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.

Received June 27, 2014. Accepted July 21, 2014.

This work was supported by the Grant Agency of the Czech Republic, project P304/11/1418. Clinical aspects are studied under the project of the Ministry of Health of the Czech Republic (NT 14206/6-2010). The laboratories at the FS MU are supported by the Centre of Experimental Biomedicine (CZ.1.07/2.3.00/20.0183) and the IAPG of the AS CR, v. v. i. is supported by RVO: 67985904.

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Folia Biologica (Praha) 60 (Suppl 1), 21-29 (2014)

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; Milewich 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

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**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).
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 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.
(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 (Shimmura 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>
<thead>
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<th>Table 1. Selected studies of tooth engineering using semi-solid medium</th>
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<td><strong>Epithelium representing</strong></td>
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<tr>
<td>Mouse dental epithelium at ED16-ED19</td>
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<tr>
<td>c-kit-positive bone marrow cells Mouse molar mesenchyme at ED14</td>
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<tr>
<td>Mouse molar epithelium at ED14</td>
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<tr>
<td>Mouse incisor or molar epithelium at ED14</td>
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<tr>
<td>Mouse dental epithelium at ED14</td>
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<tr>
<td>Mouse dental epithelium at ED14</td>
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<tr>
<td>Cell suspension of mouse molar epithelium at ED14</td>
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<tr>
<td>Cell suspension of mouse molar epithelium at ED14</td>
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</table>
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

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

<table>
<thead>
<tr>
<th>Epithelium representing</th>
<th>Mesenchyme representing</th>
<th>Type of experiment</th>
<th>Cultivation conditions</th>
<th>Experimental procedure</th>
<th>Results</th>
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<tbody>
<tr>
<td>Mouse oral/dental epithelium at ED11</td>
<td>Stage 23 of chick lateral mandibular mesenchyme</td>
<td>Heterospecific recombination</td>
<td>Filters supported by metal grids</td>
<td>Epithelial and mesenchymal components separated and constructed into recombinations</td>
<td>Interactions of mouse odontogenic epithelium with chick mandibular mesenchyme</td>
<td>Wang et al., 1998</td>
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<tr>
<td>Mouse oral/dental epithelium at ED10 (GFP mice)</td>
<td>Mouse ESCs Mouse neural SCs Mouse adult BMDCs</td>
<td>Stem cell-based tissue engineering</td>
<td>Membrane filters supported by metal grids</td>
<td>Cell pellets placed on membrane filters and covered by three or four pieces of epithelium, subsequently, explants were cultured in host kidney</td>
<td>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</td>
<td>Ohazama et al., 2004</td>
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<td>Cell suspension of mouse incisor epithelium at ED14.5</td>
<td>Cell suspension of mouse incisor mesenchyme at ED14.5</td>
<td>Engineering of tooth from dissociated cells</td>
<td>High-density cell compartmentalization on culture inserts</td>
<td>Mesenchymal and epithelial cells injected into collagen drop, subsequently, transplanted into mouse upper first molar cavity</td>
<td>After transplantation in the mouse tooth cavity, bioengineered germ formed a correct tooth structure</td>
<td>Nakao et al., 2007</td>
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<td>Mouse dental epithelium at stage ED14</td>
<td>Mouse OLCs</td>
<td>Induction of OLCs to specialized dental tissues</td>
<td>Cell culture inserts</td>
<td>Epithelium and OLCs were recombined and cultured in collagen drop or implanted in host kidney</td>
<td>Tooth structures developed in 32% of recombinations</td>
<td>Arany et al., 2009</td>
</tr>
<tr>
<td>Cell suspension of mouse molar epithelium at ED14.5</td>
<td>Cell suspension of mouse molar mesenchyme at ED14.5</td>
<td>Engineering of tooth from dissociated cells</td>
<td>Cell culture inserts</td>
<td>Cells re-associated in collagen drop and transplanted into mouse upper first molar cavity</td>
<td>Engineered tooth had correct structure including periodontal ligamentum and displayed normal hardness and responses to movement, tooth was smaller in comparison to normal tooth</td>
<td>Ikeda et al., 2009</td>
</tr>
<tr>
<td>Epithelium representation</td>
<td>Mesenchyme representing</td>
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<td>Heterogeneous cell population of third molar of 6-month-old pig</td>
<td>Heterogeneous cell population of third molar of 6-month-old pig</td>
<td>Scaffold-based engineering of tooth tissues</td>
<td>Collagen-coated PGA scaffolds</td>
<td>Cell population seeded onto scaffold and placed into rat omentum</td>
<td>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</td>
<td>Honda et al., 2005</td>
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<tr>
<td>Heterogeneous cell population of third molar of 6-month-old pig</td>
<td>Osteoblasts derived from porcine bone marrow progenitors</td>
<td>Scaffold-based engineering of tooth-bone complex</td>
<td>PGA and PLGA scaffolds</td>
<td>Cells seeded onto scaffolds separately, then combined together and implanted into athymic rats</td>
<td>Primary and reparative dentin-like, enamel-like, periodontal-like structures and bone were observed</td>
<td>Young et al., 2005</td>
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<tr>
<td>Heterogeneous cell population of third molar of 6-month-old pig</td>
<td>Heterogeneous cell population of third molar of 6-month-old pig</td>
<td>Effect of shear stress on tissue-engineered odontogenesis</td>
<td>Collagen-coated PGA scaffolds, shear stress was generated by bi-directional fluid flow</td>
<td>Cell population seeded onto scaffold and placed into rat omentum</td>
<td>Shear stress facilitated the process of tooth tissue engineering in vivo</td>
<td>Honda et al., 2006a</td>
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<tr>
<td>Epithelial cells of third molar of 6-month-old pig</td>
<td>Mesenchymal cells of third molar of 6-month-old pig</td>
<td>Scaffold-based engineering of tooth tissues</td>
<td>Collagen sponges</td>
<td>First, mesenchymal cells, second, epithelial cells seeded onto scaffold, then implanted into rats</td>
<td>Tooth morphology in vivo was developed similarly as in natural tooth</td>
<td>Honda et al., 2006b</td>
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<tr>
<td>Heterogeneous cell population of canine first molar</td>
<td>Heterogeneous cell population of canine first molar</td>
<td>Scaffold-based engineering of tooth-bone complex</td>
<td>Collagen-coated PGA scaffolds</td>
<td>Cell population seeded onto scaffold and transplanted into canine alveolar socket after tooth extraction</td>
<td>Regeneration of hard tissues was detected but enamel-like structure was not observed</td>
<td>Honda et al., 2006c</td>
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<tr>
<td>Heterogeneous cell population of third molar of 6-month-old pig</td>
<td>Heterogeneous cell population of third molar of 6-month-old pig</td>
<td>Testing of scaffold materials</td>
<td>Collagen sponges, PGA fiber mesh scaffolds</td>
<td>Cell population seeded onto scaffolds and implanted into athymic rats</td>
<td>Collagen sponge scaffold allowed tooth production with a higher degree of success than PGA fibre mesh</td>
<td>Sumita et al., 2006</td>
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<tr>
<td>Heterogeneous cell population of 1.5-month-old pig</td>
<td>Heterogeneous cell population of 1.5-month-old pig</td>
<td>Scaffold-based engineering of tooth tissues</td>
<td>GCHT</td>
<td>Cell population seeded onto scaffold and scaffold implanted into pig socket after tooth extraction</td>
<td>Formation of tooth was observed in 30% of re-associations, enamel structure was not evident and teeth were smaller</td>
<td>Kuo et al., 2008</td>
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<td>Epithelial cell rests of Malassez (ERM) from 6-month-old pig incisor</td>
<td>Dental pulp cells from porcine third molar</td>
<td>Differentiation of ERM to ameloblast-like cells</td>
<td>Collagen sponge scaffolds, co-cultivation of ERM on 3T3-J2 feeder layer</td>
<td>Dental pulp cells seeded on scaffold and ERM cells seeded on the top of dental pulp cells, then transplanted into athymic rat omentum</td>
<td>Formation of enamel-like tissues at 8 weeks after transplantation</td>
<td>Shinmura et al., 2008</td>
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<td>Tooth bud-derived cells of 6-month-old pig</td>
<td>Tooth bud-derived cells of 6-month-old pig</td>
<td>Testing of scaffold materials</td>
<td>Collagen and fibrin gel</td>
<td>Cells mixed in gel, transplanted into nude mice</td>
<td>Tooth germ-like structures were more evident in collagen and fibrin groups than in control group (PGA fibre and β-tricalcium phosphate porous block)</td>
<td>Ohara et al., 2010</td>
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</table>

D) Biodegradable scaffolds

Various natural and synthetic materials have been tested for seeding of dental cells or stem cells on bio-degradable scaffolds, such as collagen (Sumita et al., 2006), fibrin (reviewed in Sharma et al., 2014), polyglycolic acid (PGA), poly(lactic-co-glycolic acid) (PLGA) (Duaillibi et al., 2004; Young et al., 2005), HA/TCP (Sonoyma et al., 2006), and gelatin-chondroitin-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/re-combined 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; Shimura 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.

**References**


Methods in Tooth Engineering


