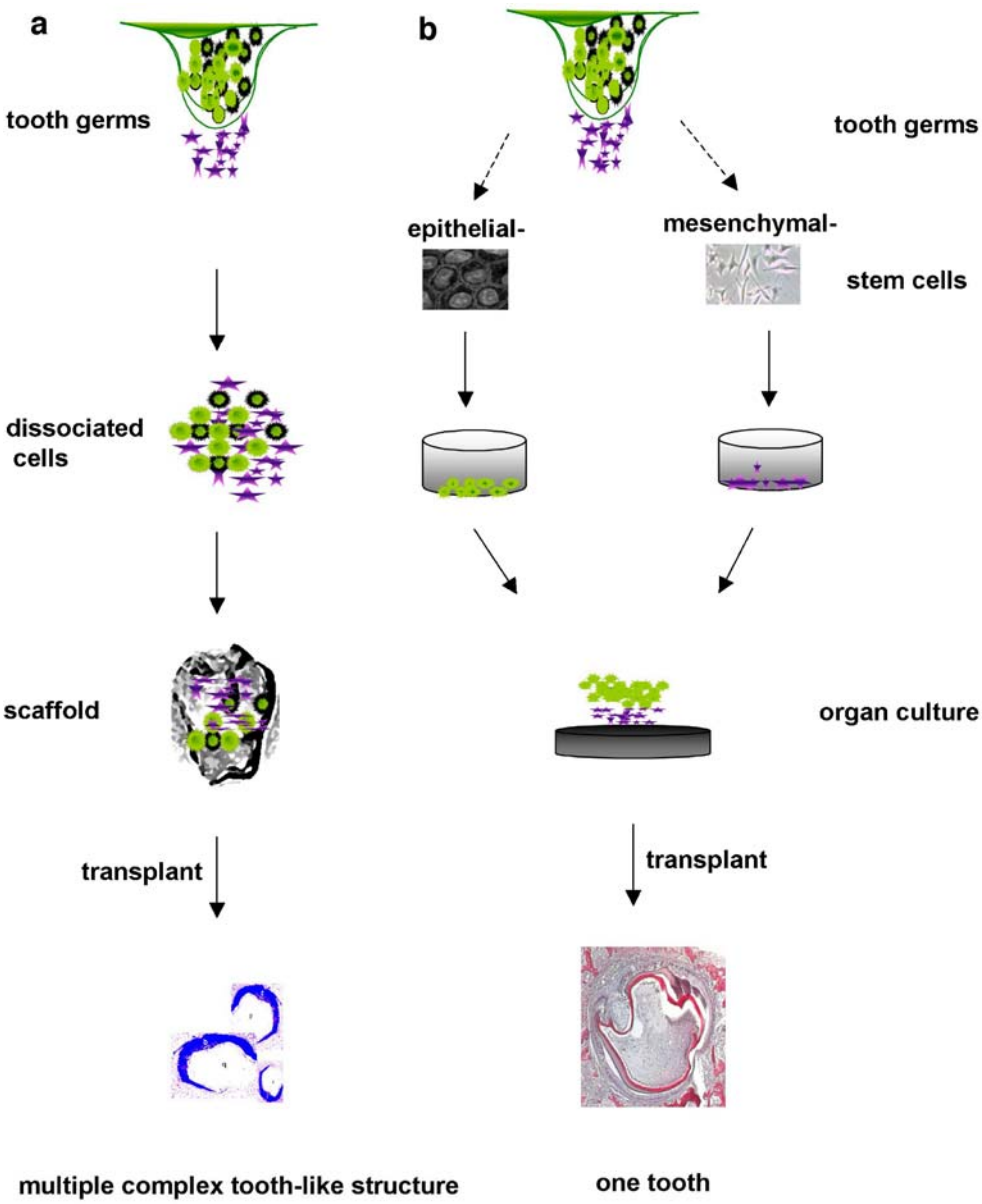


**Fig. 4** Two current ways of tooth tissue engineering. **a** Dissociated tooth germs grow on a tooth-shaped scaffold and produce small complex tooth-like structures. **b** Epithelial and mesenchymal (stem) cells, either from tooth germs or other sources, are grown in organ culture and, through epithelial-mesenchymal interactions, can form organized teeth

nized tooth structures derived from dissociated postnatal canine and porcine molar tooth germs (Honda et al. 2006, 2007).

Although in vitro cell culture methods are being progressively developed and improved, the task of finding stem cell populations to replace embryonic dental epithelium and dental mesenchyme continues. The search for possible sources of dental epithelium and dental mesenchyme is based upon the finding that dental epithelium can be created from non-teeth-bearing areas and that non-dental mesenchyme can become competent for odontogenesis on interaction with inductive dental epithelium (Kollar and Fisher 1980; Mina and Kollar 1987). These experiments have established the feasibility of instructing non-dental tissues to develop into teeth. Presumptive dental stem cells such as DPSCs have been used for partial teeth structures but not a whole biological tooth germ with the equivalent function (Gronthos et al. 2000). Because of the limited in vitro expansion ability of DPSCs, SHED and other dental stem cells, efforts have been directed into establishing cell lines; several lines have been created by the insertion of transgenes, including human telomerase reverse transcriptase, SV40 T antigen and human papillomavirus genes or by the spontaneously immortalization of dental follicle cells, HERS, cementoblasts, dental papilla, PDL, cervical loop epithelium, ameloblast and odontoblast lineage cells (Arany et al. 2006; D'Errico et al. 2000, 1999; Fujii et al. 2006; Kawano et al. 2004; Komine et al. 2007; Nakata et al. 2003; Saito et al. 2005; Somerman et al. 1999; Thonemann and Schmalz 2000; Yokoi et al. 2007; Zeichner-David et al. 2003). One of the major technical advantages is that cells can be produced, characterized and controlled relatively easily without the need to derive material repeatedly from primary tissue. These lines could therefore be used for generating dental structures in vivo.

A bigger challenge is to identify non-dental stem cells as possible replacements for dental epithelium and dental mesenchyme. ES cells, neural stem cells and bone-marrow-derived cells have been demonstrated to respond to the inductive signals from dental epithelium and to express odontogenic genes and in some cases form tooth crowns (Modino and Sharpe 2005; Ohazama et al. 2004). Recombinants between odontogenic inducing epithelium and non-dental stem cells (neural stem cells, mouse ES cells and mouse bone-marrow-derived cells) have been shown to express early mesenchymal odontogenic makers such as Pax9, Msx1 and Lhx7 (Ohazama et al. 2004). The ability of bone-marrow-derived cells to develop further into teeth with organized enamel, dentin and pulp formation surrounded by bone has been established following transplants into mouse kidney capsules. The teeth produced are of the appropriate size and shape for mouse molars. These results reinforce the idea that odontogenic signals might instruct tooth crown formation in embryonic or adult stem cells without a scaffold. Additional studies involving the use of c-kit-enriched bone-marrow-derived cells have demonstrated their ability to differentiate into ameloblast-like cells (Hu et al. 2006b). These experiments suggest bone-marrow-derived cells, possibly BMSCs, have the potential to become both dental mesenchyme and dental epithelium.

For biological teeth to perform adequately, they must be able to grow and integrate into the existing alveolar bone of the jaws. Amazingly, implanted embryonic tooth germs can develop normally and form teeth when transplanted into the adult mouth (Ohazama et al. 2004). Further analyses using 3D imaging have shown that these teeth not only have normal morphology, but also can erupt into the oral cavity and form functional roots (P. Sharpe, unpublished; Fig. 5)

## Concluding remarks

From recent experiments, basic principles have been established that teeth can be produced from stem cells of either dental or non-dental origin. Organ culture makes it possible to grow mouse tooth germs but further efforts are required to establish optimal conditions for growing human tooth germs, which are larger and take a longer time to develop than those of mouse. Whether teeth derived from autologous cells, such as DPSCs and SHED, which can be deposited in cell banks, can be produced remains to be established. Immune responses to the cells used are unknown but these have to be understood before any clinical trials can be performed. Although human and mouse BMSCs do not seem to express co-stimulatory antigens, DPSCs may not elicit humoral immune responses and may thus be immunoprivileged (Caplan and Bruder 2001; Pierdomenico et al. 2005).

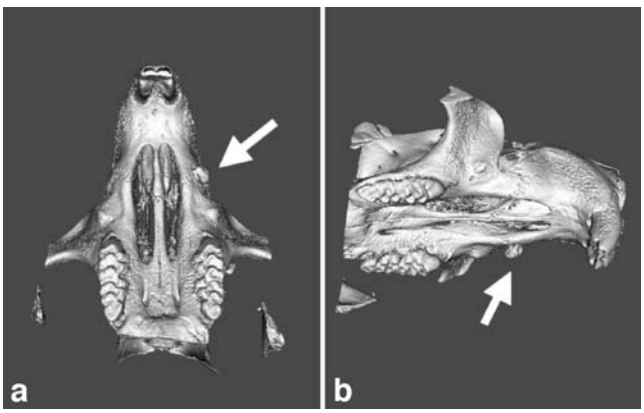


**Fig. 5** Implanted tooth germ grown in the diastema. Transplanted mouse incisor tooth germ (E13) after 20 days in the maxillary diastema of an adult mouse. The tooth has erupted. **a** Horizontal view. **b** Lateral view

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