

Tissue Engineering of Craniofacial Tissues - A Review

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Abstract

Engineering of craniofacial tissues would have a profound impact on various treatment strategies in dentistry. The role of mesenchymal stem cells in the formation of all craniofacial and dental structures has been well demonstrated. Stem cells from various craniofacial structures like dental epithelium, dental follicle, apical papilla, exfoliated deciduous teeth, periodontal ligament and dental pulp are readily accessible and have immense potential in craniofacial research for regenerative and tissue engineering applications. Various signalling molecules that pattern the morphogenesis of craniofacial tissues have been explored. This review article epitomises how dentistry should evolve and highlights the need for close partnerships between basic and clinical scientists.

Keywords: Tissue engineering, dentistry, stem cell, dental papilla, dental follicle, dental pulp stem cells

Introduction

A cadre of craniofacial tissue engineers with interdisciplinary skills in stem cell biology, genetics and molecular biology, materials science and mechanical engineering, as well as a clinical knowledge of dental, oral and craniofacial disorders is needed to advance the field of craniofacial tissue engineering. Biologically, mesenchymal cells are primarily responsible for the formation of virtually all dental, oral and craniofacial tissues and have been demonstrated to generate these key structures. Many dental and craniofacial structures are readily accessible, thus presenting a convenient platform for biologists, bioengineers and clinicians to test tissue-engineered prototypes [1,2]. Strategically, tissue engineering technologies pioneered outside the dental community have a profound implication on dental practice.

When shifting rehabilitation strategies from prosthetic to regenerative—such as with a tissue engineering approach—one must deal with the uniqueness of the craniofacial structures in their development and function. In the fields of maxillofacial surgery and periodontics, tissue engineering has been used to generate alveolar bone [3] and to regenerate oral tissues lost to cancer, decay, and periodontitis [4]. Cultured human dental pulp and gingival fibroblasts adhere to biodegradable scaffolds, and proliferate and differentiate *in vitro* [5,6] and *in vivo* [7]. While aiming high at tadpole-style regeneration [8], a cell-based therapy could bring about the regeneration of a complete fully functional tooth composed of enamel instead of ceramic, dentin instead of composite, pulp instead of gutta-percha and root/periodontium instead of a titanium fixture. Successful bioengineering of whole tooth crowns composed of accurately formed enamel, dentin, and pulp tissues has been reported [9]. A tissue engineering approach could treat

devastating craniofacial congenital malformations, such as clefts and craniosynostosis, freeing children from the morbidity of the actual treatment [10].

Tissue engineering is generally considered to consist of three key components: (i) stem/progenitor cells, (ii) signaling molecules and (iii) a scaffold or extracellular matrix (ECM) which has been represented in **Figure 1**.

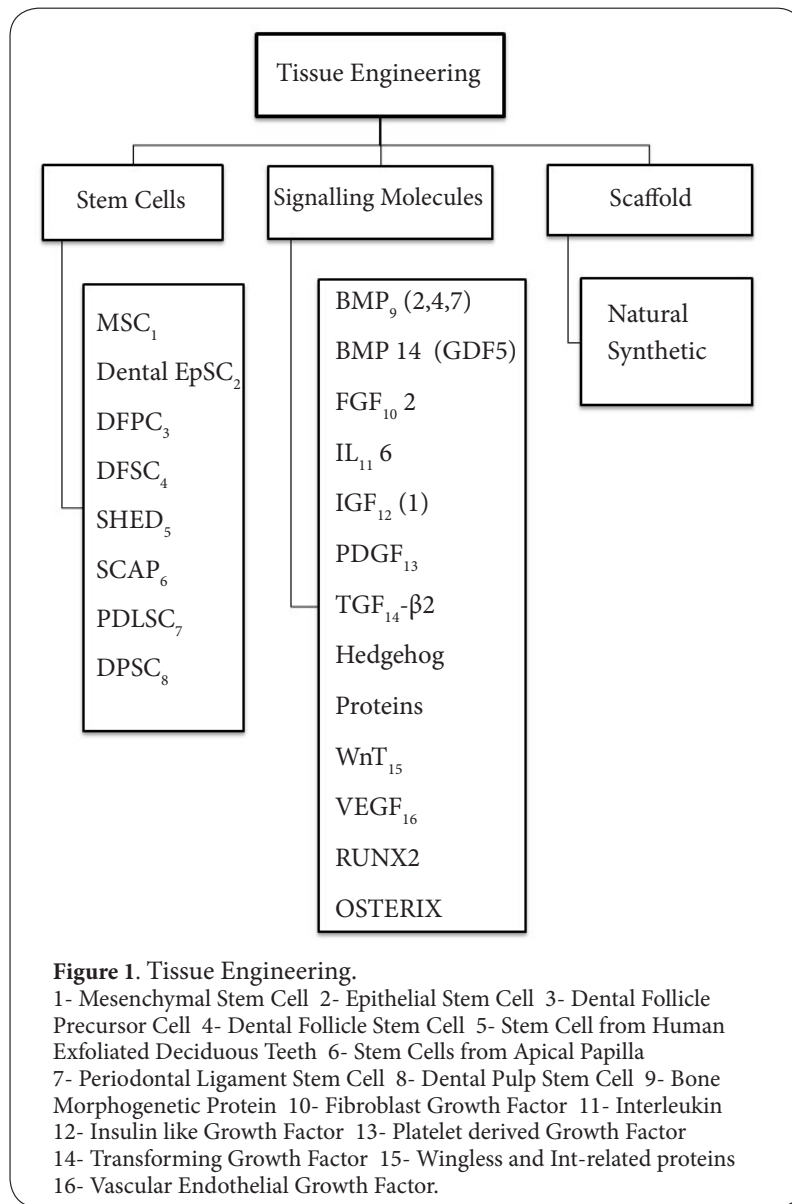
Stem cells

Mesenchymal Stem Cells (MSCs)

MSCs are self-renewable and have been experimentally differentiated into all mesenchymal or connective tissue lineages [11,12] and, in many cases, have been used to engineer the craniofacial structures that their prenatal predecessors, mesenchymal cells, are capable of generating during development.

The capacity of MSCs in the *de novo* formation and/or regeneration of craniofacial structures is too natural an endeavour to make one wonder why this potential has not been exploited substantially up until the past decade. MSCs have been reported to differentiate into hepatic [13], renal [14], cardiac [15], and neural cells [16,17].

Whether highly purified or cloned MSC populations are necessarily needed for the engineering of craniofacial structures is not clear. Stem cell populations that generate native craniofacial structures, such as the mandibular joint, are heterogeneous and likely include both mesenchymal and hematopoietic stem cells. The morphogenesis of the articular condyle requires stem cells, chondrocytes and osteoblasts in addition to angiogenesis [18]. Second, host cell invasion and stem cell homing are likely inevitable in porous biomimetic scaffolds that are used as carriers for delivering stem cells and/



or stem-cell-derived tissue-forming cells [19,20].

Dental Epithelial Stem Cells (EpSC)

The concept that teeth can be formed from primordia derived *in vitro* from non-dental cultured cells is an important step in the tissue engineering of teeth for replacement. However, this approach relies on the ability of such embryonic primordia to develop into the complete organ when transferred to the adult. The development of an embryonic primordium into a tooth in the adult jaw can be a unique idea [21].

The embryonic oral epithelium is a simple, two-cell-thick ectoderm. This epithelium can be engineered to express the appropriate signals to initiate odontogenesis to engineer a complete tooth primordium entirely from cultured cells. The identification of stem cells in dental pulp and from exfoliated

deciduous teeth also raises the possibility that a patient's own tooth cells could be used to generate new tooth primordia [22,23]. The ability to tissue-engineer an organ rudiment such as a tooth primordium constitutes a major component of a regenerative medicine procedure [24]. However, such organ primordia must be capable of developing into the complete organ *in situ*, in the appropriate site in the adult body. The renal capsule and anterior chamber of the eye are two adult sites that have been routinely used to support ectopic organ and tissue development, because they are immune-compromised and can provide an adequate blood supply to the transplanted tissue. To date, there have been no demonstrations of development of a complete organ at its normal location in the adult body following transplantation of an embryonic primordium. However, seeding cultured

tooth germ cells on bio-degradable scaffolds and then implanting them has successfully resulted in bioengineering tooth tissues [25].

Non-dental mesenchymal cells, when placed in contact with embryonic oral epithelium and transplanted to an ectopic site, generated tooth structure [26]. This report is pivotal in that it demonstrates that uncommitted MSC, in association with oral epithelium, can be instructed to mimic developmental events leading to growth of a tooth structure comprised of enamel, dentin, and pulp with a morphology resembling that of a natural tooth. These observations offer exciting opportunities for replacement of natural teeth damaged through disease or trauma and for those missing in hypodontia. There are obvious practical obstacles still to be overcome before this might be available as a routine clinical treatment, but it provides an elegant example of the translation of basic science research to the clinical arena.

The rodent incisor is a unique model for studying dental EpSC since, in contrast to human incisors or other vertebrates, this tooth grows throughout life. An EpSC niche, which is located in the apical part of the rodent incisor epithelium (cervical loop area), is responsible for a continuous enamel matrix production [27-31]. In this highly proliferative area, undifferentiated epithelial cells migrate toward the anterior part of the incisor and give rise to ameloblasts [32].

Dental EpSC can be isolated from post-natal teeth but exhibit complex problems that strongly limit their clinical application in humans. Ideally alternative sources should be easily accessible, available from adult individuals and the derived cells must have potential for enamel matrix production. The use of non-dental EpSC will only be possible with the transfer of genes, creating an odontogenic potential to non-dental epithelia prior to any association with mesenchymal cells. This is certainly one of the most exciting goals of the next decade in tooth engineering [32].

Making entire teeth with enamel and dentin structures *in vivo* is a reality and not a utopia. However, these bioengineered teeth have been produced in ectopic sites and are still missing some essential elements such as the complete root and periodontal tissues that allow correct anchoring into the alveolar bone. In a unique study proposed for growing teeth in the mouse mandible, epithelial and mesenchymal cells were sequentially seeded into a collagen gel drop and then implanted into the tooth cavity of adult mice. With this technique the presence of all dental structures such as ameloblasts, odontoblasts, dental pulp, blood vessels, crown, PDL, root and alveolar bone could be observed [33]. Thus, the implantation of these tooth germs in the mandible allowed their development, maturation and eruption indicating that stem cells could be used in the future for the replacement of missing teeth in humans.

Dental Follicle Precursor Cells (DFPCs)

Dental follicle (DF) contains progenitor cells that form the

periodontium, i.e., cementum, periodontal ligament (PDL) and alveolar bone. Precursor cells have been isolated from human DFs of impacted third molars. These cells form low numbers of adherent clonogenic colonies when released from the tissue following enzymatic digestion [34].

DFPCs express the MSC marker STRO-1 and human DFPCs have multipotential mesenchymal precursor cell properties. DFPCs differentiated toward multiple mesenchymal-derived cell types, such as cementoblasts, chondrocytes and adipocytes [35].

Cells in dental follicles (i) express markers such as Notch-1 and Nestin, suggesting the presence of undifferentiated cells, (ii) form colony forming unit-fibroblast (CFU-F) when attached onto plastic surface, (iii) show a typical fibroblast-like morphology, (iv) express Nestin, Notch-1, collagen type I, bone sialoprotein (BSP), osteocalcin (OCN), and fibroblast growth factor receptor (FGFR)1-IIIc, [34] (v) demonstrate osteogenic differentiation capacity *in vitro* after induction, (vi) express STRO-1 and bone morphogenetic protein (BMP) receptors and express cementum attachment protein (CAP) and cementum protein-23 (CP-23) [35].

After transplantation into immunocompromised mice, DFPC transplants (i) express human specific transcripts for BSP, OCN, and collagen type I, (ii) genetic expression for BSP and OCN was increased more than 100 times, (iii) genetic expression was decreased for collagen type I transcripts, (iv) dentin, cementum or bone formation was not observed in the transplant *in vivo* [36].

DFPCs can also differentiate, form robust connective tissues and produce clusters of mineralized tissue [37,38]. These connective tissues form biological diaphragms. Here, typical markers for PDL and cementum were expressed, such as collagen type XII and the CAP [37].

Dental Follicle Stem Cells (DFSC)

Most people have an impacted third molar that does not occlude and subsequently have the impacted tooth extracted to avoid inflammation or orthodontic therapy. Such extracted teeth usually contain DF and are commonly discarded as medical waste. Hence, the DF is a candidate source for isolating MSC. Recent evidence has suggested that MSCs are present in the DF of the tooth germ at various stages of development in different species [34,39-43].

Appropriately triggered DF can differentiate toward a cementoblast, osteoblast, or PDL lineage. It has been demonstrated that human DFSCs are capable of differentiating into periodontal tissues *in vivo* [34], while immortalized DFSCs isolated from mouse incisor tooth germs have the capacity to generate periodontal tissue *in vivo* [40]. Gene expression patterns of DFSCs are consistent with those of PDL fibroblasts after culturing on collagen I matrix [44]. Several key genes, including DLX3 [37], transcription factor ZBTB16 and NR4A3, have been implicated in the switch from undifferentiated status to differentiation lineage [45]. The exact mechanisms of

elaborate differentiation from stem cells to PDL, cementoblasts, and osteoblasts is expected to be elucidated in the future.

The existence of progenitor cells in bovine DF after transplantation has been reported [42]. Cultured bovine DFSCs at the root-forming stage, in combination with hydroxyapatite, successfully generated cementum tissues and fibroblasts *in vivo*. Interestingly, cementum formation was observed only at the interface of the hydroxyapatite beads in the implants. Unlike alveolar osteoblasts, DFSCs do not form bone tissue by this method. Although it is unclear whether DFSCs have the characteristics of MSC, they are clearly progenitor cells for cementoblasts [42]. Human DF progenitor cells were also reported in the DF of wisdom teeth at the root-formation stage [34]. Subsequently, mouse DFSCs at the crown-formation stage were shown to differentiate into osteogenic, adipogenic and chondrogenic lineages [46], while rat DFSCs have the capacity for osteogenesis, adipogenesis and neurogenesis but not chondrogenesis [39]. Thereafter, postnatal MSCs from human dental tissue such as DF at the apex of tooth root, PDL and dental pulp were isolated and compared to various other dental stem cells. The results showed that DFSCs have excellent proliferation rates and a capacity for osteogenesis and adipogenesis [43]; however, their capacity for chondrogenesis remains unknown.

DFSCs have been isolated from the follicle of human third molars and express the stem cell markers Notch1, STRO-1 and Nestin [34]. STRO-1 positive DFSC can differentiate into cementoblasts *in vitro* [35] and are able to form cementum *in vivo*. [42]. In addition, immortalized dental follicle cells were transplanted into immune-deficient mice and were able to recreate a new PDL-like tissue after 4 weeks [40]. It has been proved that enamel matrix derivatives (EMD) activated human dental follicle stem cells (hDFSCs) toward the cemento-blastic phenotype. hDFSCs acquired cementoblast features under stimulation of BMP-2/-7 and EMD *in vitro* [35]. The three main lineages were highly undifferentiated state of PDL-type lineage and cementoblastic or alveolar bone osteoblastic lineage. The profound cellular heterogeneity of DFSCs suggests that heterogeneous cellular constituents might play a role in tissue regeneration as much as the individual lineages might do [46].

Stem Cells from Human Exfoliated Deciduous Teeth (SHED)

The isolation of post-natal stem cells from an easily accessible source is indispensable for tissue engineering and clinical applications. The isolation of mesenchymal progenitors from the pulp of human deciduous incisors were named SHED (Stem cells from Human Exfoliated Deciduous teeth) and exhibited a high plasticity since they could differentiate into neurons, adipocytes, osteoblasts and odontoblasts [23]. The discovery of SHED sheds a light on the intriguing possibility of using DPSC (Dental Pulp Stem Cells) for tissue engineering [47,48].

The obvious advantages of these cells are (i) higher proliferation rate compared with stem cells from permanent teeth

[23], (ii) easy to be expanded *in vitro*, (iii) high plasticity since they can differentiate into neurons, adipocytes, osteoblasts and odontoblasts, and (iv) readily accessible in young patient [23], especially suitable for young patients with mixed dentition [49]. SHEDs induced bone formation and produced dentin under *in vivo* conditions; and they were able to survive and migrate in murine brain after transplantation into immune-compromised animals [23].

SHEDs are distinctive with osteoinductive ability and high plasticity. When SHEDs are seeded in porous poly L-lactic acid (PLLA) prepared within human tooth slice scaffolds and transplanted into the subcutaneous tissue of immune-deficient mice, it is observed that they differentiated into odontoblast-like cells and showed morphologic characteristics resembling those of odontoblasts. Moreover, an increase in micro-vessel density was found in the co-implantation. It is also verified that the transplanted SHEDs were capable of differentiating into blood vessels that anastomosed with the host vasculature [50]. Thus SHEDs might be an ideal resource of stem cells to repair damaged tooth structures and induce bone regeneration.

The striking feature of these cells is that they are capable of inducing recipient murine cells to differentiate into bone-forming cells, which is not a property attributed to DPSCs following transplantation *in vivo*. When single-colony-derived SHED clones were transplanted into immune-compromised mice, only one-fourth of the clones had the potential to generate ectopic dentin-like tissue equivalent to that generated by multicolony-derived SHED [23]. However, all single-colony derived SHED clones tested are capable of inducing bone formation in immune-compromised mice. While SHED could not differentiate directly into osteoblasts, they appeared to induce new bone formation by forming an osteoinductive template to recruit murine host osteogenic cells [23].

Ex vivo expanded SHED expressed STRO-1 and CD146 (MUC18), two early cell-surface markers for bone-marrow-derived MSCs [51]. In addition, SHED expressed a variety of osteoblast/odontoblastic markers including Runx2, alkaline phosphatase (ALP), matrix extracellular phosphoglycoprotein (MEPE), BSP and dentin sialophosphoprotein (DSPP). After implantation into immunocompromised mice, with hydroxyapatite/tricalcium phosphate (HA/TCP) as a carrier, SHED differentiated into odontoblast-like cells that formed small dentin-like structures. These results suggest that SHEDs are distinctive from DPSCs with respect to odontogenic differentiation and osteogenic induction [23].

SHED proliferate faster with greater population doublings than DPSCs and BMMSCs (Bone marrow derived mesenchymal stem cells) (SHED > DPSCs > BMMSCs). SHED form sphere-like clusters when cultured in neurogenic medium that either adhere to the culture dish or float freely in the culture medium, which can be dissociated by passage through needles and subsequently grown on 0.1% gelatin-coated dishes as

individual fibroblastic cells. This phenomenon suggests a high proliferative capacity analogous to that of neural stem cells [23]. In a tooth slice model (horizontal section, 1 mm thick), it was shown that SHED seeded onto synthetic scaffolds seated into the pulp chamber space and formed odontoblast-like cells that localized against the existing dentin surface [50]. Investigators have isolated SHED and termed the cells 'immature DPSCs' (IDPSCs). IDPSCs express the embryonic stem cell markers Oct 4, Nanog, stage-specific embryonic antigens (SSEA-3, SSEA-4), and tumor recognition antigens (TRA-1-60 and TRA-1-81) [52].

Stem Cells from Apical Papilla (SCAP)

Apical papilla refers to the soft tissue at the apices of developing permanent teeth [53,54]. Apical papilla is more apical to the epithelial diaphragm, and there is an apical cell-rich zone lying between the apical papilla and the pulp [55]. Human SCAP have been isolated and their potential to differentiate into odontoblasts was compared to that of the PDL stem cells (PDLSCs) [53]. SCAPs exhibit a higher proliferative rate and appear more effective than PDLSC for tooth formation. Importantly, SCAP are easily accessible since they can be isolated from human third molars.

Ex vivo expanded SCAP (i) undergo odontogenic differentiation *in vitro* (ii) express lower levels of DSPP, MEPE, transforming growth factor β receptor II (TGF β RII), FGFR3, Flt-1 (VEGF receptor 1), Flg (FGFR1), and melanoma-associated glycoprotein (MUC18) in comparison with DPSCs, (iii) express CD24 which is down-regulated in response to osteogenic stimulation (iv) demonstrate the capacity to undergo adipogenic differentiation following induction *in vitro* [53,56], (v) show positive staining for several neural markers [56] and (vi) generate a typical dentin-pulp like complex when transplanted into immunocompromised mice.

SCAP show characteristics similar to, but at the same time different from, those of DPSCs. SCAP appear to be the source of primary odontoblasts that are responsible for the formation of root dentin, whereas DPSCs are likely the source of replacement of odontoblasts that form reparative dentin. In a study with minipigs as a model, the surgical removal of the root apical papilla at an early developing stage halted root development, despite the pulp tissue being intact, whereas other roots of the tooth, containing apical papilla, maintained normal growth and development. Interestingly, only a combination of SCAPs and PDL stem cells induced the formation of a dental connective tissue, namely, the attachment of an artificial tooth crown in the alveolar bone [53].

SCAP along with PDLSCs are capable of generating a bio-root with PDL tissues. A mini-swine model was used, and the autologous SCAP and PDLSCs were loaded onto HA/TCP and gelfoam scaffolds, respectively, and implanted into sockets of the lower jaw. Three months later, the bio-root was formed in the porcine [53]. The bio-root structure was comprised of dentin randomly deposited by the SCAP. The bio-root was

encircled with PDL tissue and appeared to have a natural relationship with the surrounding bone.

Periodontal Ligament Stem Cells

PDL itself contains progenitor cells; human stem cells have been isolated from PDL. They express stemness markers such as STRO-1, CD146 and they are able to form alizarin red-positive nodules and cementoblastic/osteoblastic markers (ALP, OCN, BSP) *in vitro*. These cells have been called PDLSC. They were the first candidate stem cells for periodontal tissue engineering [57] and have the potential to form collagen fibres and generate cementum/PDL-like structures *in vivo* serving as reliable sources for periodontal tissue reconstruction [57].

In vitro expanded PDLSCs were transplanted subcutaneously into the dorsal surfaces of immunocompromised mice using HA/TCP as a carrier and showed the capacity to differentiate into cementoblasts and to form cementum/PDL-like structures [58]. PDLSCs show the capacity to form collagen fibres connecting to the cementum-like tissue; these fibres are similar to Sharpey's fibres and they suggest the potential to regenerate PDL attachment.

An alternative line of research being actively pursued in periodontal regenerative research is the possibility of utilizing stem cell implantation to enhance regenerative outcomes. The principle of this approach is the isolation of MSC from bone marrow stroma or dental tissues, expansion of cell numbers *ex vivo*, and re-implantation of cells into the wound seeded into a suitable porous scaffold material or other matrix material, including collagen matrices, β -TCP and combined β -TCP-ECM scaffolds. The suggestion that cell re-implantation could be used in this way was first raised many years ago, although only recent advances have suggested that this could be a feasible approach. A number of animal studies have now reported the isolation, *ex vivo* expansion, and reimplantation of such cells derived from bone marrow or dental sources into periodontal wounds, where they appear to have been able to contribute to the regenerative processes [57-59]. Further studies are required to determine the full potential of this therapeutic approach. Studies may need to address the potential differences between differently derived stem cell sources, such as marrow stroma, PDL, and dental pulp cells. In addition, recent studies suggest that cells may retain their embryonic positional programming, and this may have profound effects on the ability of implanted cells to contribute appropriately to tissue regeneration at heterotopic sites [60,61]. This observation may have important implications for regenerative stem cell therapies in general, but its significance remains to be investigated further.

PDLSCs implanted into nude mice generated cementum/PDL-like structures that resemble the native PDL as a thin layer of cementum that interfaced with dense collagen fibres, similar to Sharpey's fibers. After a three-week culture with an adipogenic-inductive cocktail, PDLSCs differentiated into Oilred-O-positive, lipid-laden adipocytes [58]. Upon four-week

osteo/odontogenic inductions, alizarin-red-positive nodules formed in the PDLSC cultures, similar to MSCs and DPSCs [58,62]. Thus, the PDLSCs have the potential for forming periodontal structures, including the cementum and PDL.

A study in miniature pigs [57] showed that autologous PDLSCs are capable of forming bone, cementum and PDL if they are transplanted onto HA/TCP carrier into surgical, created periodontal defects. Precursor cells from human DFs of wisdom teeth have been isolated and characterized for their periodontal regeneration potential. These cells formed *in vitro* a membranous consisting of a connective-like matrix and a mesothelium-like cellular structure with nuclei of granular calcification. It has been demonstrated that immortalized mouse dental follicle cells are able to re-generate *in vivo* a PDL-like tissue [40].

Nondental stem cells such as embryonic stem cells [63], BMMSC [64], induced pluripotent stem cells (iPS) [65], adipose-derived stem cells and cultured periosteum [66] have also been investigated for periodontal regeneration. These cells all have the potential for differentiating into osteoblasts, cementoblasts, and fibroblasts and to form cementum/PDL-like structures under appropriate conditions.

PDLSCs are also capable of differentiation to the cementoblastic lineage. The conditioned medium from developing apical tooth germ cells (APTG-CM) was shown to be able to provide a cementogenic micro-environment and induce differentiation of PDLSCs along the cementoblastic lineage. When transplanted into immunocompromised mice, the induced PDLSCs showed tissue-regenerative capacity to produce cementum/PDL-like structures, characterized by a layer of cementum-like mineralized tissues connected with PDL-like collagen fibres. This has important implications for periodontal engineering [67].

Dental Pulp Stem Cells

Although the regenerative capacity of the human dentin/pulp complex is not well-understood, it is known that, upon injury, reparative dentin is formed as a protective barrier for the pulp [68]. Accordingly, one might anticipate that dental pulp contains the dentin progenitor cells that are responsible for dentin repair. Dental pulp contains proliferating cells that are analogous to bone cells, because they express osteogenic markers and respond to many growth factors for osteo/odontogenic differentiation [69-71]. In addition, dental pulp cells are capable of forming mineral deposits with distinctive dentin-like crystalline structures [22,72,73]. DPSCs have also been isolated from extracted human third molars [22,74].

The dental pulp consists of a population of cells that contain lineage specific progenitor cells, as well as stem cells with multi-lineage differentiation capabilities [75,76]. Dental pulp tissue is expected to be a cellular source for bone tissue repair and engineering [77] because when it is cultured in osteogenic-inducing media phenotypes similar to those of osteoblasts, as represented by the production of collagen

type I, osteopontin, and BSP as well as mineralizing capability are formed [74,78].

Following physiological stimulation or injury, such as caries and operative procedures, stem cells in pulp may be mobilized to proliferate and differentiate into odontoblasts by morphogens released from the surrounding dentin matrix [79]. Tissue engineering with the triad of dental pulp progenitor/stem cells, morphogens and scaffolds may provide a useful alternative method for pulp-capping and root canal treatment [80].

Dental pulp contains highly proliferative cells that can be activated upon injury and undergo proliferation and differentiation toward osteoblastic phenotypes to provide for dentin repair. It is demonstrated that DPSCs can generate a dentin/pulp-like structure *in vivo*. When DPSCs are transplanted in conjunction with HA/TPC powder, a scaffold "odonto-conductive", into immune-compromised mice, a collagenous matrix deposit is observed after 6 weeks [22]. The dentin matrix contains a reservoir of bioactive growth factors which can mediate these processes and the functional roles of members of the TGF- β super-family in reparative dentinogenesis suggests a possible role in novel therapeutic mediators or tissue engineering solutions to dental regeneration. During dental disease or trauma, tissue damage and inflammation at sites of injury may compromise the ability of the pulpal ECM to mediate reparative events, and there may be advantages to providing a suitable matrix to encourage cell migration and differentiation at such sites. Recent studies using the tooth slice *ex vivo* culture system have demonstrated the ability of bioactive growth factor TGF-ss1 contained within alginate hydrogels, to induce odontoblast-like cell differentiation within the dentin-pulp complex, with subsequent up-regulation of dentin matrix secretion [81].

An osteo-dentin like matrix was observed two weeks after subcutaneous implantation in rabbits, when poly (lactic-co-glycolic acid) polymeric porous scaffolds grafted with DPSC were engineered. It is observed that cells formed mineralised-like structures even without the addition of any differentiation chemicals. These cells may undergo differentiation into hard tissue forming cells when provided with an appropriate substrate [82]. One important feature of pulp cells is their odontoblastic differentiation potential. Human pulp cells can be induced *in vitro* to differentiate into cells of odontoblastic phenotype, characterized by polarized cell bodies and accumulation of mineralized nodules [72,73,83]. DPSCs isolated with enzyme treatment of pulp tissues form CFU-Fs with various characteristics [22,84]. There are different cell densities of the colonies, suggesting that each cell clone may have a different growth rate, as reported for BMMSCs [75]. Within the same colony, different cell morphologies and sizes may be observed. If seeded onto dentin, some DPSCs convert into odontoblast-like cells with a polarized cell body and a cell process extending into the existing dentinal tubules [84,85].

To elucidate the self-renewal ability, investigators isolated

cells from DPSC implants at two months post-subcutaneous implantation, by enzymatic digestion and subsequent expansion *in vitro*. The isolated cells from the DPSC implants were sorted by fluorescent-activated cell-sorting (FACS) with human beta1-integrin monoclonal antibody. The isolated human cells were re-implanted into immunodeficient mice for two months. The recovered secondary implants yielded the same dentin/pulp-like structures as the primary implants. Human DSPP was expressed in dentin-like structures, confirming the human origin of the odontoblast/pulp cells in the secondary DPSC implants [86].

Signalling molecules

Various growth factors and cytokines are conjugated to the ECM, such as BMP, fibroblast growth factor-2, interleukin-6, insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), transforming growth factor- β 1, etc. [87-90]. This co-localization acts as a storage pool of growth factors and may reduce growth factor degradation [91], protecting them from the local microenvironment, while facilitating the presentation of the growth factors to cell surface receptors [92]. Several studies have reported on roles for the ECM in cell proliferation, differentiation, migration, and apoptosis through interaction with cells and cell receptors [93-97].

During development, families of protein molecules govern the signaling that patterns the morphogenesis of tissues where cells become enmeshed into an ECM. Among the identified families, fibroblast growth factors (FGFs) (potent angiogenic factors and key players in cellular proliferation and differentiation) [98-100], hedgehog proteins (fundamental regulators of animal development) [101], members of the BMP superfamily (potent molecules for bone and cartilage induction) [102], Wingless- and Int-related proteins (Wnts) (believed to promote the maintenance and proliferation of stem cell) [103], platelet-derived growth factors (PDGF) (regulators of growth, division, and angiogenesis) [104], and vascular endothelial growth factors (VEGF) (signaling proteins for the *de novo* formation of blood vessels and the growth of blood vessels from pre-existing vasculature) [105] were evaluated for their contributions to craniofacial development, and avenues are currently being explored for their value when partnered with the stem-cell-based engineering of craniofacial defects [106,107].

Among the various growth factors (GF), BMPs (mainly BMP-2 [108], BMP-4 [109], and BMP-7 [110]) have been more frequently applied in orofacial reconstruction. Since BMPs possess a well-established bone inducing effect *in vivo*, it is only recently that their effects have been exploited at the cellular level, where members of the family have been found to induce adipose-tissue-derived stem cells [111,112]. Major bone defects have been engineered by a combination of bone marrow stromal cells and BMP-7 [113], or by exclusive reliance on the growth factors [114,115].

Platelet-rich plasma (PRP), used as a growth-enhancing

substance, has been widely applied [116]. When PRP was used alone, the assorted mixture of wound-healing GFs demonstrated a solid foundation for soft-tissue healing [117], but not for hard-tissue healing, where its contribution to either alveolar bone or maxillary sinus augmentation continues to generate controversy [118]. While the GFs contained in the PRP are mitogenic, osteogenic, and angiogenic [119], the blend could lack the osteoinductive ingredient that, in the form of osteogenic cells, improved bone regeneration [120]. A controlled study in a canine model reported that the PRP/cell construct regenerated alveolar defects comparably with a cancellous autograft [121].

BMPs have been implicated in tooth development, and the expression of BMP2 is increased during the terminal differentiation of odontoblasts [122,80]. BMP2 also induces a large amount of reparative dentin on the amputated pulp *in vivo* [123]. It has been suggested that BMP2 may regulate the differentiation of pulp cells into odontoblastic lineage and stimulate reparative dentin formation [80]. After treatment with BMP2, when human pulp progenitor/stem cells with HA/TCP as a scaffold were implanted into immunocompromised mice, tubular dentin was formed [22,75]. Beads soaked in human recombinant BMP2 induce the mRNA expression of DSPP, the differentiation marker of odontoblasts after implantation onto dental papilla in organ culture. The expression of DSPP was increased later, but not that of enamelysin/MMP20 (matrix metalloproteinase) and PHEX (phosphate-regulating gene with homologies to endopeptidases on X-chromosome). The enhanced expression of enamelysin/MMP20 and Phex on day 21 in pellet cultures, compared with monolayer cultures, suggested that the differentiation of pulp cells into odontoblastic lineage was more advanced. BMP-2 and BMP-7 have been shown to stimulate regeneration of periodontal tissues, particularly bone and cementum, in a range of animal models including rodents, dogs, and non-human primates [124-127]. Larger amounts of reparative dentin formation on the amputated pulp with a BMP2-supplemented pellet compared with a control pellet. The ECM of the pellet functions as a natural scaffold, which retains and releases BMPs [128].

Treatment of monolayer cultures of bovine pulp cells with rhBMP2 significantly increased the expression of α 1(I)collagen and ALPase activity [122]. The increase in ALPase activity by rhBMP2 was higher in pellet cultures than in monolayer culture on day 14. The expression of DMP1 (dentin matrix protein), DSPP, enamelysin, and PHEX was more increased by rhBMP2 in pellet cultures than in monolayer cultures. The expression of Cbfa1 and Cbfa3 transcription factor has been reported in the dental papillae of mouse tooth germ at cap and bell stages, and later is limited in the odontoblastic layer [129].

Indeed, animal studies have demonstrated that the addition of a whole range of different factors, including BMP-2, BMP-7, GDF-5 (BMP-14), PDGF, FGFs, IGF-1 and TGF- β may be able to stimulate regeneration of the periodontal tissues [124,126,130-133].

Table 1. Functions of ECM in tissues and of scaffolds in engineered tissues.

	Functions of ECM	Architectural, biological and mechanical features of scaffolds
1	To provide structural support for cells to attach and proliferate	(i) Biomaterials with binding sites for cells; (ii) porous structure with interconnectivity for cell migration and for nutrients diffusion; (iii) biodegradation after some time upon implantation
2	Mechanical properties to support tissues	Scaffolds with adequate mechanical properties fill up the defect and simulate that of the native tissue
4	Reservoirs of growth factors	Microstructures in scaffolds retain bioactive agents i.e., growth factors in scaffold. Slow release of growth factors over a prolonged duration.
5	To provides a flexible physical environment to allow remodelling	(i) Matrix design with controllable degradation mechanisms and rates (ii) Porous microstructures for nutrients and metabolites diffusion

The possible application of GDF-5 (BMP-14) for periodontal regeneration has been tested. Although GDF-5 is a member of the BMP family and shows osteoinductive potential, it has also been shown to be able to stimulate soft tissue repair in tendons and ligaments [134,135]. Initial animal studies of GDF-5-stimulated periodontal regeneration suggest that it may be able to increase regeneration without associated ankylosis [135,136].

Pre-clinical investigations have revealed encouraging results with respect to the repair of periodontal defects with growth factors such as PDGF [137-140], BMPs [110,127], and fibroblast growth factor-2 [132,141,142]. PDGF has demonstrated the promise to regenerate bone in periodontal osseous defects, as shown in clinical trials when used alone [130,143,144] or in combination with IGF-1 [145,146]. BMPs have been shown to induce bone regeneration in peri-implant and maxillary sinus-floor augmentation procedures [147,148], extraction socket defects, [149-151] and mandibular discontinuity defects [152,153]. The use of BMPs for oral and craniofacial reconstruction has recently been reviewed [80,154].

The local regulation of cellular function during wound healing is carried out by the interplay of cytokines and growth factors. Initial granulation tissue formation and neovascularization are regulated by release of factors including the platelet-derived growth factor (PDGF), together with expression of other factors that may include vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF-β) [155,156]. The osteoblastic differentiation cascade may be of particular importance when considering regeneration of alveolar bone and the other periodontal tissues. The key factor in the initiation of osteoblast differentiation is the commitment of osteogenic stem cells to an osteoblastic lineage. Osteoblastic commitment has been known to be regulated by the action of BMPs and more recently it has become clear that the BMP signalling pathway is intimately associated with the Wnt signalling pathway. Differentiation of these committed

osteoprogenitor cells are then regulated by a range of factors that include fibroblast growth factors (FGFs) and PDGF, TGF-β, and the insulin-like growth factors, undergoing a series of amplifying divisions followed by increasing expression of genes regulating osteoblast differentiation, including the tissue-specific transcription factors runx2 and osterix [157].

Thus, physiologically, the balance between signalling molecules and their inhibitors is a key factor for the regulation of tissue homeostasis, repair, and regeneration. It is postulated that expression of growth factor inhibitors may be a limiting factor in the regenerative potential of the periodontium, particularly as the different tissue compartments (bone, PDL, gingivae) encroach on each other.

Scaffolds

During tissue engineering, cells and growth factors are combined with a porous biodegradable scaffold to repair and regenerate tissue. The scaffold acts as a temporary matrix while the cells secrete the ECM that is required for tissue regeneration. Scaffolds can be used to induce the formation of desired tissue following the in growth of cells from surrounding areas and as carriers for seeded autogeneous cells that are cultured in bioreactors and subsequently reimplanted in the patient [158]. The greatest challenges faced in tissue-engineered devices, regardless of tissue type, is promoting healing in three dimensions. Allowing angiogenesis throughout the scaffold is one of the most critical factors to the success of the scaffold.

Scaffolds have been fabricated for dental applications with a variety of natural and synthetic biomaterials, such as proteins [159], ceramics [160], metals [161], and polymers [162]. An appropriate scaffold for tissue engineering will be one that is created with biology in mind. The goal is the integration of the new tissue grown in the scaffold with the host tissue. Ideally, the scaffold provides a temporary pathway for regeneration and will degrade either during or after healing, thereby obviating the need to remove the material later and eliminating possible side effects associated with foreign materials in the body.

For tissue engineering, the ECM is required to act as a scaffold for cell attachment and to modulate cell proliferation and differentiation. The ECM is a mixture of proteins including collagens, proteoglycans, and laminins forming an elastic network surrounding most cells and tissue structures [163,164]. The composition, distribution and organization of the ECM is diverse, depending on tissue types and developmental stage [165,166]. The ECM binds to cell surface receptors, triggering changes in gene expression in the nucleus resulting in feedback mechanisms, which in turn modulate the ECM. The functions of the ECM and those of scaffolds in engineered tissues are tabulated in Table 1.

The ideal scaffold for generating new tissue should (i) be biocompatible, biodegradable and non-toxic, (ii) support the attachment, migration, proliferation and differentiation of cells and the synthesis of new matrix, (iii) have adequate

mechanical strength to withstand physiological stresses and minimise stress shielding in the surrounding host bone [167], (iv) have an integral, interconnected porous network, (v) permit neovascularisation for tissue growth. Various other properties of scaffolds like pore size, pore shape, pore-wall thickness, pore interconnectivity, pore-wall surface area, porosity, surface morphology, rate of degradation, surface chemistry, and mechanical stability affect bone healing.

Biomaterials Applied for Tissue Engineering

Over the last century, biocompatible materials such as metals, ceramics and polymers have been extensively used for surgical implantation. However, metals and ceramics are not biodegradable and their capability to be processed is very limited. Thus they cannot be used as effective scaffolds. Polymer materials have been widely used for tissue engineering because of easy control over biodegradability and processability. There are two kinds of polymer materials: synthetic polymer and naturally derived polymers [168-173]. The main biodegradable synthetic polymers include polyesters, polyanhydride, polycaprolactone, polycarbonate, polyfumarate and polyorthoester [174-179]. The polyesters such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and their copolymer of poly [lactic-co-(glycolic acid)] (PLGA) are most commonly used for tissue engineering. The naturally derived polymers include proteins of natural extracellular matrices such as collagen and glycosaminoglycan, alginate, chitosan, Matrigel and polypeptides, etc. [180-184].

The naturally derived polymer materials are biodegradable and possess known cell-binding sites. Potential disadvantages include the level of immunogenicity, the speed of degradation, and a perceived limited ability to tailor native matrix for specific properties. Matrigel, a well-known extract from the Engelbreth-Holm-Swarm sarcoma that is rich in basement membrane components [185], is widely used in basic science for the study of cell-matrix interactions and increasingly in tissue engineering, particularly in areas such as nerves [186], adipose tissue [187] and skeletal muscle [188]. Collagens synthesized by several cell types [189], are used in various formats such as gels, meshes, and sponges or in combination with slow-release gelatine microspheres as cell scaffolds for the engineering of tissues such as adipose tissue [190], blood vessels [191], and skin [192]. The mechanical strength of collagen scaffolds and the rate of absorption have been of concern [189,193], resulting in the use of cross linking agents to alter the biomedical, thermal, and mechanical properties of collagen [193]. Other extracellular matrices used as scaffolds include fibrin and fibrinogen. [187,194,195]. A recent study focusing on the development of fibrin-based matrices reported that a modified fibrin/L1Ig6 matrix could induce angiogenesis activity in human umbilical vein endothelial cells *in vitro* [196]. Studies looking at the addition of fibronectin to tissue engineering scaffolds suggest that fibronectin may be critical for scaffold vascularisation [197,198].

Chitosan exhibits a wide range of properties that make it appropriate for tissue engineering applications, namely, its biodegradability, [199-201] biocompatibility, [202-204] antibacterial activity, [205,206] wound healing properties, [207,208] and bioadhesive character [209]. One of the most important characteristics of chitosan, for tissue engineering applications, is its ability to be shaped into various structures, such as microspheres, [210] paste, [211] membranes, [212] sponges, [213-215] fibers, [216,217] and porous scaffolds [218-221]. It is a potent wound-healing accelerator, [222] possesses immunological activity, by activating macrophages, [223] produces cytokines [224] and inhibits infection [225].

Bone tissue engineering is the most important aspect of craniofacial tissue engineering. Porous scaffolds are designed to support the migration, proliferation, and differentiation of osteo-progenitor cells and aid in the organization of these cells in three dimensions. These scaffolds may be made from a wide variety of both natural and synthetic materials. Aside from autografts and allografts of cancellous and cortical bone, [226-228] naturally derived materials include cornstarch-based polymers, [229] chitosan (a polysaccharide derived from chitin, found in crab shells), [230,231] collagen, [232] and coral [233,234]. Of these materials, coral has proven to be an effective clinical alternative to autogenic and allogenic bone grafts for certain applications [235,236]. Scaffolds created from marine coral exoskeletons that are hydrothermally converted to hydroxyapatite, the mineral component of bone, are used for the repair of metaphyseal long bone cyst and tumour defects, which occur at the metaphysis of long bones. Synthetic materials include inorganic materials such as calcium phosphates [237-239] and organic materials such as poly(phosphazenes), [240] poly(tyrosine carbonates), [241] poly(caprolactones) [242], poly(propylene fumarates) [243], and poly(α -hydroxy acids) [244-247]. Composites of inorganic and organic materials have also been successfully used to create scaffolds for bone grafts [248-250].

Poly(α -hydroxy acids) are the most commonly used polymeric materials for the creation of tissue-engineering scaffolds for bone. The most common of the poly(α -hydroxy acids) are poly(glycolic acid), poly(lactic acid) (PLA), and copolymers of poly(lactic-co-glycolic acid) (PLGA). The degradation products of these materials are easily metabolized and excreted.

Hybridization of Scaffold Materials

The synthetic biodegradable polymers are easily formed into desired shapes with relatively good mechanical strength. Their periods of degradation can also be manipulated by controlling the crystallinity, molecular weight, and copolymer ratio. The scaffolds derived from synthetic polymers however lack cell-recognition signals, and their hydrophobic property hinders smooth cell seeding. In contrast, naturally derived collagen has the potential advantages of specific cell interactions and hydrophilicity, but scaffolds constructed entirely of collagen

have poor mechanical strength. Therefore, these two kinds of biodegradable polymers have been hybridized to combine the advantageous properties of both constituents [251-254].

The wettability of a polymer scaffold is considered very important for homogeneous and sufficient cell seeding in three dimensions. Because biodegradable synthetic polymers are relatively hydrophobic, it is difficult to deliver a cell suspension in a manner that uniformly distributes transplanted cells throughout their porous scaffolds. Numerous techniques have been used to evenly seed cells in PLA- and PLGA-based porous scaffolds, such as prewetting the scaffold with ethanol solution and then replacing it with distilled water and cell suspension, introducing hydrophilic poly(ethyl glycol) into the hydrophobic PLA and PLGA network, or hydrolysis [255,256]. Hybridization with collagen improved the wettability of synthetic sponges with water, which facilitated cell seeding.

Three-dimensional scaffolds for bone tissue engineering should be osteoconductive so that osteoblasts and osteoprogenitor cells can adhere, migrate, differentiate, and synthesize new bone matrix. Hydroxyapatite has frequently been used for implants, coatings, and scaffolds in bone tissue engineering [257,258]. Hydroxy-apatite particulates have been incorporated into poly (α-hydroxyl acids) to construct hybrid three-dimensional porous scaffolds of poly(α-hydroxyl acids) and hydroxyapatite. The incorporated hydroxyapatite shows a good degree of biocompatibility and osteoconductivity. The osteoconductivity of hydroxyapatite can be further improved by associating a protein matrix with the mineral structure [259]. To mimic the unique structure of collagen and hydroxyapatite in natural bone, several kinds of hybrid biomaterials of collagen and hydroxyapatite have been developed [260-262].

Nanofibrous Scaffolds

Native ECM is a hierarchically organized nanocomposite. Understanding tissue organization from the molecular to the macroscopic level will likely guide the rational design of synthetic ECM substitutes [263]. Tissue engineering scaffolds should recapitulate this hierarchical organization. The ECM is the nanofibrous protein network that surrounds cells in all tissues to support their many functions [264] and can be emulated with a nanofibrous polymer scaffold. The nanofibers can be designed to promote cell functions such as migration, adhesion, proliferation, differentiation, and tissue neogenesis [265]. The scaffold should have an internal interconnected porous network to allow for cellular integration into the scaffold among other functions. It can even be designed to release growth factors to tailor tissue development [266].

With recent advances in nanotechnology, insight into nanoscale organization is accumulating, and nanofibrous and nanocomposite scaffolds that attempt to mimic the nanoscale morphological features of natural ECM are being developed [267-269]. A recent development in calcium phosphate-based scaffolds has been the fabrication of nanofibres [270].

Nanometre-sized fibres have a large surface-area to volume ratio and can be processed so that they have high porosity. These features are advantageous because they permit the delivery of drugs or growth-factors and allow cell migration and nutrient diffusion. Nanofibrous scaffolds have been fabricated by three techniques: electrospinning, self-assembly, and phase separation. Nanofibrous scaffolds provide a cellular platform for bone formation. However, although calvarial defect models exist [271], bone regeneration research is often geared toward the engineering of long bones. Because of differing developmental origins and ossification processes of the cranial skeleton and appendicular skeleton [272], current approaches to the engineering of long bones may require some optimization to restore craniofacial bones.

Huang et al., used a nanofibrous self-assembling peptide amphiphile to support enamel formation [273]. Following injection of the peptide into a mouse incisor, the incisor was cultured in a kidney capsule for 8 weeks. Ordered crystalline hydroxyapatite was found in "pearls" near the incisor. These results suggest that self-assembling peptides could form initial stages of tooth development.

Ongoing research has begun to address the implantation of an engineered mandibular construct into a functional load-bearing model. Since both mass transport and mechanical properties depend upon 3D scaffold architecture, computational design techniques are needed to predict and ultimately optimize a microstructure to achieve the desired balance [274]. Architectural 3D scaffold design with the desired anatomical shape can be produced from image-based [275,276] or computer-aided design (CAD)-based approaches [277]. Scaffolds from these design approaches can then be built directly or indirectly by Solid Free-Form Fabrication (SFF) [277,278,274], and have been applied in craniofacial reconstruction [279,280].

The ultimate goal is to use integrated design/fabricated scaffolds for functional mandibular condyle and other craniofacial reconstruction. Integrated design/fabrication methods not only make functional reconstruction possible, but also permit the testing of design hypotheses concerning scaffold/cell carrier combinations, eventually leading to optimal reconstruction methods.

DPSCs when seeded onto a phase-separated nanofibrous PLLA scaffold, nanofibers promoted attachment and proliferation of human DPSCs *in vitro* [281]. Electrospun nanofibrous polymer scaffolds have also supported adhesion of DPSCs [282,283]. Odontogenic differentiation of DPSCs was enhanced on the phase-separated nanofibrous scaffold compared with the solid-walled scaffold *in vitro*, shown by increased alkaline phosphatase (ALP) activity, dentin marker gene expression, and calcium deposition [281]. These nanofibers promoted collagen matrix deposition, dentin sialoprotein secretion, blood vessel in-growth, and mineralization in a subcutaneous implantation study of cultured DPSC-scaffold constructs in nude mice [281]. Similar to osteogenic

differentiation, odontogenic differentiation was enhanced by nanohydroxyapatite electrospun nanofibers [284]. A mineral phase such as hydroxyapatite (HA) can also be incorporated into the nanofibrous scaffolds to form a composite bone matrix-mimicking scaffold [285]. Nano-Ha has been incorporated into a 3D phase-separated nanofibrous gelatine scaffold [286].

Studies on PDSC have shown good attachment and proliferation of periodontal ligament cells on electrospun PLGA [287] and electrospun gelatin [288] scaffolds, as well as periodontal ligament cells' osteogenic differentiation potential [287]. Furthermore, human periodontal ligament cells cultured on self-assembled peptide nanofibrous scaffolds promoted deposition of the main periodontal ligament ECM components, collagen type I and type III [159].

Strategies that involve using the scaffold itself to deliver cell growth factors such as vascular endothelial growth factor, [289] platelet-derived growth factor, [290] and recombinant human bone morphogenic protein [291,292] have been investigated to enhance and accelerate the wound healing process. A biomimetic approach to improving cell attachment has been the modification of biomaterials with the arginine-glycine-aspartic-acid (RGD)-peptide sequence [293]. Proteins with these sequences bind to cell surface receptors, facilitating the attachment of cells to substrata.

A range of materials have been suggested and employed as sustained delivery materials for growth factors [294]. These particularly include use of collagen matrices [124,295], gelatin pellets [296], alloplastic calcium phosphate materials such as β -TCP and HA [130], and resorbable polymers such as PLA [297]. All of these materials tend to result in rapid initial loss of growth factor together with a slower prolonged release of factor over several days. Furthermore, it is clear that a delivery system can also serve as a scaffold or guided tissue regeneration membrane, and that manipulation of the material's properties can alter drug release kinetics, which may in turn affect biological responses. In addition to the testing and development of rhPDGF for use in periodontal regeneration, much of the research into growth factor application for periodontal regeneration has focused on the use of BMPs.

Many biodegradable biomaterials have been used and fabricated into various shapes for tissue engineering. These scaffolds showed promising results, guiding tissue development. The processing of the chosen materials into appropriate three-dimensional scaffolds with desired shapes and pore structures will be critical. Thus, scaffolds play a pivotal role in tissue engineering and are the focus of research in regenerative medicine.

Conclusion

The last few decades have seen rapid advances in our understanding of the genetic control of cellular behaviour during embryonic tooth development, leading to the

programming and deprogramming of genes responsible for development of the dental tissues. This has provided for the foundation of knowledge by robust mechanistic data upon which we can build strategies for the tissue engineering and regeneration of teeth.

Most of the dental and craniofacial tissues are easily and readily accessible, thus presenting a convenient platform for, biologists, bioengineers, scientist and clinicians to test and apply tissue-engineered prototype. The impact of craniofacial tissue engineering extends beyond dental practice.

The optimal cell source, scaffold design, and the search for use of an appropriate multipotent or pluripotent stem cell in tissue engineering is an emerging concept. Certainly, many areas of stem cell research and their potential clinical applications are associated with controversies; therefore, it is important to address the ethical, legal, and social issues early. As more scientific knowledge will be gained from stem cell research, hopefully, some of the current ethical and technical concerns will be answered or removed in the future. Conversely, craniofacial tissue engineering could not have advanced to the current stage without the incorporation of interdisciplinary skill sets of stem cell biology, bioengineering, polymer chemistry, mechanical engineering, robotics, etc. with the ultimate goal of functional tissue restoration. Thus, craniofacial tissue engineering and regenerative dental medicine are integral components of regenerative medicine.

The scope of regenerative and tissue-engineering applications to dental science is immense; capable of bringing quantum advances in treatment strategies for patients. The need for high quality research in the basic sciences is imperative to ensure development of novel clinical treatment modalities that will revolutionise patient health care in the future.

The field of regenerative medicine is here to stay, as exemplified by the nascent but exponential growth of examples of translation from bench to bed side. The current hurdles are by no means insurmountable and, therefore, it is reasonable to assume to look forward to a more mature and highly rewarding field.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	AP	YM	PN
Research concept and design	--	--	--
Collection and/or assembly of data	√	√	√
Data analysis and interpretation	--	--	--
Writing the article	√	√	√
Critical revision of the article	√	√	√
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