Review Article

Recent Approaches in Tooth Engineering Research

(engineering / tooth / stem cells /culture techniques)

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Abstract. Tooth absence and defects caused by various reasons are frequent events in humans. They are not life threatening but may bring about social consequences. Recent dentistry provides solutions in the form of prosthetics or dental implants; however, several complications and distinct limitations favour bioengineering of dental and periodontal structures. At least two types of cells (epithelial and mesenchymal) have to be recombined to produce a new functional tooth. Moreover, the tooth must be vascularized, innervated and properly anchored in the bone. To study these issues, different approaches have been established in both basic and applied research. In this review, recent strategies and techniques of tooth engineering are comprehensively summarized and discussed, particularly regarding manipulation using stem cells.

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Abbreviations: ASCs – adult stem cells, BMDCs – bone marrow-derived cells, CL – cervical loop, DFSCs – dental follicle stem cells, DMEM – Dulbecco's Modified Eagle's Medium, DPSCs – dental pulp stem cells, ED – embryonic day, ERM – epithelial rests of Malassez, ESCs – embryonic stem cells, GCHT – gelatin-chondroitin-hyaluronan-tri-copolymer, HA/TCP – hydroxyapatite/tricalcium phosphate, HERS – Hertwig's epithelial root sheath, iPSCs – induced pluripotent stem cells, OLCs – odontoblast lineage cells, PDLSCs – periodontal ligament stem cells, PGA – polyglycolic acid, PLGA – poly(lactic-co-glycolic acid), SCs – stem cells, SCAPs – stem cells from the apical part of the papilla, SHEDs – stem cells from human exfoliated deciduous teeth.

Introduction

Dental pathologies such as carries, pulp infection, traumas or periodontitis are the most frequent reasons for tooth loss in adult human population. Furthermore, congenital dental defects leading to lower tooth number are not negligible events (reviewed e.g. in Matalova et al., 2008). Although the tooth/teeth deficiency in humans is not a life-threatening event, it may lead to difficulties with food intake, speech and individual appearance that often affects self-confidence and negatively influences social interactions. Recent dentistry provides solutions in the form of prosthetic devices and application of dental implants consisting particularly of titan fixtures and ceramic crowns. However, these materials are not always compliant with the native tissues (reviewed e.g. in Hacking and Khademhosseini, 2009) and their application or healing may also be complicated by the alveolar bone loss and/or inflammatory processes (reviewed in Hsu et al., 2014; Peake, 2012). Since the implants attach to the bone, the mastication forces are transmitted directly to the bone, and this is another reason for implant failures. Recently, molecular dentistry, regenerative endodontics and tissue engineering make efforts to shift from artificial restoration to natural regeneration (Modino and Sharpe, 2005). This review provides a brief overview of the most important aspects of tooth engineering with focus on recent techniques used in tooth reconstruction.

Tooth engineering – basic know-how

The principles of tooth engineering come out from physiological processes and molecular pathways known from odontogenesis *in vivo* (Ohazama et al., 2004). Tooth development is based on reciprocal interactions between the dental epithelium and ectomesenchyme derived from the cranial neural crest (reviewed in Tucker and Sharpe, 1999; Miletich and Sharpe, 2004). The odontogenic potential arises in the dental epithelium, and later shifts to the mesenchyme (Thesleff and Sharpe, 1997). During the tooth development (formation of epi-

thelial thickening, tooth bud, cup, bell, secretory stage and eruption), a large network of molecular signals is involved (reviewed in Bei, 2009; Tummers and Thesleff, 2009). Among others, FGFs, BMPs, TGFβ and SHH have been revealed as the most important molecular players (Tucker et al., 1998). The final tooth consists of complex hard tissues of different origin (epithelial enamel, mesenchymal dentin and cementum), mesenchymal cells of dental pulp, and periodontal fibres. Gradually, nerves and vasculature arise. Based on the knowledge of *in vivo* development, tooth engineering generally demands two cell types: epithelium and mesenchyme.

Tooth engineering – source of cells

Several types of cells have been suggested to be capable of response to odontogenic signals. Either dental or non-dental pluripotent/multipotent cells are considered for usage (reviewed in Yen and Sharpe, 2006). Embryonic stem cells (ESCs) isolated from blastocysts or adult stem cells (ASCs) residing in organ niches have been described as the potential players in tooth engineering. Since the application of human ESCs faces the ethical barriers as well as potential risks, ASCs appear to be more promising for future tooth-related tissue engineering. Additionally, umbilical cord stem cells, induced pluripotent stem cells (iPSCs) and bone marrow-derived cells (BMDCs) seem to have certain importance (Arien-Zakay et al., 2010; Tamaoki et al., 2010).

A wide range of progenitor cells residing in dental tissues have been investigated: 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), and periodontal ligament stem cells (PDLSCs) (reviewed in Volponi et al., 2010; Demarco et al., 2011; Estrela et al., 2011; Volponi and Sharpe, 2013; Han et al., 2014; Otsu et al., 2014). These progenitors perform from moderate to high proliferation rate and also plasticity and can be easily obtained e.g. from extracted third molars. However, alterations of their potential with development have to be taken into account (Balic et al., 2010; Duailibi et al., 2011).

Although several sources of mesenchymal dental progenitors are available as listed above, no replacement of dental epithelial cells capable of odontogenic induction has been identified. Therefore, recent tooth engineering has to rely on the endogenous dental epithelium from early-staged embryos (Volponi et al., 2010). Nevertheless, there are elegant techniques for epithelium separation from the embryonic mandible (Fig. 1A-D), widely and successfully used in odontogenic studies (e.g. Ohazama et al., 2004).

Additionally, there are limited internal sources of epithelial stem cells in the teeth, at least in some species. Rodent incisors are characterized by their ever-growth enabled by the epithelial stem cell niche located in the cervical loop (CL) (Harada et al., 1999; Mitsiadis et al., 2007). Recently, the potential of progenitor cells to differentiate into ameloblast-like cells has been investigated in analogical structures to the incisor CL: Hertwig's epithelial root sheet (HERS) and epithelial cell rests of

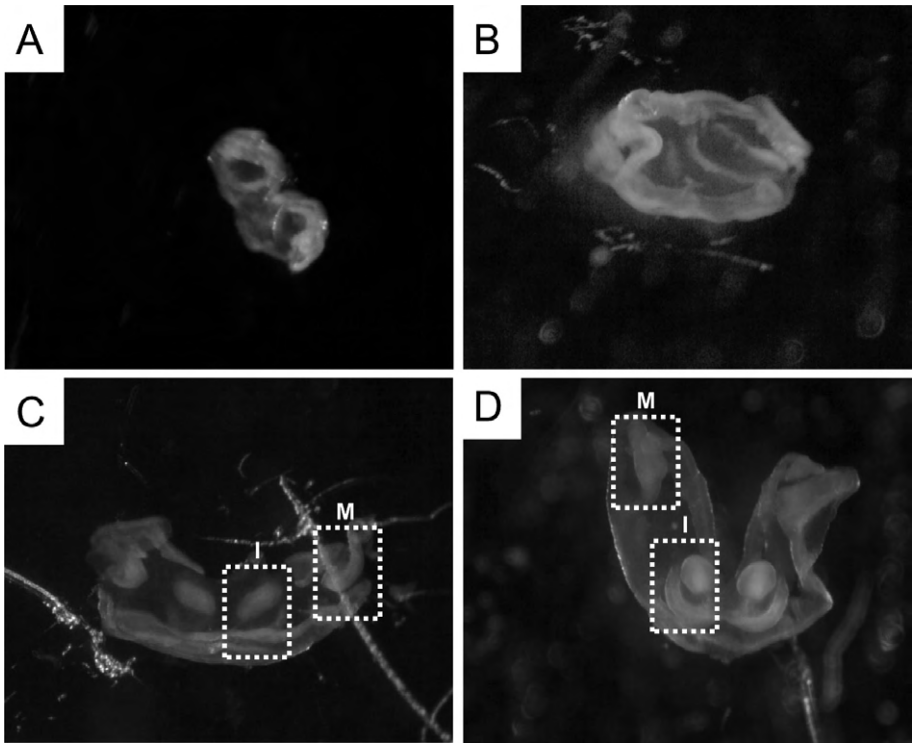

Fig. 1. Oral/dental epithelium separated from mandibles of murine embryos at different stages of development. Oral epithelium at ED 10.25 (**A**), oral epithelium at ED 11 (**B**), oral epithelium at ED 12, molars reached the bud stage (**C**), oral epithelium at ED 14, molars reached the cap stage (**D**); M (molar), I (incisor).

Malassez (ERM) (Shimonishi et al., 2007; Shinmura et al., 2008). Similarly, the odontogenic potential has also been examined in oral mucosa (Takahashi et al., 2010) and palatal epithelium (Nakagawa et al., 2009). Other trends focus on establishment of dental epithelial cell lines (Komine et al., 2007).

Tooth engineering – approaches

In the recent approaches, several procedures were established to create a biotooth:

A) Dental cell/tissue re-associations

In these types of experiments, cells/tissues of dental origin are dissociated and subsequently re-associated to demonstrate the ability of dissociated cells to re-aggregate and reform the tooth (Iwatsuki et al., 2006). The cells/tissues are usually obtained from tooth primordia, but due to massive cell losses, more primordial cells are necessary to produce one tooth. These experiments may be performed in semi-solid medium (Hu et al., 2005, 2006a; Nait Lechguer et al., 2009), modified Trowel's system (see Fig. 2B) (Nakao et al., 2007) or scaffolds (Young et al., 2002; Kuo et al., 2008). The importance of these studies is to provide the basic developmental knowledge used in advanced tooth engineering, such as tooth morphology, vascularization, innervation, mineralization and mechanical properties of the engineered tooth (Hu et al., 2006a; Ikeda et al., 2009; Nait Lechguer et al., 2008, 2009; Kökten et al., 2014). Importantly, the vascularization of the engineered tooth comes from the host (Nait Lechguer et al., 2008). A recent study showed that innervation is achieved only in the case when extra trigeminal ganglia were added and suppression of immunity was induced in the mouse model (Kökten et al., 2014). Regarding tooth morphology, the crown shape has been described to be controlled by the number of mesenchymal cells (Hu et al., 2006a; Nait Lechguer et al., 2009). Further, tooth germ re-associations have been observed to be more successful at earlier stages of tooth development (Yoshikawa and Kollar, 1981; Nakao et al., 2007; Keller et al., 2011). Additionally, the odontogenic potential has been observed to fade in the course of cultivation of mesenchymal dental cells *in vitro* (Keller et al., 2011).

B) Recombination of inducing dental tissues/ cells with inducible progenitor cells

The concept of using dental tissues to induce stem cells to differentiate into the odontogenic lineages was introduced by Ohazama et al. (2004). The study proved that the engineered tooth might be formed with contribution of different stem cells. Recombination experiments using tissues or cells are commonly performed in the systems of semi-solid media (Hu et al., 2006b; Nait Lechguer et al., 2009), modified Trowel's system (Ohazama et al., 2004) or scaffolds (Yang et al., 2005). Recent reports have shown that the bone marrow cells can be used in re-associations instead of the dental mesenchyme at ED14.5 to achieve odontoblast differentiation (Ohazama et al., 2004; Nait Lechguer et al., 2009). Similarly, these cells can give rise to ameloblast-like cells when re-associated with the dental mesenchyme

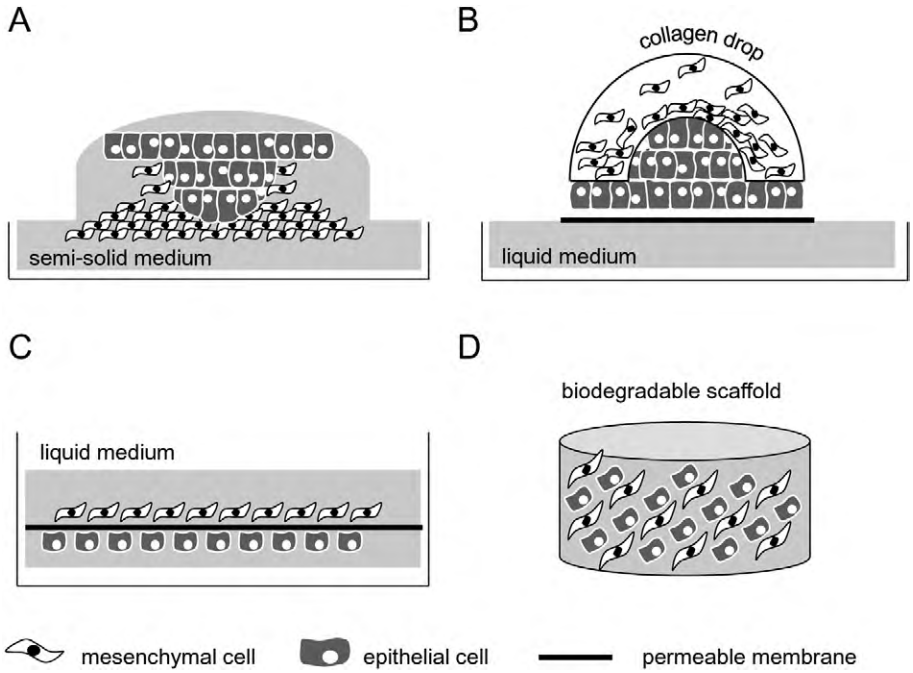

Fig. 2. Schematic illustrations of recently used techniques in tooth tissue engineering. Mesenchymal cells and early-stage dental epithelium are recombined in a semi-solid medium system (**A**), mesenchymal cells and early-stage dental epithelium are recombined in a collagen drop on a permeable membrane (**B**), populations of epithelial and mesenchymal cells are co-cultured in a system separated by a permeable membrane (**C**), heterogenous population of epithelial and mesenchymal cells is seeded on a biodegradable scaffold (**D**).

(Hu et al., 2006b). Immortalized odontoblasts (OLCs) were used for recombination with the dental epithelium (ED14.5) to develop dental structures (Arany et al., 2009).

C) Induction of odontogenic potential by molecular and other factors

Instead of using cells, the induction of odontogenic properties may also be achieved by different stimuli such as molecular factors. The influence of distinct molecular players has already been studied in odontogenic differentiation (e.g. Wang et al., 1998, 2010). Novel *in vivo* trends in reparative dentistry suggest a relief of minor defects by cell homing using certain types of molecules (Kim et al., 2010; Mao et al., 2010).

Experiments *in vitro* have also focused on co-cultivations of dental progenitors with supporting cell lines (Shinmura et al., 2008; Arakaki et al., 2012). Alternatively, differentiation of adult stem cells has been described to be influenced by the developmental environment; SCAPs and DPSCs mixed with a hydroxyapatite/tricalcium phosphate (HA/TCP) carrier and SHEDs placed in a scaffold covered by dentin slices have differentiated into dental structures after implantation in animal models (Gronthos et al., 2000; Sonoyama et al., 2006; Cordeiro et al., 2008). Further, mechanical loads may facilitate the process of odontogenic differentiation (e.g. Honda et al., 2006a).

Tooth engineering – *in vitro* techniques

Several systems for tooth tissues engineering have been developed (Fig. 2A-D):

A) Semi-solid medium

This method is based on cultivation of re-associated/recombined cells/tissues in medium consisting of agar, Dulbecco's Modified Eagle's Medium (DMEM) and standard media supplements. All liquid components of the medium are mixed, divided into single Petri dishes and heated up. Simultaneously, the agar is dissolved in

Table 1. Selected studies of tooth engineering using semi-solid medium

Epithelium representing	Mesenchyme representing	Type of experiment	Cultivating conditions	Experimental procedure	Results	Reference
Mouse dental epithelium at ED16-ED19	Mouse dental mesenchyme at ED16-ED19	Re-association of dental tissues at different ED	Agar-solidified medium	Dental tissues re-associated and implanted in ocular grafts	Re-associations formed teeth in 50-80 % of cultured grafts	Yoshikawa and Kollar, 1981
c-kit-positive bone marrow cells	Mouse molar mesenchyme at ED14	Induction of BMDCs by dental mesenchyme	Semi-solid medium	Dental mesenchyme recombined with BMDCs	c-kit-positive bone marrow cells differentiated into ameloblast-like cells and performed characteristics of ameloblasts	Hu et al., 2006b
Mouse molar epithelium at ED14	Mouse BMDCs	Induction of BMDCs by dental epithelium	Semi-solid medium embedded in matrigel	Intact enamel organ recombined with a large amount of BMDCs	Teeth were formed in 10 % of recombination experiments	Nait Lechguer et al., 2009
Mouse incisor or molar epithelium at ED14	Mouse incisor or molar mesenchyme at ED14	Heterotopic re-association	Semi-solid medium	Molar mesenchyme re-associated with incisor epithelium, incisor epithelium re-associated with molar mesenchyme	In both cases, a characteristic dental epithelial histogenesis was observed and odontoblasts became functional	Nait Lechguer et al., 2009
Mouse dental epithelium at ED14	Clonal mesenchymal cell lines (17IA4 and 705IC5) derived from ED18 mouse molars	Testing ability to form tooth in dental mesenchymal cell lines	Semi-solid medium	Re-associations cultured and implanted *in vivo* in 8-week-old mice	Cell lines failed in mediating tooth formation	Keller et al., 2011
Mouse dental epithelium at ED14	Mouse dental mesenchymal cells at ED14, ED16, ED18	Re-association of dental tissues at different ED	Semi-solid medium	Epithelium re-associated with freshly dissociated or pre-cultured mesenchymal cells	The potential of embryonic dental mesenchymal cells decreased from ED14 to ED16 and were lost at ED18	Keller et al., 2011
Cell suspension of mouse molar epithelium at ED14	Cell suspension of mouse molar mesenchyme at ED14	Re-association and analysis of differentiation	Semi solid medium	Re-associations cultivated in a semi-solid medium and then implanted into mice	Implantation of re-associations allowed for complete functional differentiation at the cell, matrix, and mineral levels	Nait Lechguer et al., 2011
Cell suspension of mouse molar epithelium at ED14	Cell suspension of mouse molar mesenchyme at ED14	Re-association and histological analysis	Semi-solid medium	Re-associations cultivated in a semi-solid medium and then implanted into mice	Similar vascularisation and cellular heterogeneity observed in the mesenchyme as in normal molars	Keller et al., 2012

PBS. Several drops of the liquid agar are dropped into the medium in the Petri dish. The mixture is stirred and incubated at room temperature for 20 min to obtain medium ready to use.

This system is suitable for usage of either cells or tissues, or combination of both. Samples are placed on the top of the medium and covered by a small amount of semi-solid medium. In general, the mesenchymal part of the culture is placed first and covered by the epithelial counterpart. In the case of early-staged tissue recombination, the mesenchymal part is applied first followed by the epithelial component and additionally, the system is covered by the mesenchyme again. The advantage of this method along with simple and fast preparation and low costs is supported by the fact that the cells may be applied in extremely high concentrations. The technique is widely used in odontogenic research (Table 1).

B) Modified Trowel's system (containing a permeable membrane)

This system consists of a Petri dish filled with the medium containing DMEM and standard media supplements, a permeable membrane (floating on the surface of the medium or supported by a metal grid), and a mixture of collagen with a medium (generally 5xDMEM/F12). In the case of manipulation with the tissues, the epithelium is placed on the top of the membrane in correct apicobasal orientation (the inner part of the epithelium has to be adjacent to mesenchymal cells). After accommodation of the epithelial part, a collagen drop is applied to the membrane and a highly concentrated suspension of mesenchymal cells is injected by a tiny capillary as fast as possible into the drop. In the case of cells, they are harvested, pelleted, and the suspensions of both components (epithelial/mesenchymal) are injected into the collagen drop. Alternatively, tissues/cells may be placed on the membrane insert without collagen.

This system is convenient for tissue/cell or cell/cell re-associations/recombination. The advantage of this technique includes a possibility to control the initial cell concentration. However, the manipulation may be technically demanding compared to the previous method. From the commercial point of view, this method is more expensive compared to the semi-solid medium. Nevertheless, the approach was applied in several odontogenic studies (Table 2).

C) Co-cultivation of cells

In this system, different populations of cells are cultured together. The method just requires basic cultivation tools. In general, two variants of the procedure may be adjusted. The populations of cells may be grown separated by a permeable membrane. Thus, the molecular factors can pass through the membrane; however, the cell populations are not in a direct contact (Arakaki et

Table 2. Selected studies of tooth engineering using Trowel's system

Epithelium representing	Mesenchyme representing	Type of experiment	Cultivation conditions	Experimental procedure	Results	Reference
Mouse oral/dental epithelium at ED11	Stage 23 of chick lateral mandibular mesenchyme	Heterospecific recombination	Filters supported by metal grids	Epithelial and mesenchymal components separated and constructed into recombinations	Interactions of mouse odontogenic epithelium with chick mandibular mesenchyme induced changes in the chick mandibular mesenchyme	Wang et al., 1998
Mouse oral/dental epithelium at ED10 (GFP mice)	Mouse ESCs Mouse neural SCs Mouse adult BMDCs	Stem cell-based tissue engineering	Membrane filters supported by metal grids	Cell pellets placed on membrane filters and covered by three or four pieces of epithelium, subsequently, explants were cultured in host kidney	Embryonic stem cells, neural stem cells, and adult bone-marrow-derived cells responded by expression of odontogenic genes, transfer of recombinations into adult renal capsules resulted in the development of tooth structures and associated bone	Ohazama et al., 2004
Cell suspension of mouse incisor epithelium at ED14.5	Cell suspension of mouse incisor mesenchyme at ED14.5	Engineering of tooth from dissociated cells	High-density cell compartmentalization on culture inserts	Mesenchymal and epithelial cells injected into collagen drop, subsequently, transplanted into mouse upper first molar cavity	After transplantation in the mouse tooth cavity, bioengineered germ formed a correct tooth structure	Nakao et al., 2007
Mouse dental epithelium at stage ED14	Mouse OLCs	Induction of OLCs to specialized dental tissues	Cell culture inserts	Epithelium and OLCs were recombined and cultured in collagen drop or implanted in host kidney	Tooth structures developed in 32 % of recombinations	Arany et al., 2009
Cell suspension of mouse molar epithelium at ED14.5	Cell suspension of mouse molar mesenchyme at ED14.5	Engineering of tooth from dissociated cells	Cell culture inserts	Cells re-associated in collagen drop and transplanted into mouse upper first molar cavity	Engineered tooth had correct structure including periodontal ligamentum and displayed normal hardness and responses to movement, tooth was smaller in comparison to normal tooth	Ikeda et al., 2009

Table 3. Selected studies of tooth engineering using biodegradable scaffolds

Epithelium representing	Mesenchyme representing	Type of experiment	Cultivation conditions	Experimental procedure	Results	Reference
Heterogeneous cell population of third molar of 6-month-old pig	Heterogeneous cell population of third molar of 6-month-old pig	Scaffold-based engineering of tooth tissues	Collagen-coated PGA scaffolds	Cell population seeded onto scaffold and placed into rat omentum	Epithelial cells in the circular aggregates differentiated into ameloblasts, enamel-covered dentin and cementum-covered dentin formed, tooth were smaller than normal with atypical structure	Honda et al., 2005
Heterogeneous cell population of third molar of 6-month-old pig	Osteoblasts derived from porcine bone marrow progenitors	Scaffold-based engineering of tooth-bone complex	PGA and PLGA scaffolds	Cells seeded onto scaffolds separately, then combined together and implanted into athymic rats	Primary and reparative dentin-like, enamel-like, periodontal-like structures and bone were observed	Young et al., 2005
Heterogeneous cell population of third molar of 6-month-old pig	Heterogeneous cell population of third molar of 6-month-old pig	Effect of shear stress on tissue-engineered odontogenesis	Collagen-coated PGA scaffolds, shear stress was generated by bi-directional fluid flow	Cell population seeded onto scaffold and placed into rat omentum	Shear stress facilitated the process of tooth tissue engineering *in vivo*	Honda et al, 2006a
Epithelial cells of third molar of 6-month-old pig	Mesenchymal cells of third molar of 6-month-old pig	Scaffold-based engineering of tooth tissues	Collagen sponges	First, mesenchymal cells, second, epithelial cells seeded onto scaffold, then implanted into rats	Tooth morphology *in vivo* was developed similarly as in natural tooth	Honda et al., 2006b
Heterogeneous cell population of canine first molar	Heterogeneous cell population of canine first molar	Scaffold-based engineering of tooth-bone complex	Collagen-coated PGA scaffolds	Cell population seeded onto scaffold and transplanted into canine alveolar socket after tooth extraction	Regeneration of hard tissues was detected but enamel-like structure was not observed	Honda et al., 2006c
Heterogeneous cell population of third molar of 6-month-old pig	Heterogeneous cell population of third molar of 6-month-old pig	Testing of scaffold materials	Collagen sponges, PGA fiber mesh scaffolds	Cell population seeded onto scaffolds and implanted into athymic rats	Collagen sponge scaffold allowed tooth production with a higher degree of success than PGA fibre mesh	Sumita et al., 2006
Heterogeneous cell population of 1.5-month-old pig	Heterogeneous cell population of 1.5-month-old pig	Scaffold-based engineering of tooth tissues	GCHT	Cell population seeded onto scaffold and scaffold implanted into pig socket after tooth extraction	Formation of tooth was observed in 30 % of re-associations, enamel structure was not evident and teeth were smaller	Kuo et al., 2008
Epithelial cell rests of Malassez (ERM) from 6-month-old pig incisor	Dental pulp cells from porcine third molar	Differentiation of ERM to ameloblast-like cells	Collagen sponge scaffolds, co-cultivation of ERM on 3T3-J2 feeder layer	Dental pulp cells seeded on scaffold and ERM cells seeded on the top of dental pulp cells, then transplanted into athymic rat omentum	Formation of enamel-like tissues at 8 weeks after transplantation	Shinmura et al., 2008
Tooth bud-derived cells of 6-month-old pig	Tooth bud-derived cells of 6-month-old pig	Testing of scaffold materials	Collagen and fibrin gel	Cells mixed in gel, transplanted into nude mice	Tooth germ-like structures were more evident in collagen and fibrin groups than in control group (PGA fibre and β-tricalcium phosphate porous block)	Ohara et al., 2010

al., 2012). Alternatively, both populations are mixed together (Yu et al., 2006; Shinmura et al., 2008; Arakaki et al., 2012). Co-cultivations allow for induction of differentiation-related processes and are used particularly in the situations when the exact molecular pathways have not yet been defined. Furthermore, the influence of the direct contact of cells versus the effect of the humoral factors themselves may be easily compared. This has a real value for studies of the mechanisms of cell interactions. Unfortunately, outcomes of these studies are limited by the 2D system.

D) Biodegradable scaffolds

Various natural and synthetic materials have been tested for seeding of dental cells or stem cells on biodegradable scaffolds, such as collagen (Sumita et al., 2006), fibrin (reviewed in Sharma et al., 2014), polyglycolic acid (PGA), poly(lactic-co-glycolic acid) (PLGA) (Duailibi et al., 2004; Young et al., 2005), HA/TCP (Sonoyama et al., 2006), and gelatin-chrondroitin-hyaluronan-tri-copolymer (GCHT) (Kuo et al., 2008). Scaffolds prepared from natural materials such as collagen

provide better cell adhesion and higher success rates for the tooth formation (Sumita et al., 2006). However, the amount of the formed hard tissue seems to be higher in the synthetic ones (Ohara et al., 2010). Furthermore, mechanical and chemical parameters (microstructure, porosity, shape, strength, rate of degradation, etc.) of the synthetic scaffolds may be easily controlled (reviewed in Sharma et al., 2014). Sterilization of scaffolds can be carried out by γ-irradiation or by 75% ethanol. Specific protocols for different scaffold preparations are described in the reports mentioned above.

The most important advantage of this technique lies in the cultivation of cells in their native-like 3D environment, convenient implantation and further manipulation, large space for optimization including desired shapes of the scaffolds. This method has been widely used in odontogenic research (Table 3).

In vivo implantations

For the initial period (1–7 days), re-associated/recombined structures may be grown *in vitro*. However, to obtain the mineralized tissues of the tooth, vasculature and/or innervation, newly formed tooth structures have to be implanted into animal models. For the purpose, mouse ocular grafts may be used (Yoshikawa and Kollar, 1981). The mouse kidney capsule is another frequently utilized option (Ohazama et al., 2004; Nakao et al., 2007). A more convenient approach for the user and also for survival of the animal hosts seems to be introduction under the mouse skin in the region behind the ears (Hu et al., 2006a; Keller et al., 2012; Kökten et al., 2014) or in the rat omentum (Honda et al., 2005, 2006b; Yang et al., 2005; Shinmura et al., 2008). In the most advanced studies, tooth germs have been implanted into alveolar sockets after tooth extraction in mice (Nakao et al., 2007; Ikeda et al., 2009), and pigs (Kuo et al., 2008) or dogs (Honda et al., 2006c). Alternatively, mouse diastema can be used for tooth introduction (Modino and Sharpe, 2005).

Concluding remarks

Great progress in dental engineering has already been achieved in animal models such as growth of the molar in the oral cavity of mice (e.g. Ikeda et al., 2009) or restoration of functional tooth roots in pigs (Sonoyama et al., 2006). Although efforts have been dedicated to the construction of engineered teeth in humans, many questions still remain unanswered.

The most important limitations of the tooth engineering research seem to be the experimental legislative, source of epithelial dental cells, formation of functional PDL, specific tooth-bone interface, and blood supply or innervations. Scientific groups around the world focus on different approaches to study the basic principles or advanced procedures of tooth engineering. Specific techniques have been developed and widely used in the field. For the future prospects, combination of stem cells, molecular factors and scaffolds may be an optimal settlement for human tooth engineering.

In most tissue-engineering approaches, the tooth has been considered as an isolated organ. However, the tooth develops in close relation with the surrounding structures, particularly the alveolar bone (e.g. Fleischmannova et al., 2010). Therefore, the recent tooth engineering emphasizes the importance of approaching the entire functional complex (Modino and Sharpe, 2005; Young et al., 2005; Mao et al., 2010). First successes in alveolar bone restoration have already been reported in humans (Fiorellini et al., 2005; Wallace et al., 2014) and hopefully indicate a promising future for tooth engineering.

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