Tissue-Engineered Teeth

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Abstract

Clinical practice in the field of dentistry has remained largely unchanged for over a century. Recently, significant advances in the fields of tissue engineering and regenerative medicine (TERM) have provided new opportunities for dental therapies to advance in ways that will provide patients with more effective therapies to regenerate living dental tissue, while at the same time preserving natural dental tissues as much as possible. Since regenerative dental therapies are based on knowledge and understanding of natural tooth development, here we first describe early tooth development, including morphogenesis of tooth crown and root structures, and review new, relevant findings in tooth development biology. With respect to regenerative approaches for dental tissue repair, we next describe the three components recognized as doctrine in tissue engineering strategies – dental stem

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cells, scaffolds, and growth factors/signaling molecules/cytokines – and recent findings in each. We next review the use of dental stem cells for applications in tooth regeneration, including highlights on the use of innovative and promising scaffold materials and growth factors. We discuss the significant breakthrough and discovery in 2006 of induced pluripotent stem cells (iPSCs), and how this stem cell technology demonstrated the possibility of using a patient's own reprogrammed cells to regenerate new tissues and organs. We next describe promising partial tooth regeneration strategies, including regeneration of the dentin-pulp complex, the periodontium, and tooth root regeneration. Finally, we describe exciting progress in whole tooth regeneration strategies, focusing on three-dimensional (3D) tissue engineering strategies.

1 Introduction

Tooth loss, the most common organ failure in humans, can result from congenital malformations or genetic disorders, from common disorders such as dental caries and periodontal disease, and from accidental or battlefield injuries to the mouth and face (Young et al. 2002). The tooth organ itself consists of highly complex, organized, and precisely patterned mineralized tissue matrices that are functionally integrated with soft dental tissues, including dental pulp, periodontal ligament, nerves, and vasculature (Ten Cate 1998) (Fig. 1a). The major hard tissues of a tooth include enamel, dentin, and cementum, while soft dental tissues include dental pulp and periodontal ligaments. Human teeth are ectodermally derived organs that exhibit only limited regeneration potential. Mineralized enamel cannot regenerate primarily due to the fact the dental epithelium, which forms enamel in a naturally developing tooth, is lost prior to tooth eruption (Moradian-Oldak 2012), while dentin exhibits a very limited capacity for reparative dentin formation in response to injury (Song et al. 2017).

Current commonly used dental therapies consist of the following. Dental implants are the principle restorative therapy used to replace lost teeth, despite the fact that synthetic implants have none of the characteristics of natural, living teeth (Ferreira et al. 2007). Dental implants are also susceptible to a variety of insults including inflammation of the hard and soft tissues surrounding the implant, called peri-implantitis, which can potentially lead to implant failure (Smeets et al. 2014). Bone grafts, commonly used to repair and reinforce load-bearing jaw bone, have been extensively investigated although this approach does not regenerate functional dental tissues (Reynolds et al. 2003).

Another traditional surgical dental interventions used for periodontal tissue repair primarily focuses on removing diseased tissues via open flap debridement, followed by scaling and root planning (Becker et al. 1986; Brayer et al. 1989). These procedures can easily result in long-term junctional epithelium detachment, leading to a compromised gingival seal around the tooth, making it highly susceptible to bacterial infection (Ivanovski 2009). Another common dental tissue regeneration therapy used in the clinic is guided tissue regeneration (GTR), most commonly used for

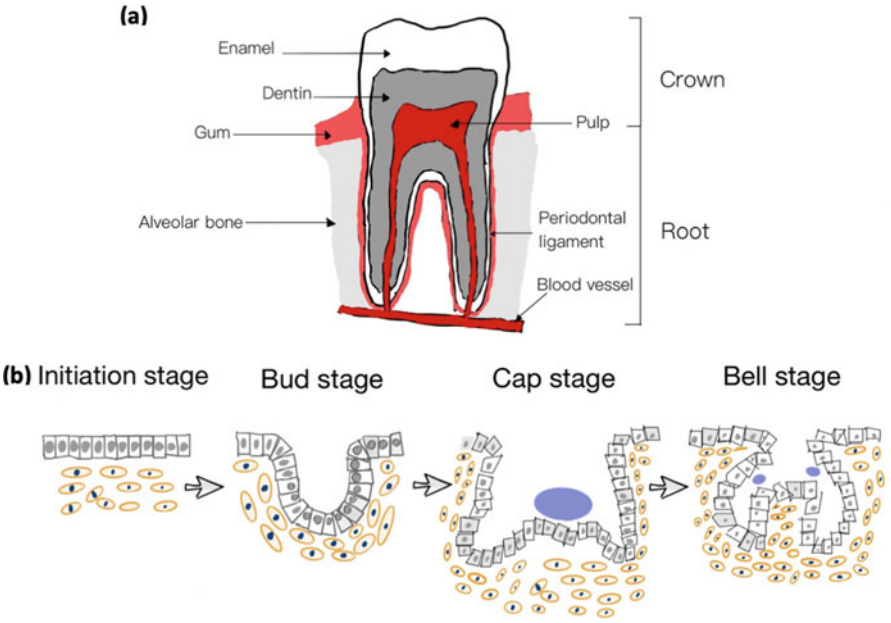

Fig. 1 **Anatomy of the tooth and developmental stages of tooth morphogenesis**. (**a**) Anatomy of the tooth. The enamel is the calcified tissue in the crown of the tooth. Pulp contains connective tissue, blood vessels, and nerves. (**b**) Four different developmental stages during tooth morphogenesis

periodontal tissue repair as first proposed by Melcher in the 1970s (Melcher 1976). The goal of GTR is to regenerate tight PDL tissue attachment to the tooth roots (Nyman et al. 1982a, b) via application of a tissue barrier membrane to guide the migration of cementogenic and osteogenic stem cells to the defect site (Ivanovski 2009). However, such membranes can become problematic, as early non-resorbable membranes required removal after transplantation, potentially leading to post-operational complications (Murphy 1995). A newer resorbable membrane consisting of collagen and polylactic/polyglycolic acid has also shown complications in that it can inhibit PDL tissue healing (Sculean et al. 2007).

Current approaches used to treat severe dental caries can include endodontic treatment, which involves completely removing all dental pulp tissue and subsequently replacing it with synthetic, inert cement (Parirokh et al. 2018; Torabinejad et al. 2018). During the tooth restoration step, two types of pulp capping methods can be used – direct or indirect pulp capping. Direct pulp capping involves the use of a protective material placed directly on the exposed pulp, while indirect pulp capping requires the presence of thin layer of residual dentin to avoid pulp exposure (European Society of Endodontology 2006; Hilton 2009). Unfortunately, neither method facilitates the regeneration of the dentin-pulp complex, and direct capping results in the permanent loss of the dental pulp tissue.

Together, the limited efficacy of these currently used dental tissue repair therapies has spurred the development of novel strategies for improved and effective partial dental tissue and whole tooth therapies that actually regenerate natural dental tissues.

2 Natural Tooth Development

For more than three decades, tooth development, called odontogenesis, has been extensively studied in the context of both developmental and evolutionary models (Jernvall and Thesleff 2012). Similar to other ectodermally derived organs such as hair and feathers, teeth are epithelial appendages that can be found throughout all vertebrate groups (Biggs and Mikkola 2014). Teeth are derived from the reiterative interactions of the dental epithelium and the dental mesenchyme (Thesleff 2003). Mammalian odontogenesis is initiated from the oral ectoderm, which subsequently signals to the neural crest cell (NCC) derived mesenchyme to direct tooth morphogenesis (Thesleff and Tummers 2008). Subsequent tooth development is mediated via crosstalk between two major tooth specific-cell types – the dental epithelium and the dental mesenchyme (Hurmerinta and Thesleff 1981).

Mammalian tooth development occurs in four stages: (1) the initiation of the tooth development; (2) the morphogenesis of the tooth crown; (3) dental cell differentiation; and (4) the maintenance of dental stem cells that support tooth development (Balic and Thesleff 2015). In the mouse, a simple dentition pattern consists of one central incisor and three molars in each quadrant, with continuously erupting incisors and no molar replacement teeth (Lumsden 1979; Peterkova et al. 1996; Viriot et al. 1997). Humans replace their baby, or deciduous, teeth with a second set of adult teeth that include additional tooth types including canines and premolars (Vastardis 2000). However, both mice and human dentition share similar development patterns during odontogenesis (Yu et al. 2015). The availability of a wide variety of transgenic mouse genetic knock out and reporter lines makes mice the most commonly used animal model to study tooth development (Kantarci et al. 2015). The signaling pathways regulating the complex interactions between dental epithelial and mesenchymal cells and tissues are very dynamic (Thesleff and Tummers 2008). As such, tooth organogenesis can be considered as a stepwise process where morphogenesis and cell differentiation occur through reciprocal and sequential interactions.

2.1 Morphogenesis of the Tooth Crown

The initiation stage of tooth development begins with the appearance of the *primary dental laminae*, also called odontogenic bands, which are essentially stripes of thickened epithelium that give rise to future teeth (Mina and Kollar 1987; Lumsden 1988). The thickened dental epithelium then invaginates into the underlying mesenchymal to form *placodes.* During the bud stage of tooth development, the dental

papilla forms as the dental mesenchyme condenses beneath the invaginating dental epithelium around mouse embryonic day 13 (E13) (Fig. 1). The expression of dental lamina genes is restricted to the placodes, where transcription factors such as *paired like homeodomain 2* (*pitx2*) are expressed (Oosterwegel et al. 1993). The dental epithelial placodal cells express four conserved signaling molecules – *Shh*, *Wnt10*, *BMP2,* and *FGF20* – marking the sites of the future teeth (Haara et al. 2012; Jussila and Thesleff 2012).

Next, tooth bud stage dental epithelium proliferates, giving rise to cap and then bell stage tooth buds (Fig. 1). During the cap stage, a cluster of undifferentiated cells located at the inner enamel epithelium, the primary enamel knot, mark the future tooth cusp (Balic and Thesleff 2015). In multi-cusped mammalian teeth, primary and then secondary enamel knots mark the sites of multi-cusped tooth patterns. The enamel organ forms during the cap stage and is comprised of two layers of cells, the inner and outer enamel epithelium (Balic and Thesleff 2015). Demarcated by the cervical loop and the dental papilla, the basal epithelial cell layer of the cervical loop bordering the dental papilla is known as the inner enamel epithelium (iee), while the epithelial layer facing the dental follicle is known as the outer enamel epithelium (oee) (Harada et al. 1999; Tummers and Thesleff 2003; Thesleff and Tummers 2008). The inner enamel epithelium differentiates into ameloblasts that will secrete enamel matrix, and the mesenchymal cells differentiate into odontoblasts that produce dentin, all prior to tooth eruption (Jernvall and Thesleff 2012).

Tooth crown morphogenesis occurs in bell stage teeth, where the enamel knot signaling centers functions direct the height, location, and the number of the developing tooth cusps (Balic and Thesleff 2015) (Fig. 1b). In the late bell stage, dental mesenchymal cell-derived odontoblasts and dental epithelial cell-derived ameloblasts secrete matrix that will produce dentin and enamel, respectively (Balic 2018).

During tooth development, the cervical loop serves as a stem cell niche that produces daughter cells that differentiate into enamel-forming ameloblasts. Growth factors such as FGF10, expressed in the dental mesenchyme, and its receptor Fgfr2b, expressed in the dental epithelium, are both required for dental stem cell proliferation (Harada et al. 2002). Shh has been shown to be essential for dental stem cell replication and recruitment, but not for dental stem cell survival (Suomalainen and Thesleff 2010). In general, the same signaling pathway networks regulating the stem cell niche of the mouse continuously erupting incisor are also active in the stem cell niche of developing molar teeth (Tummers and Thesleff 2003), implying that largely conserved signaling pathways regulate the development of all teeth (Jernvall and Thesleff 2012).

It is worth noting that mammals have a very limited capacity to replace their teeth, as compared to other vertebrates such as reptiles and fish (Davit-Beal et al. 2009; Jernvall and Thesleff 2012). The majority of mammals, including humans, can replace their teeth only once. However, the renewal and maintenance of many dental tissues is supported by stem cells, which in turn are regulated by a variety of growth factor signaling families (Jernvall and Thesleff 2012).

2.1.1 Cells and Signaling Pathways During Tooth Development

Tooth organogenesis can be considered a stepwise process where tooth morphogenesis and dental cell differentiation occur through reciprocal and sequential dental epithelial-mesenchymal cell interactions (Thesleff and Tummers 2008). Five major signaling pathways are involved in odontogenesis including Wnt, bone morphogenetic protein (BMP), fibroblast growth factor (FGF), Sonic Hedgehog (Shh), and Ectodysplasin (Eda) – see Table 1. Together, these signaling pathways mediate the tissue interactions that lead to tooth formation.

BMP Signaling

BMPs are members of a very large family of the transforming growth factor β (TGF-β) superfamily, that consist of homodimer proteins (Valera et al. 2010). BMPs are involved in both the early and later developmental stages of tooth organogenesis (Yuan et al. 2015; Graf et al. 2016). Specifically, BMP2 is expressed in dental epithelium and BMP7 is expressed in dental mesenchyme of initiation stage tooth buds (Malik et al. 2018). Both BMP2 and BMP7 also promote early tooth mineralization (Malik et al. 2018), while BMP4 regulates the formation of the epithelial root sheath (Hosoya et al. 2008). BMP9 is notably responsible for promoting odontoblastic and osteogenic differentiation (Huang et al. 2019).

Shh Signaling

Sonic hedgehog (Shh) signaling is required for embryonic mouse tooth initiation, as well as to maintain differentiation of dental epithelial cells into ameloblasts (Gritli-Linde et al. 2002). Shh is expressed in the oral epithelium prior to invagination and in the dental epithelium during tooth development. Together with Wnt10, BMP2,

Table 1 Five major signaling pathways regulating tooth development

Signaling pathways	Key cytokines	Functions	References
BMP	BMP2	Early tooth morphogenesis and mineral secretion, found in odontoblasts	Malik et al. (2018)
	BMP7	Similar to BMP2 but also found in ameloblasts	Gao et al. (2018)
	BMP9	Promotes odontoblast differentiation and osteogenic differentiation	Huang et al. (2019)
WNT	WNT3a	Stimulates cementoblasts formation	Nemoto et al. (2016)
	WNT7b	Positions the site of tooth formation	Sarkar et al. (2000)
FGF	FGF3	Regulate proliferation of epithelial stem cell progeny	Wang et al. (2007)
	FGF8	Required for tooth initiation	Trumpp et al. (1999)
SHH	SHH	Promotes epithelial cell proliferation and are expressed throughout dental mesenchyme and epithelium	Bitgood and McMahon (1995), Cobourne et al. (2001)
EDA	EDA	Required for the development of ectodermal organ during initiation from placodes	Mustonen et al. (2004)

and FGF20, Shh expression is restricted to a cluster of placodal cells of the early signaling center (Haara et al. 2012; Jussila and Thesleff 2012). During the bud stage, the expression of Shh becomes restricted to the enamel knot, while during the cap stage, it is expressed in tissues surrounding the inner enamel epithelium (Vaahtokari et al. 1996). Loss of Shh results in arrested development of cap stage teeth and the formation of only a rudimentary tooth bud (Dassule et al. 2000). Together with the BMP signaling pathway, BMP/SHH signaling networks dictate the fate of dental epithelial stem cells in mouse molars and incisors (Li et al. 2015). Conditional Shh null mice exhibit severe craniofacial defects, including shortened height of molar placodes, indicating roles for Shh in organizing dental placodal cells. The canonical Shh pathway includes key transcription factors Gli1–3 (Hardcastle et al. 1998), and mice lacking Gli2 and Gli3 lack all molar tooth development, and form only a primitive central incisor tooth bud.

WNT Signaling

Both the canonical WNT/β-catenin pathway and the noncanonical Wnt signaling pathway play important roles in the early embryonic tooth development (Wang et al. 2014). WNT family members are expressed in the dental epithelium, and WNT7b is expressed in the oral epithelium when tooth patterns become clearly defined (Sarkar et al. 2000). Wnt/Shh signaling interactions define where the tooth is formed. In cap stage teeth, WNT 4 and WNT6 are expressed in the dental epithelium, while WNT5a, and signaling partners sFrp2 and sFrp3 are expressed in the dental mesenchyme (Sarkar and Sharpe 1999). WNT/β-catenin signaling pathways also mediate a variety of downstream signaling pathways in tooth development (Huang et al. 2020).

FGF Signaling

FGF signaling also plays important roles in odontogenesis. FGF8 is credited as the dental epithelial cell-originating factor (Trumpp et al. 1999), while FGF9 is essential for dental epithelial invagination, and initiates dental ectodermal organogenesis (Tai et al. 2012). In addition, FGF8 induces the expression of Pax9 in mouse tooth development, implying its essential role in tooth development beyond odontogenesis (Neubuser et al. 1997; Huang et al. 2020). FGF3, FGF4, FGF9, FGF15, and FGF20 are all expressed in the primary enamel knot signaling center (Porntaveetus et al. 2011).

EDA Signaling

Eda, a member of the tumor necrosis family (TNF), is a signaling molecule responsible for regulating the development of a variety of ectodermal appendages, including teeth and hair (Biggs and Mikkola 2014; Balic and Thesleff 2015). The receptor for Eda, Edar, is expressed in the dental *placode* (Haara et al. 2012; Balic and Thesleff 2015). Together with the intracellular adaptor protein Edaradd, they form a pathway leading to the downstream activation of the transcription factor NF-κB (Mikkola 2008). Eda is involved in the initial development of the tooth placode, where the Wnt/β-catenin pathway upregulates the expression of Edar, and Edar/NF-κB are required to maintain the expression of WNT10a/b (Zhang et al. 2009). In addition,

Eda has been shown to play a role in tooth bud morphogenesis, since Eda null embryos exhibit very small tooth buds (Pispa et al. 1999), although Eda is not required in later stages of tooth development (Swee et al. 2009). Eda is vital for the formation of placode during early tooth development (see Table 1), (Mustonen et al. 2004).

2.2 Tooth Root Development

2.2.1 Tooth Root Morphogenesis

Tooth root development is also regulated by crosstalk between the dental epithelium and mesenchyme (Thesleff and Sharpe 1997). Tooth root morphogenesis can be divided into root initiation and root elongation stages. After tooth crown formation, the cervical loop continues to grow and elongate after tooth crown formation, and eventually forms a double-layered epithelial structure called Hertwig's epithelial root sheath (HERS) (Ten Cate 1998). HERS is located between the dental papilla and the dental follicle and is generally thought to be the signaling center responsible for tooth root formation (Ten Cate 1998).

The cervical loop gives rise to the Hertwig's epithelial root sheath (HERS) via fusion of the outer and inner enamel epithelial cell layers, and serves as an important signaling center for tooth root formation (Huang et al. 2009a). Tooth root initiation occurs as the mesenchymal cell layer of the apical papilla comes in contact with the inner layer of the HERS, which signals mesenchymal cell differentiation into odontoblasts that form the radicular dentin that covers the tooth root (Li et al. 2017).

Interactions between the dental follicle and the newly formed dentin induce the differentiation of cementoblasts, which will produce both cementum and cementum-specific extracellular matrix such as collagen fibers (Zeichner-David 2006). HERS can give rise to cementoblasts by epithelial-to-mesenchymal transition (EMT) (Huang et al. 2009a), and also becomes the epithelial cell Rests of Malassez (ERM), which participates in cementum regeneration and repair (Xiong et al. 2013). The HERS is also responsible for the number of roots formed by a tooth, by forming protrusions downwards from the dental pulp cavity, which join horizontally to form a bridge, and then become divided to form individual tooth roots. As such, tooth root development is directed by the apical growth of HERS (Orban and Bhaskar 1980).

HERS is also heavily involved in periodontal ligament (PDL) formation, due in part to both HERS formation and degeneration (Li et al. 2017). As migrating dental follicle cells contact the HERS, PDL is formed between the tooth root and surrounding alveolar bone (Cho and Garant 2000). The PDL supports and anchors the root to the alveolar bone via collagen fibers secreted by dental follicle cells, thereby stabilizing the tooth for the forces of mastication.

2.2.2 Tooth Root Development Signaling Pathways

The discovery of a unique transcription factor, called *nuclear factor I C (Nfic),* revealed that distinct mechanisms mediate tooth crown and root formation (Steele-Perkins et al. 2003). Nfic was found to be required for tooth root formation

but not crown formation, and NFIC-dependent and NFIC-independent signaling pathways have been characterized in recent years (Wang and Feng 2017). Nfic belongs to the nuclear factor I family and functions as a master regulator gene during tooth root dentin formation. The expression of Nfic is restricted to odontoblasts and pre-dontoblasts in developing molars in human and mice, where it participates in odontoblast differentiation by modulating TGFβ signaling pathway (Gao et al. 2014).

The canonical Wnt signaling pathway plays a crucial role in tooth root formation, where β-catenin-mediated Wnt signaling mediates odontoblast differentiation (Kim et al. 2013). It was determined that the integrity of Wnt signaling affects the formation of HERS, and in turn can compromise the vital epithelial-mesenchymal interactions necessary for proper tooth root development (Li et al. 2017). The Wnt signaling pathway also mediates tooth root formation through interactions with other conserved signaling pathways, including the canonical BMP signaling pathway, where BMP signaling is required to maintain the expression of Wnt signaling inhibitors (Li et al. 2011). In addition, Wnt10 was found to induce the expression of the odontoblast differentiation marker dentin sialo-phospho protein (Dspp), suggesting roles for Wnt10 in regulating tooth root dentinogenesis (Li et al. 2011, 2017). Interactions between Bmp and Wnt signaling also define the transition between tooth crown and tooth root formation (Yang et al. 2013).

Other conserved signaling pathways such as SHH and FGF are also crucial for tooth root development by their ability to mediate dental epithelial-mesenchymal cell interactions. Fgf10 regulates HERS formation during molar tooth development (Tummers and Thesleff 2003; Yokohama-Tamaki et al. 2006). Fgf2 is expressed in the apical furcation end of the tooth root where it directs odontoblast differentiation, and also in cementoblasts and fibroblasts of the PDL where it is thought to regulate tooth root development (Gao et al. 1996).

3 Cells and Scaffolds

3.1 Dental Stem Cells

Embryonic stem cells (ESCs), which are derived from the undifferentiated cells of the blastocyst, are capable of differentiating into all types of cell lineages derived from endoderm, mesoderm, and ectoderm (Thomson et al. 1998). It is not possible to use human ESCs in dental clinic applications due to serious concerns over their ethical use in nonlethal diseases (Yildirim et al. 2011). Therefore, stem cells currently available for applications in the dental clinic include dental mesenchymal cells, since the majority of dental epithelial cells disappear prior to tooth eruption (Huang et al. 2009b). Dental mesenchyme is also referred to as “ectomesenchyme,” due to its derivation from the neural crest during early embryonic development (Huang et al. 2009b).

Currently available human dental stem/progenitor cells that can be harvested for applications in regenerative dentistry include: dental pulp stem cells (DPSCs); stem

cells from human exfoliated deciduous teeth (SHED); periodontal ligament stem cells (PDLSCs); stem cells from apical papilla (SCAP); and dental follicle progenitor cells (DFPCs) (Huang et al. 2009b). These non-embryonic, postnatal cell populations exhibit mesenchymal-stem-like characteristics, including the ability to differentiate into multilineage cell populations (Huang et al. 2009b). Here we describe each of these five major dental stem cell types and their potential applications in regenerative dentistry.

3.1.1 Dental Pulp Stem Cells: DPSCs

DPSCs are a type of dental mesenchymal stem cell that can be isolated from dental pulp tissue (Alongi et al. 2010). The dental pulp is a highly vascularized and innervated tissue comprised of soft connective tissues, located in the central pulp cavity of the tooth crown and tooth roots (Ten Cate 1998). The dental pulp is encapsulated and protected by the highly mineralized dental tissues enamel, dentin, and cementum. DPSCs can proliferate and renew themselves, differentiate into odontoblasts, osteoblasts, and neurocytes both in vivo and in vitro under the right conditions (Gronthos et al. 2002), and were demonstrated to form both dentin and bone (Gronthos et al. 2000). One of the most prominent early studies of DPSCs by Gronthos and Batouli found that when seeded ex vivo onto hydroxyapatite/tricalcium phosphate (HA/TCP) and transplanted into immunocompromised mice, DPSCs showed formation of what closely resembled a dentine-pulp complex (Gronthos et al. 2000).

SHED, a type of stem cell isolated from human exfoliated deciduous teeth, possess similar osteogenic and odontogenic characters as DPSCs, but are more highly proliferative (Huang et al. 2009b). When expanded ex vivo and transplanted into immunocompromised mice, the formation of odontoblast-like cells associated with dentin-like structures was observed (Miura et al. 2003). In addition to higher proliferative and clonogenic ability, SHEDs can also differentiate into mesenchymal derivatives including neural cells and adipocytes (Miura et al. 2003). The neural-crest cell (NCC) origin of the dental pulp is reflected in the fact that SHED express neuronal and glial cell markers (Chai et al. 2000). Cultured SHED readily express neural cell markers such as βIII-tubulin, GAD, and NeuN, whose expression increases upon stimulation with neurogenic medium (Huang et al. 2009b). The neurogenic potential of SHED was highlighted by the fact that SHED survived transplantation into a mouse brain microenvironment for more than 10 days while expressing various neural markers (Miura et al. 2003). In addition, similar to BMSCs, SHED is capable of differentiating into neural-like cells after transplanted into mice brain (Azizi et al. 1998).

3.1.2 Periodontal Ligament Stem Cells: PDLSCs

PDLSCs, stem cells derived from human periodontal ligament, can be found in the PDL connective tissue and cementoblasts precursors, and are potential candidates to regenerate the soft tissue PDL, as well as cementoblasts and osteoblasts

(Sharpe 2016). PDLSCs can adopt adipogenic, chondrogenic, cardiomyogenic, and neurogenic cell fates (Huang et al. 2009b). When provided with lineage-specific cocktail during culture, PDLSCs have also been shown to exhibit hepatogenic cell differentiation by the expression of critical hepatic markers for glycogen storage, albumin and urea secretion, suggesting potential therapeutic roles for regenerating organs such as the liver (Vasanthan et al. 2016). A new subpopulation of hPDLSCs successfully harvested from the inner surface of alveolar bone sockets (a-PDLSCs) were reported to display enhanced multilineage differentiation potential (Wang et al. 2011). When compared with cells isolated from tooth root (r-PDLSCs), a-PDLSCs showed an increased ability to repair periodontal defects.

PSLCSs are studied clinically due to their potential roles in restoring diseased and damaged PDL tissues. For example, in a miniature swine model, surgical introduction of PDLSCs were shown to improve the restoration of periodontal lesions generated by surgical removed bone in the third molar (Liu et al. 2008). The PDLSCs used in this study were obtained from teeth extracted from miniature pigs and subsequently expanded ex vivo. In a mouse model, PDLSCs exhibited enhanced expression of the osteogenic markers ALP, BSP, OCN, and RUNX2, and transplantation of GelMA microgel encapsulated hPDLSCs into immunocompromised mice showed increased levels of vascularized, mineralized tissue formation (Chen et al. 2016a). In a recent randomized clinical trial, autologous PDLSCs in combination with bovine bone-derived mineralized tissue matrix were used to test guided tissue regeneration in periodontal intrabony defects (Chen et al. 2016b). Enrolled patients were randomly assigned to either a group treated with PDLSC-derived cell sheet, or a control group without stem cells. Although this study has yielded preliminary results, including that PDLSCs are safe to employ with no adverse reactions from patients in a 12-month follow-up, the efficacy of this therapy for repairing boney defects will require further validation.

3.1.3 Stem Cells of the Apical Papilla: SCAPs

The apical papilla, located apical to the dental epithelial diaphragm and next to the dental pulp (Fig. 1), is an abundant stem cell source that is highly proliferative and migratory (Rubio et al. 2005). SCAPs also have the potential of multilineage differentiation when expanded ex vivo, including odontogenic differentiation (Huang et al. 2008). In addition to expressing several growth factors including DSP, matrix extracellular phosphoglycoprotein (MEPE), FGFR3, the VEGF receptor 1 (Flt-1), and melanoma-associated glycoprotein (MUC18), all of which are also expressed in DPSCs. SCAPs express the unique marker CD24, which is downregulated in response to osteogenic stimulation (Huang et al. 2009b). While SCAPs exhibit similar characteristics to DPSCs in vitro, their distinction lies in the fact that the apical papilla is the natural precursor tissue of the radicular pulp, raising speculation that SCAPs may be a superior cell source for tissue regeneration (Huang et al. 2009b). The differentiation capacity of SCAP has been explored in various in vivo experiments in animal models, where the formation of a dentin-pulp-like complex was observed when SCAPs were transplanted into immunocompromised mice (Huang et al. 2006, 2008).

3.1.4 Follicle Stem Cells: DFCs

The dental follicle is a fibrous structure surrounding the developing tooth germ (Mantesso and Sharpe 2009), which gives rise to the PDL by differentiating into collagen secreting PDL fibroblasts that attach to adjacent alveolar jaw bone and cementum surrounding the tooth root (Mantesso and Sharpe 2009). DFCs can differentiate into PDL cells, osteoblasts and cementoblasts, although the critical factors regulating DFC differentiation remain to be determined (Zhai et al. 2019). DFCs express markers found in other stem cells, such as Notch-1 and Nestin (Morsczeck et al. 2005a; b). Transplanted DFCs are capable of differentiating into collagen-secreting PDL fibroblasts, and can interact with cementum and adjacent bone to generate functional cementum/PDL tissue in a nude mice model (Handa et al. 2002). Recently, in addition to roles in PDL tissue regeneration, DFCs combined with treated dentin matrix (TDM) formed tooth root-like structures, suggesting versatile and multidirectional differentiation potential in bio-root tissue engineering applications (Guo et al. 2012).

3.1.5 Induced Pluripotent Stem Cells: iPSCs

In 2007, Japanese scientist Shinya Yamanaka was awarded the Nobel Prize for the discovery of induced pluripotent stem cells (iPSCs), created by reprogramming mouse skin fibroblasts to their embryonic state in order to generate a putative patient-specific, autologous embryonic stem cell source that could be used to regenerate all tissues and organs (Takahashi et al. 2007). iPSCs are functionally superior to, and exhibit enhanced proliferation and differentiation potential, as compared to traditional somatic stem cells (Takahashi et al. 2007; Morsczeck 2012). Similar to ESCs, iPSCs can differentiate into all lineages, including endoderm, mesoderm, and ectoderm (Hu et al. 2018). iPSCs can be generated from both non-dental and dental cell types including DPSCs, SHEDs, PDLSCs, and SCAPs (Yan et al. 2010, Wada et al. 2011).

An exciting new model is that of epithelial cell sheets created from human urine cell derived-iPSCs (ifhU-iPSCs), which were shown to regenerate a whole tooth when transplanted into mouse subrental capsules (Cai et al. 2013). Furthermore, transgene-free iPSCs (TF-iPSCs), generated from the dental apical papilla, could provide an unlimited stem cell source (Zou et al. 2012). TF-iPSCs showed improved characteristics including better recovery after cryopreservation, a frequently encountered obstacle for both iPSCs and ESCs (Yan et al. 2010; Morsczeck 2012; Zou et al. 2012). Furthermore, TF-SCAP-iPSCs expressed neuro-makers without the need for added neurogenic differentiation stimuli (Zou et al. 2012), although this preferential neurogenic differentiation potential has not been further validated (Arthur et al. 2008; Vollner et al. 2009; Morsczeck 2012).

3.2 Cell Transplantation and Cell Homing

Stem-cell based tooth regeneration approaches primarily focus on cell transplantation and cell homing (Mao et al. 2010). Cell transplantation approaches rely on the

regenerative potentially of stem cells such as DPSCs, with or without bioengineered scaffolds. In canonical models of cell delivery, stem cells are seeded onto bio-scaffolds as a vehicle of delivery, and the cell-seeded construct is then implanted to the host with or without added growth factors (Hu et al. 2018).

In contrast, cell homing can be broadly defined as a process in which endogenous stem/progenitor cells are actively recruited to migrate to different anatomical compartments (Mao et al. 2010). Cell homing involves local stem-cell based activation approaches, and primarily relies on the recruitment of endogenous progenitor cells to migrate to, repair and restore damaged tissue (Mao et al. 2010). The cell-homing approach offers advantages such as augmenting the host's own regenerative capacity while utilizing bio-cues such as cytokines and chemokines delivered in a single surgery (Mao et al. 2010). In contrast to cell delivery, cell homing does not require the lengthy process of cell isolation and in vitro expansion. Cell homing has been used in a variety of animal models, for example, via recruitment by a biological molecules such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), or bFGF and NGF in combination with BMP7 (Kim et al. 2010). Cell-homing approaches demonstrated the regeneration of dental-pulp tissue in an ectopic implantation model (Cordeiro et al. 2008; Huang et al. 2010). In their chemotaxis-based approach, Kim et al. reported robust re-cellularization and revascularization of teeth treated via root canal in vivo (Kim et al. 2010).

3.3 Scaffold Fabrication

Biomaterials with adequate physical strengths, porosity, and bioactivity can also be used for tooth regeneration (Sharma et al. 2014). Scaffold materials can broadly be categorized into natural versus synthetic materials, with innovative and novel 3D printing approaches employed in both types of materials. Current efforts focus on designing biomaterials that replicate native organ-specific extracellular matrix (ECM) environments, in order to facilitate the micro-environmental cell-cell and cell-scaffold interactions that are required for functional tissue regeneration (Sharma et al. 2014).

Natural biomaterials such as collagen, fibrin, and hyaluronic acid (HA) offer good cellular compatibility and biocompatibility (Lee and Kurisawa 2013). Collagen scaffolds, incorporated with growth factors such as VEGF and/or PDGF, have been shown to re-cellularize and revascularize connective tissues in the root canal; however, concerns remain with respect to batch to batch fabrication variability, potential immunogenicity, and lack of precise control over pore size (Kim et al. 2010). Fibrin-based scaffold fabrication methods allow for facile manipulation of mechanical properties, physical characteristics, and cell invasion, where increased fibrinogen content can enhance the stiffness of the scaffold while reducing porosity (Sharma et al. 2014). Another study showed that injectable fibrin hydrogels of specified 3D shapes could be used as suitable and promising materials for tooth regeneration (Linnes et al. 2007). HA also offers good biocompatibility with low immunogenicity (Delmage et al. 1986). HA hydrogels have been extensively used in

regeneration studies of various organs including bone, cartilage, vocal cords, and even the brain (Sharma et al. 2014). The low mechanical strength of HA scaffolds can be overcome by the addition of stiff cross-linking chemical polymers such as alginate and RGD peptides (Lee and Kurisawa 2013).

Synthetic polymers such as polylactic acid (PLA), polyglycolic acid, (PGA) and poly(lactide-co-glycolide) (PLGA) have been broadly employed in regenerative dental applications due to their biocompatibility with a variety of dental stem cells including SHED, DPSCs, and dental pulp fibroblasts. Synthetic polymer scaffolds are highly tailorable and degrade into products that can be removed via a wide variety of metabolic pathway. However, limitations to synthetic scaffolds lie in the fact that they lack many physiological chemical components present in natural tissue ECM (Moussa and Aparicio 2019).

Both natural and synthetic scaffold materials are capable of performing basic drug delivery tasks when seeded with stem cells. However, due to the highly complex nature of human tissues and organs, we have yet to create biomaterials that are capable of enabling organ-specific, cell-cell, and cell-scaffold interactions that would be required for efficient regenerative dental therapies. Thus, there remains a strong need for novel and more advanced biomaterial fabrication methods and materials.

3.3.1 Innovative and Advanced Scaffolds

Nanotechnology has been a revolutionary force in the field of TE (Monteiro and Yelick 2017). Nanoparticles are smaller than microparticles and as such can increase the surface area per unit volume, enabling the fabrication of multiple innovative scaffolds such as composite scaffolds with nanofibrous components (Bhanja and D'Souza 2016). Nanometer-sized particles allow for increased cell migration due to increased porosity, which can also facilitate the delivery of growth factors, promote the interactions between growth factors, ECM, and cells, thereby mimicking the environments of naturally formed tissues (Bottino et al. 2017).

Recent advances have been made to more precisely control scaffold material fabrication method to facilitate applications in translational medicine and dentistry. For example, polymer nanofibers (Fig. 2) have recently emerged as new and innovative synthetic materials (Stojanov and Berlec 2020). After undergoing electrospinning and phase separation, polymer nanofibers can be modified to add growth factors, and then seeded with stem cells for tissue regeneration (Stojanov and Berlec 2020).

Advanced scaffold fabrication methods use innovative synthetic materials with exceptional abilities to mimic the native extracellular matrix (ECM), while also providing mechanical and structural support, and regulating chemical, cellular, and tissue level interactions. Novel nanofibrous spongy microspheres (NF-SMS) were used as a delivery mechanism for human DPSCs into the dental cavity (Kuang et al. 2015, 2016). These biodegradable polymer microspheres have been used to repair tissue defects based on its superior injectability. The spongy, nanoparticle structure of NF-SMS allows for self-assembly capability as well as drug delivery (Kuang et al. 2015, 2016). Dental pulp tissue formation was observed, where NF-SMS was shown

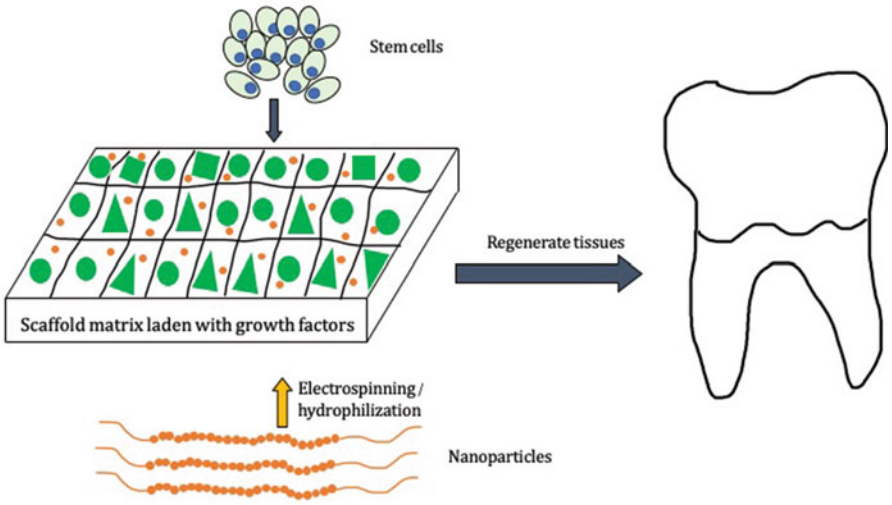

Fig. 2 Nanoparticle coated scaffold matrix stem cell delivery mechanism. Nanometer-sized particles/fibers can be incorporated into a scaffold to create a desired environment that best accommodates cell adhesion, proliferation, migration, and nutrient diffusion. Nanofibrous scaffolds provide a large surface area and high porosity, permitting efficient delivery of cells, drugs, and growth factors

to improve the attachment of and promote VEGF expression in hDPSCs cultured using 3D hypoxic conditions.

Nanostructured microspheres were also recently reported to direct growth factor release and suppress cellular inflammation while enhancing cellular differentiation (Niu et al. 2016). In addition to demonstrating growth factor-induced dentin regeneration, this group showed that microspheres could successfully deliver both hydrophobic and hydrophilic biomolecules in a controlled time-release fashion, while at the same time repressing inflammation, thereby promoting odontogenesis. More recently, nanostructured spongy microspheres demonstrated their potential as a novel delivery system to meet a variety of challenges for tooth regeneration (Bottino et al. 2017).

In addition to the whole-tooth regeneration approach, nanotechnology can also aid in the simultaneous regeneration of the complete periodontal structure including cementum, PDL, and alveolar bone (Sowmya et al. 2017). In this study, a dental follicle stem cell seeded tri-layered nanocomposite hydrogel scaffold containing tissue-specific layers to direct the formation of cementum, PDL, and alveolar bone, respectively, directed the formation of all three layers as designed, and exhibited complete defect closure and healing (Sowmya et al. 2017).

3.3.2 The Use of 3D Printing for Tooth Tissue Engineering

Three-dimensional printing is a method by which objects are fabricated by adding materials, layer by layer, in order to render a construct of predetermined 3D

volumetric structure (Derby 2012). In contrast to nonbiological-based 3D printing approach, 3D printed constructs for applications in tissue engineering and regenerative medicine and dentistry requires that 3D printed construct can be manufactured to direct cells to designated spatial positions in a highly precise manner, provide good biocompatibility and controllability, while also maintaining internal and external reproducibility and accuracy (Amrollahi et al. 2016). With respect to whole tooth tissue engineering, 3D bioprinted cell-laden hydrogels have been demonstrated as good candidates for applications in tooth tissue engineering strategies (Park et al., Biofabrication, In Press). In addition, hydrogel encapsulated MSCs for efficient ECM production can be fabricated by micro-extrusion techniques used in 3D printing (Tao et al. 2019).

Hydrogels with photo-crosslinkable polymers such as gelatin methacrylate (GelMA) have been investigated extensively for their exceptional ability to be used for cell encapsulation and for adaptability for varying conditions (Monteiro et al. 2016; Smith et al. 2017). The formation of functional and patent host-derived blood vessels was observed in in vivo implanted 3D GelMA constructs consisting of human umbilical vein endothelial cells (HUVECs) and DPSCs encapsulated within GelMA (Khayat et al. 2017) (Fig. 3). Although very promising, GelMA hydrogel scaffolds present limitations with respect to polymer concentration, where higher polymer concentrations yield better mechanical properties, while encapsulated cells demonstrate increased cell proliferation in softer, lower polymer concentration scaffolds (Tao et al. 2019). To achieve better control over scaffold stiffness while also promoting cell differentiation, thermoplastic polymers such as PCL can be printed by coextrusion to act as a frame to reinforce the softer co-printed hydrogel 3D printed constructs (Morrison et al. 2018). For example, using 3D bio-printing and bioinks such as alginate, it has been found that alginate bioink composed of 3% with a mixture of low and high alginate in 1:2 ratio showed superior performance in terms of viscosity, printability, and in vitro cell responses with optimal cell proliferation and distribution (Park et al. 2017). A limitation for the use of PCL is that its

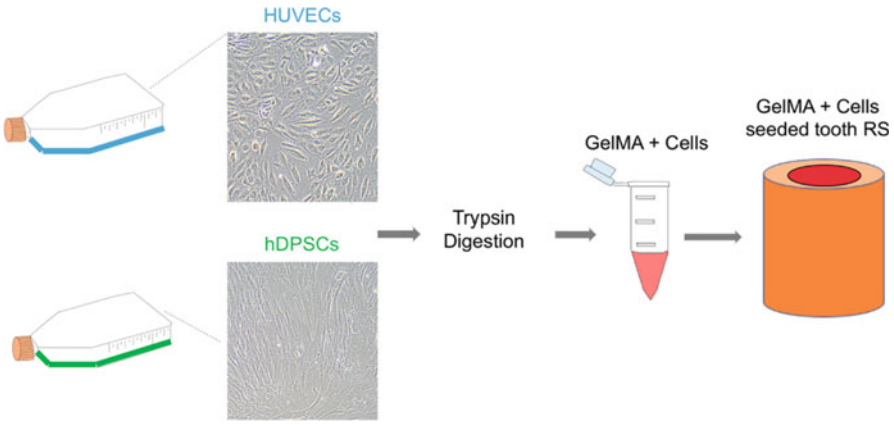

Fig. 3 Schematic of GelMA encapsulated hDPSC/HUVEC-filled tooth root segment (RS)

degradation window of 2–3 years can be a barrier to tissue formation (Tao et al. 2019).

For periodontal tissue regeneration, PCL-HA scaffolds were 3D printed using different sized microchannels to create a multiphasic scaffold (Lee et al. 2014). When seeded with hDPSCs, presumed PDL complex structures were observed. In another PDL-alveolar bone regeneration study, using an electrospun membrane on the biphasic scaffold of the periodontal compartment, scaffolds with PDL cell sheets were shown to better attach to the dentin surface as compared to those of without cell sheets (Vaquette et al. 2012).

4 Tooth Regeneration

4.1 Partial Tooth Regeneration

4.1.1 Dentin-Pulp Complex Regeneration

The dentin-pulp tissue complex is a highly organized tissue complex that offers nutrition, sensation, and defense against various pathogens (Hu et al. 2018). Irreversible pulpitis is clinically defined as vital inflamed pulp incapable of healing (Lin et al. 2020). The traditional clinical therapy for treating pulpitis is root canal therapy, which can subsequently cause tooth fragility and fractures, requiring tooth extraction (Andreasen et al. 2002). Conventional pulp chamber fillings used in endodontic treatment fail to revitalize the pulp (Ingle and Bakland 2002; Dammaschke et al. 2003). Furthermore, removal of pulp tissue results in a loss of pulpal sensation to cold/hot stimulus and the ability to detect secondary infections. (Ingle and Bakland 2002; Dammaschke et al. 2003; Caplan et al. 2005). Therefore, strategies to regenerate vital dental pulp regeneration have become the focus of many research laboratories (Kim et al. 2010). For example, using the aforementioned GelMA hydrogel seeded robust pulp-like structure has been observed in tooth root segments injected with human dental pulp stem cells (hDPSCs) and human umbilical vein endothelial cells (HUVECs) (Fig. 3).

Dentin-pulp complex regeneration strategies include scaffold-dependent stem cell therapy combined with appropriate signaling cues (Zhai et al. 2019). In addition to the aforementioned traditional scaffold materials, novel scaffold-free DPSC self-assembled spheroids have been reported (Dissanayaka et al. 2014). Using hDPSCs combined with human umbilical vein endothelial cells (HUVCEs), microtissue spheroid formation was observed in vitro (Dissanayaka et al. 2014). When microtissue spheroids were loaded within tooth root slices and implanted subcutaneously and grown in immunodeficient mice, histological analyses showed the formation of highly vascularized, dental pulp-like tissue.

Other prominent human pilot studies to regenerate dental pulp tissue include those carried out by the Nakashima group, who used mobilized hDPSCs (MDPSCs) (Nakashima et al. 2017), and the Xuan group, which used deciduous hDPSCs implanted in a randomized clinical trial (Xuan et al. 2018) (see Table 2). By using granulocyte colony-stimulating factor (G-CSF) to mobilize DPSCs harvested from

Table 2 Recent scaffold-independent tooth regeneration

Year	Author	Cell source	Regenerated tissue	Condition	Outcome
2011	(Oshima et al. 2011)	Mouse molar tooth germ cells (embryonic cells)	Whole tooth regeneration	Periodontal disease	Tooth developed periodontal tissue; alveolar bone regenerated; tooth responded to mechanical stimuli
2013	(Cai et al. 2013)	Integration-free urine-derived iPSCs (ifhU-iPSCs), mouse molar mesenchyme cells	Whole tooth regeneration/		iPSCs can functionally replace tooth germ epithelium to regenerate whole tooth-like structure
2014	(Iohara et al. 2014)	DPSCs with G-CSF (granulocyte colony-stimulating factor)	Dentin-pulp complex	Whole pulpotomy	Neurogenesis and angiogenesis
2015	(Murakami et al. 2015)	DPSCs/ BMMSCs/ ADSCs treated with G-CSF	Dentin-pulp complex	Pulpotomy	Neurogenesis and angiogenesis
2017[a]	(Nakashima et al. 2017)	DPSCs pretreated with G-CSF	Dentin-pulp complex	Pulpitis	Neurogenesis
2018[a]	(Xuan et al. 2018)	Human deciduous pulp stem cell (hDPSC)	Dentin-pulp complex	Patients with traumatized incisor tooth	Neurovascularization observed in regenerated pulp tissue with odontoblast layer and connective tissues. Incisor received implants showed functional response to stimuli

[a]Human clinical trials

discarded teeth, clinical-grade human MDPSCs were created in an isolator and expanded following good manufacturing practices (Xuan et al. 2018). All five patients involved in this clinical study had irreversible pulpits requiring pulpectomy and had undergone caries treatment including composite resin wall restoration (Xuan et al. 2018). MDPSCs were then seeded at the root position of the empty pulp chamber, followed by tooth closure and final restoration. In a 24-week follow-up,

clinical and evaluation of all five patients showed no toxicity, and electric pulp test (EPT) at week 4 demonstrated a strong positive response. Magnetic resonance imaging (MRI) of the regenerated pulp tissue in the root canal was similar to the untreated control. Dentin-formation was observed in three out of five patients, as detected by cone bean computed tomography.

In addition to stem cell-based technologies, direct pulp capping with a new commercially available biomaterial, Biodentin® (Septodont, Saint-Maur-des Fossés, France), has been made available for patients needing root canal treatment (Zafar et al. 2020). Biodentin®, a synthetic material composed of calcium silicate that provides mechanical support for native dentin tissue, also supported the growth of vital DPSCs (Kaur et al. 2017). One recent case report of patients treated with Biodentin® excellent adhesion properties to the inner dentin wall where it appeared to anchor dentin microtubules, prevent microleakage and pulpal inflammation, and exhibited excellent biocompatibility with no observed cytotoxicity or DPSC cell death (Laslami et al. 2017).

4.1.2 Periodontal Bone-PDL-Cementum Complex Regeneration

Alveolar Bone Regeneration

Periodontitis is an inflammatory oral disease induced by chronic bacterial infection of the gingiva or/and the periodontium, which if left untreated can lead to tooth loss (Kinane and Marshall 2001). Periodontitis is also associated with a strong host inflammatory response that may confer elevated risk of cardiovascular disease and premature low birth weight (Holmlund et al. 2006). Clinically, the ultimate goal of periodontal treatment is to control the infection and regain the functions of the periodontal tissues (Sculean et al. 2015).

Full restoration of normal periodontal functions includes restoring structural support and functions of the alveolar bone, periodontal ligament (PDL), and the cementum, which secures the tooth via the PDL to the surrounding alveolar bone. Conventional regeneration therapies include guided tissue regeneration (GTR) to repair cementum (Goncalves et al. 2006) and PDL complex (Needleman et al. 2006), topical application of enamel matrix derivatives to treat patients with class II furcation defects (Hoffmann et al. 2006), all of which demonstrated partial regeneration of periodontal tissues. Still, challenges remain for our ability to successfully regenerate an integrated PDL apparatus, including the simultaneous formation of the entire bone-PDL-cementum complex (Sowmya et al. 2017).

Current periodontal bone regeneration therapies utilize injectable and absorbable scaffolds including calcium phosphate cement (CPCs) pastes that can subsequently harden in situ to form a solid scaffold that can also serve as a delivery mechanism (Xu et al. 2017). Recently, a tri-culture system including human-induced pluripotent stem cell-MSC (hi-PSC-MSCs), HUVECs, and pericytes delivered via CPC scaffold (Zhang et al. 2017). This novel tri-layer constructs actively promoted both angiogenesis and osteogenesis, demonstrating promise for periodontal complex regeneration.

PDL-Cementum Complex Regeneration

The PDL complex mediates mechanical forces of mastication, which in turn regulate alveolar bone remodeling (Fuks 2008). PDL tissues house immunoglobulins that form a local defense mechanism against bacteria. In one study, regenerated cementum, produced by dental follicle-derived cementoblasts, developed into a thin layer around the root neck, covering the lower part of the root up the apex (Foster et al. 2012). Cell sheet techniques have also been used for PDL-cementum complex regeneration, based on the ability to culture PDL cells to hyper-confluency which induces the formation of a cell sheet layer rich with secreted ECM that promotes extensive cell-cell interactions (Liu et al. 2019). Tri-layered PDL cell sheets grown on thin PGA scaffolds were delivered to the exposed tooth root surfaces, and any intrabony wounds were filled with microporous β-TCP. Subsequent histometric analysis showed complete periodontal regeneration with collagen fibers connecting newly formed cementum with newly generated bone (Liu et al. 2019).

In addition to stem-cell based cell delivery techniques, endogenous cell homing of resident stem cells has shown potential for recruiting endogenous stem cells to the periodontium (Yin et al. 2017). Gene therapy-based approaches have also been explored for advantages in achieving greater biocompatibility of growth factors at the defected periodontal sites (Vhora et al. 2018). Gene therapy in oral regenerative medicine rely on the delivery of genes that direct an individual's own cells to produce a therapeutic agent while minimizing patient risk (Mitsiadis and Smith 2006). For periodontal tissue repair, gene delivery vectors can be injected directly into the periodontal defect where they can be incorporated into resident stem cells (Rios et al. 2011). Gene therapy using PDGF-B has gained attention as a future promising approach for periodontal tissue engineering through modulating the microenvironment of the defect to improve periodontal regeneration (Jin et al. 2004; Cai et al. 2013; Liu et al. 2019).

4.1.3 Tooth Root Regeneration and Bio-root Engineering

In addition to PDL and dental pulp regeneration, the tooth root is probably the most important tooth structure for tooth regeneration, since it anchors and supports the tooth crown in the mouth. Ideally, bioengineered tooth roots would exhibit similar biomechanical properties such natural tooth roots, and consist of supporting periodontal ligament-like tissues, cementum, and dentin-like matrix structure, and be capable of supporting post-crown prostheses.

SCAP and PDLSCs have been extensively investigated for their utility in engineering bio-roots. Sonoyama et al. first demonstrated that a combination of SCAP and PDLSCs could generate a bio-root with periodontal ligament tissues in miniature swine model (Huang et al. 2008). In this study, HA/TCP scaffolds seeded with autologous SCAP and PDLSCs constructs were implanted to the sockets of the swine jaws, followed by placement of a porcelain crown for stability. Subsequent analyses of harvested constructs showed the formation of PDL tissues that adhered to the surrounding bone, although the tooth root showed decreased mechanical strength and integrity over time (Huang et al. 2008).

Another recent report used a sandwich model consisting of human-treated dentin matrix (hTDM) and the growth factors VEGF1, osteoclaicin (OCN), and dentin sialophosphoprotein (DSPP) (Meng et al. 2020). In this study, the authors used Matrigel, an ECM Matrigel (references) to provide a neurogenic environment for the seeded hDPSCs (Meng et al. 2020). Implanted MSCs, combined with SHED aggregates regenerated a complete dental pulp tissue with intricate and highly organized physiological patterns (Xuan et al. 2018).

4.2 Whole Tooth Regeneration

Thanks to recent advances in bioengineering technologies, the past three decades have shown great progress towards the goal to create a fully functional bioengineered tooth that meets the desired aesthetic, functional masticatory, and physiological performance of a natural tooth. Here we will review seminal studies which have brought us closer to achieving this lofty goal.

4.2.1 Scaffold-Based Methods for Whole Tooth Regeneration

Scaffold-based tooth regeneration approaches use biodegradable and biocompatible scaffolds as a delivery mechanism for cells, to facilitate the diffusion of nutrient and metabolites (Young et al. 2002; Sharma et al. 2014; Liu et al. 2020a). Ideal scaffolds are expected to facilitate not only the delivery of cells in an organized fashion but also to facilitate infiltration of a blood supply, oxygen, and nutrition (Loh and Choong 2013; Liu et al. 2020b). In the early 2000s, collagen/gelatin sponges and polyglycolic acid/poly-L-lactate-co-glycolide copolymers (PLA/PLGA) were proposed as biodegradable scaffolds that could control the size and shape of a bioengineered tooth. However, the bioengineered teeth were very small and consisted of accurately shaped tooth crown structures without tooth roots (Young et al. 2002; Honda et al. 2010).

4.2.2 Scaffold-Free Methods for Whole Tooth Regeneration

The first scaffold-free whole tooth regeneration using a tooth organ germ method was first report by Nakao group (Nakao et al. 2007). Using single-cell suspensions generated from dental epithelial and mesenchymal tissues isolated from the E14.5 mouse incisor tooth germs, the authors created a reconstitute tooth germ that generated tooth-like structures in an in vitro organ culture, and also in the oral cavity when transplanted to a mandibular incisor tooth extraction site (Nakao et al. 2007).

Building upon on Nakao group's three-dimensional organ germ method (Nakao et al. 2007), Oshima et al. coined the term "organ germ method" to describe their newly proposed 3D approach to regenerate a functional tooth (Oshima et al. 2011, 2012). In this method, embryonic dental mesenchymal (DM) cells at high density were introduced into collagen gel to form mesenchymal cell aggregates (Hirayama et al. 2013). Next, embryonic dental epithelial (DE) cells were injected to the collagen gel, and within a day, the organ culture showed a compartmentalization between the epithelial and mesenchymal layer, and DM cell compaction as seen in

natural embryonic tooth development (Hirayama et al. 2013). This method yielded the subsequent creation of a full-sized bioengineered tooth and alveolar bone. Importantly in this model, bone remodeling occurred in response to mechanical street (Ikeda et al. 2009; Oshima et al. 2011).

For human applications, such a method would require appropriate and sufficient postnatal dental cell sources, scaling up to full-sized human teeth, and a complete recapitulation of the odontogenic developmental program, as well as the ability for the resulting structure to integrate with host vasculature and nervous systems (Monteiro and Yelick 2017).

5 Conclusions

The prospect of repairing and regenerating dental tissue holds great promise due to the possibilities provided by recent advances in tooth tissue engineering. However, for both partial- and whole tooth regeneration, several fundamental challenges remain. In terms of cell manipulation, regardless of the cell source, concerns regarding the nature of cell transplantation include the fact that allogenic and xenogeneic dental cells can cause immune rejection. In order to circumvent a deleterious immunogenic response, cell-homing method is currently being extensively studied as an alternative method that does not require cells to be transplanted. Other obstacles include the high cost of cell culture and handling, ethical concerns, and challenges in fabricating GMP grade scaffolds and materials (Mao and Prockop 2012; Yildirim et al. 2011).

Although iPSCs are envisioned to acquire such odontogenic potential via reprogramming approaches (Zhang and Chen 2014), the ground-breaking discovery of induced pluripotent stem cells (iPSCs) in 2006 and their use in dental therapy applications raise concerns with respect to tumorigenicity (Bhanja and D'Souza 2016). In addition, none of the currently available human dental postnatal mesenchymal cells can form teeth on their own – they need an available and suitable dental epithelial cell source as well. Conferring cells with odontogenic potential thus remains a challenge. Clearly, further research in stem cell biology, combined with state of the art tissue engineering techniques and strategies, will eventually facilitate methods to create reliable bioengineered teeth as a new and widely applicable therapy.

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