Expert Opinion

Growth factor delivery for oral and periodontal tissue engineering

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The treatment of oral and periodontal diseases and associated anomalies accounts for a significant proportion of the healthcare burden, with the manifestations of these conditions being functionally and psychologically debilitating. Growth factors are critical to the development, maturation, maintenance and repair of craniofacial tissues, as they establish an extracellular environment that is conducive to cell and tissue growth. Tissue-engineering principles aim to exploit these properties in the development of biomimetic materials that can provide an appropriate microenvironment for tissue development. These materials have been constructed into devices that can be used as vehicles for delivery of cells, growth factors and DNA. In this review, different mechanisms of drug delivery are addressed in the context of novel approaches to reconstruct and engineer oral- and tooth-supporting structures, namely the periodontium and alveolar bone.

Keywords: biomimetics, gene therapy, osteogenesis, periodontal regeneration, tissue repair, wound healing

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1. Introduction

Oral diseases and oral manifestations of systemic diseases can create major functional deficits and impair patients' abilities to eat, drink and speak. The consequences of these conditions can also be profoundly debilitating aesthetically, with the resultant loss of facial symmetry and, in some instances, the ability to smile. The treatment of craniofacial hard and soft tissue anomalies accounts for a significant proportion of the total US healthcare burden, with dental caries (tooth decay) and periodontal disease (gum disease) representing two of the most prevalent diseases worldwide. In fact, tooth extraction secondary to periodontal disease, dental caries or trauma is universally one of the most commonly practiced surgical procedures [1]. Full-mouth tooth loss or complete edentulism was once a relatively common condition among middle-aged adults, but is now most prevalent in older persons, affecting approximately one-third of adults who are ≥ 65 years of age [2-4]. For those adults in the US who have teeth, it is estimated that at least 35% who are ≥ 30 years of age possess periodontal disease, with 21.8% having a mild form and 12.6% having a moderate or severe form [5]. Additionally, by the age of 17, > 80% of the adolescent population is affected by dental caries [5,6].

The standard treatment for edentulism involves prosthetic replacements, such as full or partial dentures, or dental implants [7]. Although these prostheses can initially restore limited function and can produce satisfactory results, because these prostheses are artificial, over time, individuals often continue to be faced with aesthetic and functional challenges relative to the fit and stability of these devices [8]. Even though continuing efforts to prevent tooth loss exist, there is a need to re-examine the current methods of tooth replacement and to pursue evolving tissue-engineering strategies and technologies that will enable the development of

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biological materials to regenerate teeth and tooth-supporting structures (e.g., the periodontium).

In examining craniofacial disease beyond the oral cavity, oral and pharyngeal cancer is the sixth most common cancer in the developed world. Each year, an estimated 28,900 Americans are diagnosed with this form of cancer and > 7400 die each year as a result of it [6]. Radiation therapy is often employed as a treatment for these individuals, but frequently aggressive resective surgery is also adjunctively performed to remove remaining affected tissues. Even if radiation treatment and resective surgery are successful in eradicating all damaged and diseased cells and tissues, the affected area is often left functionally compromised. Although prosthetic replacements and plastic reparative procedures are often performed in the reconstructive phases of therapy, regenerative outcomes are often undesirable relative to the restoration of function and aesthetics.

Craniofacial deformities are among the most common of all birth defects, with cleft lip and cleft palate among the more common birth defects in the US, occurring in ~ 1 to 2 of 1000 births. Adding to the burden of treatment of the condition itself is the burden of the lifetime cost of treating children born with cleft lip or cleft palate, which is estimated to be US$697 million [9]. Surgery, dental care, psychological counselling and rehabilitation may help ameliorate the physical and social problems, but these also pose a tremendous financial cost to individuals and caregivers. Innovative advances in molecular biology are providing great insight on the function of genes that are important in forming the craniofacial structures. This information may ultimately build the necessary foundation for developing better treatments.

The science of tissue engineering is a multidisciplinary field that aims to restore and regenerate tissues and organs that have been compromised, damaged or lost [10]. Clinical success is highly dependent on the development of new naturally derived materials and synthetic biomimetic scaffolds that offer renewed hope for the treatment of craniofacial hard and soft tissue defects and disorders that are secondary to disease or injury. This review addresses the fundamental process of wound healing and the important growth factors involved, the development of biomaterials that are capable of delivering these growth factors to regenerative sites, and novel tissue-engineering approaches for oral and periodontal tissue engineering and regenerative medicine.

2. Growth factors for wound repair

Understanding the complex processes of wound healing has been a challenge for researchers for many years. Recently, advances in the areas of cellular and molecular biology have allowed the elucidation of functions of growth factors and their participation in the different phases of wound healing. Restoration of normal form and function is the ultimate goal of regenerative approaches for tissues disrupted by trauma, surgical resection or infectious disease. However, if the functional integrity of the tissue is not achieved, the process of repair will take place and a fibrous tissue will replace the original tissue [11]. Recent *in vitro* and *in vivo* studies have confirmed that growth factors can improve the capacity of tissues to regenerate, improving cellular chemoattraction, differentiation and proliferation. Growth factors are natural biological mediators that regulate important cellular events involved in tissue repair by binding to specific cell surface receptors [12]. After reaching specific target cells, growth factors induce intracellular signalling pathways, which result in the activation of genes that change cellular activity and phenotype [13]. However, the effect of each growth factor is regulated through a complex system of feedback loops that involve other growth factors, enzymes and binding proteins [14,15]. The study of many different growth factors and other cytokines, each one with several functions during the different phases of wound healing, have been performed in order to define their proper application for therapeutic purposes [14,15] (Table 1).

Healing of osseous tissue is regulated by growth factors and other cytokines in a sequence of overlapping events similar to cutaneous wound repair. In ideal circumstances, this process mimics embryonic bone development, allowing the replacement of damaged bone with new bone, rather than with fibrous scar tissue. This process is driven by cellular and molecular mechanisms controlled by the TGF-β superfamily of genes, which encodes a large number of extracellular signalling growth factors [16]. Bone morphogenetic proteins (BMPs) are a well-studied group of these growth factors involved in the processes of bone healing; and the human genome encodes at least 20 of these multifunctional polypeptides [17]. Among several of its functions, BMPs induce the formation of both bone and cartilage by stimulating the cellular events of mesenchymal progenitor cells. However, only a subset of BMPs, most notably BMP-2, -4, -7 and -9, have osteoinductive activity, a property of inducing *de novo* bone formation by themselves [18]. Studies involving mutations of BMP ligands, receptors and signalling proteins have shown important roles of BMPs in embryonic and postnatal development. Severe skeletal deformation, development of osteoporosis, reduction in bone mineral density and bone volume are all aberrations associated with disrupted and dysregulated BMP signalling [19,20]. Further extraskeletal effects of BMPs have been examined, with mice deficient for BMP-2, -4 or -7 having severe organ deficiencies and dying shortly after birth [21-23].

Several other growth factors produced by osteogenic cells, platelets and inflammatory cells participate in bone healing, including IGF-1 and -2, TGF-β_1, PDGF and FGF-2 [24]. The bone matrix serves as a reservoir for these growth factors and BMPs, and are activated during matrix resorption by matrix metalloproteases [25,26]. Additionally, the acidic environment that develops during the inflammatory process leads to activation of latent growth factors [27], which assist in the chemoattraction, migration, proliferation and differentiation of mesenchymal cells into osteoblasts or chondroblasts [27]. All of these functions are driven by a complex mechanism of

Table 1. Effects of growth factors in the different phases of wound healing.

Wound-healing phase	Growth factor	Cell of origin	Functions
Inflammatory	PDGF	Platelets	Increases chemotaxis of neutrophils and monocytes
	TGF-β	Platelets leukocytes, fibroblasts	Increases chemotaxis of neutrophils and monocytes Autocrine expression: generation of additional cytokines (TNF-α, IL-1β, PDGF and chemokines)
	VEGF	Platelets, leukocytes, fibroblasts	Increases vascular permeability
Proliferative	EGF	Macrophages, mesenchymal cells, platelets	Stimulates epithelial proliferation and migration
	FGF-2	Macrophages, endothelial cells	Stimulates fibroblasts proliferation and ECM synthesis Increases chemotaxis, proliferation and differentiation of endothelial cells
	KGF (FGF-7)	Keratinocytes, fibroblasts	Stimulates epithelial proliferation and migration
	PDGF	Macrophages, endothelial cells	Stimulates fibroblast proliferation and ECM synthesis Increases chemotaxis, proliferation and differentiation of endothelial cells
	TGF-β	Macrophages, leukocytes, fibroblasts	Stimulates epithelial proliferation and migration Stimulates fibroblasts proliferation and ECM synthesis Inhibits proteases and enhances inhibitor production
	VEGF	Macrophages	Increases chemotaxis of endothelial progenitor cells Stimulates endothelial cell proliferation
Bone remodelling matrix synthesis	BMPs 2 – 4	Osteoblasts	Stimulates mesenchymal progenitor cell migration
	BMP-7	Osteoblasts	Stimulates osteoblast and chondroblast differentiation
	FGF-2	Macrophages, endothelial cells	Stimulates mesenchymal progenitor cell migration
	IGF-2	Macrophages, fibroblasts	Stimulates osteoblast proliferation and bone matrix synthesis
	PDGF	Macrophages	Stimulates differentiation of fibroblasts into myofibroblasts Stimulates proliferation of mesenchymal progenitor cells
	TGF-β	Fibroblasts, osteoblasts	Induces endothelial cell and fibroblast apoptosis Induces differentiation of fibroblasts into myofibroblasts Stimulates chemotaxis and survival of osteoblasts
	VEGF	Macrophages	Chemotaxis of mesenchymal stem cells, antiapoptotic effect on the bone-forming cells, angiogenesis promotion

BMP: Bone morphogenetic protein; ECM: Extracellular matrix; EGF: Epidermal growth factor; FGF: Fibroblast growth factor; IGF: Insulin-like growth factor; KGF: Keratinocyte growth factor; PDGF: Platelet-derived growth factor; TGF: Transforming growth factor; VEGF: Vascular endothelial growth factor.
Information from [11,154-156].

interaction among growth factors and other cytokines, which are influenced by several regulatory factors [28].

3. Approaches for growth factor delivery

A key variable for successful tissue regeneration and engineering is the environment in which cells and tissues grow. The concepts of guided tissue regeneration (GTR) and guided bone regeneration aim to optimise this variable, in that different membranes are used in these procedures as barriers. These barriers support the ingrowth of more favourable tissue regenerative cells (i.e., periodontal ligament [PDL] cells, bone cells), whilst selectively excluding non-desirable cells (i.e., gingival epithelial cells, connective tissue cells) from reconstruction sites [29]. These membranes also support the establishment of other critical parameters that are paramount to successful periodontal and bone regeneration, including: tenting and isolation (adaptation of the membrane to ensure adequate space will be maintained for tissue regeneration); scaffolding (the membrane serves as a matrix-enabling organisation of tissue progenitor cells and newly forming vasculature); and stabilisation (the membrane protects the area from being mobilised during initial phases of healing and regeneration) [30-34]. Both resorbable and non-resorbable membranes have been used for these applications with comparable success, although a key advantage in the use of resorbable materials is that the need for re-entry into the regenerated site is eliminated [35,36]. Membranes derived from natural compounds (i.e., collagen) are degraded by an enzymatic process (biodegradation), catalysed by cells in the local microenvironment and within

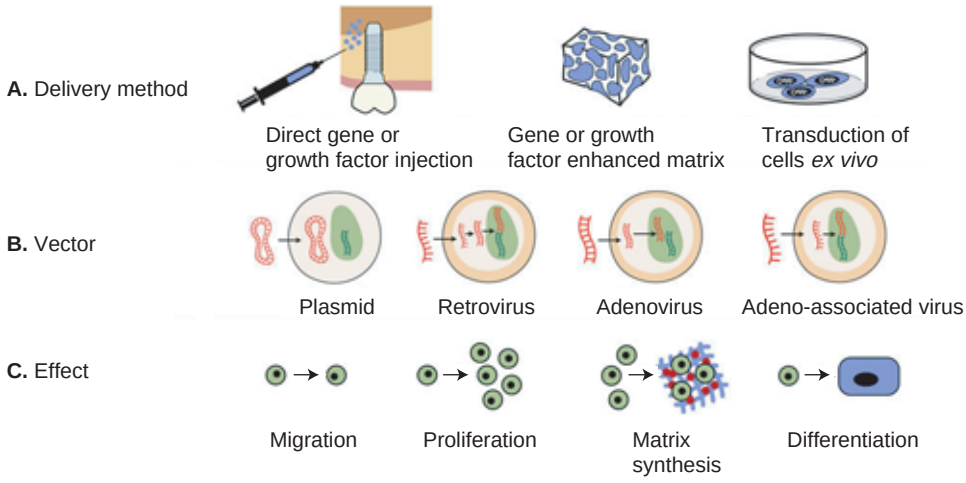

Figure 1. Gene therapy approaches. A. DNA and growth factors can be delivered to cells through different mechanisms, including direct injection to an *in vivo* site, transport to a site via a carrier matrix, or introduced *ex vivo* prior to cell transplantation. **B.** Genetic material can be transferred into cells using different vectors, the most common of which are plasmids, retroviruses, adenoviruses and adeno-associated viruses. **C.** Growth factor delivery by gene therapy strategies aim to modulate cell proliferation, migration, matrix synthesis and differentiation.

the developing tissue [37,38], but synthetic polymers (i.e., derivatives of polylactic and polyglycolic acid) are hydrolysed (bioabsorption) into the natural metabolites lactic acid and glycolic acid [39,40]. The polylactic scaffolds are popularly employed due to familiarity with their favourable biocompatible properties, which helps minimise an undesirable inflammatory response. In addition, the development of both naturally derived and synthetic materials allows the degradation properties to be controlled by the composition and ratios of compounds that comprise the material. A key advantage of this, particularly with more recently developed polymers, is that these properties can be tailored to meet the specific needs of the application with regard to how long one desires to have the material maintained [34,41-43].

The development and maintenance of an appropriate environment for tissue regeneration and engineering is critical not only for GTR and guided bone regeneration approaches, but also in the use of gene, protein and cell therapy approaches. Thus, these tissue-engineering strategies involve the use of different polymer systems to serve as synthetic extracellular matrices. In these strategies, polymers serve as delivery depots not only for growth factors and DNA molecules, but also for cells. Specifically, cells can be delivered after *ex vivo* expansion to be combined with biomaterials to deliver cells, but also as a scaffold template to allow cells to continually proliferate and differentiate into new tissues (Figure 1). Successful regeneration and engineering of a wide variety of oral structures have been demonstrated with this cell transplantation approach, and these tissues include bone [44,45], PDL [46], oral mucosa [47], skin [48] and teeth [49]. Cells can also be genetically modified *ex vivo* prior to implantation, creating a combined gene therapy–cell transplantation approach [50].

Two common types of polymeric materials used in growth factor delivery strategies are natural collagen-derived materials [37,38,51] and synthetic polymers of lactic and glycolic acid (i.e., poly[lactide-co-glycolide]) [40,52]. Extracellular matrix-derived macromolecules such as collagen have been used for many years in biomaterial application [53], and it is now possible to create artificial analogues of extracellular matrix proteins using recombinant DNA technology [54]. Collagen is degraded by cells within regenerating tissue as it develops, and biodegradable synthetic polymers such as poly(lactide-co-glycolide) are hydrolysed into natural metabolites, lactic acid and glycolic acid by the action of water at regenerated sites. The poly(lactide-co-glycolide) scaffolds are popularly employed due to familiarity with their functional properties and uses in other applications (e.g., biodegradable sutures). Additionally, growth factors and DNA can be incorporated into these materials and released in a controlled, sustained manner to enhance tissue regeneration [44,55].

A variety of new injectable materials such as hydrogels are also being developed for growth factor delivery applications and have been of special interest [56,57]. These injectables are especially attractive because, in clinical application, they can allow for minimally invasive delivery of inductive molecules. A great deal of insight has been gained in evaluating the *in vivo* performance of engineered biomaterials consisting of polysaccharides. As an example, alginate hydrogels bearing cell-adhesion ligands have been used as scaffolds for cell

encapsulation and transplantation, and have yielded promising results in experiments directed toward the engineering of bone tissue [58]. An alternative to synthesising polymers composed of these natural components is the synthesis of biomimetic polymers. These polymers combine the information content and multifunctional character of natural materials with the tailorability (ability to impart appropriate mechanical properties) of synthetic polymers. This hybrid concept has been used in the binding of polymers with specific amino acids (such as the tri-peptide sequence RGD) that are capable of regulating cell adhesion [59,60].

Another area of increasing attention has been the development of shape-memory materials that have one shape at one temperature and another shape at a different temperature [61,62]. These materials have the ability to memorise a permanent shape that can be substantially different from an initial temporary shape. As an example, a bulky device could potentially be introduced into a surgical site as a temporary shape (such as a string or freely flowing material), penetrate through a small area of the site, and then be expanded in response to different cues into a permanent shape (i.e., a stent or a sheet). The response signals that stimulate the changes in shape in response to environmental cues are incorporated within the material during its fabrication. These materials have been designated as 'smart' materials, having the ability to appropriately change their structural and functional material properties in response to environmental cues [63]. These materials have also demonstrated great promise and the ability to control their built-in signalling is what makes them attractive for growth factor delivery strategies.

4. Growth factor applications for oral and periodontal tissue engineering

Therapeutic application of growth factors to restore damaged tissues aims at regeneration through biomimetic processes, or mimicking the processes that occur during embryonic and post-natal development [15]. The complexity of these events suggests that creating an optimal regenerative environment requires the combination of different growth factors as found in natural reparative processes. The use of a single recombinant growth factor may also induce several molecular, biochemical and morphological cascades that will result in tissue regeneration [14].

A number of studies (Table 2) have evaluated the application of recombinant growth factors alone or associated with other growth factors or biomaterials for regeneration of different oral tissues, including maxillary/mandibular bone [15], salivary glands [64], dentin–pulp complexes [65,66] and periodontal structures [67]. In the periodontium, regenerative treatment has been a challenge due to morphological and functional specificities of each component of tooth-supporting tissues. The most studied growth factors for periodontal regeneration have been PDGF, IGF, FGF-2, TGF-β and different BMPs.

PDGF was the first growth factor to be evaluated in preclinical periodontal and peri-implant regenerative studies. Proliferation, migration and matrix synthesis were observed on cultures of periodontal cells stimulated by PDGF, including gingival and PDL fibroblasts, cementoblasts, preosteoblasts and osteoblastic cells [68-73]. These effects were shown to be time- and dose dependent [73]. The PDGF family is composed of four growth factors: PDGF-A, -B, and the most recently discovered PDGF-C and -D [74]. All of these participate in the wound-healing process, but, until now, only the three isoforms PDGF-AA, BB and AB were evaluated in periodontal therapy. PDGF-BB is the most effective on PDL cell mitogenesis and matrix biosynthesis [75,76]. Several preclinical studies were performed using the combination of PDGF-BB and IGF-I for periodontal and peri-implant bone regeneration [77-82], culminating in the first study in humans using growth factors for periodontal regeneration [83]. In a human Phase I/II clinical trial, PDGF/IGF-I were considered safe when applied topically to periodontal osseous lesions, resulting in a significant improvement in bone growth and fill of periodontal defects, compared with standard therapy [83]. The evaluation of PDGF alone was demonstrated in two preclinical studies in dogs. Alveolar bone defects of critical size were completely regenerated after treatment with PDGF-BB associated to GTR [84,85]. The results were superior to the same treatment without PDGF. The authors concluded that PDGF stimulated formation of fibrous connective tissue in an early stage of repair, filling and stabilising the wound. In a subsequent regenerative stage, the fibrous tissue was substituted with new bone and PDL [84]. This conclusion is supported by another study that shows enhanced fibroblast proliferation in an early periodontal wound healing, after treatment of alveolar bone defects in dogs with PDGF [86]. PDGF-BB alone was evaluated in humans in three recent studies [87-89]. The first two studies [87,88] evaluated PDGF-BB associated with demineralised freeze-dried bone allograft in the treatment of different types of critical size periodontal bone defects. Histological evidence of periodontal regeneration was provided by these proof-of-principle studies. The most recent study [89], a large multi-centre Phase III clinical trial, evaluated the benefits of recombinant human PDGF-BB (rhPDGR-BB) associated with synthetic β-tricalcium phosphate in the treatment of periodontal bone defects in 180 patients. The study demonstrated that the use of rhPDGF-BB is safe and improves bone fill and attachment of gingival tissue to root surfaces of involved teeth.

The angiogenic and fibroblast stimulatory properties of FGF-2 during wound healing and its chemotactic and proliferative effects on PDL cells [90,91] suggest its use for periodontal regenerative therapeutic approaches. In preclinical studies, this growth factor was evaluated for the treatment of different types of periodontal bone defects, in dogs [92-95] and non-human primates [96]. Despite different concentrations of FGF-2 and different delivery systems used in the studies, all

Table 2. Preclinical and clinical studies using growth factors in oral and periodontal healing.

Growth factor	Pre-clinical studies	Clinical studies
BMP-2	Periodontal defects: stimulated alveolar bone regeneration [17,101,103-105,107-110,157-159] Mandibular bone defects: induced new bone growth [106,116,160] Sinus floor: stimulated bone augmentation [114,161] Peri-implant defects: stimulated bone regeneration [106,111-113,115] Dentin–pulp complex: stimulation of dentin regeneration [65]	Alveolar bone: induced bone ridge augmentation [119] Sinus floor: induced bone formation [117,120] Peri-implant bone defects: accelerated bone regeneration [118]
BMP-7	Periodontal furcation defects: stimulated significant cementum and bone regeneration [121-123] Peri-implant defects: stimulated bone regeneration [124,125] Sinus floor: similar to bone grafts in bone augmentation [126] Dentin–pulp complex: stimulation of dentin regeneration [65]	Sinus floor augmentation: variable amount of bone regeneration after implantation in three patients [127]
BMP-12	Periodontal defects: new PDL with improved fibre orientation when compared with BMP-2 [129]	
FGF-2	Furcation and intrabony periodontal defects: improved periodontal regeneration (new bone, cementum and PDL) [92-95]	
PDGF	Periodontal furcation and fenestration defects: accelerated healing and improved PDL and bone regeneration [84-86]	Furcation and intrabony defects: histological evidence of periodontal regeneration in proof-of-principle studies [87,88]; improved clinical attachment level gain and radiographic bone filling [89]
PDGF plus IGF	Periodontal defects: improved periodontal regeneration (new bone, cementum and PDL) [78-81] Peri-implant defects: improved bone regeneration [77,82]	Periodontal defects: improved bone growth and filling of defects [83]
TGF-β	Periodontal defects: insignificant improvement in cementum formation; conflicting results regarding alveolar bone regeneration [98-100] Dentin–pulp complex: results for dentin regeneration [65]	

BMP: Bone morphogenetic protein; FGF: Fibroblast growth factor; IGF: Insulin-like growth factor; PDGF: Platelet-derived growth factor; PDL: Periodontal ligament; TGF: Transforming growth factor.

showed an improvement in the periodontal tissue regeneration, compared with control groups. Studies that evaluated more than one concentration of FGF-2 suggested that its effects are dose dependent [92,96].

The involvement of TGF-β in several important functions during wound healing makes it an important growth factor in this process and suggests its use for therapeutic approaches. However, TGF-β can control gene expression either positively or negatively, a factor that can interfere with its therapeutic use [97]. TGF-β_1, the most abundant isoform of the TGF-β family and found primarily in the platelets and osseous tissue, has been used for this application. The results of rhTGF-β_1 for periodontal regeneration have not been consistent preclinically as shown in canine and ovine investigations [98-100]. Two studies in supra-alveolar critical bony defects in dogs showed little advantages in new bone formation and no improvement in cementum regeneration when treated with rhTGF-β_1 with or without GTR [98,99]. In another application, TGF-β_1 was demonstrated to increase the amount of bone healing adjacent to dental implants in minipigs [97]. The limited number of studies and inconsistent results suggest that more study is needed to confirm the beneficial effects of rhTGF-β1 on periodontal regeneration.

An important group of proteins for therapeutic applications, of the well-studied TGF-β superfamily, is the BMPs. BMPs -2, -4, -7 and -12 have all been evaluated for periodontal and peri-implant bone regeneration [67]. From this group of proteins, BMP-2 has been the most studied for bone and periodontal regenerative treatment [101-106].

Several preclinical studies demonstrate significant improvement of alveolar bone regeneration in different types of periodontal defects after treatment with rhBMP-2 via different carriers. Cementum regeneration was also stimulated by rhBMP-2, although not significantly in some reports [104,107,108]. Ankylosis was also occasionally reported to affect periodontal regeneration associated with rhBMP-2 treatment [102,103,109]. Ankylosis (fusion of the tooth and the alveolar bone) is more frequent in the cervical area of the exposed root and seems to be associated with rapid osteogenesis, especially in submerged tooth models. However, a recent study demonstrated a tendency of resolution of the ankylosis over time when evaluated 12 weeks after treatment [110].

Another important therapeutic application of BMPs is for maxillary bone regeneration to allow replacement of lost teeth by osseointegrated dental implants. This approach involves the regeneration of peri-implant bone after implant fixation or bone height improvement in areas below the maxillary sinus. Preclinical [111-116] and clinical [117-120] studies have shown improved bone formation after treatment with BMP-2. However, the use of different carriers and the association of barrier membrane (GTR technique) or other biomaterials seem to be critical factors in influencing the therapeutic outcome.

BMP-7 or osteogenic protein-1 is a potent modulator of osteogenesis and bone cell differentiation [121]. Its effect in periodontal-regenerative treatment was evaluated in bony defects around tooth roots in preclinical studies [121-123]. Significant improvement on bone and cementum regeneration was observed in dogs [121]. An extensive cementogenesis was considered the most significant effect of BMP-7 in bony defects in baboons [122,123]. Improvement of bone formation around titanium implants was also demonstrated by studies in animals [124,125], although no significant benefit was observed when rhBMP-7 was compared with bone grafts for maxillary bony defects in preclinical [126] and pilot human clinical studies [127].

The potential of BMP-12 to repair tendon and PDL tissues has been shown *in vitro* and *in vivo* studies [128]. A recent preliminary study in dogs compared rhBMP-12 with rhBMP-2 for the treatment of periodontal defects [129]. The results showed less bone and more functionally oriented PDL between the new bone and new cementum after BMP-12 treatment, contrasting with a more parallel fibre arrangement than BMP-2-treated defects.

The results from preclinical and initial clinical studies using growth factors are encouraging; however, some limitations exist with respect to bone volume and predictability. Although *in vitro* studies have elucidated the role of growth factors in the cellular events of the different type of cells, several factors may influence the results *in vivo*. Limitations that restrict optimal responses indicate growth factor delivery included the short half-life of growth factors after being delivered *in vivo*. This may be due to proteolytic degradation, rapid diffusion and the solubility of delivery vehicles in chronic periodontal wounds [12].

Enamel matrix derivative (EMD), an alternative approach for periodontal regeneration, uses proteins that are derived from embryonic enamel matrix with the purpose of mimicking the specific events that occur during the development of the periodontal tissues [130]. EMD uses propylene glycol alginate as a vehicle in a viscous formulation. After coating the tooth root close to the periodontal defect, the propylene glycol alginate viscosity is reduced under physiological conditions and facilitates EMD release and precipitation [131]. Recent systematic reviews were published on the efficacy of this product in the treatment of periodontal bony defects supporting its use [132-134].

5. Next generation approaches for periodontal tissue engineering

The development of new delivery devices has improved the efficacy of growth factor delivery *in vivo*. Bioabsorbable controlled-release scaffolds have been fabricated to sequester growth factors and release them at optimal doses in a timely manner depending on the biological demand of the target tissue [135]. Several studies have shown release of growth factors for up to 15 days when associated with poly(lactate-coglycolide) scaffold-containing microspheres [136,137].

However, even with optimal scaffolds, the local application of growth factors often requires a large amount of protein to stimulate significant effects *in vivo*, which increases the risk of unwanted side effects [138]. An alternative approach using gene transfer may have the advantage of transferring into specific cells with specific promoters, using appropriate vectors, to attain a sustained gene expression and more efficient way of delivering *in vivo* [67]. A method of treating salivary gland functional deficiencies makes use of this gene therapy approach. The aim of this method is to drive existing non-secretory ductal epithelial cells into secretory cells capable of fluid movement, and success in animal models has been demonstrated [139,140].

6. Gene delivery for alveolar bone engineering

The improvement in the knowledge of the genetic and cellular mechanisms of human diseases allowed the development of a new therapeutic approach for congenital and acquired diseases via gene therapy. This new clinical strategy can be defined as an introduction of specific genetic changes by homologous vector sequences [141]. Although initially designed to permanently correct a single gene in monogenetic disorders, gene therapy methods have included modification or elimination of malignant cells, modulation of host defences and re-engineering of diseased organs or tissues. With this approach is the potential to genetically modify the cells to express the required growth factors for bone regeneration and, more specifically, periodontal regeneration [142] (Figure 2).

A vector is a carrier that helps to circumvent the natural barriers to DNA internalisation to the cell nucleus, where it can use the cellular machinery to express the exogenous gene [143]. In general, gene delivery can be approached by using viral or non-viral vectors. Viral vectors consist of viruses that are transformed into gene-delivery vehicles by replacing part of their genome with a therapeutic gene. Usually, the gene of interest is subcloned in a plasmid under the control of the cytomegalovirus promoter before recombination with the viral backbone DNA. The substitution of the original gene promoter has the purpose of enhancing transcription efficiency [144]. The most commonly employed vectors are retrovirus, lentivirus, adenovirus or adeno-associated virus.

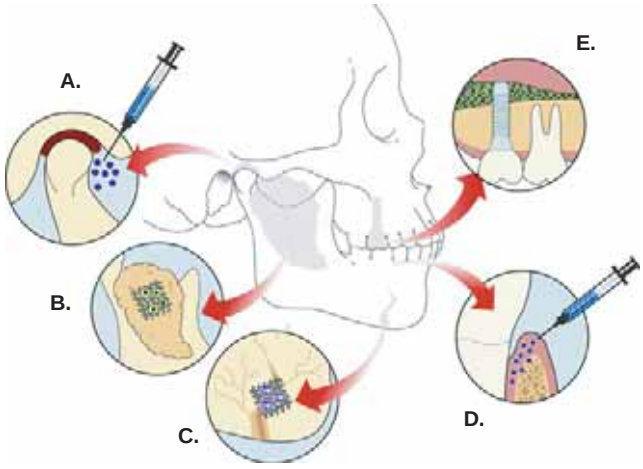

Figure 2. Specific examples of different strategies for tissue engineering craniofacial tissues. A. Growth factor delivery via an injectable system to stimulate regeneration of the cartilaginous articular disc of the temporomandibular joint. **B.** Cell transplantation of salivary gland cells on polymer scaffolds to restore a functionally deficient salivary gland. **C.** Combined approach of cell transplantation and growth factor delivery for bone regeneration using a growth factor-releasing scaffold as a cell carrier for osteo-progenitor cells. **D.** Gene therapy approach employed to regenerate the periodontium of a periodontal defect resulting from periodontal disease. **E.** Cell therapy approach using bone precursor cells as an autogenous bone graft to enable the placement of dental implants in an area that is commonly deficient in bone (below the maxillary sinus).

Each of these viral vectors has characteristics that make it more or less appropriate for specific applications. Retrovirus, lentivirus and adeno-associated virus have their genomes integrated into host chromosomes and are more indicated for applications requiring persistent and stable genetic changes [145]. Adenovirus (Ad) instead persists in a non-integrated form and produces a more transient transgene expression, which is not an issue for some applications, such as bone regeneration. Ad is also characterised by a high transduction efficiency and, although it has been the most-used vector, has the disadvantage of eliciting an immunological response. In general, safety is the primary concern and safety issues include not only immunogenical reactions, but also the risk of conversion of a non-replicative vector to a wild-type virion and tumorigenesis [146].

Non-viral vectors include plasmid DNA and synthetic vectors that consist of complexes of plasmid DNA with cationic lipids and polymers, known as lipoplexes and polyplexes, respectively. Although they present improved safety and are more easily manufactured than viral vectors, they have low gene-transfer efficiency and, in some cases, toxicity and *in vivo* instability [147].

The application of gene therapy for tissue engineering has proved to be effective and has extended to multiple areas of medicine. Ad has been largely employed for this purpose as it is non-integrating and is a relatively safe virus, whilst inducing high levels of transient gene expression and transduction of multiple cell types. In the oral complex, gene therapy has been evaluated in the regenerative treatment of bony anomalies, salivary gland injuries, dental pulp repair and periodontal diseases [142]. Table 3 displays recent applications of gene therapy in oral and craniofacial disorders.

In periodontology, the role of PDGF in periodontal regeneration has been discussed in the context of bone [148] and other tissues [58], highlighting the potential of PDGF gene delivery for periodontal engineering. The initial studies evaluated the ability of Ad-PDGF-A to affect cells derived from the periodontia [149]. Osteoblasts, PDL fibroblasts, gingival fibroblasts and cementoblasts displayed effective expression of the *PDGF-A* gene for up to 7 days following gene delivery, which resulted in enhanced mitogenic and proliferative responses in these cells [149]. Dermal fibroblasts also presented prolonged signalling events and downregulation of $PDGF_{\alpha}R$ up to 96 h after Ad-PDGF-A delivery [150].

Simulating a clinical condition, a three-dimensional *ex vivo* wound-healing model used human gingival fibroblast cultures to evaluate the effects of gene transfer of Ad-PDGF-A and Ad-PDGF-B on cell repopulation and wound fill [50]. The expression of PDGF genes was prolonged for up to 10 days. Ad-PDGF-B resulted in a twofold increased rate of defect fill and fourfold greater cell densities inside the defect than Ad-PDGF-A or control groups. The upregulation of genes associated with PDGF signalling (PI_3 kinase) and fibroblast migration (integrin α_5) suggested modulation of cellular and molecular events by Ad-PDGF-B therapy [50].

An early evaluation of gene therapy for periodontal regeneration *in vivo* used *ex vivo* gene transfer in alveolar bone wounds in rats [151]. Syngeneic dermal fibroblasts were transduced *ex vivo* with Ad-BMP-7, seeded onto gelatin carriers, then transplanted to mandibular alveolar bone defects in a wound-repair model. The treatment stimulated periodontal wound healing, including bone, PDL and cementum. However, the *ex vivo* gene transfer has the limitations of cell procurement issues and the need for an additional surgical procedure for biopsy harvest. To overcome these disadvantages, an *in vivo* viral gene delivery approach was evaluated [152]. A collagen matrix containing Ad-PDGF-B was applied in a similar osseous defect model. Localised transgene expression was observed for up to 3 weeks, resulting in proliferative and regenerative effects on periodontal cells. A fourfold increase in bridging bone and a sixfold increase in cementum repair was observed in the Ad-PDGF-B-treated sites in comparison to controls [152].

Another approach using *in vivo* gene delivery evaluated bone regeneration after the treatment of large defects surrounding dental implants using Ad-BMP-7 that were associated with a collagen matrix [153]. The treatment resulted in enhancement of alveolar bone defect fill, coronal new bone formation and new bone-to-implant contact.

The initial preclinical studies with gene delivery point to the feasibility of using gene therapy for periodontal tissue

Table 3. Gene therapy studies for craniofacial applications.

Tissue	Vector	Purpose	Ref.
Cranium	Ad–BMP-2	Bone regeneration (craniofacial defects)	[138,162]
	Ad–BMP-2 and -9	Bone regeneration (mandible)	[163]
	Ad–VEGF	Angiogenesis: tissue ischaemia	[164]
	Ad–BMP-7	Repair of skull defects	[165]
	Retro–BMP-4 plus VEGF	Repair of skull defects	[166]
Salivary gland	Ad–aquaporin-1 (Ad-AQP1)	Stimulate salivary secretion	[139,140,167]
	AAV–IL-10	Autoimmune epithelialitis (Sjögren's syndrome)	[168,169]
	Liposome/plasmid complex: 'gene cocktail'	Reduce levels of superoxide radicals and hydrogen peroxide (protection against irradiation damage)	[170]
Tooth pulp	GDF 11	Stimulate dentin production (pulp capping)	[67,171,172]
	Ad–BMP-7	Same purpose	[173]
TMJ	Ad–*Lac*Z reporter	TMJ articular surface targeting	[174]
Periodontal tissues	Ad–PDGF-B	Periodontal regeneration	[175]
	Ad–BMP-7	Periodontal regeneration	[50]
Peri-implant bone	Ad–BMP-7	Bone regeneration	[153]

AAV: Adeno-associated virus; BMP: Bone morphogenetic protein; Ad: Adenovirus; GDF: Growth/differentiation factor; *Lac*Z: β-Galactosidade/*Lac*Z; Platelet-derived growth factor; Retro: Retrovirus; TMJ: Temporomandibular joint; VEGF: Vascular endothelial growth factor.

engineering. Future studies need to be conducted to better evaluate the advantages and disadvantages of different vectors and elucidate safety concerns.

7. Expert opinion

Major advances have been made over the past decade in the reconstruction of complex periodontal and alveolar bone wounds that have resulted from disease or injury. For future growth and development of the field over the next 5 – 10 years, several key areas will need to be addressed to promote greater predictability of oral tissue-engineering procedures. These include the following: i) improved delivery methods (including use of natural, polymeric and ceramic biomaterials); ii) consideration of dual and multi-factor release; iii) anatomical defect reconstruction three-dimensionally; and iv) combined efforts to deliver anti-infective molecules with regenerative growth factors. Developments in scaffolding matrices for cell, protein and gene delivery have demonstrated significant potential to provide 'smart' biomaterials that can interact with the matrix, cells and bioactive factors. The targeting of signalling molecules or growth factors (via proteins or genes) to craniofacial structures and periodontia has led to significant new knowledge generation using factors that promote cell replication, differentiation, matrix biosynthesis and angiogenesis. A major challenge that needs to be addressed in order to take oral tissue engineering to the next level is the modulation of the exuberant host response to microbial contamination of the periodontal and oral wound microenvironment. For improvements in the outcome of oral and periodontal regenerative medicine, scientists will need to examine dual delivery of host modifiers or anti-infective agents to optimise the results of therapy. Further advancements in the field will continue to rely heavily on multidisciplinary approaches combining engineering, dentistry, medicine and infectious disease specialists in repairing the complex periodontal wound environment.

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